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Two new sesquiterpenes from *Artemisia sieversiana*

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ABSTRACT

Two new sesquiterpenes, together with 32 known compounds(**3**–**34**), were isolated from *Artemisia sieversiana* Ehrhart ex willd. and the compounds **3**–**21** were isolated from this plant for the first time. The new compounds were elucidated as 2α,9α-dihydroxymuurol-3(4)-en-12-oic acid (**1**) and 13α-methyl-(5αH,6αH,7αH,8αH)-austricin 8-*O*-β-D-glucopyranoside (**2**), respectively. The structural identification of these compounds was mainly achieved by spectroscopic methods including 1D and 2D NMR techniques, and the structure of compound **1** was confirmed by a single crystal X-ray diffraction experiment. Compounds **1**–**2** were evaluated for cytotoxic activity *in vitro* against MCF-7, NCI-H460 and Hep-G2 cell lines, respectively.

1. Introduction

The genus *Artemisia* belongs to the tribe Anthemideae, family of Compositae, comprising more than 186 species native to China [1]. Many species have been used as folk medicines in China. *Artemisia sieversiana* Ehrhart ex willd. is an annual herb that is for the most part abundantly distributed in Qinghai, Gansu, Ningxia, Shaanxi and Sichuan provinces. It grows on the roadside, wasteland, floodplains, steppe and forest edges in altitudes of 500–4500 m [1], and it has been used for detumescence, hemostasis and relieving heat in Tibetan medicines.

However, there have been few reports[2] on phytochemical investigations of this plant up to now. To ascertain its chemical composition and medicinal value, the petroleum ether-Et_2O-MeOH (1:1:1) extract of *A. sieversiana* was investigated. Herein, the isolation and structural elucidation of the two new sesquiterpenes as well as their cytotoxic effects against MCF-7, NCI-H460 and Hep-G2 cancer cells are described in this paper.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer-343 spectropolarimeter. IR spectra were recorded on a NICOLET IR200 FT-IR spectrophotometer. NMR spectra were scanned on a Bruker Avance DRX-500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). HR-ESI-MS was carried out on an Agilent Technologies 6224 TOF LC-MS apparatus. Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Marine Chemical, Inc.). MCI GEL CHP20p (75–150 μm, Mitsubishi Kasei Corporation), or Sephadex LH-20 (20–100 μm, Pharmacia) was also used for CC. TLC was conducted on silica gel GF_{254} plates (10–40 μm; Qingdao Marine Chemical, Inc.). Petroleum ether (30–60 °C), ethyl acetate and other reagents were purchased from Nanjing Wanqing Reagent, Inc. Spots were observed by UV light as well as by spraying with 10% H_2SO_4–EtOH followed by heating. The cell lines (MCF-7, NCI-H460 and Hep-G2) were purchased from the center cell

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resources of Shanghai Institute of Life Science Chinese Academy of Sciences. Tumor cells were incubated in an HF-212UV CO_2 incubator and observed under an OLYMPUS CKX41 inverted microscope. Optical density (OD) values were read under a BIO-RAD Model 680 microplate reader. Cisplatin (HPLC >98%) was purchased from the National Institutes for Food and Drug Control. The purity of the two new sesquiterpenes (**1**: 99.84%; **2**: 99.12%) was analyzed by HPLC (Agilent 1260: DEACA01043), which was carried out on a column (Agilent ZORBAX SB-C18; 4.6 × 250 mm, 5 μm) using methanol and 0.1% glacial acetic acid–water soln. (*v*/*v* 80/20) at a flow rate of 1 mL/min within about 35 min.

2.2. Plant material

The dried aerial parts of *A. sieversiana* were collected in July, 2012, Heka town of Xinghai County in Qinghai Province, China. It was identified by Prof. Hong-Fa Sun of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China, and a voucher specimen (No. 12-07-01) was deposited at the laboratory of Zhi-Xin Liao, Southeast University, Nanjing, China.

2.3. Extraction and isolation

The dried and powdered plant material (2.40 kg) of *A. sieversiana* was percolated four times (7 days each time) with petroleum ether–Et_2O–MeOH (1:1:1) (8 L × 4) at room temperature. The filtrates were consolidated and evaporated in vacuum to give a concentrate (204 g).

The resultant extract (204 g) was subjected to column chromatography (CC) on silica gel (2000 g, column: 10 × 100 cm) with a petroleum ether–ethyl acetate gradient (*v*/*v* 50:1–0:1). Five crude fractions (Fr.1–Fr.5) were obtained, which were combined according to TLC data. Specifically, Fr.1 (55.4 g, petroleum ether-ethyl acetate 50:1–20:1) was discarded without further separation because it was mainly comprised of volatile oil components of low polarity. Fr.2 (25.4 g, petroleum ether–ethyl acetate 10:1–8:1) was further separated by column chromatography over silica gel (250 g, column: 5 × 100 cm) with a petroleum ether–ethyl acetate gradient (*v*/*v* 20:1–4:1) to yield **22** (203 mg). For Fr.3 (6.70 g, petroleum ether–ethyl acetate 6:1–4:1), it was rechromatographed over silica gel (100 g, column: 2.5 × 100 cm) with petroleum ether–ethyl acetate (*v*/*v* 14:1–1:1) mixtures to give two fractions (Fr.3.1–Fr.3.2, TCL data). In detail, Fr.3.1 (2.10 g, petroleum ether–ethyl acetate, 14:1–8:1) was further purified by column chromatography over silica gel (40 g, column: 2 × 80 cm) with a petroleum ether–ethyl acetate gradient (*v*/*v* 16:1–4:1) to yield **3** (3.00 mg) and **9** (5.00 mg) while Fr.3.2 (3.20 g, petroleum ether-ethyl acetate 6:1–1:1) being further separated over Sephadex LH-20 with chloroform–methanol (2:1) to yield **31** (3.00 mg), **26** (4.00 mg) and **32** (3.00 mg). In terms of Fr.4 (37.0 g, petroleum ether–ethyl acetate 3:1–1:1), it was firstly purified over MCI GEL eluted with 80% ethanol to remove the pigments. The remaining materials were evaporated for further isolation. It (20.4 g) was rechromatographed over silica gel (200 g, column: 5 × 100 cm) with petroleum ether–ethyl acetate (*v*/*v* 6:1–1:1) mixtures to give two fractions (Fr.4.1–Fr.4.2 TCL data). Fr.4.1 (5.40 g, petroleum ether–ethyl acetate 6:1–3:1) was further separated by repeated column chromatography over silica gel (100 g, column: 2.5 × 100 cm) with a petroleum ether–ethyl acetate gradient (*v*/*v* 8:1–2:1) to yield **28** (6.00 mg), **15** (105 mg), **23** (100 mg) and **4** (3.00 mg) while Fr.4.2 (10.6 g, petroleum ether–ethyl acetate 2:1–1:1) was subjected to separation repeated by silica gel (150 g, column: 3 × 100 cm) column chromatography eluted with petroleum ether–ethyl acetate (*v*/*v* 4:1–0:1) to yield **5** (200 mg), **11** (10.0 mg), **10** (305 mg) and **14** (4.00 mg). After that, a repeated separation of the residue over Sephadex LH-20 with chloroform–methanol (1:1) resulted in **6** (5.00 mg), **7** (3.00 mg), **8** (4.00 mg), **33** (3.00 mg) and **36** (6.00 mg). Concerning to Fr.5 (60.4 g, petroleum ether-ethyl acetate 0:1), it was fractionated by column chromatography over silica gel (600 g, column: 6 × 100 cm) with the gradient system of increasing polarity with chloroform–methanol (*v*/*v* 50:1–0:1). In this case, four fractions (Fr.5.1–Fr.5.4, TCL data) were obtained. Compounds **19** (2.00 mg), **27** (2.00 mg), **25** (3.00 mg) and **24** (3.00 mg) were subsequently eluted from Fr.5.1 (14.4 g, chloroform–methanol 50:1–20:1) when eluted with a chloroform–methanol of increasing polarity (*v*/*v* 50:1–10:1) by column chromatography over silica gel (150 g, column: 4 × 100 cm), and compounds **16** (4.00 mg), **1** (25.0 mg) and **17** (12.0 mg) were precipitated successively from Fr.5.2 (20.6 g, chloroform–methanol 10:1–8:1) with increasing polarity of chloroform–methanol (*v*/*v* 12:1–7:1) by column chromatography over silica gel (200 g, column: 5 × 100 cm). Fr.5.3 (13.8 g, chloroform–methanol 6:1–4:1) was subjected to a silica gel column (150 g, column: 4 × 100 cm) eluting with chloroform–methanol (*v*/*v* 8:1–3:1) to yield **29** (15.0 mg), **2** (20.0 mg), **12** (15.0 mg) and **13** (26.0 mg), while Fr.5.4 (13.4 g, chloroform–methanol 3:1–0:1) was further purified on silica gel (150 g, column: 4 × 100 cm) with chloroform–methanol (*v*/*v* 4:1–1:2) to give **18** (108 mg), **20** (11.0 mg), **30** (15.0 mg) and **21** (21.0 mg).

2.4. Compound characterization

2α,9α-dihydroxymuurol-3(4)-en-12-oic acid (**1**): Colorless crystals ($CHCl_3$–MeOH, 8:1), mp: 203–204 °C, R_f 0.60, silica gel 40 F_{254}, $CHCl_3$/MeOH (7:1), $[\alpha]^{20}_D = -75.3$ (c = 1.00, MeOH), IR (KBr):3400, 2920, 2880, 1700 cm^{-1}. ^1H and ^{13}C NMR (DMSO) (see Table 1). HR-ESI-MS: *m*/*z* 267.1599 ($[M - H]^-$, $C_{15}H_{23}O_4^-$, calc. 267.1596).

13α-methyl-(5αH,6αH,7αH,8αH)-austricin 8-*O*-β-D-glucopyranoside (**2**): white amorphous powder, mp: 306–307 °C, R_f 0.50, silica gel 40 F_{254}, $CHCl_3$/MeOH (7:1), $[\alpha]^{20}_D = +12.2$ (c = 0.11, MeOH), IR (KBr): 3527, 1760, 1688, 1621 cm^{-1}. ^1H and ^{13}C NMR (DMSO) (see Table 1), HR-ESI-MS: *m*/*z* 425.1809 ($[M + H]^+$, $C_{21}H_{29}O_9^+$, calc. 425.1813).

2.5. Cytotoxicity experiments

The cytotoxicity effects of compounds **1**–**2** against MCF-7, NCI-H460 and Hep-G2 cells were tested using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and Cisplatin was used as a positive control. The cell suspensions were distributed into 96-well cell culture plates and cultured at 36–37 °C in a 5% CO_2 incubator for 24 h and each sample was dissolved with a limited amount of DMSO, diluted to five different concentrations with a culture medium and then added to the corresponding well. The blank controls consisting of microbial culture were

Table 1
^{1}H, ^{13}C-NMR data of 1 and 2 at 500 and 125 MHz in DMSO at 30 °C (δ in ppm; J in Hz).

Position	**1**		**2**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1α	1.53(1H, dd,	30.9		132.5
1β	13.20,3.70)			
	1.46(1H, d, 12.95)			
2	3.72(1H, t, 4.35)	66.8		194.8
3		135.6	6.16(1H, s)	134.7
4	5.73(1H, d,5.40)	128.2		170.4
5	2.34(1H, t, 5.55)	34.9	3.62(1H, d,10.05)	50.4
6	2.54(1H, dd,2.70,	39.7	3.95(1H, t, 10.35)	80.7
	7.00)			
7α	1.29(1H, m)	22.9	2.70(1H, dd,3.25,	55.8
7β	1.51(1H, m)		10.40)	
8α	1.26(1H, m)	34.8	3.54(1H, t, 10.55)	74.6
8β	1.40(1H, m)			
9α		70.1	2.61(1H, dd,1.65,	45.0
9β			11.30)	
			2.94(1H, dd, 7.35,	
			9.25)	
10	1.38(1H, m)	41.7		146.3
11	1.72(1H, m)	39.9	2.50(1H, m)	40.0
12		176.7		178.2
13	0.99(3H, d, 7.05)	15.0	1.15(3H, d, 7.60)	9.3
14	1.05(3H, s)	29.6	2.33(3H, s)	20.7
15	1.67(3H, s)	21.7	2.20(3H, s)	19.2
1'			4.29(1H, d, 7.80)	104.9
2'			2.95(1H, m)	73.6
3'			3.16(1H, m)	76.8
4'			3.05(1H, m)	70.1
5'			3.15(1H, m)	76.6
6'α			3.45(1H, m)	61.1
6'β			3.69(1H, m)	

also incubated with 0.02% DMSO under the same conditions, and DMSO was not toxic at these limited amounts under the experimental conditions. After 48 h of cultivation, MTT was added to each well for another 4 h cultivation. Finally, the supernatant was discarded, and 150 μl DMSO was added to each well to completely dissolve the blue–violet crystals, then the optical density (OD) values were then read on a microplate reader. Origin7.5 computer programme (Data analysis and Graphics Software, USA) was used to determine the median inhibitory rate (IC_{50}), and the results are presented in Table 2.

3. Results and discussion

Compound **1** was obtained as a colorless crystals ($CHCl_3$–MeOH, 8:1). The molecular formula of **1** was deduced to be

Table 2
The median inhibitory rate (IC_{50}) (mean ± S.D., $n = 5$) of compounds 1 and 2.

Sample	Median inhibitory rate(IC_{50}) (μM)		
	MCF-7[a]	NCI-H460[a]	Hep-G2[a]
1	26.8 ± 0.6	48.7 ± 1.2	34.5 ± 0.9
2	31.4 ± 0.8	43.4 ± 1.2	40.0 ± 1.1
Cisplatin[b]	27.3 ± 0.5	30.9 ± 0.7	15.2 ± 0.2

[a] Clinical strain.
[b] Positive control.

$C_{15}H_{24}O_4$ based on the ^{1}H- and ^{13}C-NMR data and the quasi-molecular ion peak at m/z 267.1599 ($[M - H]^-$, calc. 267.1596) in the HR-ESI-MS. The assignment was confirmed with the aid of 2D-NMR (HSQC, HMBC and ROESY) spectra.

The NMR data further secured the structure. There were three signals corresponding to methyl groups (δ 1.67, 1.05 and 0.99) in the ^{1}H-NMR spectrum and 15 carbon signals in the ^{13}C-NMR spectrum. An olefinic proton at δ 5.73 (d, J = 5.40 Hz, 1H) with a pair of olefinic carbon signals at δ 128.2 and 135.6 indicated a double bond in the structure. The proton signal at δ 11.90 (s, 1H) together with the carbon signal at δ 176.7 revealed the presence of a carboxylic group. The ^{13}C-NMR and HSQC of **1** also indicated that three methylene (δ_C 30.9, C-1; 22.9, C-7; 34.8, C-8), four methine (δ_C 34.9, C-5; 39.7, C-6; 41.7, C-10; 39.9, C-11), one oxygen-bearing methine (δ_C 66.8, C-2), and one sp3 oxygen-bearing quaternary C-atom (δ_C 70.1, C-9) existed in structure **1**. These data revealed that **1** was a muurolane-type sesquiterpene acid [3], and the proton and carbon signals of **1** are shown in Table 1.

In the HMBC spectrum (Fig. 1), the correlations of H-1 with C-3 and C-5; H-4 with C-2, C-6 and C-15 indicated that the double bond was located between C-3 and C-4 and that the hydroxyl group was linked to C-2. The correlation of H-14 with C-8 revealed that the methyl group (δ_C 29.6) and the additional hydroxyl group were located at C-9. In addition, the correlation of H-13 with C-12 suggested the presence of a $-CH(CH_3)-COOH$ moiety, and the attachment position was deduced to be C-6 because of the correlations of H-6 with C-8, C-10, C-12 and C-13.

The relative configuration of **1** was determined by a ROESY experiment. In the ROESY spectrum (Fig. 2), H-5 was coupled with H-10, which suggested that H-5 and H-10 were α-axial [3]. The ROESY correlations of H-1α and H-8α with H-10, H-1β and H-8β with H-14, H-1β with H-2 in compound **1** indicated that H-14 and H-2 were β-side. Consequently, the hydroxyl group at C-2 and C-9 was α-equatorial. The structure of **1** was

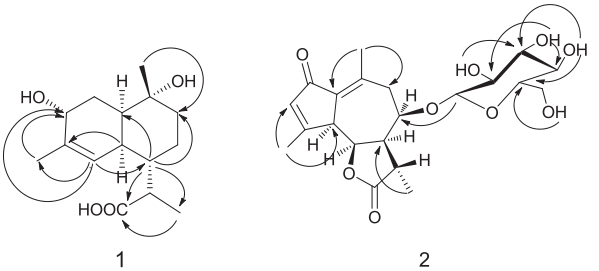

Fig. 1. Key HMBC (H → C) correlations of compounds 1–2.

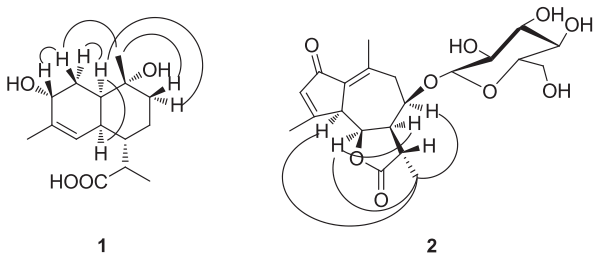

Fig. 2. Key ROESY correlations of compounds 1–2.

confirmed by X-ray crystallography (Fig. 3). On the basis of the above analysis, compound **1** was identified as 2α,9α-dihydroxymuurol-3(4)-en-12-oic acid.

X-ray crystallographic analysis of **1**: Colorless blocks, $C_{15}H_{24}O_4$, $M_r = 268.34$, tetragonal, space group $P4_32_12$, a = 8.8251(10)Å, $b = 8.8251(10)$Å, $c = 38.147(9)$Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, $V = 2971.0\ (10)$Å^3, $Z = 8$, $D_x = 1.200$ mg/m^3, $F(000) = 1168$, μ (Mo Kα) = 0.085 mm^{-1}. Data collection was performed on a Gemini SUltra using graphite-monochromated Mo Kα radiation. λ = 0.71073 Å at 296 K. 20830 unique reflections were collected to $\theta_{max} = 24.99°$, in which 2442 reflections were observed [$F^2 > 4\sigma(F^2)$]. The structure was solved by direct methods using the SHELXS-97 program and refined by the program SHELXL-97 and full-matrix least-squares calculations. The final refinement gave $R_1 = 0.0418$, $wR_2 = 0.1152$ and S = 1.050. CCDC 978094 contains the supplementary crystallographic data for **1**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre www.ccdc.cam.ac.uk/data_request/cif.

Compound **2** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{21}H_{28}O_9$ based on the ^{1}H- and ^{13}C-NMR data and the quasi-molecular ion peak at m/z 425.1809 ($[M + H]^+$, calc. 425.1813) in the HR-ESI-MS.

The ^{1}H NMR spectrum (Table 1) of **2** displayed the signal of an anomeric proton of D-glucose, the anomeric proton doublet was well separated at δ 4.29 and its large coupling constant ($J = 7.80$ Hz) indicated the β-D-glycosidic linkage of the sugar moiety [4]. The ^{1}H NMR spectrum (Table 1) of **2** displayed the signal of an anomeric proton of sugar, and acid hydrolysis of **2** gave D-glucose identified by direct comparison with the authentic sample. The J value of the anomeric proton ($J = 7.80$ Hz) indicated the β-D-glycosidic linkage. In addition, the ^{1}H NMR spectrum showed three methyl protons signals at δ 2.33 (3H, s), 2.20(3H, s) and 1.15 (3H, d, $J =$ 7.60 Hz). ^{13}C NMR data (Table 1) revealed the presence of 21 carbon signals, in which, the following functionalities of two double bond (δ_C 134.7, C-3; 170.4, C-4; 132.5, C-1; 146.3,

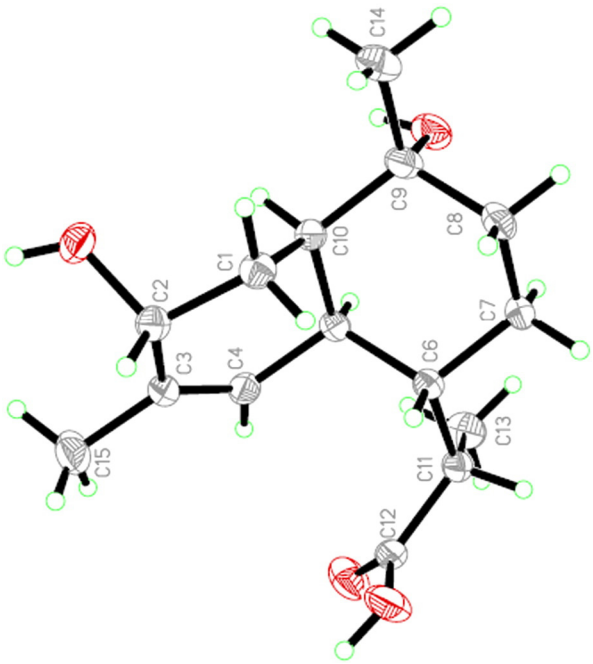

Fig. 3. Perspective drawing of the X-ray structure 1.

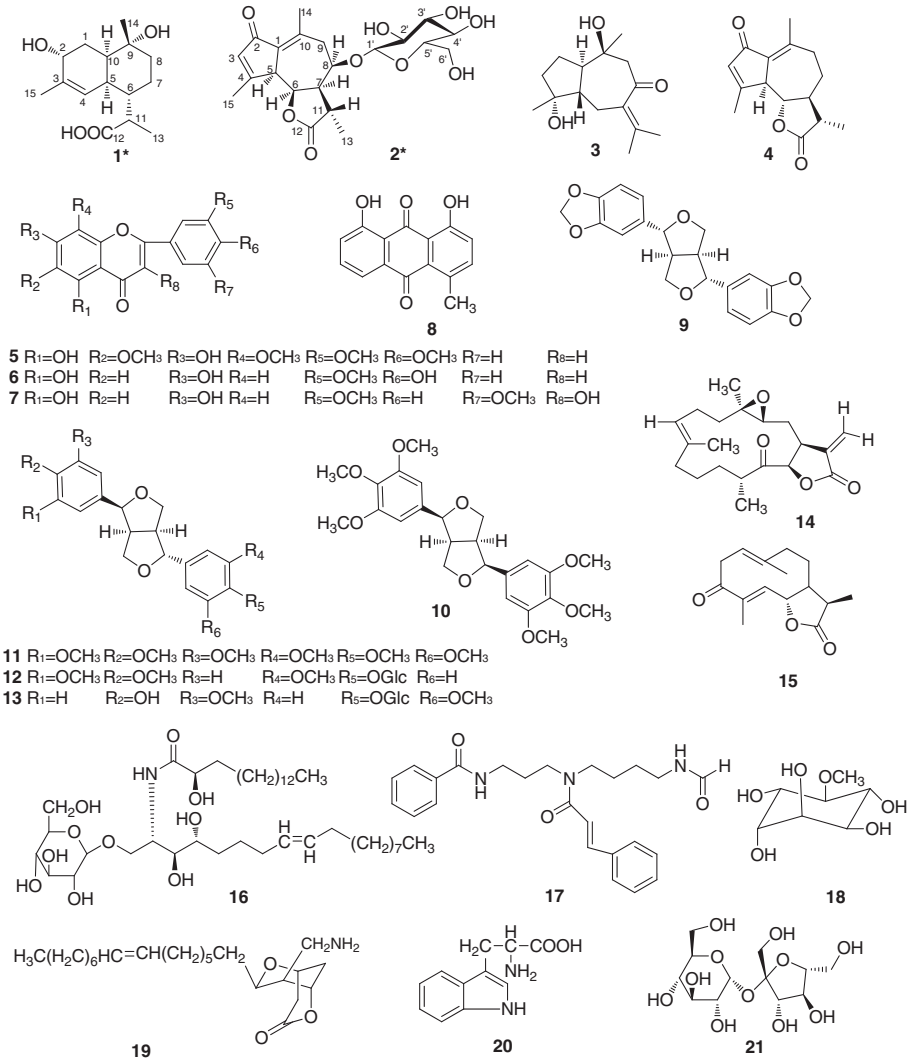

Fig. 4. Chemical structures of compounds 1–21.

C-10), two oxygenated methine carbons (δ_C 80.7, C-6; 74.6, C-8), two carbonyl (δ_C 194.8, C-2; 178.2, C-12), three methyl (δ_C 9.3, C-13; 20.7, C-14; 19.2, C-15) and D-glucose carbon (δ_C 104.9, C-1′; 73.6, C-2′; 76.8, C-3′; 70.1, C-4′; 76.6, C-5′; 61.1, C-6′) were distinguishable.

In the HMBC spectrum (Fig. 1), the proton signal of H-1′ (δ 4.29) (the anomeric proton of D-glucose) correlated with the carbon signal at δ 74.6 (C-8) revealed the presence of a D-glucose at C-8. The planar structure of **2** was thus worked out.

Compound **2** possessed the same planar structure as that of austricin 8-*O*-β-D-glucopyranoside as reported by Xu (2000) [5]. However, the chemical shift of C-7, C-8 and C-13 was clearly different. The ROESY (Fig. 2) correlations of H-7α [5] with H-8 and H-13; H-7α with H-6 and H-5; H-5 with H-13 in compound **2** indicated that they were located mutually on the same α-side; consequently, H-11 was β-equatorial. Thus, compound **2** was identified as 13α-methyl-(5αH,6αH,7αH, 8αH)-austricin 8-*O*-β-D-glucopyranoside. The structure having a *cis*-fused γ-lactone ring at C-6 and C-7, which was reported in literatures was rather rare [6].

Based on the comparison of the NMR data as well as the physicochemical properties of the known compounds, the following compounds were identified as follows: 4,10-*epi*zedoarondiol (**3**) [7]; leukodin (**4**) [8]; 5,7-dihydroxy-6,8, 3′, 4′-tetramethoxy flavone (**5**) [9]; chrysoeriol (**6**) [10]; 5,7-trihydroxy-3′,5′-dimethoxylflavone (**7**) [11]; 1,8-dihydroxy-4-methylanthraquinone (**8**) [12]; sesamin (**9**) [13]; diayangambin (**10**) [14]; *epi*yangambin (**11**) [14]; phillyrin (**12**) [15]; simplocosin (**13**) [16]; 12-*epi*-eupalmerone (**14**) [17]; 3-oxo-11α-H-germacra-1(10) *E*, 4*Z*-dien-12,6α-olide (**15**) [18]; 1-*O*-β-D-glucopyranosyl-(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2′*R*)-2′-hydroxypalmitoylamino]-8-octadecene-1,3,4-triol (**16**) [19]; chisitine 2 (**17**) [20]; D-3-*O*-methyl chiroinositol (**18**) [21]; (1*S*,5*S*,7*R*,8*S*)-8-(aminomethyl)-7-(pentadec-7-en-1-yl)-2,6-dioxabicyclo [3.3.1] nonan-3-one (**19**) [22]; tryptophan (**20**); sucrose(**21**) (Fig. 4); and additionally stigmasterol (**22**); achillin (**23**) [8]; 3α,4α,10β-trihydroxy-8α-acetoxy-11*B*h-guai-

1-en-12,6α-olide (**24**) [2]; 3α,4α,10β-trihydroxy-11 β H-guai-1-en-12,6α-olide (**25**) [2]; sieversol (**26**) [2]; 2α,4α,8α,-trihydroxy-3α-acetoxy-11βH-guai-1(10)-en-12,6α-olide(**27**) [2]; *epi*ashchantin(**28**) [14]; 3α,4α-dihydroxy-8α-acetyloxy-11βH-guai-1,9-dien-12,6α-olide(**29**) [2]; rutin(**30**) [23]; 5-methoxysesamin(**31**) [2]; 11,14,15-trimethoxy-6-one-1(10), 3-diene-12,5-olide (**32**) [24]; 3,5-dihydroxy-6,7,3′,4′-tetramethoxy flavone (**33**) [25] and tricin (**34**) [26]. Compound **14** was first isolated from the soft coral *Sarcophyton crassocaule* [27]. Such cembranes diterpene was often as an evidence in chemotaxonomy found in some marine organisms(soft corals) [28], but cembranes are not restricted to marine organisms. Novel cembranes have been also obtained from frankincense [29], *tobacco* [30,31], the bark of *Croton oblongifolius* [32] and *Anisomeles indica* [33] of the terrestrial plant. The cembrane diterpenoid was first found in the genus *Artemisia*.

In an anti-proliferative activities test, compound **1** was found to show moderate cytotoxicity in MCF-7 cell lines with an IC_{50} value of 26.8 μM and weak cytotoxic activity in NCI-H460 (48.7 μM) and Hep-G2(34.5 μM). However, compound **2** showed to have weak cytotoxic activity in MCF-7 (31.4 μM), NCI-H460 (43.4 μM) and Hep-G2(40.0 μM) cell lines. Additionally, compounds **1**–**2** were evaluated for cytotoxic activity *in vitro* against MCF-7, NCI-H460 and Hep-G2 cell lines, respectively. The choice of the cell lines was established on the basis of cell line data with literature values of similar compounds [34–39].

Conflict of interest

There are no conflicts of interest of all authors with respect to this work.

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Appendix A. Supplementary data

1D and 2D NMR spectra, as well as HR-ESI-MS spectra and HPLC data for the new compounds (1–2) are available as Supporting Information. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.05.007.

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