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Three new dinormonoterpenoid glucosides from pericarps of *Myriopteron extensum*

Qin Dai [a,b], Yue-Hu Wang [a], Hong-Xia Zhang [a], Guo Sun [a,b], Zhi-Zhi Du [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China

[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

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ABSTRACT

Three new dinormonoterpenoid glucosides, rel-(3*R*,4*R*)-3-(1-hydroxypropan-2-yl)-3,4-epoxypentane-1,5-diol-1-*O*-*β*-D-glucopyranoside (**1**), rel-(3*R*,4*S*)-3-(1-hydroxypropan-2-yl)-3,4-epoxypentane-1,5-diol-1-*O*-*β*-D-glucopyranoside (**2**), and rel-(3*R*,4*S*)-3-(1-hydroxy-2-propen-2-yl)-3,4-epoxypentane-1,5-diol-1-*O*-*β*-D-glucopyranoside (**3**), were isolated from the edible pericarps of *Myriopteron extensum* (Wight & Arn.) K. Schum. (Asclepiadaceae). Their structures were elucidated by chemical and spectroscopic methods including HRESIMS, 1D and 2D NMR. Dinormonoterpenoid glucosides were reported from Asclepiadaceae for the first time. Compounds **1**–**3** were evaluated for their cytotoxicity against five human cancer cell lines HL-60, SMMC-7221, A-549, MCF-7, and SW-480, but they did not exhibit cytotoxicity on the tested cell lines.

1. Introduction

Myriopteron extensum (Wight & Arn.) K. Schum. is the single species of the genus *Myriopteron* (Asclepiadaceae), and has its main distribution in China, India, Indonesia, Myanmar, Thailand and Vietnam (Li et al., 1995). In the Yunnan Province of China, the aerial part of the plant in folk medicine is used for cough and tuberculosis, and the root is used as a remedy for cough, cold, pulmonary tuberculosis, menorrhagia, uterine prolapse and prolapse (Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 1998). During our ethnobotanical investigation on medicinal and edible plants used by minority groups in the Yunnan Province, we found that the Yao people in the county of Xinping collect fruits of *M. extensum* in December each year and eat the pericarps as pickled vegetable. To date, no study on neither chemistry nor bioactivity of the pericarp of *M. extensum* have been published. For those reasons, a chemical study on the pericarp of *M. extensum* was conducted, which led to the isolation of three new dinormonoterpenoid glucosides. The chemical studies on compounds with this type of structure were rare (Abe and Yamauchi, 1996; Abe et al., 1996), and no bioactivity study on them was reported by now. As previous studies revealed that the methanol extract of the whole plant has cytotoxic activity (Li and Zhang, 2003), and that two new steroidal saponins, extensumside A with cytotoxic activity and extensumside B, were obtained in the earlier chemical investigation of the whole plant (Yang et al., 2004), we conducted the cytotoxic assay of the three new dinormonoterpenoid glucosides to explore whether this type of compounds having cytotoxic. In this paper, we report the structure elucidation and the results of cytotoxicity of the three new dinormonoterpenoid glucosides.

2. Results and discussion

The combined and concentrated extracts of pericarps of *M. extensum* (MeOH and 60% MeOH) was partitioned with petroleum ether and water five times to yield three portions: a petroleum ether portion, an emulsified portion, and an aqueous portion. The aqueous portion was concentrated and chromatographed over a macroporous resin HP-20, silica gel, and Sephadex LH-20 to obtain compounds **1**–**3** (Fig. 1).

Compound **1**, a colorless gum, had a molecular formula $C_{14}H_{26}O_9$ determined on the basis of HRESIMS peak of $[M+Na]^+$ at *m/z* 361.1482 (calcd. for $C_{14}H_{26}O_9Na$, 361.1475), with two degrees of unsaturation. In ^1H NMR spectrum, an anomeric proton signal (δ_H 4.25, d, J = 7.8 Hz) as well as signals (δ_C 104.3, CH; 75.0, CH; 77.9, CH; 71.5, CH; 77.8, CH; 62.7, CH_2) in ^{13}C NMR and DEPT spectra (Table 1) showed the component sugar to be *β*-linked

* Corresponding author. Tel.: +86 871 65223224; fax: +86 871 65216335.
E-mail address: duzhizhi@mail.kib.ac.cn (Z.-Z. Du).

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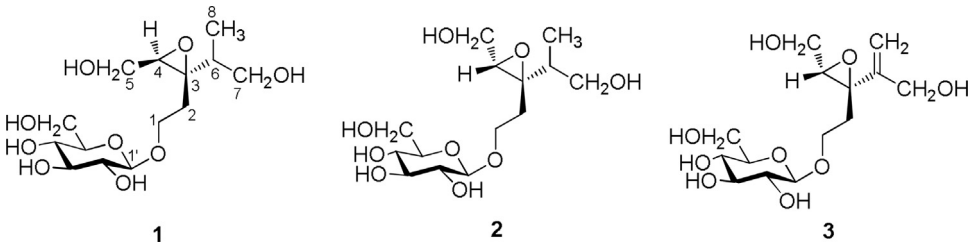

Fig. 1. The structures of compounds **1**–**3**.

glucopyranose (Abe and Yamauchi, 1996). The aglycone moiety was considered to be composed of eight carbons, with one degree of unsaturation. In ^{13}C NMR and DEPT spectra, two carbon signals ($\delta_{C\text{-}4}$ 62.6 and $\delta_{C\text{-}3}$ 64.7) were observed at slightly higher field than those of usual carbinols, suggesting the presence of an epoxide (Abe and Yamauchi, 1996). The correlations signals in ^1H–^1H COSY spectrum showed three fragments: –O–CH_2–CH–CH_3 ($\delta_{H\text{-}6}$ 1.59–1.65 and $\delta_{H\text{-}7}$ 3.54, 3.72; $\delta_{H\text{-}6}$ 1.59–1.65 and $\delta_{H\text{-}8}$ 1.02), –CH_2–CH_2–O– ($\delta_{H\text{-}1}$ 3.49, 3.89 and $\delta_{H\text{-}2}$ 1.89, 2.14), –O–CH_2–CH–O– ($\delta_{H\text{-}4}$ 3.16 and $\delta_{H\text{-}5}$ 3.63, 3.81). The plane structure of aglycone was suggested to be a dinormonoterpenoid: 3-(1-hydroxypropan-2-yl)-3,4-epoxypentane-1,5-diol, by the correlations signals ($\delta_{C\text{-}3}$ 64.7 and H-2, 4, 5, 6, 7, 8; $\delta_{C\text{-}2}$ 30.4 and H-4, 6) in the HMBC spectrum (Fig. 2). The glucosidic linkage was determined by the HMBC correlations between the anomeric proton at δ 4.25 and C-1 at δ 66.1 (Fig. 2).

The relative configuration of the 1-hydroxypropan-2-yl group and hydroxymethyl group was determined to be *trans* by the correlations signals of H_2-2/H_2-5 and H-4/H-6 in the ROESY spectrum of **1** (Fig. 3). The absolute configuration of the sugar was determined to be D-glucose by GC analysis to compare of the retention time of the derivative obtained from the acylation reaction of hydrolyzed **1** with its authentic sugar derivatized in a similar way, which showed retention time at 21.985 min. Thus, the structure of **1** was characterized as rel-(3*R*,4*R*)-3-(1-hydroxypropan-2-yl)-3,4-epoxypentane-1,5-diol-1-*O*-β-D-glucopyranoside.

Compound **2** was isolated as a colorless gum. It has the same molecular formula $C_{14}H_{26}O_9$ as that of compound **1**, determined by peak of $[M+Na]^+$ at *m/z* 361.1482 (calcd. for $C_{14}H_{26}O_9Na$, 361.1475) in the HRESIMS spectrum. In the 1D-NMR spectra, the signals of **2** were similar to those of **1** (Table 1). According to the correlations of ^1H–^1H COSY and HMBC, compound **2** has the same planar structure as **1**. The relative configuration of the 1-hydroxypropan-2-yl group and hydroxymethyl group of **2** was to be *cis* determined by correlations signals of H_2-2/H-4 and H_2-5/H-6 in ROESY spectrum of **2** (Fig. 3). Thus, the structure of **2** was characterized as rel-(3*R*,4*S*)-3-(1-hydroxypropan-2-yl)-3,4-epoxypentane-1,5-diol-1-*O*-β-D-glucopyranoside.

Compound **3** was isolated as a colorless gum. The molecular formula is $C_{14}H_{24}O_9$, determined by the $[M+Na]^+$ peak at *m/z* 359.1318 (calcd. for $C_{14}H_{24}O_9Na$, 359.1318) in the HRESIMS spectrum, indicating that compound **3** has two protons less and one degree of unsaturation more than compounds **1** and **2**. On comparing the 1D-NMR data (Table 1) of compound **3** with those of **1** and **2**, compound **3** showed two typical signals for a terminal double bond (δ_C 145.9, qC; 114.2, CH_2, δ_H 5.13, s, 5.28, s), rather than the signals for a –$CHCH_3$ group in **1** and **2**. Further confirmed by ^1H–^1H COSY and HMBC spectra, the planar structure of **3** was predicted to be 3-(1-hydroxy-2-propen-2-yl)-3,4-epoxypentane-1,5-diol-1-*O*-β-D-glucopyranoside. The relative configuration of the 1-hydroxy-2-propen-2-yl group and hydroxymethyl group of compound **3** was to be *cis* by the correlation signals of H_2-2/H-4 and H_2-5/H_2-8 in the ROESY spectrum of **3** (Fig. 3). Thus, the structure of **3** was characterized as rel-(3*R*,4*S*)-3-(1-hydroxy-2-propen-2-yl)-3,4-epoxypentane-1,5-diol-1-*O*-β-D-glucopyranoside.

Compounds **1**–**3** were evaluated for their cytotoxicity against HL-60, SMMC-7221, A-549, MCF-7, and SW-480 human cancer cell

Table 1
^1H (600 MHz, CD_3OD) and ^{13}C (100 MHz) NMR data of compounds **1**–**3**, δ in ppm.

No. of C/H	**1**		**2**		**3**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	66.1	3.49 (ddd, 9.8, 7.5, 6.4) 3.89 (dt, 10.1, 6.1)	66.3	3.49 (m) 3.92 (dt, 9.9, 6.0)	66.8	3.62 (dt, 9.9, 7.2) 3.97 (ddd, 9.9, 7.7, 5.9)
2	30.4	1.89 (dt, 15.1, 5.9) 2.14 (dt, 14.6, 7.0)	29.7	1.87 (dt, 15.1, 5.8) 2.17 (ddd, 14.6, 7.8, 6.5)	36.2	1.83 (dt, 14.5, 7.2) 2.30 (ddd, 13.7, 7.5, 5.9)
3	64.7		63.9		64.0	
4	62.6	3.16 (dd, 6.3, 4.7)	63.9	3.29 (m)	64.0	3.14 (m)
5	61.0	3.63 (dd, 12.0, 6.4) 3.81 (dd, 12.0, 4.5)	61.6	3.64 (dd, 12.0, 6.6) 3.81 (dd, 12.1, 5.1)	61.5	3.44 (dd, 12.0, 6.3) 3.55 (dd, 12.0, 5.3)
6	40.4	1.61 (m)	39.9	1.75 (m)	145.9	
7	65.0	3.54 (dd, 10.8, 7.4) 3.72 (dd, 10.7, 6.1)	64.9	3.49 (m) 3.56 (m)	63.8	4.11 (d, 14.1) 4.19 (d, 14.1)
8	14.1	1.02 (d,7.1)	12.5	1.00 (d, 6.9)	114.2	5.13 (s) 5.28 (s)
1′	104.3	4.25 (d, 7.8)	104.4	4.23 (d, 7.9)	104.5	4.23 (d, 7.8)
2′	75.0	3.17 (m)	75.0	3.17 (dd, 9.0, 7.9)	75.0	3.16 (m)
3′	77.9	3.36 (m)	77.9	3.35 (m)	77.9	3.34 (m)
4′	71.5	3.28 (m)	71.6	3.27 (m)	71.6	3.31 (m)
5′	77.8	3.28 (m)	77.9	3.27 (m)	77.9	3.27 (m)
6′	62.7	3.67 (dd, 11.9, 4.8) 3.85 (dd, 11.6, 4.4)	62.7	3.67 (m) 3.86 (d, 12.0)	62.7	3.67 (dd, 11.9, 5.3) 3.86 (m)

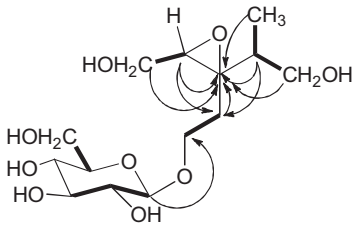

Fig. 2. Selected ^{1}H–^{1}H COSY (bold lines) and HMBC (H → C) correlations of compound **1**.

lines. However, they were all inactive under the tested concentrations ($IC_{50} > 40$ μM). The IC_{50} values of cisplatin against the five cancer cell lines are 2.22 μM (HL-60), 10.21 μM (SMMC-7221), 7.25 μM (A-549), 12.16 μM (MCF-7), and 10.07 μM (SW-480), respectively. The IC_{50} values of paclitaxel against the five cancer cell lines are less than 0.008 μM (cisplatin and paclitaxel as positive controls).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Horiba Sepa-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A tensor 27 spectrophotometer was used for scanning IR spectroscopy using KBr pellets. 1D and 2D NMR spectra were measured on Brucker AM-400 and DRX-500, Avance III 600 with tetramethylsilane (TMS) as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESIMS was obtained on a Bruker HTC/Esquire spectrometer. HRESIMS was recorded on an Agilent G6230 TOF MS spectrometer.

Column chromatography (CC) was done using silica gel (200–300 mesh, Qingdao Marine Chemical Ltd. Co., China) and Sephadex LH-20 (GE Healthcare bio-sciences AB, Sweden), TLC was performed on silica gel GF254 (Qingdao Marine Chemical Led. Co., China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. GC analysis was performed on an Agilent 7890A gas chromatography equipped with an H_2 flame ionization detector (FID).

3.2. Plant material

The fresh fruits of *M. extensum* were collected from Xinping County of Yunnan Province, China in December 2011 and were identified by Prof. Li-Song Wang. A voucher specimen (KUN0309000) has been deposited with the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried and powered pericarps (without seeds and tomenta inside) of *M. extensum* (1.4 kg) were extracted with MeOH at room temperature (15 L × 3 days × 3 times), then with 60% MeOH under ultrasonic waves (8 L × 30 min × 3 times). The combined extract was concentrated under reduced pressure. The residue was partitioned with petroleum ether and water five times to yield petroleum ether portion (24.45 g), emulsified portion (45.24 g), and aqueous portion (about 300 g). The aqueous portion was chromatographed over a macroporous resin HP-20 column (2 kg), and eluted with H_2O, 30% MeOH, 50% MeOH, 70% MeOH, and MeOH, successively. The 30% MeOH–H_2O fraction (10.26 g) was chromatographed on silica gel column (200–300 mush, 500 g), eluting with $CHCl_3$–MeOH (9:1 → 8:2 → 7:3) to get fractions 1–10. Fr. 7 (1.811 g) was subjected to silica gel column chromatography (200–300 mush, 150 g) again, eluted with $CHCl_3$–MeOH–H_2O (9:1:0.05) to Fr. 7-1 to 7-8. Fr. 7-3 (97 mg), Fr. 7-5 (60 mg), and Fr. 7-8 (723 mg) were further purified by Sephadex LH-20 column chromatography, eluted with methanol to obtain compounds **2** (20 mg), **3** (18 mg), and **1** (126 mg), respectively.

3.3.1. *rel-(3R,4R)-3-(1-Hydroxypropan-2-yl)-3,4-epoxypentane-1,5-diol-1-O-β-D-glucopyranoside (**1**)*

Colorless gum; $[\alpha]_D^{21} = -31.0$ ($c = 0.20$, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 209 (2.23), 194 (1.92); IR (KBr) ν_{max} cm^{-1}: 3418, 2929, 1636, 1384, 1036, 630; ^{1}H and ^{13}C NMR data see Table 1; HRESI-MS *m/z* 361.1482 $[M+Na]^+$ (calc. 361.1475 for $C_{14}H_{26}O_9Na$).

3.3.2. *rel-(3R,4S)-3-(1-Hydroxypropan-2-yl)-3,4-epoxypentane-1,5-diol-1-O-β-D-glucopyranoside (**2**)*

Colorless gum; $[\alpha]_D^{21} = -34.0$ ($c = 0.19$, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 204 (2.67); IR (KBr) ν_{max} cm^{-1}: 3427, 2928, 1635, 1384, 1035, 582; ^{1}H and ^{13}C NMR data see Table 1; HRESI-MS *m/z* 361.1471 $[M+Na]^+$ (calc. 361.1475 for $C_{14}H_{26}O_9Na$).

3.3.3. *rel-(3R,4S)-3-(1-Hydroxy-2-propen-2-yl)-3,4-epoxypentane-1,5-diol-1-O-β-D-glucopyranoside (**3**)*

Colorless gum; $[\alpha]_D^{21} = -61.4$ ($c = 0.14$, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 223 (2.47), 201 (3.14); IR (KBr) ν_{max} cm^{-1}: 3427, 2929, 1634, 1034, 576; ^{1}H and ^{13}C NMR data see Table 1; HRESI-MS *m/z* 359.1318 $[M+Na]^+$ (calc. 359.1318 for $C_{14}H_{24}O_9Na$).

3.4. Acid hydrolysis of compounds ***1*–*3***

Compounds **1**–**3** (2 mg) were dissolved in 1 M HCl (dioxane–H_2O, 1:1, 2 mL) and heated at 95 °C for 2 h, respectively. After drying under a stream of nitrogen, the reaction mixtures were extracted with EtOAc (×3). The aqueous layer was neutralized with $NaHCO_3$ and evaporated under vacuum to furnish a neutral residue (Wang et al., 2006). The residue was dissolved in anhydrous

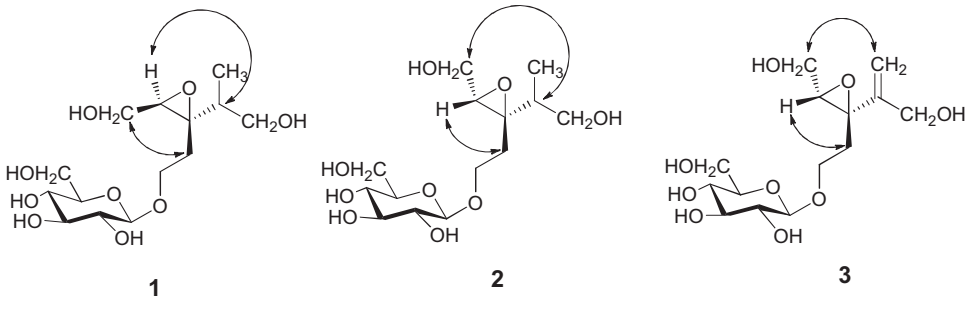

Fig. 3. Selected ROESY correlations of compounds **1**–**3**.

pyridine (1 mL), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h, and after evaporation in vacuo to dryness, 0.2 mL of *N*-trimethylsilylimidazole was added; the mixture was kept at 60 °C for another 2 h (Dong et al., 2011). The reaction mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane extract analyzed by GC under the following conditions: capillary column, HP-5 (5% phenyl methyl siloxan, 50 m × 0.32 mm, with a 0.52 μm film, Agilent); detection, FID; detector temperature, 250 °C; injection temperature, 250 °C; initial temperature 160 °C, then raised to 280 at 5 °C/min; carrier, He gas; flow rate: 2.219 mL/min.

3.5. Cytotoxicity assay

The cytotoxicity assay was performed using the MTT method, and the following human tumor cell lines were used: HL-60, SMMC-7221, A-549, MCF-7, and SW-480. Each tumor cell line was exposed to each test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) used as positive controls (Alley et al., 1988).

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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.2015.03.018.

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