Article Type: Full Paper

Four new diterpene glucosides from *Perovskia atriplicifolia*

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Abstract:

Four new diterpene glucosides, namely perovskiaditerpenosides A-D (**1**-**4**), were isolated from the butanol extract of *Perovskia atriplicifolia*. Their structures were well elucidated by chemical methods and comprehensive spectroscopic analyses including MS, IR, and NMR (1D and 2D). The newly isolated compounds were screened for their cytotoxic activity against HepG2, NB4 Hela, K562, HL60, MCF7 and HL60. The obtained results indicated that the new compounds possessed

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/cbdv.201700071

considerable cytotoxic activity.

Keywords:

Perovskia atriplicifolia, diterpene glucosides, perovskiaditerpenosides A-D, cytotoxic activity

Introduction. - The *Perovskia* is a small genus from Labiatae family, and had been reported to possess leishmanicidal, antiplasmodial, and cytotoxic activity [1]. In China, there are only two species (*P. atriplicifolia* and *P. abrotanoides*), distributed mainly in Tibet and Xinjiang [2]. These two species are used in folk medicine for the treatment of leishmaniasis by local people. It was also been reported to possess antibacterial and anti-inflammatory activities [3,4]. Some investigation on this plant had been conducted and many compounds had been isolated [5-7]. Our previous investigation suggested the petroleum ether extract of *P. atriplicifolia* showed anti-HBV (Hepatitis B virus) activity and some bioactive chemicals had been isolated from the petroleum ether extract [8-12]. But the butanol extract showed none anti-HBV activity during the preceding investigation. In consideration that no investigation on the butanol fraction was reported, a subsequent bioassay screening was conducted and the butanol extract was found to exhibit moderate cytotoxic activity. Further investigation of the butanol extract of *P. atriplicifolia* led to the isolation of four new diterpene glucosides. By chemical methods and comprehensive analyses of their MS, IR, 1D-and 2D-NMR data, the structures of the four new diterpene glucosides were determined (*Fig* 1). The cytotoxic activity of the newly isolated compounds was investigated against HepG 2, NB4, Hela, K562, MCF7, PC3 and HL60 cell lines. Results suggested that compounds **1**-**4** possessed noticeable

cytotoxicities. Described herein were the isolation, structural elucidation and cytotoxic activities of compounds **1**-**4**.

Results and Discussion. - Compound **1** was obtained as a white amorphous powder from Fraction C2. The positive ESI-MS gave a quasi-molecular ion peak at *m/z* 649, in agreement with the molecular formula $C_{32}H_{50}O_{12}$ revealed by the HR-ESI-MS at *m/z* 649.3192 $[M+Na]^+$ (calc. for $C_{32}H_{50}O_{12}Na^+$ 649.3199). The IR spectrum displayed absorptions attributable to OH (3443 cm^{-1}) and aromatic ring (1606, 1497, 1448 cm^{-1}) functions. After the hydrolysis of **1** with 10% HCl in MeOH, D-glucose was identified by a comparison with the authentic sugar sample on TLC (developed by *n*-BuOH/AcOH/H_2O 4:1:5, upper layer; and PhOH/H_2O 4:1). The ^{1}H-NMR spectrum (CD_3OD, 400 MHz) (*Table* 1) displayed two aromatic proton signals at δ_H 7.03 (1H, *s*, H-11), 6.84 (1H, *s*, H-14), three singlet methyls (δ_H 1.19, 1.05, 0.88), in addition to signals that indicated the presence of one isopropyl group at δ_H 3.49 (1H, *sept*. *J* = 6.8 Hz, H-15), 1.18 (3H, *d*, *J* = 6.8 Hz, H-16), and 1.13 (3H, *d*, *J* = 6.8 Hz, H-17). The two anomeric proton signals at δ_H 4.93 (1H, *d*, *J* = 7.6 Hz, H-1") and 4.79 (1H, *d*, *J* = 7.6 Hz, H-1') suggested there were two *β*- orientation sugar moieties in the molecule. The ^{13}C-NMR spectrum (CD_3OD, 100 MHz) (*Table* 2) revealed 32 carbon signals, indicating the presence of a diterpene skeleton and two sugar moities. The ^{1}H and ^{13}C-NMR spectral data were in total agreement with those of hinokiol [8], suggesting that compound **1** might be a glucoside derivative of hinokiol. The position of the additional two *β*-D-glucose units was deduced as 1→2 linkage by comparing the NMR data with those of lucihirtin A [13], and was further confirmed by the HMBC correlations between H- 1" and C-2'. The obvious HMBC cross peak H- 1' /C-12

established the location of the inner glucose at C-12 of hinokiol. The absolute configuration of D-glucose was finally verified by GC analysis of the trimethylsilyl L-cysteine derivative. The other HSQC, HMBC, ^{1}H-^{1}H COSY (*Fig.* 2) and ROESY (*Fig.* 3) correlations allowed the full proton and carbon assignments of compound **1**. Consequently, the structure of compound **1** was determined as shown in *Fig. 1* and named as perovskiaditerpenoside A (**1**).

Compound **2** was obtained as a white amorphous powder from Fraction C2. The molecular formula was deduced as $C_{32}H_{50}O_{13}$ based on its HR-ESI-MS which showed a molecular ion peak at *m/z* 665.3141 $[M+Na]^+$ (cald. for $C_{32}H_{50}O_{13}Na^+$ 665.3149). The IR spectrum of compound **2** showed absorption peaks that indicated the presence of OH (3442 cm^{-1}) and aromatic ring (1603, 1498, 1449 cm^{-1}). The presence of D-glucose in compound **2** was corroborated by TLC and GC analyes of the acid hydrolysate. In the ^{1}H-NMR spectrum (C_5D_5N, 400 MHz), an aromatic proton signal at δ_H 6.75 (1H, *s*, H-14), three singlet methyls at δ_H 1.47, 1.29, 1.08, one isopropyl group atδ_H 4.18 (1H, *sept*. *J* = 6.8 Hz, H-15), 1.44 (3H, *d*, *J* = 6.4 Hz, H-16), and 1.10 (3H, *d*, *J* = 6.8 Hz, H-17), and two anomeric proton signals ascribable to two β-linked sugar units at δ_H 5.64 (1H, *d*, *J* = 7.8 Hz, H-1") and 5.21 (1H, *d*, *J* = 8.0 Hz, H-1') were displayed. Detailed analysis of the NMR data suggested compound **2** was also a diterpene glucoside, with a similar sugar chain at C-12. A comparison of the MS and NMR spectrum indicated that compound **2** contained one more OH than **1**. The additional OH was determined at C-11 by the HMBC correlations observed between H-14 (δ_H 6.75, δ_C 117.7) and each of C-7 (δ_C 33.4), C-8 (δ_C 134.4) and C-9 (δ_C 133.8) in addition to the correlations observed between H-15 (δ_H 4.18) and C-12 (δ_c 141.7). Accordingly, the structure of compound **2** was deduced as depicted in *Fig.*1 and named as perovskiaditerpenoside B (**2**).

Compound **3** was obtained as a white amorphous powder from Fraction C2 and had the molecular formula $C_{32}H_{48}O_{13}$ deduced by the HR-ESI-MS at *m/z* 663.2989 $[M+Na]^+$ (calc. for $C_{32}H_{48}O_{13}Na^+$ 663.2992). Similar to the previous compounds, the IR spectrum displayed absorptions for OH (3442 cm^{-1}), olefin (1642 cm^{-1}), and aromatic ring (1606, 1497, 1448 cm^{-1}) functions. The NMR spectral data of compound **3** were similar to those observed for compound **2** but with the presence of one more double bond. The location of this double bond was deduced from the different 2D NMR spectra of compound **3**, including COSY and HMBC experiments. The long range HMBC correlations observed between H-6 (δ_H 5.93, δ_C 129.0) and each of C-10 (δ_C 41.9), C-8 (δ_C 133.3); H-7 (δ_H 6.44, δ_C 129.9) and each of C-5 (δ_C 52.9) and C-9 (δ_C 131.5) clearly indicated the position of the double bond between C-6 and C-7 of the diterpene skeleton. This was also confirmed by the COSY correlations observed between H-5 and H-6. Important correlations observed in the different 2D spectra of compound **3**, including HMBC, H-H COSY and ROSEY are indicated in Figures 2 and 3, respectively. Based on these data, compound **3** was characterized and named as perovskiaditerpenoside C (**3**).

Compound **4** was obtained as a white amorphous powder from Fraction C2, with the molecular formula $C_{32}H_{50}O_{13}$ revealed by the HR-ESI-MS at *m/z* 665.3138 $[M+Na]^+$ (calc. for $C_{32}H_{50}O_{13}Na^+$ 665.3149). The IR spectrum was almost the same as compound **2**, indicating both compounds were structurally similar. A comprehensive comparison of the NMR spectra of compounds **4** and **2** suggested both compounds had the same diterpene aglycone. The main difference between compounds **4** and **2** was the locations of the sugar moieties. The HMBC correlations observed between H-1' (δ_H 4.96, δ_C 107.0) and C-3 (δ_C 89.0), H-1" (δ_H 5.16, δ_C 108.4) and C-12 (δ_C 142.5) established the linkage of the two

β-D-glucopyranose at C-3 and C-12, respectively. Lastly, the structure of compound **4** was deduced as shown in *Fig.* 1 and named as perovskiaditerpenoside D (**4**).

Compounds **1**-**4** were evaluated for their cytotoxic activity against Hep G 2, NB4, Hela, K562, MCF7, PC3 and HL60 cell lines with cisplatin as the positive control. Results suggested all compounds showed noticeable in *vitro* cytotoxic activity (*Table* 3). Compound **1** had the highest cytotoxic activity against HepG-2 and HL60. Compound **3**, on the other hand, had significant cytotoxic activity against K562 and MCF7 cell lines (IC_{50} values of 0.35 and 0.96 μM, respectively.).

In conclusion, this paper described the isolation, structural elucidation and cytotoxic activities of four novel diterpene glucosides. To the best of our knowledge, only few diterpene glycosides were reported before. Our investigation suggested all the tested diterpene glycosides possessed notable anti-tumor activity in *vitro*, manifesting that glycosidation of some diterpenes could result in the improvement of their bioactivity.

Experimental Part

General

Column chromatography (CC): silica gel (200-300 mesh; Qingdao Marine Chemical Inc., China); Lichrospher Rp-18 gel (40-63μ; Merck, Germany); MCI gel CHP-20P (70-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). HPLC was performed on an Agilent 1260 liquid chromatograph equipped with a Venusil XBP C18 (10 × 250 mm, 5μm) column. Optical

rotations were carried out on a HORIBA SEPA-300 High Sensitive Polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets, ν in cm^{-1}. MS data were obtained on a VG Auto Spec-3000 instrument. NMR spectra were recorded on a Bruker AM-400 ($^1H/^{13}C$, 400 MHz/100 MHz) spectrometer, and chemical shifts were given in δ (ppm) with TMS as internal reference. The authentic sugar sample (D-glucose) was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

Plant Material. The whole plant of *P. atriplicifolia* was collected from Tibet in September, 2010, a flower season of this plant, and was identified as *Perovskia atriplicifolia* Benth. by Prof. Dr. Li-Gong Lei from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (TSYJ-201093) was deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University.

Extraction and Isolation. The air-dried *P. atriplicifolia* (whole plants, 9.0 kg) were powdered and extracted with 90% ethanol (54 L) under reflux conditions (3 times, 2 hours each). After concentrated in *vacuo*, the extract was suspended in water and successively partitioned with petroleum ether, chloroform and n-BuOH to give petroleum ether (A). chloroform (B), n-BuOH (C) and water (D) fractions. The n-BuOH extract (C, 95 g) was chromatographed on a silica gel (1.0 kg, 200-300 mesh; 8 × 170 cm; packed in chloroform/MeOH, 95:5, v/v) chromatography column (CC) and eluted with gradient chloroform/MeOH (95:5, 90:10, 80:20; v/v; each 2.0 L; 500 mL/flask) to afford twelve fractions. These fractions were further grouped into 5 major groups (Frs.C.1-5) according to their TLC behavior. The Fr.C2 were subjected to CC on Silica gel (packed in chloroform/MeOH, 95:5, v/v) with an eluant of chloroform/MeOH (93:7) to give six subfractions (Frs.C2.1- Frs.C2.6). Fr.C.2.3 (3.5 g) was performed on a MCI CC (packed in MeOH/H_2O, 85:15; eluted with MeOH/H_2O, 85:15) to afford

five subfractions (Frs.C.2.3.1- Fr.C.2.3.5). The Fr.C2.3.2 (850 mg) was purified on an HPLC (Agilent 1260; Column: Venusil XBP C18 ,10 × 250 mm, 5μm) with the eluant of CH_3CN/H_2O (70:30) to yield compounds **1** (31 mg), **3** (9 mg) and **4** (12 mg). The Fr.C2.3.3 (723 mg) was purified on HPLC (CH_3CN/H_2O; 65:35) to yield compound **2** (25 mg).

*Perovskiaditerpenoside A (**1**)*: white amorphous powder. $[\alpha]_D^{16.2}$ +18.9 (*c* 0.20, MeOH). IR (KBr): 3443, 2998, 1606, 1497, 1448, 1066, 1011. ^{1}H- and ^{13}C-NMR (CD_3OD, 400 MHz and 100 MHz) data, see *Tables* 1 and 2; HR-ESI-MS (pos.): *m/z* 649.3192 ($[M + Na]^+$, $C_{32}H_{50}O_{12}Na^+$; calc. 649.3199).

*Perovskiaditerpenoside B (**2**)*: white amorphous powder. $[\alpha]_D^{16.9}$ +23.3 (*c* 0.21, MeOH). IR (KBr) 3442, 2998, 1603, 1498, 1449, 1063, 1010. ^{1}H- and ^{13}C-NMR (C_5D_5N, 400 MHz and 100 MHz) data, see *Tables* 1 and 2. HR-ESI-MS (pos.): *m/z* 665.3141 ($[M + Na]^+$, $C_{32}H_{50}O_{13}Na^+$; calc. 665.3149).

*Perovskiaditerpenoside C (**3**):* white amorphous powder. $[\alpha]_D^{16.3}$ +19.5 (*c* 0.15, MeOH). IR (KBr) 3442, 2995, 1642, 1606, 1497, 1448, 1065, 1011. ^{1}H- and ^{13}C-NMR (CD_3OD, 400 MHz and 100 MHz) data, see *Tables* 1 and 2. HR-ESI-MS (pos.): *m/z* 663.2989 ($[M + Na]^+$, $C_{32}H_{48}O_{13}Na^+$; calc. 663.2992).

*Perovskiaditerpenoside D (**4**)*: white amorphous powder. $[\alpha]_D^{16.8}$ +28.4 (*c* 0.20, MeOH). IR (KBr) 3443, 2998, 1602, 1499, 1451, 1061, 1011. ^{1}H- and ^{13}C-NMR (C_5D_5N, 400 MHz and 100 MHz) data, see *Tables* 1 and 2; HR-ESI-MS (pos.): *m/z* 665.3138 ($[M + Na]^+$, $C_{32}H_{50}O_{13}Na^+$; calc. 665.3149).

Acidic Hydrolysis. Each solution of compounds **1-4** (each 4 mg) in a mixture of MeOH (1.0 mL) and

10% HCl (1.0 mL) was stirred at reflux for 4 h. The hydrolysate was allowed to cool, diluted 2-fold with H_2O, and extracted with EtOAc (3 × 2 mL). The aqueous layer was neutralised with 2 M ammonium hydroxide and concentrated *in vacuo* to give a residue in which D-glucose was identified by comparison with authentic sugar sample (*n*-BuOH/AcOH/H_2O 4:1:5, upper layer; PhOH/H_2O, 4:1) on TLC (sprayed with aniline phthalate reagent, followed by heating).

Determination of Absolute Configuration of D-glucose. After being dried over P_2O_5 for 48 h, the above-mentioned aqueous residue was dissolved in anhydrous pyridine (1.0 mL), and 5 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h. The reaction mixture was then concentrated *in vacuo* to furnish a dry residue. 0.5 mL of *N*-trimethylsilylimidazole was added, and the reaction mixture was heated at 60 °C for 1 h. Subsequently, the mixture was partitioned between *n*-hexane and H_2O (1:1; v/v). The *n*-hexane extract was directly subjected to GC analysis under the following conditions: capillary column, HP-5 (30 m × 0.25 mm, with a 0.25 μm film, Dikma); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature 160 °C, raised to 280 °C at 5 °C·min^{-1} with the final temperature being maintained for 10 min; N_2 gas as carrier. By comparing the retention times (Rt.) of the derivatives with that of authentic sugar (D-glucose) prepared in a similar way, D-glucose (Rt. 20.6 min) from **1** - **4** were detected.

Cytotoxic Assay. Cytotoxic activities were evaluated by the MTT (3- (4, 5- dimethylthiazol- 2-yl) -2, 5- diphenyltetrazolium bromide) method [14] using HepG 2, NB4, Hela, K562, MCF7, PC3 and HL60 cell lines. Briefly, the cell suspensions (200 mL) at a density of 5 × 10^4 cells·mL^{-1} were plated in 96

well microtiter plates and incubated for 24 h at 37 °C in a humidified incubator containing 5% CO_2. The test compound solution (2 mL in DMSO) at different concentrations was added to each well and further incubated for 72 h under the same conditions. The MTT solution (20 mL) was then added to each well and incubated for 4 h. The old medium (150 mL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. The absorbance was then determined with a Spectra Max Plus plate reader at 540 nm. Dose-response curves were generated and the IC_{50} values were defined as the concentration of compound required to inhibit cell proliferation by 50%. Cisplatin, an approved agent for the treatment of many tumours, was used as the positive control.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China (NSFC No. 81560708), the Reserve Talent Project of Middle and Young Academic Leaders in Yunnan Province (2015HB050), and the program for Innovative Research Team (Science and Technology) in University of Yunnan Province (IRTSYN, 2014).

Supplementary data

Supplementary data (^{1}H, ^{13}C-NMR, HSQC, HMBC, ^{1}H-^{1}H COSY, ROESY spectra of compounds **1**-**4**) associated with this article can be found.

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Table 1. The ^{1}H NMR (400 MHz) data of compounds **1**-**4**, *J* in Hz.

No.	**1**[a]	**2**[b]	**3**[a]	**4**[b]
1	2.34 (1H,m)	3.53(1H,overlap)	3.12 (1H,m)	3.60(1H,overlap)
	1.45-1.53 (1H,m)	1.38(1H,m)	1.75 (1H,m)	1.43(1H,overlap)
2	1.78(2H,overlap)	1.94(1H,m)	1.72-1.79 (2H,m)	2.39(1H,m)
		1.83(1H,m)		2.03(1H,m)
3	3.23(1H, overlap)	3.53(1H,overlap)	3.26 (1H,overlap)	3.57(1H,m)
5	1.26(1H,brd,11.6)	1.30 (1H,brd, 12.0)	2.09 (1H,brs)	1.37(1H,overlap)
6	1.88 (1H,m)	1.81 (1H,overlap)	5.93(1H,dd,9.7,2.4)	1.78(1H,m)
	1.71-1.79 (1H,m)	1.58 (1H,m)		1.57(1H,m)
7	2.87(1H,m)	2.90(2H,m)	6.44 (1H,dd,9.6,2.8)	2.90(2H,m)
	2.70-2.79(1H,m)			
11	7.03 (1H, s)	-	-	-
14	6.84(1H, s)	6.75(1H,s)	6.49(1H,s)	6.73(1H,s)
15	3.49(1H,sept,6.8)	4.18(1H,sept,6.8)	3.65-3.70 (1H,overlap)	4.17(1H,sept,6.8)
16	1.18(3H,d,6.8)	1.44(3H,d,6.4)	1.18(3H,d,6.8)	1.35(3H,d,6.8)
17	1.13(3H,d,6.8)	1.10(3H,d,6.8)	1.13(3H,d,6.8)	1.15(3H,d,6.8)
18	1.05(3H,s)	1.29(3H,s)	1.06(3H,s)	1.42(3H,s)
19	0.88(3H,s)	1.08(3H,s)	0.98(3H,s)	1.09(3H,s)
20	1.19(3H,s)	1.47(3H,s)	1.08(3H,s)	1.44(3H,s)
11-OH	-	8.45(1H,s)	-	9.89(1H,s)
Glc-				
1′	4.93(1H,d,7.6)	5.21 (1H, d,8.0)	4.56(1H,d,7.6)	4.96(1H,d,7.6)
2′	3.85(1H,overlap)	4.53 (1H,t)	3.81(1H,overlap)	4.07(1H,m)
3′	3.69(1H,overlap)	4.36 (1H,overlap)	3.67(1H,overlap)	4.26(1H,overlap)
4′	3.36-3.42(1H,overlap)	4.37 (1H,overlap)	3.53(1H,t)	4.28(1H,overlap)
5′	3.42(1H,overlap)	3.78 (1H,m)	3.21-3.26(1H,overlap)	3.96(1H,m)
6′	3.89(1H,overlap)	4.36-4.44 (2H,overlap)	3.73-3.83(2H,overlap)	4.61(1H,brd,10.4)
	3.68(1H,overlap)			4.45(1H,overlap)
Glc-				
1″	4.79(1H,d,7.6)	5.64(1H,d,7.8)	4.84(1H,d,7.6)	5.16(1H,d,7.4)
2″	3.24(1H,overlap)	4.16-4.26(1H,overlap)	3.37(1H,overlap)	4.36(1H,overlap)
3″	3.31(1H,overlap)	4.26(1H,overlap)	3.40(1H,overlap)	4.34(1H,overlap)
4″	3.21-3.31(1H,overlap)	4.27(1H,overlap)	3.35(1H,overlap)	4.44(1H,overlap)
5″	3.22(1H,overlap)	3.96（1H,m）	3.35(1H,overlap)	3.88(1H,m)
6″	3.59-3.70(2H,overlap)	4.25-4.35(2H,overlap)	3.79(1H,overlap)	4.45(2H,overlap)
			3.65-3.70(1H,overlap)	

[a]Measured in CD_3OD; [b]Measured in C_5D_5N.

Table 2. The ^{13}C NMR (100 MHz) data of compounds **1**-**4**.

No.	**1**[a]	**2**[b]	**3**[a]	**4**[b]
1	38.2(t)	35.2(t)	36.1(t)	35.2(t)
2	28.8(t)	29.0(t)	28.7(t)	27.7(t)
3	79.5(d)	78.1(d)	79.2(d)	89.0(d)
4	40.1(s)	40.0(s)	39.9(s)	40.2(s)
5	51.6(d)	53.2(d)	52.9(d)	53.5(d)
6	20.2(t)	19.5(t)	129.0(d)	19.2(t)
7	31.3(t)	33.4(t)	129.9(d)	33.4(t)
8	130.0(s)	134.4(s)	133.3(s)	134.2(s)
9	148.9(s)	133.8(s)	131.5(s)	133.4(s)
10	38.8(s)	39.7(s)	41.9(s)	39.4(s)
11	112.4(d)	148.5(s)	147.5(s)	148.8(s)
12	154.0(s)	141.7(s)	143.2(s)	142.5(s)
13	136.3(s)	140.6(s)	141.8(s)	140.2(s)
14	127.1(d)	117.7(d)	117.0(d)	117.1(d)
15	26.6(d)	26.0(d)	26.8(d)	26.1(d)
16	23.5(q)	24.1(q)	24.1(q)	24.1(q)
17	24.0(q)	24.4(q)	24.3(q)	24.3(q)
18	28.8(q)	29.2(q)	28.7(q)	28.8(q)
19	16.2(q)	16.9(q)	17.1(q)	17.4(q)
20	25.5(q)	19.9(q)	18.2(q)	19.9(q)
Glc-				
1′	101.5(d)	105.7(d)	105.5(d)	107.0(d)
2′	81.2(d)	84.2(d)	83.4(d)	75.8(d)
3′	78.5(d)	78.0(d)	77.9(d)	78.8(d)
4′	71.4(d)	70.6(d)	70.7(d)	71.7(d)
5′	78.0(d)	78.7(d)	78.1(d)	78.3(d)
6′	62.6(t)	62.0(t)	62.0(t)	62.9(t)
Glc-				
1″	104.4(d)	106.5(d)	105.7(d)	108.4(d)
2″	76.0(d)	76.2(d)	75.9(d)	75.6(d)
3″	77.8(d)	78.1(d)	77.7(d)	78.5(d)
4″	71.5(d)	71.3(d)	71.3(d)	71.1(d)
5″	78.1(d)	78.8(d)	78.4(d)	79.0(d)
6″	62.6(t)	62.5(t)	62.6(t)	62.2(t)

[a]Measured in CD_3OD; [b]Measured in C_5D_5N.

Table 3. The cytotoxic activities (mean±SD, n=3) of compounds **1**-**4**.

Compounds	IC_{50} (μM)						
	HepG 2	NB4	Hela	K562	MCF7	PC3	HL60
1	0.37±0.01	7.85±0.22	1.42±0.26	>10	8.72±0.41	>10	0.23±0.02
2	6.29±0.17	8.16±0.39	3.57±0.19	1.24±0.03	5.43±0.27	>10	5.88±0.51
3	>10	1.27±0.04	1.02±0.08	0.35±0.01	0.96±0.02	>10	5.17±0.36
4	2.26±0.13	7.13±0.54	8.36±0.32	>10	>10	8.25±0.55	>10
Cisplatin[a]	0.20±0.01	0.03±0.001	0.05±0.001	0.20±0.01	0.12±0.01	0.20±0.01	0.18±0.01

[a]positive control.

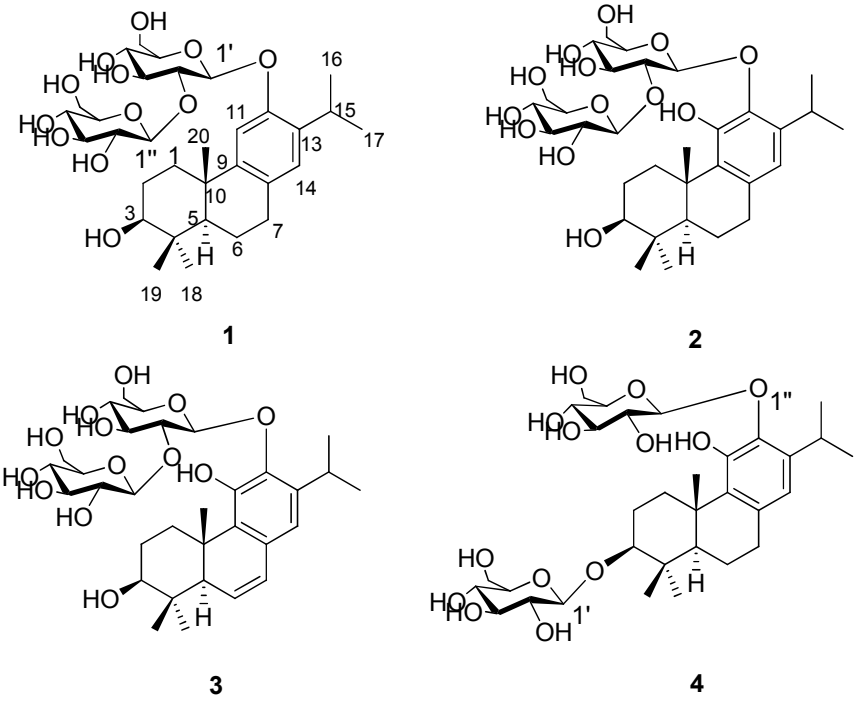

Figure 1. The structures of compounds **1**-**4**.

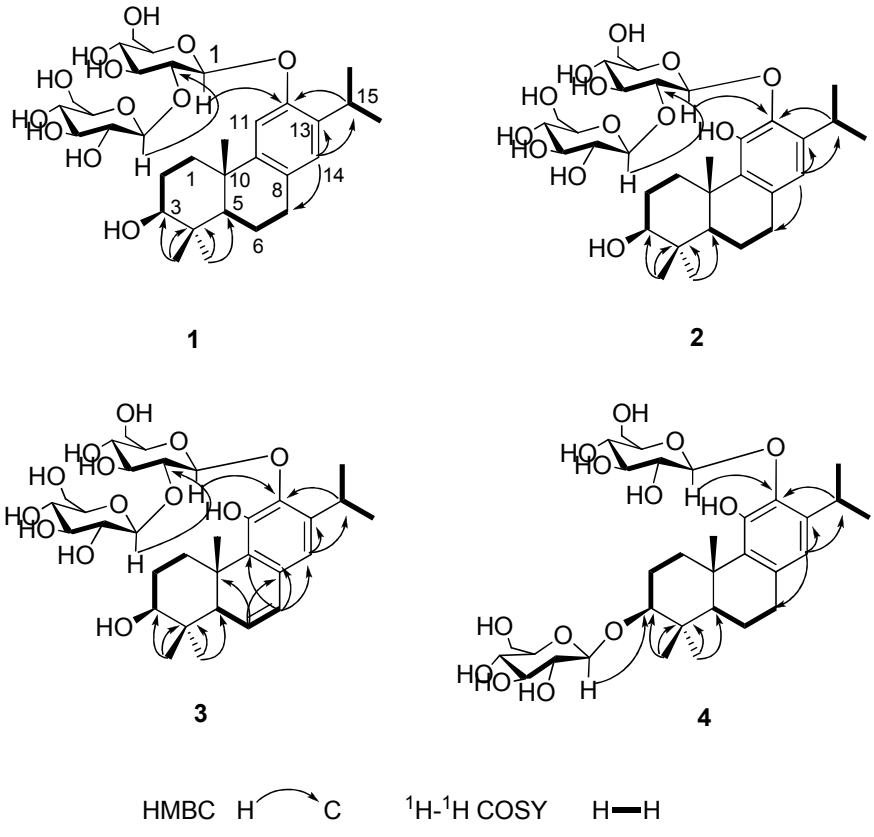

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds **1**-**4**.

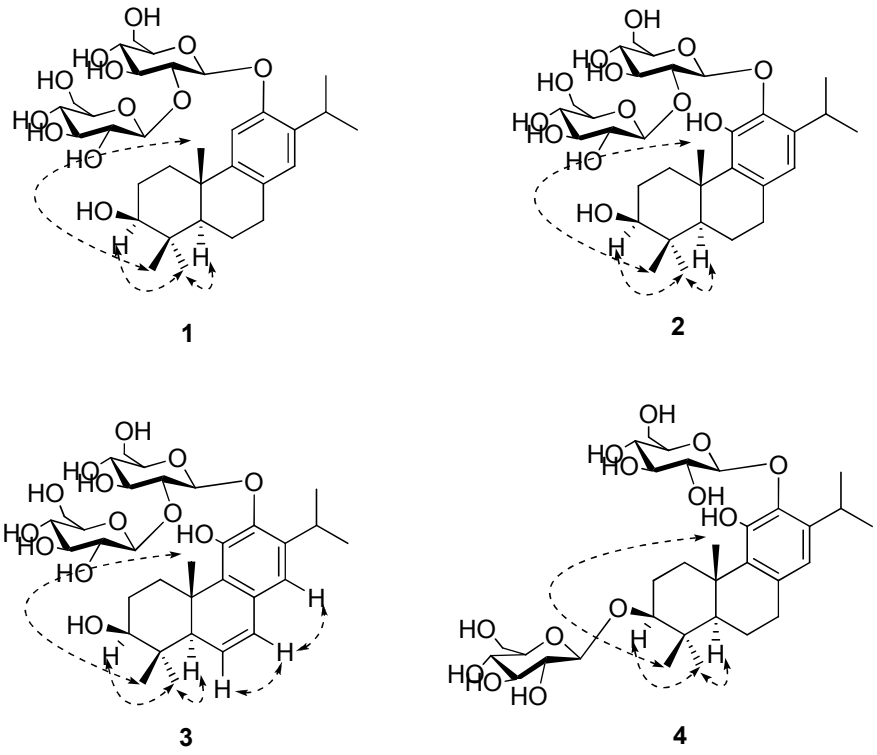

Figure 3. Key ROESY correlations (H⇢⇠H) of compounds **1**-**4**.