A New Alkaloid Glycoside from the Stems of Zanthoxylum dissitum Hemsl.

Xian Wei†1, Yang Tan†2, Yuhe Shi2, Yuan Yuan2,3* and Shunmin Li2*  

1Department of Nephrology, Shenzhen Traditional Chinese Medicine Hospital, No. 3015 Jiefang Road, Shenzhen, Guangdong, 518034, P. R. China  
2Pharmacy of College, Hunan University of Chinese Medicine, No. 300 Xueshi Road, Changsha, Hunan, 410208, P. R. China  
3Hunan Provincial Engineering Technology Center of Standardization and Function of Chinese Herbal Medicine, No. 300 Xueshi Road, Changsha, Hunan, 410208, P. R. China  

(Received May 07, 2020; Revised July 07, 2020; Accepted July 13, 2020)

Abstract: A new alkaloid glycoside (1) and six known alkaloids (2–7) were isolated from the stems of Zanthoxylum dissitum Hemsl. The structure of compound 1 was elucidated by UV, IR, 1H NMR, 13C NMR and mass spectroscopic analyses. All compounds obtained in this research were evaluated for their inhibitions against NO release from LPS-activated RAW264.7 macrophages. Compounds 1-3, 6 and 7 showed significant inhibition activities with IC50 values of 26.12±0.81, 8.41±0.23, 13.75±0.54, 6.97±0.77, and 5.78±0.42 μM, respectively.

Keywords: Zanthoxylum dissitum Hemsl.; alkaloid; macrophages; NO. © 2020 ACG Publications. All rights reserved.

1. Introduction

The genus Zanthoxylum belongs to the Rutaceae family, including more than 250 species worldwide, ranges from the subtropical to the tropic zones. Among all such species, 45 are distributed in China. Some fruits of these species are well known spices, while some species are widely used as folk medicine [1,2]. In addition, part of plants belong to Zanthoxylum are economically important for food, timber industries and wood working [3,4].

Zanthoxylum dissitum Hemsl. (Z. dissitum), a climbing or sprawling shrub woody vine in the forest and mountains, is a member of Zanthoxylum plants, which are found in the southwest of Shanxi, Guangxi and Hunan provinces [5]. The roots, stems and leaves of Z. dissitum have been used as Chinese folk medicine for women’s disorders, pains in the loin, limbs and arthritis. Previous phytochemical studies have reported that the stem extracts from Z. dissitum exhibit powerful antimicrobial and anti-tumor activities [6,7]. Some interesting secondary metabolites such as alkaloids with anti-inflammatory, anti-microbial, and anti-tumor activities have also been found in this genus [8,9]. To search for structurally unique and biologically natural components [10], we have isolated and

† These authors contributed to this work equally.
* Corresponding authors: E-Mail: zyxilishunmin@yeah.net (Shunmin Li) and 49464565@qq.com (Yuan Yuan).

The article was published by ACG Publications  
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 2020 EISSN:1307-6167  
DOI: http://doi.org/10.25135/rnp.191.20.08.1643
A new alkaloid glycoside
identified compounds from the stems of *Z. dissitum*. During this process, a new alkaloid glycoside (1), along with six known alkaloids (2–7), were obtained (Figure 1). Herein, the process of isolation, structural determination, and biological detection of these compounds are described.

![Figure 1. The structures of compounds 1-7](image)

2. Materials and Methods

2.1. General

A Bruker Avance III-600 spectrometer was used for NMR detections (Bruker, Shanghai, China). An Agilent Cary 3500 UV-visible spectrometer (Agilent, Santa Clara, CA, USA) and Bruker VERTEX 80/80v Fourier transform infrared spectrometer were used to detect UV and IR spectra of compound 1, respectively. A Bruker Impact II mass spectrometer was used to obtain ESI-MS spectra. The MCI gel was purchased from Mitsubishi Chemical Holdings (Tokyo, Japan). Macroporous adsorption resin was purchased from Dalian Meilun Biotechnology (Dalian, China). Both column chromatography (CC) silica gel and thin layer chromatography (TLC) silica gel were purchased from Qingdao Haiyang Chemical (Qingdao, China). Sephadex LH-20 was purchased from Pharmacia (Rockville, MD, USA). All organic solvents used in this research were purchased from Tianjin Henxing Chemical Reagent (Tianjin, China).

Mouse mononuclear RAW264.7 macrophages were kindly provided by the Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China). Consumables for cell experiments were purchased from NEST Biotech (Wuxi, China). Both high-glucose Dulbecco’s-Modified eagle medium (DMEM) and fetal calf serum were purchased from Gibco by Thermo Fisher Scientific (Waltham, MA, USA).
Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). NO detection kit was purchased from Beyotime Biotechnology (Shanghai, China).

2.2. Plant Material

The stems of *Zanthoxylum dissitum* were collected in September 2018 from Zhangjiajie, Hunan Province, China, and authenticated by Professor Bingmei Xiao, School of Pharmacy, Hunan University of Chinese Medicine. A voucher specimen (No. DMZ201809) was deposited with Hunan Provincial Engineering Technology Center of Standardization and Function of Chinese Herbal Medicine.

2.3. Extraction and Isolation

The air-dried stems of *Z. dissitum* Hemsl. (10 kg) were cut into small pieces and extracted three times with 70% ethanol by refluxing for 2 hours each time. After evaporation under reduced pressure, a dark-brown fluid extract (1.2 kg) was obtained. The extracts were suspended in H2O and then successively extracted with CHCl3, EtOAc, and n-BuOH.

The CHCl3 portion (40.3 g) was subjected to silica CC and eluted with a gradient system from C6H6/CHCl3 (10:1–0:1, V:V) to 100% MeOH with 10% increments. Based on the TLC detection, similar fractions were combined to obtain 11 fractions (C1–11). Fraction C8 (3.8 g) was applied to silica CC using a [C6H6/CHCl3 (8:1) to CHCl3/MeOH (1:1), v:v] wash to obtain six fractions (C8-1–C8-6). Fraction 8-4 (2.1 g) was loaded on a MCI gel and eluted with H2O and 30%, 50%, 70%, 90% and 100% EtOH to obtain four parts (C8-4-1 to C8-4-4) and compound 2 (8.4 mg). Fraction C8-4-2 (1.1 g) was further repeatedly purified by Sephadex LH-20 CC [CHCl3/MeOH (1:1), v:v] and preparative TLC [CHCl3/MeOH (5:1), v:v] to obtain compounds 3 (8.5 mg) and 4 (9.6 mg). Fraction C10 (8.1 g) was also successively separated and purified by a MCI gel [MeOH/H2O (0:100-100:0), v:v], then further purified by CC (C6H6/CHCl3 5:1, V:V) with 5% increment and Sephadex LH-20 [CHCl3/MeOH (1:1), v:v] to obtain compound 5 (10.4 mg).

The n-BuOH portion (65.2 g) was suspended with H2O to obtain two parts. The water soluble part was subjected to macroporous adsorption resin (AB-8) with a step-gradient of H2O and 30%, 50%, 70%, 90% and 100% MeOH to yield four subfractions (B-1 to B-6). B-2 (35.3 g) was further purified by a silica gel column, using the gradient systems of CHCl3/EtoAc (2:1, v:v) to 100% MeOH with a final MeOH/H2O (75:15, v:v) wash to obtain compound 1 (36.8 mg). Compounds 6 (33.7 mg) and 7 (22.0 mg) were obtained from B-4 (6.2 g) and B-5 (10.0 g), respectively.

2.4. Spectroscopic Data

Colorless needle crystal: UV (H2O) λmax: 223, 298 nm; IR (KBr) max 3300, 2964, 1692, 1603, 1513, 1445, 832.14, 982.96 cm⁻¹; 1H NMR (CDCl3, 600 MHz), see Table 1; 13C NMR (CDCl3, 150 MHz), see Table 1; HR-ESI-TOF-MS (positive ion model) m/z 420.1774 [M+H]+, (calculated for C20H26N4O3, m/z 419.1693).

2.5. Determination of Inhibition NO Release

RAW264.7 cells were cultured in DMEM with 10% fetal calf serum in a humidified atmosphere of 5% CO2 at 37 °C. Cells under logarithmic phase were suspended, and then plated in 96-well plates at 1x10^5 cells/well. The old culture medium was removed after the cells were incubated for 24 h at 37°C. A total of 200 ng/mL LPS, combined with various concentrations of test samples, was added for intervening RAW264.7 cells. At 24 h after drug intervention, the cell culture medium in each well was collected centrifuged at 300g for 10 min, and the liquid supernatant was used to test the concentrations of NO using an assay kit. Dexamethasone was used as a positive control. Each experiment was run in triplicate and averaged [11].
3. Results and Discussion

3.1. Structure Elucidation

Compound I had a colorless needle crystal form. The molecular composition was determined as C_{26}H_{32}N_{2}O_{5}, from the ESI-MS with a molecular ion peak found m/z 420.1774 [M+H]^{+}, (calculated for C_{26}H_{32}N_{2}O_{5}, m/z 419.1693). This molecule had 10 degrees of unsaturation. The UV spectrum showed the existences of a conjugated system (223 nm) and a carbonyl group (298 nm). The IR spectrum showed a functional hydroxyl group 1 (3300 cm^{-1}), para-substituted and conjugated benzene ring (2964, 1603, 1513, 1445, and 832 cm^{-1}), carbonyl (1692 cm^{-1}), and trans-double bond (982 cm^{-1}). In the $^{13}$C NMR spectrum, it had resonated 20 carbons, which were classified as three methylenes, thirteen methines, and four quaternary carbons. A phenolic proton signal of δ: 9.82 (1H, s, H-1) was observed in the ¹H NMR spectrum. By reviewing the literature, this data was proved to be consistent with the hydroxyl signal in compounds containing 4-hydroxycinnamic acid amide [12]. There is a set of adjacent coupled proton signals of δ: 7.37 (2H, d, J=8.6 Hz) and 6.77 in the aromatic region (2H, d, J=8.5 Hz), combined with two strong carbon signals in the $^{13}$C NMR spectrum (δ: 129.2 and 115.7), which indicated that the compound had para-disubstituted benzene ring. Peaks at δ: 7.31 (1H, d, J=15.7) and δ: 6.39 (1H, d, J=15.7 Hz) were related in the ¹H-¹H COSY spectrum, which showed a trans-double bond exist in this compound. The connection between the benzene ring and the double bond was determined by the correlation of CH-13, 17 (δ_{H}: 7.37) to C-11 (δ_{C}: 138.6) in the HMBC spectrum (Figure 2). In addition, the HMBC spectrum also showed the proton CH-11 (δ_{H}: 7.30) connected with the double bond coupled with carbonyl C-9 (δ_{C}: 163.5). The hydrogen atom NH-8 (δ_{H}: 8.04) was correlated with C-9 (δ_{C}: 163.5) and C-7 (δ_{C}: 38.8). The correlation of CH$_{2}$-6 (δ_{H}: 2.61) to CH$_{2}$-7 (δ_{C}: 3.38) was observed in the ¹H-¹H COSY spectrum. In the HMBC spectrum, CH$_{2}$-6 (δ_{H}: 2.61) to C-7 (δ_{C}: 38.8), C-4 (δ_{C}: 138.6) and C-5 (114.2) in the imidazole ring showed the connection of CH$_{2}$CH$_{2}$ and the imidazole ring. The NMR spectrum of the compound had a similar structure a aglycone. dissolved in CDCl$_{3}$, the chemical shifts of C-2, 4 and 5, which made up the imidazole ring combine with two nitrogen atoms, were 136.1, 136.1, and 117.9, respectively [13]. Combined with the data of the HMBC spectrum, we speculated that the imidazole ring exists in the structure of compound 1. In the $^{13}$C NMR spectrum, δ_{C}: 85.3, 72.5, 77.2, 69.3, 79.6, and 60.9 belonged to glycosyl signals. The correlation of CH-1' (δ_{H}: 5.03) to C-4 (δ_{C}: 138.9) and C-5 (δ_{C}: 114.2) indicated the part where the glycosyl connected with the aglycone in the imidazole ring. The signal of the anomeric carbon in the glycosyl was δ_{C}: 85.3, which implied that the glycosyl was connected with the nitrogen atom in the heterocyclic ring. In the spectral data of the compound with similar structure, the chemical shift of carbon at the same position was 87.2, which also supported our speculation [14] (See supporting information Table S1). The chemical shifts of the carbon and protons of the glycosyl in compound 1 were close to the relevant data of casimiroedine [13]. The coupling constant of the proton connecting with the anomeric carbon in the glycosyl was 9.06 Hz, indicating that the glycosyl belonged to a β configuration. The structure of compound I was determined and named as disittumine.

![Figure 2. Key HMBC and ¹H-¹H COSY correlations of compound 1](image1.png)

(----- HMBC,       ¹H-¹H COSY.)
By querying the SciFinder database, we found there are two compounds with a similar structure to compound 1. The chemical shifts of their benzene ring and the double bond connected to the benzene ring were different from that of dissitumine, and there was an additional signal of a methyl group connected to the nitrogen atom in those known compounds. Thus, we speculate that compound 1 was a new alkaloid glycoside. Additionally, known compounds were identified as dictamnine (2), γ-fagarine (3), 4-methoxy-1-methyl-2-quinolone (4), norchelerythrine (5), sanguinarine (6), and nitidine (7). The structures of the above mentioned compounds are shown in Figure 1.

3.2. The Assay of NO Release Inhibition

All compounds (1–7) were assessed for their activities of inhibiting NO release from LPS-stimulated RAW264.7 mouse macrophage cells [11]. Compounds 4 and 5 did not show significant results at high concentrations (50 μM). Compounds 1–3, 6, and 7 were evaluated for their activities to the inhibit macrophage release of inflammatory cytokines, and showed IC50 values of 26.12±0.81, 8.41±0.23, 13.75±0.54, 6.97±0.77, and 5.78±0.42 μM, respectively. Among those isolated compounds, dictamnine (2), sanguinarine (6), and nitidine (7) showed strong activity with IC50 values lower than 10.00 μM. Moreover, the activity of γ-fagarine (3) is weaker than dictamnine (2), which may imply that adding a methoxy group to the benzene ring of a uinolone alkaloid will down-regulate its activity to inhibit NO release from LPS-stimulated macrophages.

Table 1. 1H (600 MHz) and 13C NMR (150 MHz) data of compound 1 (δ in ppm, J in Hz) in DMSO-d6

| Position | δH | δC | HMBC | COSY |
|----------|----|----|------|------|
|          |    |    |      |      |
| 2        | 7.60 (1H, s) | 136.5 | C-2 and 5 | - |
| 4        | - | 138.6 | - | - |
| 5        | 7.03 (1H, s) | 114.2 | C-2 and 4 | - |
| 6        | 2.61 (2H, t, 7.6) | 28.4 | C-4, 5 and 7 | - |
| 7        | 3.38 (2H, m) | 38.8 | C-4, 6 and 9 | H-6 and 8 |
| 8        | 8.04 (1H, t, 5.6) | - | C-7 and 9 | H-7 |
| 9        | - | 163.5 | - | - |
| 10       | 6.39 (1H, d, 15.7) | 118.8 | C-9 and 12 | H-11 |
| 11       | 7.30 (1H, d, 15.7) | 138.9 | C-9,10, 13 and 17 | H-10 |
| 12       | - | 125.9 | 163.5, 118.8, 129.2 | - |
| 13,17    | 7.37 (2H, d, 8.6) | 129.2 | C-11, 13, 14, 16 and 17 | H-14 and 16 |
| 14,16    | 6.78(2H, d, 8.5) | 115.7 | C-12, 14, 15 and 16 | H-13 and 17 |
| 15       | - | 158.8 | - | - |
| 1'       | 5.03 (1H, d, 9.1) | 85.3 | C-2, 5, 2', 3' and 5' | H-2' |
| 2'       | 3.46 (1H, m) | 72.5 | C-3' and 1' | H-1' and OH-2' |
| 3'       | 3.28(1H, m) | 77.2 | C-3' | OH-3' |
| 4'       | 3.16(1H, m) | 69.3 | C-2' | OH-4' |
| 5'       | 3.32(1H,m) | 79.6 | C-4' and 6' | - |
| 6'       | 3.68, 3.42 (each 1H, m) | 60.9 | C-4' and 5' | H-6' and OH-6' |

Acknowledgments

This work was partially supported by Hunan University of Chinese Medicine First-class Disciple Construction Project of Chinese Material Medica (China).
A new alkaIold glycoside

Supporting Information

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

ORCID
Xian Wei: 0000-0003-0539-2675
Yang Tan: 0000-0002-4834-6292
Yuhe Shi: 0000-0001-8456-2585
Yuan Yuan: 0000-0001-7543-5808
Shunmin Li: 0000-0001-5524-4238

References
[1] S. K. Adesina (2005). The nigerian Zanthoxylum: chemical and biological values, Afr. J. Trad. Complement. Alterna. Med. 2, 282-301.
[2] M. Anza, E. Haile, S. Tadesse, F. Mammo, M. Endale (2014), A coniferyl alcohol derivative from the roots of Zanthoxylum chalybeum, J. Coast. Life Med. 2, 970-974.
[3] F. Alam, Q. N. Saqib and M. Ashraf (2018). Zanthoxylum armatum DC extracts from fruit, bark and leaf induce hypolipidemic and hypoglycemic effects in mice- in vivo and in vitro study, BMC Complement. Alternat. Med. 18, 68. doi:10.1186/s12906-018-2138-4.
[4] X. Yang (2008). Aroma constituents and alkylamides of red and green huajiao (Zanthoxylum bungeanum and Zanthoxylum schinifolium), J. Agric. Food Chem. 56, 1689-1696.
[5] State Administration of Traditional Chinese Medicine, Chinese Materia Medica Editorial Board (1999). Chinese Materia Medica, Shanghai Scientific and Technical Publishers.
[6] S. P. Xiao, J. H. Zhan, Y. L. Zhang, Y. Tan, Y. C. Meng and G. Pei (2016). Antibacterial and inhibitory effects of different extracts from stem of Zanthoxylum dissitum Hemsl on MCF-7 Cell, Nat. Prod. Res. Dev. 28,1460-1463, 1469.
[7] Y. Yuan, C. Xiao, X. H. Liao, Y. Z. Ding and G. Pei (2011). Inhibitory action of nitidine and zanthobungeanine on lung adenocarcin adenocarcinoma A549 cell, Anti-tumor Pharm. 1, 30-32.
[8] C. Xiao, Y. Yuan, Y. Z. Ding, T. F. Jin and G. Pei (2011). Study on the alkaloids from the stem of Zanthoxylum dissitum, J. Chin. Med. Mater. 34,551-553.
[9] S. Liu, W. Zhang, G. X. He, P. Lei, X. Z. Li and Y. Z. Liang (2009). Study on chemical constituents from the stem of Zanthoxylum dissitum, China J. Chin. Mate. Med. 34, 571-573.
[10] D. B. Zhang, J. G. Yu, Z. Zhang, Y. N. Liang, Z. S. Tang and Z. Wang (2020). Structure, absolute configuration and biological evaluation of a new labdane diterpenoid from Jatropha podagrica, Rec. Nat. Prod. 14, 335-360.
[11] L. Li, W. Du and W. Wang (2019). Evaluation of the antioxidative and anti-inflammatory effects of the extract of Ribes mandshuricum (Maxim.) Kom. leaves, Rec. Nat. Prod. (2019) 13, 141 - 155
[12] P. Beisenhan, T. Zhang, B. Makabel and Z. M. Zou (2018). Chemical constituents of Pothos chinensis, J. Init. Pharm. Res. 45, 61-65.
[13] R. L. Arslanian, B. Mondragon, F. R. Stermitz and K. L. Marr (1990). Acyl histamines and a rare protepine-type alkaloid from leaves of Zanthoxylum dipetalaum, Biochem. System. Ecol. 18, 345-347.
[14] W. F. Reynolds, R. G. Enriquez, G. A. Magos and D. Gnecco (2000). Total assignment of the 1H and 13C NMR spectra of casimiroedine and its peracetylated derivative, Magn. Reson. Chem. 38, 366-369.