SHORT REPORT

ACG publications

Rec. Nat. Prod. 16:4 (2022) 404-408

records of natural products

A New Isoflavan Glucoside from the Roots of *Astragalus membranaceus* var. *mongholicus*

Yadong Wang [1], Chao Wang [2], Yuanhao Wang [*2], Qing Hu [*2] and Fenghuan Wang [1]

[1]*School of Light Industry, Beijing Technology and Business University, Beijing 100048, China*
[2]*SUSTech Engineering Innovation Center, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China*

(Received September 09, 2021; Revised October 18, 2021; Accepted October 24, 2021)

Abstract: A new isoflavan glucoside, namely astramemside A (**1**), together with six known compounds (**2**–**7**) were obtained from the roots of a traditional Chinese medicine, *Astragalus membranaceus* var. *mongholicus*. Their structures were elucidated by spectroscopic analyses (HRESIMS, UV, IR, 1D and 2D NMR), and the absolute configuration of **1** was determined by combination of chemical transformation and single-crystal X-ray diffraction. Compound **1** showed moderate inhibition on nitric oxide (NO) production induced by lipopolysaccharide in RAW264.7 cells with an IC_{50} value of 38.98 ± 5.28 μM.

Keywords: *Astragalus membranaceus* var. *mongholicus*; Leguminosae; isoflavan glucoside.

1. Plant Source

In this phytochemical study of the roots of *Astragalus membranaceus* var. *mongholicus* (Leguminosae) (Plant materials see supporting information), a new isoflavan glucoside, astramemside A (**1**), together with six known compounds (**2**–**7**) were isolated. Herein, we report the isolation and structural elucidation of these compounds.

2. Previous Studies

Astragalus membranaceus var. mongholicus, also known as “Huang-Qi” in traditional Chinese medicine (TCM), is a perennial herb widely distributed in North, Northeast, and Northwest China [1]. Its roots have long been used in TCM for various purposes, such as antiperspirant, antimicrobial, anti-inflammatory, diuretic and tonic, etc [2]. Particularly, the extract of this plant can enhance immune system of human [3]. Previous studies on chemical composition of this species showed that isoflavonoids and triterpene saponins were major constituents [4-10]. Some of these constituents

* Corresponding author: E- Mail: wangyh2020@mail.sustech.edu.cn (Y. Wang); huq@sustech.edu.cn (Q. Hu)

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products July-August 2022 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.292.2109.2203
Available online: November 8, 2021

exhibited diverse pharmacological properties such as triglyceride accumulation inhibitory and anti-inflammatory activities [4,11].

3. Present Study

The EtOAc fraction of *A. membranaceus var. mongholicus* was separated by repeated column chromatography over silica gel, reversed phase C_{18} (RP-C_{18}), LH-20 gel, and finally HPLC to obtain compounds **1**–**7** (Figure 1) (detailed separation process see supporting information).

*Astramemside A (**1**):* white amorphous powder, $[\alpha]^{25}_{D}$ –30.8 (*c* 0.2, MeCN); UV (MeCN) λ_{max} (log ε) 280 (2.87), 206 (4.11) nm; IR (KBr) ν_{max} 3395, 2921, 2850, 1712, 1619, 1505, 1464, 1171, 1095 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 555.1838 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_{11}Na^+$, 555.1837).

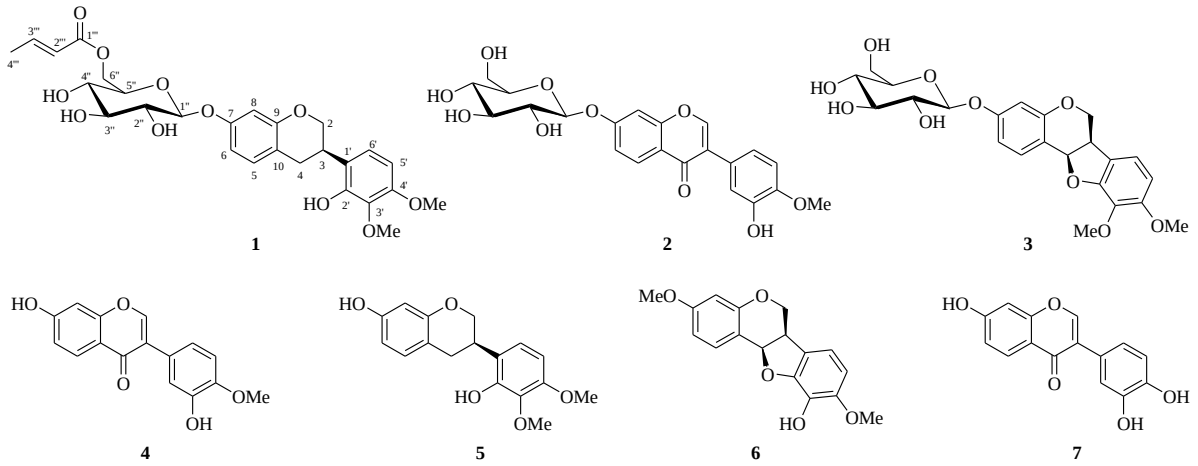

Figure 1. Structures of compounds **1**–**7**

Compound **1**, white amorphous powder, displayed a negative optical rotation ($[\alpha]^{25}_{D}$ –30.8, in MeCN). Its molecular formula, $C_{27}H_{32}O_{11}$, was established by the HRESIMS ion peak at *m*/z 555.1838 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_{11}Na^+$, 555.1837). In the IR spectrum, the absorption bands of hydroxy (3395 cm^{-1}), carbonyl group (1712 cm^{-1}) and aromatic ring (1619, 1505 and 1464 cm^{-1}) were observed. The ^{1}H NMR spectroscopic data (Table 1) displayed signals for a 1,3,4-trisubstituted benzene ring [δ_H 6.97 (1H, d, J = 8.3 Hz, H-5), 6.59 (1H, dd, J = 8.3, 2.5 Hz, H-6) and 6.56 (1H, d, J = 2.5 Hz, H-8)], a 1,2,3,4-tetrasubstituted benzene ring [δ_H 6.77 (1H, d, J = 8.7 Hz, H-6′) and 6.46 (1H, d, J = 8.7 Hz, H-5′)], two *trans*-olefinic protons [δ_H 7.03 (1H, dq, J = 15.6, 6.9 Hz, H-3‴) and 5.90 (1H, dd, J = 15.6, 1.6 Hz, H-2‴)], two methoxy groups [δ_H 3.81 (3H, s, 4′-OMe) and 3.79 (3H, s, 3′-OMe)], two oxygenated methylenes [δ_H 4.51 (1H, dd, J = 11.8, 2.1 Hz, H-6″a), 4.22 (1H, dd, J = 11.8, 7.5 Hz, H-6″b), 4.27 (1H, brd, J = 9.9 Hz, H-2a) and 4.02 (1H, t, J = 9.9 Hz, H-2b)], five oxygenated methines [δ_H 4.81 (1H, d, J = 7.5 Hz, H-1″), 3.66 (1H, m, H-5″), 3.46 (1H, m, H-3″), 3.45 (1H, m, H-2″) and 3.35 (1H, m, H-4″)], a methyl group [δ_H 1.85 (3H, dd, J = 6.9, 1.6 Hz, Me-4‴)]. The ^{13}C NMR spectrum of **1** showed 27 carbon signals, which can be classified by DEPT and HSQC spectra as a carbonyl (δ_C 168.0), two benzene rings (δ_C 158.3, 156.4, 153.2, 149.6, 137.6, 131.1, 122.9, 122.3, 117.8, 110.6, 105.6 and 104.4), a disubstituted double bond (δ_C 146.9 and 123.2), a hexosyl group (δ_C 102.5, 77.9, 75.5, 74.9, 71.9 and 64.7), two methoxy groups (δ_C 61.6 and 56.3), two sp^3 methylenes (one oxygenated at δ_C 71.1), a sp^3 methine and a methyl. The aforementioned spectroscopic data suggested that compound **1** was an isoflavan glucoside.

The 2D structure of **1** was determined by analysis of its 2D NMR data (Figure S1). The ^{1}H–^{1}H COSY correlations of H_2-2/H-3/H_2-4, H-5/H-6, H-5′/H-6′, in combination with the HMBC correlations of H_2-2/C-9; H_2-4/C-5, C-9 and C-10; H-5/C-7 and C-9; H-8/C-6; H-5′/C-3′; H-6′/C-3, C-

2′ and C-4′ demonstrated the existence of 7,2',3',4'-tetrahydroxyisoflavan moiety. The HMBC correlations from 3′-OMe (δ_H 3.79) to C-3′ and from 4′-OMe (δ_H 3.81) to C-4′ suggested that the methoxy groups were attached to C-3′ and C-4′, respectively. The hexosyl group was determined to be a β glucose by comparison of its 1D NMR data and coupling constant ($J_{1''/2''}$ = 7.5) with reported data [12]. This glucosyl was located at C-7 of the isoflavan moiety as indicated by the HMBC correlation from the anomeric proton (δ_H 4.81, H-1′′) to the C-7 (δ_C 158.3). The ^{1}H–^{1}H COSY correlations of H-2′′′/H-3′′′/H$_3$-4′′′ and HMBC correlation of H-3′′′/C-1′′′ suggested the presence of a (*E*)-but-2-enoyl group, and this group was linked to 6′′-OH by HMBC from H$_2$-6′′ to C-1′′′. Thus, the planar structure of compound **1** was determined.

The absolute configuration of **1** was confirmed by chemical transformation. The alkaline hydrolysis of **1** yielded a known product, (3*R*)-(–)-7,2′-dihydroxy-3′,4′-dimethylisoflavan-7-*O*-β-D-glucopyranoside (**1a**), whose absolute configuration was determined by single-crystal X-ray diffraction [with a Flack parameter of 0.05(5)] (Figure 2). Thus, the structure of compound **1** was established as depicted and named astramemside A.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** (δ in ppm) in CD$_3$OD

No.	δ_H (*J* in Hz)	δ_C	No.	δ_H (*J* in Hz)	δ_C
2a	4.27, brd (9.9)	71.1	5′	6.46, d (8.7)	104.4
2b	4.02, t (9.9)		6′	6.77, d (8.7)	122.9
3	3.48, m	33.4	1′′	4.81, d (7.5)	102.5
4	a 3.00, dd (15.8, 10.4)	31.2	2′′	3.45, m	74.9
	b 2.86, dd (15.8, 4.5)		3′′	3.46, m	77.9
5	6.97, d (8.3)	131.1	4′′	3.35, m	71.9
6	6.59, dd (8.3, 2.5)	110.6	5′′	3.66, m	75.5
7		158.3	6′′a	4.51, dd (11.8, 2.1)	64.7
8	6.56, d (2.5)	105.6	6′′b	4.22, dd (11.8, 7.5)	
9		156.4	1′′′		168.0
10		117.8	2′′′	5.90, dd (15.6, 1.6)	123.2
1′		122.3	3′′′	7.03, dq (15.6, 6.9)	146.9
2′		149.6	4′′′	1.85, dd, (6.9, 1.6)	18.2
3′		137.6	3′-OMe	3.79, s	61.1
4′		153.2	4′-OMe	3.81, s	56.3

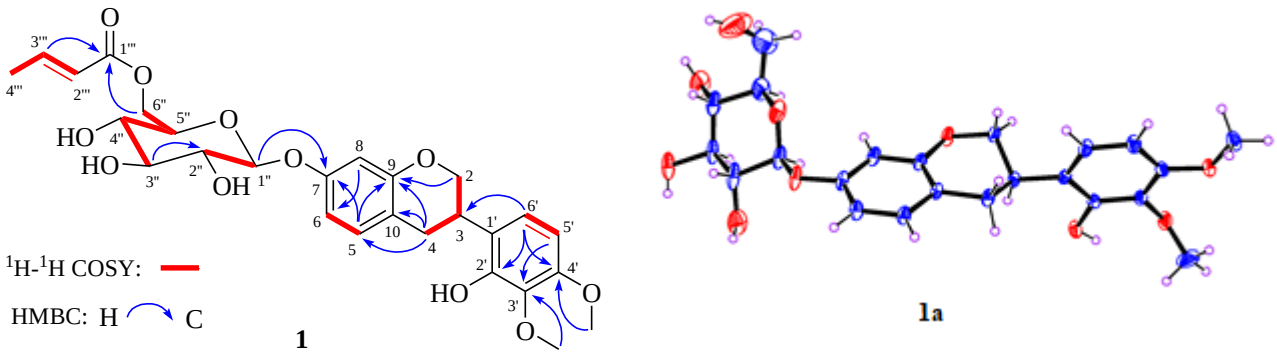

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1** (left); X-ray structure of **1a** (right)

The known compounds were identified as 7-*O*-*β*-D-glucopyranosyl 7,3′-dihydroxy-4′-methoxy isoflavone (**2**) [13], (6a*R*,11a*R*)-9,10-dimethoxypterocarpan-3-*O*-*β*-D-glucoside (**3**) [14], calycosin (**4**) [15], isomucronulatol (**5**) [16], (6a*R*, 11a*R*)-10-hydroxy-3,9-dimethoxypterocarpan (**6**) [17], and 3′-hydroxydaidzein (**7**) [18], by comparison of their spectroscopic data with the published data.

Most of these co-isolated known compounds have been previously reported to possess inhibitory activity on the nitric oxide (NO) production. Among them, compounds **2** and **3** showed strong inhibitory activity, with with IC_{50} values of 4.10 ± 0.10 and 14.70 ± 0.90 μM, respectively [19]. Compound **4** had moderate inhibitory activity (IC_{50} = 39.56 ± 2.43 μM), while compound **7** was inactive (IC_{50} > 100 μM [20]. The new compound **1** was tested for its inhibitory effect on the NO production induced by lipopolysaccharide in RAW264.7 cells. Quercetin was used as a positive control (IC_{50} = 17.86 ± 2.13 μM). The result showed that compound **1** showed moderate inhibitory activity (IC_{50} = 38.98 ± 5.28 μM).

Acknowledgments

This work was financially supported by the National Key R&D Program of China (grant number 2020YFC1712700).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Yadong Wang: 0000-0002-8815-6397
Chao Wang: 0000-0002-6671-7050
Yuanhao Wang: 0000-0003-4135-7359
Qing Hu: 0000-0002-6532-9718
Fenghuan Wang: 0000-0003-2498-3673

References

[1] Editor Committee for Flora of China of Chinese Academy of Science (1997). Science Publishing House, Beijing. PP. 133.

[2] X. Li, L. Qu, Y. Dong, L. Han, E. Liu, S. Fang, Y. Zhang and Tao Wang (2014). A review of recent research progress on the Astragalus genus, *Molecules* **19**, 18850-18880.

[3] X. Du, B. Zhao, J. Li, X. Cao, M. Diao, H. Feng, X. Chen, Z. Chen and X. Zeng (2012). *Astragalus* polysaccharides enhance immune responses of HBV DNA vaccination via promoting the dendritic cell maturation and suppressing Treg frequency in mice, *Int. Immunopharmacol.* **14**, 463-470.

[4] Y. Zhang, X. Li, J. Ruan, T. Wang, Y. Dong, J. Hao, E. Liu, L. Han, X. Gao and T. Wang (2016). Oleanane type saponins from the stems of *Astragalus membranaceus* (Fisch.) Bge. *var. mongholicus* (Bge.) Hsiao, *Fitoterapia* **109**, 99-105.

[5] Y. Pei, R. Li, H. Fu, J. Wang and Y. Zhou (2007). A new isoflavone glucoside from *Astragalus membranaceus* var. *mongholicus*, *Fitoterapia* **78**, 602-604.

[6] L. M. Xiao, P. H. Cao, Z. H. Luo, X. F. Bao, Z. Q. Zhou, S. Li, S. S. Zhao, J. X. Zheng, H. Gao and H. Zhi (2020). Cycloartane-type triterpenoids from the root of *Astragalus membranaceus* var. *Mongholicus*, *J. Asian Nat. Prod. Res.* **22**, 905-913.

[7] Z. Q. He and J. A. Findlay (1991). Constituents of *Astragalus membranaceus*, *J. Nat. Prod.* **54**, 810-815.

[8] Q. H. Wang, N. R. C. K. T. Han, N. Y. T. Dai, X. L. Wang and W. L. J. Ao (2014). Anti-inflammatory effects and structure elucidation of two new compounds from *Astragalus membranaceus* (Fisch) Bge. var. *mongholicus* (Bge) Hsiao, *J. Mol. Struct.* **1074**, 284-288.

[9] X. Du, Y. Bai, H. Liang, Z. Wang, Y. Zhao, Q. Zhang and L. Huang (2006). Solvent effect in ^{1}H NMR spectra of 3′-hydroxy-4′-methoxy isoflavonoids from *Astragalus membranaceus* var. *mongholicus*, *Magn. Reson. Chem.* **44**, 708-712.

[10] Q. T. Yu, P. Li, Z. M. Bi, J. Luo and X. D. Gao (2007). Two new saponins from the aerial part of *Astragalus membranaceus* var. *mongholicus*, *Chin. Chem. Lett.* **18**, 554-556.

[11] D. Y. Lee, H. J. Noh, J. Choi, K. H. Lee, M. H Lee, J. H. Lee, Y. Hong, S. E. Lee, S. Y. Kim and G. S. Kim (2013). Anti-inflammatory cycloartane-type saponins of *Astragalus membranaceus*, *Molecules* **18**, 3725-3732.

[12] L. J. Zhang, H. K. Liu, P. C. Hsiao, L. M. Y. Kuo, I. J. Lee, T. S. Wu, W. F. Chiou and Y. H. Kuo (2011). New isoflavonoid glycosides and related constituents from Astragali Radix (*Astragalus membranaceus*) and their inhibitory activity on nitric oxide production, *J. Agric. Food Chem.* **59**, 1131-1137.

[13] N. I. Baek, Y. S. Kim, J. S. Kyung and K. H. Park (1996). Isolation of anti-hepatotoxic agent from the root of *Astragalus membranaceus*, *Korean J. Pharmacogn.* **27**, 111-116.

[14] J. Sun, M. Zhao, H. Liang and P. Tu (2010). Isolation and identification of flavonoids from Baoyuan Decoction, *Chin. Tradit. Herb. Drugs* **41**, 696-700.

[15] R. Y. Yang, Y. S. Lan, Z. J. Huang, C. L. Shao, H. Liang, Z. F. Chen and J. Li (2012). Isoflavonoids from *Sophora tonkinensis*, *Chem. Nat. Compd.* **48**, 674-676.

[16] E. J. Lee, M. H. Yean, H. S. Jung, J. S. Kim and S. S. Kang (2008). Phytochemical studies on Astragalus root (2)-flavonoids and a lignan, *Nat. Prod. Sci.* **14**, 131-137.

[17] X. Dong, Y. Liang, B. Wang and X. Long (2010). Simultaneous separation of four flavonoids and two astragalosides from *Radix Astragali* by semi-preparative LC, *Chromatographia* **71**, 225-231.

[18] Y. C. Chen, Y. Sugiyama, N. Abe, R. Kuruto-Niwa, R. Nozawa and A. Hirota (2005). DPPH radical-scavenging compounds from dou-chi, a soybean fermented food, *Biosci. Biotechnol. Biochem.* **69**, 999-1006.

[19] L. J. Zhang, H. K. Liu, P. C. Hsiao, L. M. Y. Kuo, I. J. Lee, T. S. Wu, W. F. Chiou and Y. H. Kuo (2011). New isoflavonoid glycosides and related constituents from astragali radix (*Astragalus membranaceus*) and their inhibitory activity on nitric oxide production, *J. Agric. Food Chem.* **59**, 1131-1137.

[20] Y. Song, L. Pan, W. Li, Y. Si, D. Zhou, C. Zheng, X. Hao, X. Jia, Y. Jia, M. Shi, X. Jia, N. Li and Y. Hou (2017). Natural neuro-inflammatory inhibitors from *Caragana turfanensis*, *Bioorg. Med. Chem. Lett.* **27**, 4765-4769.