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Hydroxyshengmanol-type triterpenoids from the aerial parts of *Cimicifuga simplex* Wormsk



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1. Introduction

Cimicifuga simplex Wormsk, as an important herb medicine, is traditionally used for the treatment of inflammatory, pyretic and pain (Li and Yu, 2006; Su et al., 2014) in eastern Asian. In our continuous study for antitumor constituents from *cimicifuga* spp., a series of cytotoxic 9,19-cycloartane triterpenes on human tumor cell lines and p53^{N236S} mouse embryonic fibroblasts were reported (Nian et al., 2010, 2013). And further investigation on the aerial parts of *cimicifuga simplex* Wormsk led to the isolation of eight new hydroxyshengmanol-type triterpenoids. Compounds (**1**, **7** and **8**) were measured for their inhibitory activities on acetylcholinesterase and cytotoxicities against five human tumor cell lines (including HL-60, SMMC-7721, A-549, MCF-7, and SW480). Herein, reported are the isolation, structure elucidation, and their bioactivities.

2. Results and discussion

Compound **1**, isolated as a white column crystal, gave the molecular formula $C_{35}H_{54}O_{10}$, as deduced from positive-ion HREIMS at m/z 634.3711 (calcd for 634.3717). An analysis of the ¹H NMR spectrum of **1** showed the presence of the characteristic

ABSTRACT

New hydroxyshengmanol-type triterpenoids (1-8) were identified from the aerial parts of *cimicifuga simplex* Wormsk by comprehensive 1D and 2D NMR, MS, and single-crystal X-ray diffraction analyses. The absolute configuration of the himeketal carbon (C-16) in hydroxyshengmanol-type constituents from *cimicifuga* spp. was initially determined as *R* using X-ray diffraction. All compounds were evaluated for their cytotoxicity in a panel of cancer cell lines and acetylcholinesterase inhibitory activity.

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cyclopropane methylene signals at $\delta_{\rm H}$ 0.45 and 0.18 (each 1H, d, J = 4.1 Hz), an anomeric proton at $\delta_{\rm H}$ 4.89 (1H, d, J = 7.6 Hz), a olefinic proton at 6.71 $\delta_{\rm H}$ (1H, dd, 7.4 Hz, 1.4 Hz), a secondary methyl at $\delta_{\rm H}$ 1.55 (d, J = 6.8 Hz), and six tertiary methyls at $\delta_{\rm H}$ 1.04–1.69 (S. Table 1). The above data suggested that **1** was a 9,19-cycloartane triterpene monoglycoside similar to 7,8-didehydro-24-O-acetylhydroshengmanol-3-xyloside (Li et al., 1993). The only difference was the absent acetyl group at C-24 and the oxygen-bearing carbon of C-15 was a carbonyl group in **1**, as deduced from the HMBC correlations (Fig. 2) of H-28 ($\delta_{\rm H}$ 1.64) with C-15 ($\delta_{\rm C}$ 212.51), and another cross-peaks from H-26/H-27 to C-24 ($\delta_{\rm C}$ 78.99). The deduction was further supported by the observation of the carbon resonances of C-14 and C-16 dramatically shifted to $\delta_{\rm C}$ 54.29 and 96.61, respectively. Thus, the planar structure was determined as shown in Fig. 1.

The relative configuration of **1** was mainly deduced from the ROESY correlations (Fig. 2). However, the configuration of the hemiketal group at C-16 was still ambiguous. Fortunately, a single crystal of **1** was obtained from a mixture of MeOH-H₂O (10:1), and following X-ray crystallographic analysis with copper radiation was successfully performed (CCDC 1024308), which determined the complete structure of **1** as deduced (Fig. 3), with the absolute stereochemistry of 3*R*, 13*R*, 14*S*, 16*R*, 17*R*, 21*S*, 23*R*, 24*S*. So compound **1** was elucidated as (23*R*,24*S*)-hydroxyshengmanol-7(8)-en-15-one-3-O- β -D-xylopyranoside.

Compounds (2-6) were all obtained as white amorphous power. Interestingly, detailed comparison of the NMR data of

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Fig. 1. Structures of new compounds (1-8) isolated from the aerial parts of *Cimicifuga simplex* Wormsk.



these compounds with **1** showed that they were similar with each other, except for the major differences ascribed to the sugar moiety, the double-bond of C-7 and C-8, and the chiral carbon of C-24. They were elucidated as (23*R*, 24*S*)-hydroxyshengmanol-15-one-3-*O*- β -D-xylopyranoside (2), (23*R*, 24*S*)-hydroxyshengmanol-7(8)-en-15-one-3-*O*- α -L-arabinopyrano-side (3), (23*R*, 24*S*)-hydroxyshengmanol-15-one-3-*O*- α -L-arabino-pyranoside (4), (23*R*, 24*R*)-hydroxyshengmanol-7(8)-en-15-one-3-*O*- α -L-arabinopyranoside (5), and (23*R*, 24*R*)-hydroxy -shengmanol-15-one-3-*O*- α -L-arabinopyranoside (5), respectively (detailed elucidation was showed in S.1.1).

Compounds **7** and **8** were normal hydroxyshengmanol-type triterpenoids with the sugar unit at C-3, a hydroxyl group at C-15, a hemiketal unit at C-16 and an epoxy cyclohexane between C-16, 17, 20, 22 and 23, which are characteristic structural features of



Fig. 3. X-ray crystal structure of compound 1.

these compounds. **7** and **8** were elucidated as 24-*epi*-24-O-acetylhydroshengmanol-3-O- β -D-glucopyranoside (7) and shengmanol-3-O- β -D-glucopyranosyl-($1'' \rightarrow 3'$)- β -D-xylopyranoside (8) (detailed elucidation was showed in S.1.1).

The bioassay results showed that none of them exhibited cytotoxic activity ($IC_{50} > 40 \ \mu$ M) and compounds **7** and **8** showed weak inhibitory activity on AChE (S. Table 3). And the X-ray diffraction result presented here supported the validity of the previous method, which deduced the configurations of C-23 and C-24 in the hydroxyshengmanol, dahurinol and isodahurinol compounds by comparison of the coupling constants with previous literature (Shao et al., 2000). Particularly, for shengmanol-type compounds, the X-ray diffraction method to clarify the absolute configuration of hemiketol group (C-16) is better than NOESY experiment or CD method (Li et al., 1993; Akiko et al., 1996).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2015.04.005.

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