

A New Ursane-Type Triterpene from the Roots of *Salvia miltiorrhiza* Bunge

Le Quoc Hung^{1,2}, Phuong Thien Thuong² and Nguyen Huu Tung^{1,*}

¹School of Medicine and Pharmacy, Vietnam National University, Hanoi (VNU); 144 Xuan Thuy St.,
Cau Giay, Hanoi, Vietnam

²National Institute of Medicinal Materials (NIMM); 3B Quang Trung St.,
Hoan Kiem district, Hanoi, Vietnam

(Received July 23, 2018; Revised September 16, 2018; Accepted September 18, 2018)

Abstract: Danshen (*Salvia miltiorrhiza*) is a one of the most well-known medicinal herbs in the Oriental medicine for treatment of certain cardiovascular disorders. Our current research on triterpenoid component in danshen roots led to the isolation of a new highly hydroxylated ursane-type triterpene, urs-12,20(30)-dien-3 β ,7 β ,24-triol-28-oic acid (**2**) and one known triterpene, epiasiatric acid (**1**). Their structures were elucidated on the basis of extensive spectroscopic analyses including NMR and MS spectra and comparison with literature data. The new compound **2** showed medium activity against HL-60 cells with the IC₅₀ value of 16.3 μ M. The obtained results of unique bioactive triterpenes from danshen contributed not only to its phytochemical profile associated with chemotaxonomy but also to biological evidence of the title plant.

Keywords: Danshen; *Salvia miltiorrhiza*; Lamiaceae; triterpene. © 2018 ACG Publications. All rights reserved.

1. Plant Source

Salvia miltiorrhiza Bunge (Lamiaceae), commonly known as red sage or danshen, is one of the most used herbs in the Oriental medicine. Danshen is widely cultivated in China and other Asian countries including Korea, Vietnam and Australia to yield typical red roots [1,2], which are highly valuable and been used for the treatment of heart disease and improving cardio-cerebral circulation [3]. We have carried out study on phytochemical profile of danshen in Vietnam and herein report the newly results of investigation on triterpenoid constituents.

2. Previous Studies

Regarding phytochemical profile, a number of secondary metabolites have been identified from the title plant, revealing that nonpolar abietane-type diterpenes such as tanshinone I, tanshinone IIA, and cryptotanshinone and polar phenolic acid including salvianolic acids A-C and lithospermic acid B are the

* Corresponding author: E-Mail: tunginpc@gmail.com

principle constituents of the danshen root [4,5]. In addition, several other compounds including common sterol and triterpenes were readily reported together during the isolation procedures for the two above mentioned-compound classes and it is noteworthy that the major constituents of the aerial parts are triterpenes and be found effective on antiatherosclerosis [6]. Recently, the newly isolated compounds have been ongoing reported from danshen [7,8].

3. Present Study

Our ongoing study on terpenoid profile of the title sample resulted in the isolation of one known compound, epiasiatric acid (**1**), and one new highly hydroxylated ursane-type triterpene, urs-12,20(30)-dien-3 β ,7 β ,24-triol-28-oic acid (**2**). This paper deals with the isolation and structural identification of the two newly isolated triterpenes.

The air-dried danshen root sample was first extracted with EtOH-H₂O (80:20, v/v) and then solvent partition procedure to obtain the EtOAc portion found to contain triterpenoid, which was followed by various column chromatography to yield the triterpenes (for the detail separation, see Supporting information, S1-S2), whose structures were elucidated on the basis of extensive physicochemical data including NMR and MS spectra together with comparison with those in the literature (Figure 1). To our knowledge, this is the first report of epiasiatric acid (**1**) in danshen.

Urs-12-ene-2 α ,3 β ,24-triol-28-oic acid (Epiasiatric acid, 1): white amorphous powder; ESI-MS: m/z 489.2 [M + H]⁺; ¹H NMR (400 MHz, CD₃OD): δ 5.22 (1H, br s, H-12), 3.74 (1H, d, J = 11.6, H-24a), 3.60 (1H, m, H-3 α), 3.29 (1H, d, J = 11.6 Hz, H-24b), 2.91 (1H, d, J = 9.6 Hz, H-2 β), 1.16 (3H, s, CH₃), 1.09 (3H, s, CH₃), 1.00 (3H, s, CH₃), 0.92 (3H, s, CH₃), 0.94 (3H, d, J = 6.0 Hz), 0.83 (3H, d, J = 6.0 Hz); ¹³C NMR (100 Hz, CD₃OD): δ 47.0 (C-1), 69.1 (C-2), 85.4 (C-3), 42.6 (C-4), 49.1 (C-5), 19.0 (C-6), 33.8 (C-7), 39.6 (C-8), 48.2 (C-9), 38.6 (C-10), 23.8 (C-11), 125.9 (C-12), 138.9 (C-13), 42.6 (C-14), 28.5 (C-15), 24.9 (C-16), 48.5 (C-17), 53.5 (C-18), 39.7 (C-19), 39.5 (C-20), 31.1 (C-21), 37.6 (C-22), 22.9 (C-23), 65.8 (C-24), 17.0 (C-25), 17.3 (C-26), 26.2 (C-27), 181.1 (C-28), 17.4 (C-29), 21.4 (C-30) [9].

Urs-12,20(30)-dien-3 β ,7 β ,24-triol-28-oic acid (2): white amorphous powder; $[\alpha]_D^{25}$ = +12 (c 0.15, CH₃OH); HR-ESI-MS: m/z 487.3431 [M + H]⁺ (calcd for C₃₀H₄₇O₅, 487.3423); ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (125 MHz, CD₃OD): see Table 1.

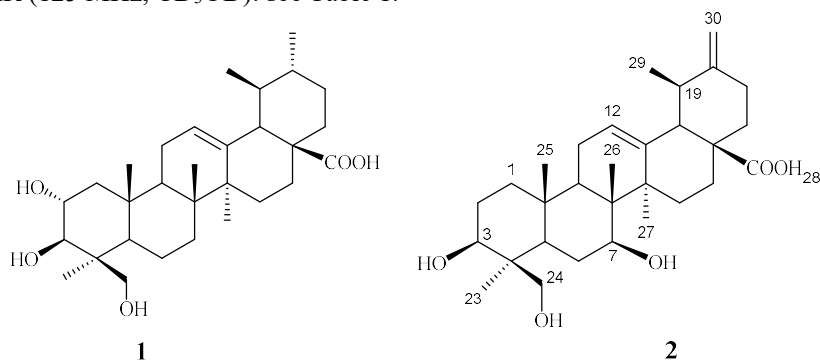


Figure 1. Structures of the two isolated triterpenes from the roots of *S. miltiorrhiza*

Compound **2** was obtained as a white amorphous powder and its molecular was determined to be C₃₀H₄₆O₅ by HR-ESI-MS (m/z 487.3431 [M + H]⁺; calcd for C₃₀H₄₇O₅, 487.3423). In consistent with those of epiasiatric acid (**1**), the NMR data suggested that **2** have structural features typical of an urs-12-en-28-oic acid [10,11]. The ¹H NMR spectrum detected an olefinic proton at δ 5.28 (1H, br t, J = 3.5 Hz), four oxygen-bearing resonances at δ 3.94 (1H, m), 3.77 (1H, br d, J = 2.5 Hz), 3.67 (1H, d, J = 11.5 Hz), and 3.41 (1H, d, J = 11.5 Hz), four singlet methyls (δ 1.22, 1.13, 1.00, 0.86), and one doublet methyl at δ 1.02 (d, J = 6.0 Hz), respectively. In addition, ¹³C and DEPT analyses detected two olefinic carbons (δ 139.6 and 126.6), three oxygenated carbons (δ 74.6 CH, 67.0 CH, and 65.9 CH₂), five methyl signals (δ 24.1, 23.1, 17.8, 17.5, 16.8), and one carboxylic carbon (δ 182.2), respectively. Moreover, the other proton and carbon signals assignable to rings A, B, C, and D were in agreement with those reported for urs-12-en-3 β ,7 β ,24-triol-28-oic acid [12],

whereas the signals assignable to ring E were different. The NMR spectra of **2**, compared with ursolic acid, lacked a doublet methyl signal (Me-30) and contained a typical pair of germinal olefilic protons at δ 4.68 and 4.63 (each 1H, br s) in the ^1H NMR as well as a quaternary carbon (δ 155.0, C) and secondary carbon (δ 104.9, CH_2) in the ^{13}C NMR and DEPT spectra proposed an *exo*-methylene group ($>\text{C}=\text{CH}_2$). The location of the exocyclic double bond at C-20 was suggested by the absence of the Me-30 signal and confirmed by the resonances of the neighboring carbons C-18, C-19, C-21, and C-22 as well as in compatible with those of the similar molecules of urs-12,20(30)-dien-28-oic acid [13,14].

Table 1. NMR spectroscopic data (500 MHz, CD_3OD) for **2**

Position	δ_{C} mult	δ_{H} (J in Hz)
1	42.8, CH_2	1.61 m, 1.30 m
2	34.6, CH_2	1.56 m, 1.36 m
3	74.6, CH	3.77 br d(2.5)
4	45.4, C	
5	50.0, CH	1.35 m
6	19.4, CH_2	1.54 m, 1.35 m
7	67.0, CH	3.94 m
8	40.9, C	
9	49.0, CH	1.76 m
10	39.2, C	
11	24.6, CH_2	2.03 m, 1.92 m
12	126.6, CH	5.28 br t(3.5)
13	139.6, C	
14	43.5, C	
15	29.4, CH_2	1.11 m, 1.03 m
16	25.5, CH_2	2.20 m, 1.75 m
17	48.5, C	
18	56.8, CH	2.32 d(12.0)
19	38.8, CH	2.40 dd(12.0, 6.5)
20	155.0, C	
21	33.5, CH_2	2.30 m, 2.20 m
22	40.6, CH_2	1.85 m, 1.66 m
23	23.1, CH_3	1.13 s
24	65.9, CH_2	3.67 d(11.5), 3.41 d(11.5)
25	16.8, CH_3	1.00 s
26	17.8, CH_3	0.86 s
27	24.1, CH_3	1.22 s
28	182.2, C	
29	17.5, CH_3	1.02 d(6.0)
30	104.9, CH_2	4.68 s, 4.63 s

Assignments were confirmed by DEPT, COSY, HSQC, HMBC, and NOESY spectra

The indication of 3β -OH was evidenced by the ^1H NMR signal at δ 3.77 (1H, br d, $J = 2.5$ Hz) and the relatively upfield ^{13}C NMR signal at δ 74.6 [12,14] in conjunction with the assignment of 24- CH_2OH evidenced by the two typical germinal protons [δ 3.67 (1H, d, $J = 11.5$ Hz) and 3.41 (1H, d, $J = 11.5$ Hz)] and in respect to those of related ursane derivatives with $3\beta,24$ -diol [10,12]. The proton signal at δ 3.94 (1H, m) was typical for the oxymethin 7β -OH as evidenced by downfield-shifted resonances of respective C-6 (δ 19.4) and C-8 (δ 40.9), the spin coupling system of H-5—H-7 observed in the COSY spectrum, and HMBC correlations of H-5/C-7 and H-9/C-7 [12] (Figure 2).

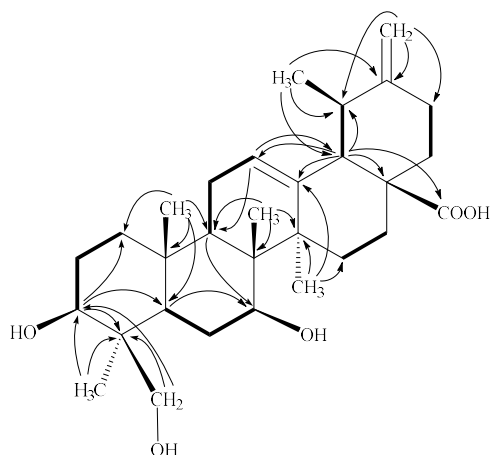


Figure 2. Key HMBC (arrow) and H-H COSY (bold line) correlations for **2**

In concern of stereochemistry, the conformation between the five fused rings of the ursane skeleton is unambiguously rigid [10,11]. The ¹H and ¹³C NMR data indicated the orientation of 3 β -OH [12,14], which was confirmed by the NOE correlations of H-3 α /H-5 in the NOESY spectrum of **2**. The hydroxyl group at C-7 was then determined as β -orientation on the basis of the NOESY correlations of H-7 α /H-3 α , H-5/H-7 α , and H-7 α /H-9, while no NOESY cross-peak of H-7/H₃-25 and H-7/H₃-26 was observed (Figure 3). The full assignments of the ¹H NMR and ¹³C NMR data of **2** were listed in Table 1. Based on the above analyses, the structure of **2** was unambiguously identified urs-12,20(30)-dien-3 β ,7 β ,24-triol-28-oic acid (**2**).

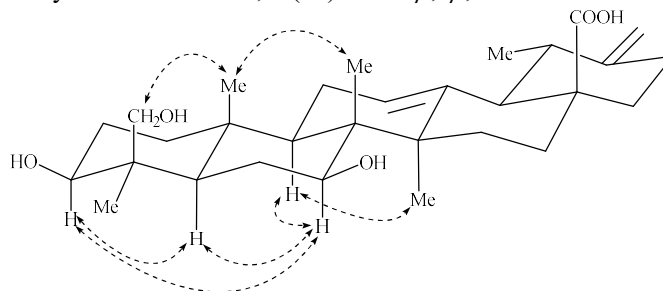


Figure 3. Stereochemistry with selected NOESY correlations of **2**

Antiproliferative activity of the two isolated compounds was tested on the leukemia HL-60 cell line using the MTT assay [15,16]. As the results, while compound **1** showed negligible effect (IC₅₀ > 30 μ M), the new compound **2** exhibited medium activity with the IC₅₀ value of 16.3 μ M in compared with mitoxantrone (positive control, IC₅₀ = 7.2 μ M).

In the viewpoint of chemotaxonomy, the abundance of pentacyclic triterpenoids has been well documented from various *Salvia* species including oleanane, ursane, lupane, and taraxerane skeletons [17,18]. Among them, ursane and oleanane contents represent more common and highly structural diversity based on unsaturated degree and oxidized positions with hydroxyl and oxo functions in their molecules. To our knowledge, the feature of 7-OH and/or $\Delta^{20(30)}$ in the triterpene molecules from *Salvia* species and nature is relatively rare and, to date, only very few such derivatives have been reported including dehydrouvaol and micromeric acid from *S. horminum*, respectively [18].

In consistent with literature review of *Salvia* spp [5,17,18] and our researches [7], various ursane and oleanane triterpenes have been identified in both aerial and root parts of danshen, and concentrated in EtOAc portion with not low yield, it appears that triterpenoid components need be cited along with hydrophobic diterpenes (tanshinones) and hydrophilic phenolic acids in the chemical profile of danshen. In conclusion, the occurrence of various ursane and oleanane triterpenes contributed in part evidence of the chemotaxonomy, biological activity and potential in medicinal use of danshen.

Acknowledgement

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-YS.05-2015.05.

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

ORCID

Le Quoc Hung: [0000-0001-8331-2859](https://orcid.org/0000-0001-8331-2859)

Phuong Thien Thuong: [0000-0003-0334-379X](https://orcid.org/0000-0003-0334-379X)

Nguyen Huu Tung: [0000-0001-6005-9280](https://orcid.org/0000-0001-6005-9280)

References

- [1] D. T. Loi (2001). *Medicinal plants and ingredients in Vietnam*. Vietnam Medical Publisher, Hanoi, 818-820.
- [2] S. J. Sheng, E. C. Pang, C. C. Xue and C. G. Li (2009). Seasonal variations in biomarker contents in Australian-grown *Salvia miltiorrhiza* roots, *Chem. Biodiver.* **6**, 551-560.
- [3] L. Zhou, Z. Zuo and M. S. Chow (2005). Dansen: An overview of its chemistry, pharmacology, pharmacokinetics, and clinical use, *J. Clin. Pharmacol.* **45**, 1345-1359.
- [4] Y. Y. Xu, R. Z. Wan, Y. P. Lin, L. Yang, Y. Chen and C. X. Liu (2007). Recent advance on research and application of *Salvia miltiorrhiza*, *Asi. J. Pharmacodyn. Pharmacokinet.* **7**, 99-130.
- [5] B. Q. Wang (2010). *Salvia miltiorrhiza*: Chemical and pharmacological review of a medicinal plant, *J. Med. Plants Res.* **4**, 2813-2820.
- [6] Q. Zhang, Z. Chang, J. Yang and Q. Wang (2008). Antiatherogenic property of triterpenoids-enriched extract from the aerial parts of *Salvia miltiorrhiza*, *Phytother. Res.* **22**, 1040-1045.
- [7] N. H. Tung, K. Nakajima, T. Uto, N. T. Hai, D. D. Long, T. Ohta, S. Oiso, H. Kariyazono and Y. Shoyama (2017). Bioactive triterpenes from the root of *Salvia miltiorrhiza* Bunge, *Phytother. Res.* **31**, 1457-1460.
- [8] H. G. Choi, P. T. Tran, J. H. Lee, B. S. Min and J. A. Kim (2018). Anti-inflammatory activity of caffeic acid derivatives isolated from the roots of *Salvia miltiorrhiza* Bunge, *Arch. Pharm. Res.* **41**, 64-70.
- [9] X. R. Sun, H. W. Luo and T. Sakai (1992). Pentacyclic triterpenes from *Salvia paramiltiorrhiza*, *Shoyakugaku Zasshi* **46**, 202-205.
- [10] S. Mahato and A. Kundu (1994). ¹³C NMR spectra of pentacyclic triterpenoids—a complication and some salient features, *Phytochemistry* **37**, 1517-1575.
- [11] S. B. Mahato, A. K. Nandy and G. Roy (1992). Triterpenoids, *Phytochemistry* **31**, 2199-2249.
- [12] K. Mazumder, E. R. O. Siwu, S. Nozaki, Y. Watanabe and K. Tanaka (2011). Ursolic acid derivatives from Bangladeshi medicinal plant, *Saurauja roxburghii*: isolation and cytotoxic activity against A431 and C6 glioma cell lines, *Phytochem. Lett.* **4**, 287-291.
- [13] A. Ulubelen, C. H. Brieskorn and N. Ozdemir (1977). Triterpenoids of *Salvia horminum* constitution of a new diol, *Phytochemistry* **16**, 790-791.
- [14] S. Passannanti, M. Paternostro and F. Piozzi (1983). Triterpene acids from *Salvia* and *Teucrium* species, *Phytochemistry* **22**, 1044-1045.
- [15] N. H. Tung, T. Uto, A. Sakamoto, Y. Hayashida, Y. Hidaka, O. Morinaga, S. Lhieochaiphant and Y. Shoyama (2013). Antiproliferative and apoptotic effects of compounds from the flower of *Mammea siamensis* (Miq.) T. Anders. On human cancer cell lines, *Bioorg. Med. Chem. Lett.* **23**, 158-162.
- [16] T. Mosmann (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *J. Immunol. Methods* **65**, 55-63.
- [17] G. Topcu (2006). Bioactive triterpenoids from *Salvia* species, *J. Nat. Prod.* **69**, 482-487.
- [18] S. K. Jash, D. Gorai and R. Roy (2016). *Salvia* genus and triterpenoids, *Int. J. Pharm. Sci. Res.* **7**, 4710-4732.