Phytochemistry Letters 5 (2012) 729–733

Contents lists available at SciVerse ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

New sesquiterpene lactone glucosides from the roots of *Ferula varia*

Shin-ichiro Kurimoto [a], Kyoko Suzuki [a], Mamoru Okasaka [a], Yoshiki Kashiwada [a,*], Olimjon K. Kodzhimatov [b], Yoshihisa Takaishi [a]

[a] Graduate School of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770-8505, Japan
[b] Institute of Botany and Botanical Garden, F. Khodzhaev, St. 32, 700143 Tashkent, Uzbekistan

ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 26 July 2012
Accepted 1 August 2012
Available online 11 August 2012

Keywords:
Ferula varia
Sesquiterpene lactone
Umbelliferae
Uzbekistan

ABSTRACT

Five new eudesmane- (**1**–**5**), two new guaiane- (**6** and **7**) and one new germacrane-type (**8**) sesquiterpene lactone glucosides were isolated from the H_2O-soluble fraction of the roots of *Ferula varia*. Their structures were elucidated by extensive spectroscopic analyses. The absolute configuration of **1** was determined by modified Mosher's method.

1. Introduction

We have been studying on medicinal plants of Uzbekistan for searching seed compounds for drug development (Kurimoto et al., 2011). We have reported several compounds with unique structure and a variety of bioactive compounds from various Uzbek medicinal plants so far, and therefore these medicinal plants are considered to be potential source for seed compounds for developing therapeutic agents (Kurimoto et al., 2011). *Ferula varia* (Schrenk) Trautv. (Umbelliferae) is one of the medicinal folk plants of Uzbekistan, whose roots have been traditionally used for the treatment of fever and intestinal parasitosis, and as a mouth rinse (Sakhobiddinov, 1948). We previously reported the isolation and structure elucidation of acylated sesquiterpene lactones from the EtOAc-soluble fraction obtained from the MeOH extract of *F. varia* roots, as well as evaluation of their cytotoxicities against human cancer cell lines (Suzuki et al., 2007). Our further study on the H_2O-soluble fraction from the MeOH extract of this plant has resulted in the isolation of eight new sesquiterpene lactone glucosides (**1**–**8**). All these compounds have *cis*-fused γ-lactone ring between C-6 and C-7. We describe herein the isolation and structure elucidation of these compounds.

2. Result and discussion

Dried roots of *F. varia* (1.4 kg) were extracted three times with MeOH at 60 °C for 4 h each time, and concentrated by rotary evaporator. The MeOH extract (227 g) was partitioned between EtOAc and H_2O. The H_2O-soluble fraction was separated by repeated column chromatography over Diaion HP-20 (MeOH/H_2O), Sephadex LH-20 (MeOH/H_2O), MCI gel CHP 20P (MeOH/H_2O), YMC ODS-A (MeOH/H_2O), Asahipak GS-310 on HPLC (MeOH), reverse-phase HPLC (MeOH/H_2O), and silica gel ($CHCl_3$/MeOH/H_2O) to give **1** (18 mg), **2** (3 mg), **3** (5 mg), **4** (1 mg), **5** (17 mg), **6** (2 mg), **7** (1 mg) and **8** (4 mg).

The molecular formula of **1** was assigned as $C_{21}H_{30}O_9$ by the HRESIMS (*m*/*z* 449.1807 $[M+Na]^+$). The ^1H NMR spectrum showed the presence of one *tert*-methyl, one *sec*-methyl, one methylene, one oxygenated methylenes, three methines, two oxygenated methines, one disubstituted olefin and one trisubstituted olefin, along with signals including an anomeric proton, arising from a sugar moiety. The ^{13}C NMR and DEPT spectra displayed, along with six carbon resonances assignable to a sugar moiety, two methyls, one sp^3 methylene, one oxygen-bearing sp^3 methylene, three sp^3 methines, two oxygen-bearing sp^3 methines, one sp^3 quaternary carbon, three sp^2 methines, one sp^2 quaternary carbon and one ester carbonyl carbon. Enzymatic hydrolysis of **1** gave an aglycone (**1a**) and glucose, confirming the sugar moiety. The ^1H–^1H COSY spectrum revealed the connectivity of C-1 to C-3, C-5 to C-9, and of C-7 to C-11. The HMBC correlations of Me-14 with C-1, C-5, C-9 and C-10, and of H_2-15 with C-3, C-4 and C-5 indicated that two olefin

* Corresponding author. Tel.: +81 88 633 7276; fax: +81 88 633 7276.
E-mail address: kasiwada@tokushima-u.ac.jp (Y. Kashiwada).

1874-3900/$ – see front matter © 2012 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.phytol.2012.08.001

units were located at C-1(C-2) and C-3(C-4), and oxygenated functional groups were attached at C-6, C-9 and C-15. The presence of γ-lactone ring between C-6 and C-7 was concluded from the HMBC correlations of H-6 with C-12, and of Me-13 with C-11 and C-12. The location of the glucosyl moiety was confirmed from the HMBC correlation of H-1′ with C-15 and its β-linkage was concluded from the coupling constant value (J = 7.8 Hz) of the anomeric proton signal. The relative configuration of **1** was elucidated by the NOESY experiment. The NOESY correlations of Me-14 with H-6, H-7 and H-8α indicated their α-orientations and the *cis* configuration of γ-lactone ring. Thus, ring B was found to adopt a half chair conformation. The β configurations of H-5, H-9 and Me-13 were assigned from the NOESY cross peaks of Me-13 with H-5 and H-9. The absolute configuration of **1** was elucidated by modified Mosher's method (Ohtani et al., 1991). Thus, the (*S*)-α-methoxy-α-trifluoromethyl phenylacetate (MTPA) ester of **1a** was obtained by treatment of **1a** with (*R*)-MTPA chloride, while treatment of **1a** with (*S*)-MTPA chloride gave (*R*)-MTPA ester of **1a** (Kusumi et al., 2006). The 5*R*, 6*R*, 7*S*, 9*R*, 10*R*, and 11*R* absolute configuration could be assigned based on the $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$) (Fig. 2). On the basis of these data, the structure of **1** was characterized as shown in Fig. 1.

Compound **2** was shown to possess the same molecular formula as **1** ($C_{21}H_{30}O_9$) by HRESIMS analysis. The ^{1}H and ^{13}C NMR spectra were quite similar to those of **1**. The analysis of 2D NMR spectra gave the same planar structure as **1**, and therefore **2** was considered to be a diastereoisomer of **1**. The NOESY correlations of Me-14 with H-6 and H-8α indicated that they were α-oriented and the ring-B adopted a chair conformation. The α configurations of Me-13 and the hydroxyl group at C-9 as well as the presence of *cis*-fused γ-lactone were concluded from the NOESY correlations of H-11 with H-5 and H-9, and of H-7 with H-6 and Me-13. From these data, **2** was assigned as the C-11 epimer of **1**.

Compounds **3** and **4** were also found to possess the same molecular formula of **1** ($C_{21}H_{30}O_9$) by the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **3** and **4** showed the presence of the same functional groups as **1**. In addition, analyses of the ^{1}H–^{1}H COSY and the HMBC spectra of **3** and **4** revealed that both had the same planar structure as **1**. The relative configuration of **3** was elucidated from the NOESY correlations. Thus, the NOESY correlations of Me-14 with H-6, H-7 and H-9, and of H-7 with H-11 disclosed the β configurations of Me-13 and the hydroxyl group at C-9 and the *cis* configuration of γ-lactone ring. The boat conformation of the ring-B was concluded from the NOESY cross peak of H-5 with H-8β. In contrast, the NOESY spectrum of **4** displayed the cross peaks of Me-14 with H-6, H-8α and H-9, and of H-7 with H-6 and Me-13, indicating **4** was the C-11 epimer of **3**. On the basis of these evidences, the structures of **3** and **4** were assigned as shown (Fig. 1).

The molecular formula of **5** was established as $C_{21}H_{32}O_{10}$ by the HRESIMS. The ^{1}H and ^{13}C NMR spectra were correlated with those of **1** except for the appearance of one methylene and one oxygenated methine instead of one of disubstituted olefins seen in **1**, indicating **5** was also an eudesmane-type sesquiterpene lactone glucoside. The ^{1}H–^{1}H COSY correlations of H-1–H-2–H-3, and of H-5–H-6–H-7–(H-11)–H_2-8–H_2-9, together with the HMBC cross peaks of Me-14 with C-1 and C-9, and of H_2-15 with C-3, C-4 and C-5 indicated that two hydroxyl group were located at C-1 and C-2, while the double bond was present at C-3(C-4). The location of the glucosyl moiety was determined to be at C-15 from the HMBC correlation of H-1′ with C-15. The β-linkage of the glucosyl moiety was concluded from the J-value (J = 7.8 Hz) of H-1′. The β-orientation of the hydroxyl group at C-1 and the *cis* configuration of γ-lactone ring were confirmed from the NOESY correlations of Me-14 with H-1, H-6 and H-7. Furthermore, the NOESY cross peak of H-5 with H-8β indicated that ring B adopts a boat conformation. The configuration of the hydroxyl group at C-2 was assigned as α by the NOE correlations of H-5 with H-2, while the β configuration of Me-13 was elucidated by the NOE correlation of H-8β with Me-13. On the basis of these observations, the structure of **5** was determined as shown in Fig. 1.

The HRESIMS of **6** showed the $[M+Na]^+$ ion peak at m/z 447.1632, indicating a molecular formula of $C_{21}H_{28}O_9$. The ^{1}H and ^{13}C NMR spectroscopic data indicated the presence of one *sec*-methyl, two sp^3 methylenes, two oxygen-bearing sp^3 methylenes, two sp^3 methines, one oxygen-bearing sp^3 methine,

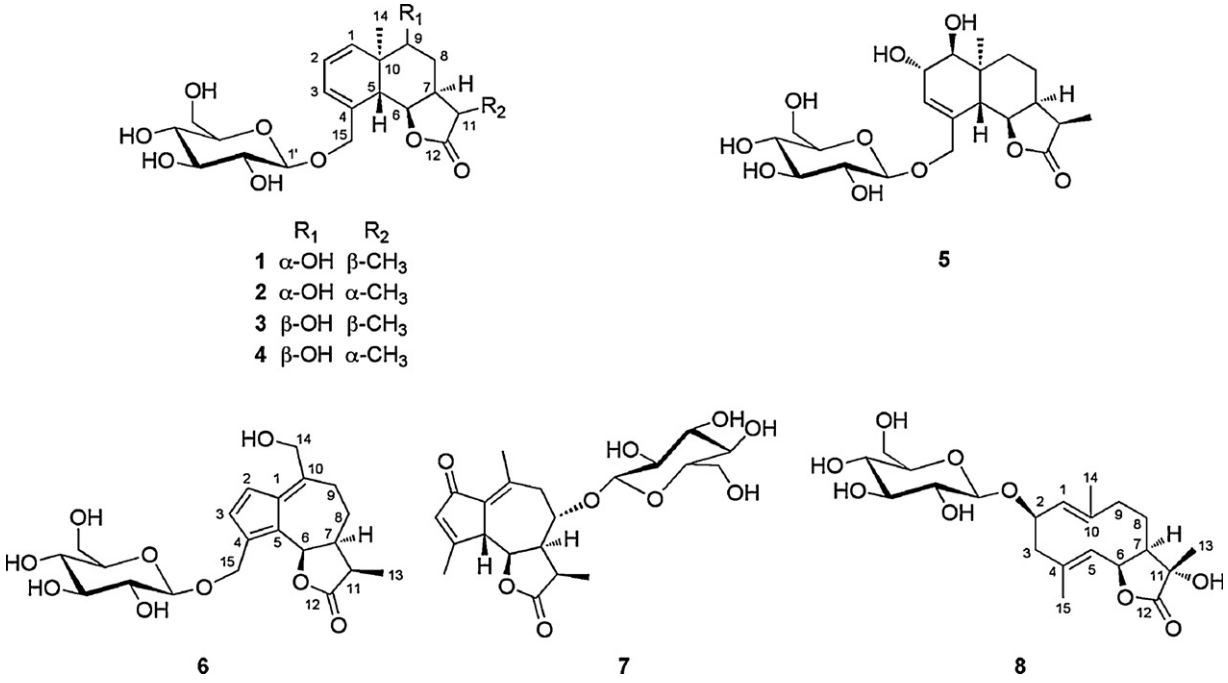

Fig. 1. Structures of new compound from *F. varia*.