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New acyclic sesquiterpenoid derivatives and a monoterpene disaccharide from *Dichondra repens* Forst.

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ABSTRACT

A new highly oxygenated acyclic sesquiterpenoid (2*E*, 6*E*)-8,10,11-trihydroxyl-7,11-dimethyl-3-hydroxymethyl-2,6-dodecadienoic acid (**1**) and its glucoside (**2**), together with a new pinane monoterpene disaccharide glucoside 6,6-dimethyl-2-methlenebicyclo [3.1.1]hept-3-*O*-(6-*O*-apiofuranosyl)-β-D-glucopyranoside (**3**) were isolated from hydrophilic extract of *Dichondra repens*. Their structures were elucidated on the basis of spectroscopic analyses and chemical methods. The three compounds did not show any cytotoxic activity (IC50 > 20 μM) against two human lung cancer cell lines (NCI-H661 and A549).

1. Introduction

The genus *Dichondra* (Convolvulaceae) comprises of eight species. *Dichondra repens* Forst., is the only species widely growing in southern China as common herbaceous cover (Cardin et al., 2005). In traditional Chinese medicine, *D. repens* was used for the treatment of jaundice and dysentery (Cardin et al., 2005). However, the chemical study on *D. repens* is rarely reported (Sheu et al., 2012). We report the isolation and structural elucidation of the compounds from the hydrophilic extract of *D. repens* in this paper. New highly oxygenated acyclic sesquiterpenoid derivatives (**1**–**2**), and a new pinane monoterpene disaccharide (**3**) (Fig. 1), along with the known compounds, adicardin (Li et al., 2009), actinidioionoside (Xu et al., 2005), eleganoside B (Zhang et al., 2011), tuberonic acid (Nonaka et al., 2010), aegineoside (Ho et al., 2003), obtusifoside A (Liu et al., 2013), gymnemasaponin IV (Yoshikawa et al., 1991) were isolated. Their chemical structures were determined by use of HRESIMS, high resolution NMR techniques and chemical methods. Moreover, the three new compounds were evaluated for their cytotoxicity and the results of which will also be presented.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The molecular formula was determined as $C_{15}H_{26}O_6$ based on the HRESIMS pseudomolecular ion at *m/z* 325.1630 ($[M+Na]^+$, $C_{15}H_{26}O_6Na$, calcd 325.1622) and ^{13}C NMR data. The IR spectrum showed the absorption band of α, β-unsaturated carboxyl group (1647 cm^{-1}). The ^1H NMR spectrum exhibited three tertiary methyl groups (δ_H 1.59, 1.13, 1.09) and two olefinic protons (δ_H 5.92, 5.44). The ^{13}C NMR and DEPT spectra of **1** displayed three methyls, four methylenes, four methines, and three quaternary carbons (two olefinic moieties), and one carbonyl group (δ_C 170.3). In the ^1H–^1H COSY, correlations between H-4/H-5/H-6, H-8/H-9/H-10 were apparent. In the HMBC (Fig. 2), spectrum revealed that the signal of the methyl groups at δ_H 1.13, 1.09 correlated to the quaternary carbon C-11 (δ_C 73.5), which further correlated to H-10 (δ_H 3.24). The remaining methyl signal (δ_H 1.59) correlated to the olefinic carbon C-7 (δ_C 138.4), which further correlated to H-8 (δ_H 4.19). The correlations between H-4 (δ_H 2.54), H-13 (δ_H 4.08) and the olefinic moiety (δ_C 115.0, 163.0) indicated the linkage of C-4-C3-C-13. Besides, the correlation between olefinic proton H-2 (δ_H 5.92) and the carbonyl group (δ_C 170.3) showed the linkage between C-1 and C-2. Thus, the planar structure of **1** was assigned. The absolute configuration of **1** was determined according to the reported method (Mondol et al., 2011). Firstly, the relative configuration of **1** was assigned by using Kishi's Universal NMR Database (Database 2) (Kobayashi et al., 2000). The ^{13}C NMR resonances of C-8/C-10 were in agreement with a *syn* arrangement of the 1,3-diol model system (Fig. 4). Secondly, the absolute configuration of the 8,10-diol of **1** was determined by modified Mosher's ester method (Freire et al., 2005). The chemical shift difference ($\Delta\delta = \delta_S - \delta_R$) implied that the configuration is 8*R*, 10*S*. Thus, the structure of compound **1** was elucidated as (2*E*, 6*E*)-8,10,11-trihydroxyl-7,11-dimethyl-3- hydroxymethyl-2,6-dodecadienoic acid.

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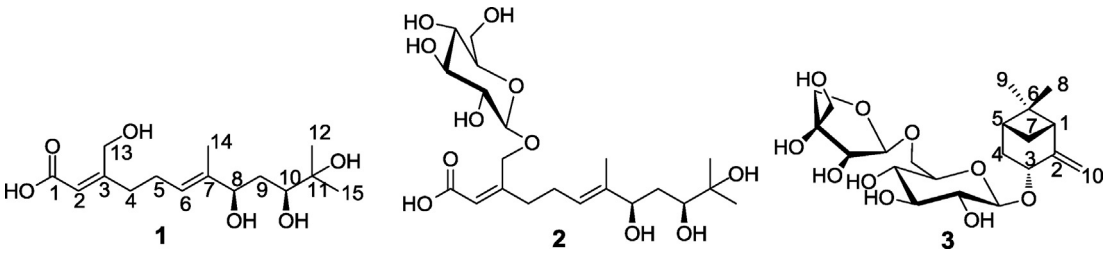

Fig. 1. Structure of compounds **1–3.**

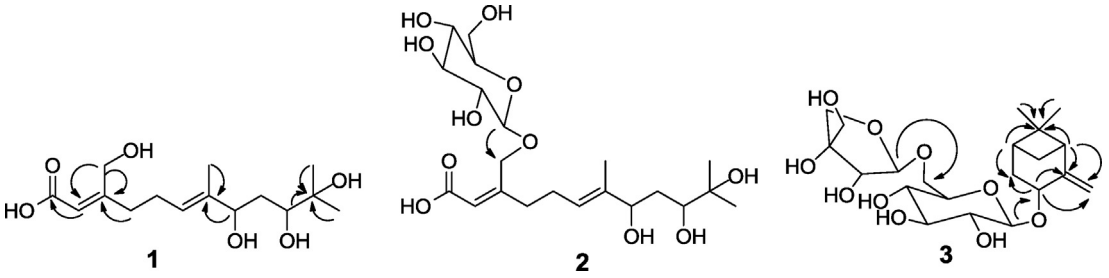

Fig. 2. Key HMBC correlations of **1–3.**

Compound **2** was obtained as a white amorphous powder. The molecular formula was determined as $C_{21}H_{36}O_{11}$ based on the HRESIMS pseudomolecular ion at *m/z* 487.2144 ([M + Na]$^+$, $C_{21}H_{36}O_{11}Na$, calcd 487.2150) and ^{13}C NMR data. Its IR spectrum revealed similar absorption bands to **1**. Comparison of the ^1H and ^{13}C data with that of **1** showed that **2** contained an additional sugar unit. After acid hydrolysis, **2** afforded **1** and D-glucose by HPLC and rotation analyses. Therefore, **2** was the glycoside of **1**. The long-range correlation between the anomeric proton δ_H 4.28 (d, J = 7.7 Hz) and C-13 (δ_C 72.2) suggested D-glucose located at C-13 (Fig. 2). The β configuration of D-glucose was deduced from the coupling constant. Thus, the structure of **2** was determined as (2*E*, 6*E*)-8,10,11-trihydroxyl-7,11-dimethyl-3-hydroxymethyl-2,6-dodecadienoic acid 13-*O*-β-D-glucopyranoside.

Compound **3** was obtained as a white amorphous powder. The molecular formula was determined as $C_{21}H_{34}O_{10}$ based on the HRESIMS pseudomolecular ion at *m/z* 469.2082 ([M + Na]$^+$, calcd. 469.2044) and ^{13}C NMR data. The ^1H NMR spectrum exhibited two tertiary methyl groups (δ_H 1.28, 0.66), two olefinic protons (δ_H 4.97) and two anomeric protons (δ_H 4.50, 5.01). The ^{13}C NMR and DEPT spectra of **3** displayed two sugar moieties, two methyls, three methylenes, three methines, and two quaternary carbon atoms. The HMBC experiments showed correlation between the methyl groups (δ_H 1.28 and δ_H 0.66) and C-6 (δ_C 42.7), which further correlated to H-1 and H-5 (δ_H 2.43 and δ_H 1.93). H-3 (δ_H 4.53) correlated to C-4 (δ_C 33.9), which further correlated to H-5 (δ_H 1.93). H-3 also correlated to C-10 (δ_C 115.3) and C-2 (δ_C 149.0),

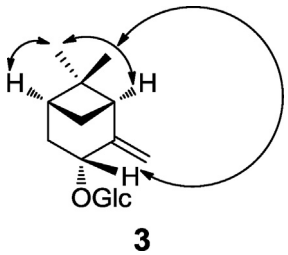

Fig. 3. Selected ROESY correlations of **3.**

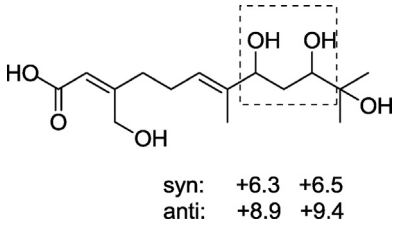

Fig. 4. Assignment of the relative configuration of **1** based on Kishi's Universal NMR Database.

which further correlated to H-1. Therefore, the aglycone of **3** was 3-hydroxy-camphene. The ROESY spectrum (Fig. 3) revealed correlations of H-1/H-9/H-5 indicated H-1, H-9, H-5 to be β-oriented. In addition, correlations of H-8/H-3/H-7 indicated H-3, H-8 to be α-oriented. After acid hydrolysis, **3** afforded D-glucose and D-apiose, which were identified by HPLC analysis and rotation analyses. *β* configuration of Glc and α configuration of Api were suggested from the *J* values of H-1 of Glc (δ_H 4.50, J = 7.8 Hz), H-1 of Api (δ_H 5.01, J = 2.5 Hz). The correlation between H-1 of Api (δ_H 5.01) and C-6 of Glc (δ_C 68.8) suggested that the sugar moiety was Apiosyl-(1 → 6)-glucosyl. Furthermore, the HMBC correlation between H-1 of Glc (δ_H 4.50) and C-3 (δ_C 72.8) showed Glc linked to C-3. Therefore, compound **3** was characterized as 6,6-dimethyl-2-methlenebicyclo[3.1.1]hept-3-*O*-(6-*O*-apiofuranosyl)-β-D-glucopyranoside.

We evaluated the cytotoxic activity of these three new compounds against two human lung cancer cell lines (NCI-H661 and A549) by a SRB method. All of the compounds showed no significant cytotoxic activity (IC_{50} > 20 μM).

3. Conclusion

The phytochemical study of the aerial part of *D. repens* led to the isolation of an unusual new highly oxygenated acyclic sesquiterpenoid (**1**) and its glucoside (**2**), along with a new pinane monterpene disaccharide glucoside (**3**). Their cytotoxic activity on NCI-H661 and A549 cell lines were evaluated and they all showed no toxic (IC_{50} > 20 μM) activity.

4. Experimental

4.1. General

Optical rotation was measured by PerkinElmer 341 polarimeter. IR was obtained on Hitachi 275-50 spectrometer. ^{1}H NMR, ^{13}C NMR, and 2D NMR spectrum were recorded on Bruker AM-500 spectrometer (^{1}H: 500 MHz and ^{13}C: 125 MHz). HRESIMS was recorded on Finnigan LCQ-DECA spectrometer. Column chromatography was done on MCI CHP-20P gel (75–150 μm; Mitsubishi Chemical Industries Co., Ltd.), and Cosmosil 75C18-OPN (20–45 μm; Nacalai Tesque Inc.). Prep-HPLC was performed on Waters 4000 Prep-HPLC using a YMC C18 column (10 × 250 mm, 5 μm).

4.2. Plant material

The aerial parts of *D. repens* Forst. were collected from Guangxi, China, in September 2011, and identified by Prof. Heming Yang. A voucher specimen (No. SIMM811) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, and People's Republic of China.

4.3. Extraction and isolation

Air-dried, powdered aerial parts of *D. repens* (5.0 kg) were extracted with H_2O/acetone 2:8 (v/v) at room temperature for 7 × 24 h. After removal of organic solvent, the sample was suspended in H_2O and sequentially extracted with petroleum ether, $CHCl_3$. The water-soluble fraction was subjected to a column of MCI and eluted with H_2O, 25%, 50%, 75%, and 100% MeOH successively. Six major fractions (A–F) were obtained. Fraction C was further subjected to a C18 column eluted with MeOH/H_2O (10–50%) and yielded **2** (40.6 mg). Fraction D was separated by a C_{18} column eluted with MeOH/H_2O (10–80%) to give fractions (D1–D3). Fraction D1 was further chromatographed on a Sephadex LH-20 with MeOH and yield **1** (12.3 mg). Fraction F was subjected to a C_{18} column eluted with MeOH/H_2O (50–100%) to give **3** (27.9 mg).

4.4. Preparation of Mosher's esters

Compound **1** (2 mg) was treated with (*S*)-MPTA chloride and (*R*)-MPTA chloride in Pyridine-d_5, separately, and stood for 8 h at room temperature. After reaction, the chemical shift difference ($\Delta\delta = \delta_S - \delta_R$) observed, which made it possible to conclude that the absolute configuration of compound **1** was 8*R*, 10*S* configuration ($\Delta\delta_{15}$ = +0.090, $\Delta\delta_{14}$ = −0.215, $\Delta\delta_{12}$ = +0.119, $\Delta\delta_{10}$ = +0.002, $\Delta\delta_8$ = −0.003).

S-MPTA ester of **1**: ^{1}H NMR (in Pyridine-d_5, 400 MHz): δ 1.439 (3H, s, Me-15), 1.602 (3H, s, Me-14), 1.429 (3H, s, Me-12), 3.377 (1H, m, CH-10), 4.873 (1H, m, CH-8).

R-MPTA ester of **1**: ^{1}H NMR (in Pyridine-d_5, 400 MHz): δ 1.329 (3H, s, Me-15), 1.817 (3H, s, Me-14), 1.348 (3H, s, Me-12), 3.375 (1H, m, CH-10), 4.876 (1H, m, CH-8).

4.5. Sugar analysis

Compounds **2** (10 mg) and **3** (10 mg) were hydrolyzed with 1 N H_2SO_4 in H_2O and heated for 4 h in an 85 °C water-bath, separately. After cooling, the reaction mixture was neutralized with 10% Na_2CO_3 and extracted with $CHCl_3$ twice. The aqueous layer was desalted by Sephadex LH-20 with MeOH to afford a sugar residue. The residue was dissolved in H_2O and directly analyzed by HPLC with authentic samples (MeCN–H_2O, 95/5): D-glucose eluted at 21.1 min from Compounds **2** and **3**, and D-apiose at 24.0 min from Compound **3**. Each of these eluates was individually collected, concentrated, and dissolved in H_2O. The elutes were identified as D-glucose $[\alpha]^{23}_D$ = +52 (*c* = 0.1, H_2O), D-apiose $[\alpha]^{23}_D$ = +6 (*c* = 0.1, H_2O) in comparisons with their specific rotations with those of the corresponding authentic samples.

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **1-2** (CD_3OD)

Position	1		2	
	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c
1		170.3		170.5
2	5.92 s	115.0	6.00 s	117.3
3		163.0		158.1
4	2.54 dd (14.0, 7.6)	30.0	2.53 m	30.0
5	2.23 dd (14.0, 7.6)	28.0	2.20 m	27.7
6	5.44 t (7.6)	127.6	5.40 t (7.0)	127.7
7		138.4		138.3
8	4.19 t (7.0)	77.8	4.14 m	77.8
9	1.74 m, 1.51 m	36.7	1.71 m, 1.56 m	36.7
10	3.24 d (10.4)	77.9	3.20 m	77.9
11		73.5		73.5
12	1.09 s	24.8	1.06 s	24.9
13	4.08 s	65.9	4.39 d (15.9)	72.2
			4.11 d (15.9)	
14	1.59 s	10.8	1.56 s	10.9
15	1.13 s	25.7	1.09 s	25.5
Glc-1			4.25 d (7.5)	103.6
2			3.17 m	75.0
3			3.23 m	77.9
4			3.20 m	71.5
5			3.30 m	77.8
6			3.81 dd (11.6, 3.3),	62.7
			3.60 dd (11.6,3.3)	

4.6. Physical and spectroscopic data of new compound

(2*E*, 6*E*)-8,10,11-trihydroxyl-7,11-dimethyl-3-hydroxymethyl-2,6-dodecadienoic acid (**1**)

White amorphous powder. $[\alpha]^{24}_D$ = +5 (*c* = 0.1, MeOH). HRESIMS: *m/z* 325.1630 ($[M+Na]^+$, $C_{15}H_{26}O_6Na^+$, calcd. 325.1622). UV λ_{max}(MeOH) nm: 206. IR(KBr): 3419, 2918, 1647, 1431, 1373, 1165, 1078 cm^{-1}. ^{1}H and ^{13}C NMR (CD_3OD): see Table 1.

(2*E*, 6*E*)-8,10,11-trihydroxyl-7,11-dimethyl-3-hydroxymethyl-2,6-dodecadienoic acid 13-*O*-β-D-glucopyranoside (**2**)

Table 2
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **3** (CD_3OD)

Position	3	
	δ_H (J in Hz)	δ_c
1	2.43 t (5.4)	51.8
2		149.0
3	4.53 d (7.3)	72.8
4	2.01 m, 2.23 m	33.9
5	1.93 m	40.9
6		42.7
7	2.27 m, 1.91 m	27.1
8	0.66 s	22.3
9	1.28 s	26.5
10	4.97 dd (17.8, 1.7)	115.3
Glc-1	4.50 d (7.8)	99.4
2	3.22 m	74.9
3	3.31 m	78.2
4	3.25 m	71.9
5	3.31 m	76.6
6	3.51 m, 3.86 m	65.6
Api-1	5.01 d (2.5)	111.0
2	3.90 d (2.5)	78.1
3		80.5
4	3.59 m, 4.00 m	68.8
5	3.76 d (9.7), 3.97 d (9.7)	74.

White amorphous powder. $[\alpha]^{24}_D = -10$ ($c = 0.1$, MeOH). HRESIMS: m/z 487.2144 ($[M + Na]^+$, $C_{21}H_{36}O_{11}Na^+$, calcd. 487.2150). UV λ_{max}(MeOH) nm: 205. IR(KBr): 3430, 2918, 1649, 1431, 1373, 1165, 1081 cm^{-1}. ^1H and ^{13}C NMR (CD_3OD): see Table 1.

6,6-dimethyl-2-methlenebicyclo[3.1.1]hept-3-*O*-(6-*O*-apiofuranosyl)-β-D-glucopyranosiDe (**3**)

White amorphous powder. $[\alpha]^{24}_D = -52$ ($c = 0.1$, MeOH). HRESIMS: m/z 469.2082 ($[M + Na]^+$, $C_{21}H_{34}O_{10}Na^+$, calcd. 469.2044). UV λ_{max}(MeOH) nm: 204. IR(KBr): 3419, 2972, 2921, 1456, 1367, 1265, 1078, 1051, 1016, 916 cm^{-1}. ^1H and ^{13}C NMR NMR (CD_3OD): see Table 2.

4.7. Cytotoxicity assay

The cytotoxicity of the test compounds against A549 and NCI-H661 cell lines were measured using a sulforhodamine B (SRB) assay as described in the literature (Skehan et al., 1990). Doxorubicin (Sigma) was used as positive control.

Acknowledgments

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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.2015.08.013.

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