Bioorganic & Medicinal Chemistry Letters 25 (2015) 795–798

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Multidrug resistance–reversal effects of resin glycosides from *Dichondra repens*

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ARTICLE INFO

Article history:
Received 24 October 2014
Revised 18 December 2014
Accepted 26 December 2014
Available online 3 January 2015

Keywords:
Dichondra repens
Resin glycoside acids
Multidrug-resistance (MDR) reversal activity

ABSTRACT

Investigation of hydrophobic extract of *Dichondra repens* (Convolvulaceae) led to the isolation of three new resin glycosides dichondrins A–C (**1**–**3**), and three known resin glycosides cus-1, cus-2, and cuse 3. All the isolated resin glycosides with an acyclic core were evaluated for their multidrug resistance reversal activities, and the combined use of these compounds at a concentration of 25 μM increased the cytotoxicity of vincristine by 1.03–1.78-fold.

Resin glycosides, primarily found in the family Convolvulaceae, are unusual amphipathic metabolites with structures including hydrophobic (fatty acid aglycone) and hydrophilic (oligosaccharide) moieties.[1] To date, hundreds of resin glycosides have been isolated from different genera of the family Convolvulaceae, and these resin glycosides exhibit various pharmacological activities, such as cytotoxicity,[2] multidrug resistance (MDR) reversal,[3] ionophoretic,[4] and phytogrowth-inhibitory activities,[5] as well as effects on the central nervous system.[6] It is worthy of note that the resin glycosides have gained increasing attention as a novel P-glycoprotein inhibitor, which is attributed to their intriguing structures and abundant natural resources. Therefore, the discovery of various structural resin glycosides from the family Convolvulaceae and the investigation of their MDR reversal activities arouse our interest.

Dichondra repens, grown alone or in association with turfgrass in subtropical and Mediterranean regions, is a perennial plant with persistent leaves, and has traditionally been used for the treatment of jaundice and dysentery in China.[7] However, as a member of family Convolvulaceae, the resin glycosides of *D. repens* are rarely reported. Therefore, we initiated the isolation and MDR reversal activities studies of resin glycosides from *D. repens*.

In this Letter, three new resin glycosides dichondrins A–C (**1**–**3**) (Fig. 1), and three known compounds cus-1, cus-2,[8] and cuse 3,[9] were isolated from acetone–H_2O (4:1, v/v) extract of *D. repens*. Their chemical structures were determined by extensive application of high resolution 2D NMR techniques, HRESIMS and chemical methods. Furthermore, all isolated compounds showed MDR reversal activity at 25 μM when combined with vincristine. The structures of the three known compounds were elucidated based on the comparison of their HRESIMS, ^{1}H, and ^{13}C NMR data with the reported data.

Dichondrin A (**1**) was obtained as white amorphous powder. The molecular formula was determined as $C_{50}H_{88}O_{25}$ based on the ^{13}C NMR data (Table 1) and the HRESIMS ion at *m*/*z* 1111.5531 $[M+Na]^+$ (calcd 1111.5507). The ^{1}H NMR spectrum of **1** showed seven methyl groups, four anomeric protons and long-chain fatty acid signals. The ^{13}C NMR and DEPT spectra of **1** showed 50 carbons including four anomeric carbons and three carbonyl carbons.

The NMR data of **1** could be divided into two parts: resonances in the anomeric region and those representative of the aglycone moieties. The ^{1}H NMR spectrum of **1** showed two methine groups (δ_H 2.80 and 2.76) due to H-2 of 3-hydroxy-2-methybutyryl (Nla) moieties, two oxygenated methine groups (δ_H 4.30 and 4.28) due to H-3 of Nla, two primary methyl groups (δ_H 1.36 and 1.35) due to H-4 of Nla, and two methyl groups (δ_H 1.29 and 1.25) due to 2-Me of Nla in the aglyconic region, which suggested **1** contained two 3-hydroxy-2-methybutyryl moieties. After alkaline hydrolysis, the 2*R*, 3*R* absolute configuration of Nla was determined by its specific rotation ($[\alpha]_D^{23}$ −28).[10] In addition, the 11-hydroxyhexadecanoyl moiety (Ag) was suggested by the diagnostic signals of the

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http://dx.doi.org/10.1016/j.bmcl.2014.12.083
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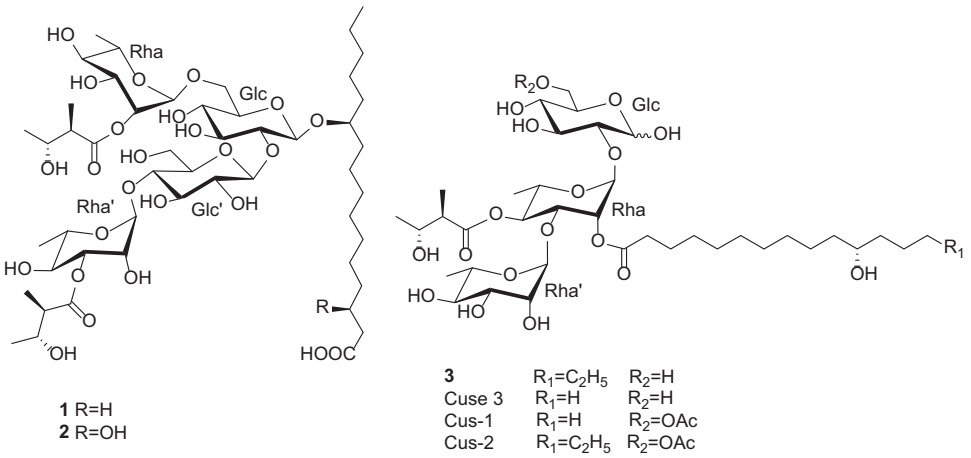

Figure 1. Chemical structures of compounds **1**–**3**.

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **1**–**3** (in ppm)

Position	1[a]		2[a]		3[b]			
					α-Anomer		β-Anomer	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
Glc-1	4.90 d (7.7)	102.1	5.24 d (7.9)	102.1	5.19 d (3.6)	93.2	4.57 d (7.7)	96.8
2	4.11 m	83.6	4.12 m	84.1	3.39 dd (9.7,3.5)	82.1	3.28 m	80.5
3	4.27 m	78.1	4.29 m	78.2	3.79 m	73.7	3.81 m	78.9
4	3.90 dd (8.5, 8.5)	71.6	3.92 m	71.7	3.75 m	72.2	3.75 m	72.2
5	3.97 m	76.3	4.00 m	76.4	3.81 m	73.5	3.27 m	77.9
6	3.99 m, 4.46 m	68.8	4.01 m, 4.55 m	68.9	3.68 m, 3.81 m	62.7	3.60 m, 3.77 m	62.8
Glc′-1	5.24 d (7.8)	105.9	5.24 d (7.9)	106.3				
2	4.08 m	77.2	4.04 m	77.5				
3	4.14 d (3.8)	76.4	4.16 m	76.5				
4	4.40 m	78.5	4.44 dd (8.7,8.7)	78.5				
5	3.72 m	77.6	3.73 m	77.9				
6	4.12 m, 4.30 m	61.7	4.32 m, 4.16 m	68.9				
Rha-1	5.32 br s	99.3	5.33 br s	99.5	4.94 d (1.4)	100.8	5.24 br s	99.0
2	5.84 dd (3.4, 1.4)	73.3	5.86 m	73.4	5.23 m	73.0	5.26 m	73.2
3	4.64 m	72.7	4.66 m	73.1	4.20 dd (9.9, 3.3)	76.5	4.11 dd (9.9, 3.3)	76.1
4	4.54 dd (9.2, 9.2)	72.9	4.57 m	70.7	5.03 dd (9.9, 9.9)	74.0	5.03 dd (9.9, 9.9)	74.0
5	4.34 m	68.5	4.35 m	67.5	4.06 dd (9.9, 6.3)	68.3	4.32 dd (9.9, 6.3)	67.9
6	1.50 d (6.2)	18.3	1.52 d (6.2)	18.5	1.16 d (6.3)	17.9	1.16 d (6.3)	17.8
Rha′-1	5.82 br s	102.9	5.86 br s	103.0	4.83 d (1.3)	104.0	4.79 (1.3)	104.0
2	4.66 m	72.7	4.68 m	72.9	3.70 m	72.1	3.70 m	72.1
3	5.66 t (9.8)	75.2	5.68 d (9.8)	75.4	3.51 dd (9.4, 3.5)	72.3	3.51 dd (9.4, 3.5)	72.3
4	4.32 m	74.1	4.34 br s	74.3	3.32 dd (9.4, 9.4)	72.8	3.32 dd (9.4, 9.4)	72.8
5	4.92 m	70.5	4.95 m	70.7	3.57 dd (9.4, 6.3)	70.6	3.57 dd (9.4, 6.3)	70.6
6	1.66 d (6.2)	18.7	1.69 d (6.0)	18.9	1.18 d (6.3)	17.9	1.18 d (6.3)	17.8
Ag-1		176.8		177.0		174.5		174.5
2	2.47 m	35.2	2.51 m, 2.87 m	44.4	2.40 m	35.2	2.40 m	35.2
3			4.53 m	68.7				
11	3.92 m	80.3	3.91 m	80.3	3.68 m	71.9	3.68 m	71.9
16	0.90 t (7.0)	14.6	0.92 t (6.8)	14.7	0.91 t (7.2)	14.2	0.91 t (7.2)	14.2
Nla-1		175.6		175.7		175.2		175.2
2	2.80 m	49.3	2.80	49.5	2.45 m	49.2	2.45 m	49.2
3	4.34 m	69.6	4.27	69.8	3.86 m	70.3	3.86 m	70.3
4	1.34 d (6.2)	21.1	1.34 d (6.3)	21.3	1.19 d (6.2)	20.8	1.19 d (6.2)	20.8
5	1.22d (7.0)	13.8	1.23 d (6.9)	13.9	1.12 d (7.2)	14.1	1.12 d (7.2)	14.1
Nla′-1		174.9		175.0				
2	2.76 m	49.1	2.77	49.4				
3	4.33 m	69.6	4.27	69.7				
4	1.32 d (6.3)	20.9	1.33 d (6.3)	21.1				
5	1.19 d (7.0)	13.7	1.20 d (7.0)	13.7				

[a] NMR data recorded in pyridine.
[b] NMR data recorded in MeOH.

methyl triplet (δ_H 0.90), methylene group (δ_H 2.47), and oxygenated methine (δ_H 3.92). After acid hydrolysis, the resulting 11-hydroxyhexadecanoic acid showed a fragment ion at m/z 201 $[M-CH_3(CH_2)_4]^+$ by the EIMS data, suggesting the 11-OH group of Ag. The *S* configuration of 11-hydroxyhexadecanoic acid was determined by Mosher's method as we reported before.[11]

In the anomeric region, the ^{1}H–^{1}H COSY showed four spin systems, which were attributed to two hexose and two 6-deoxyhexose

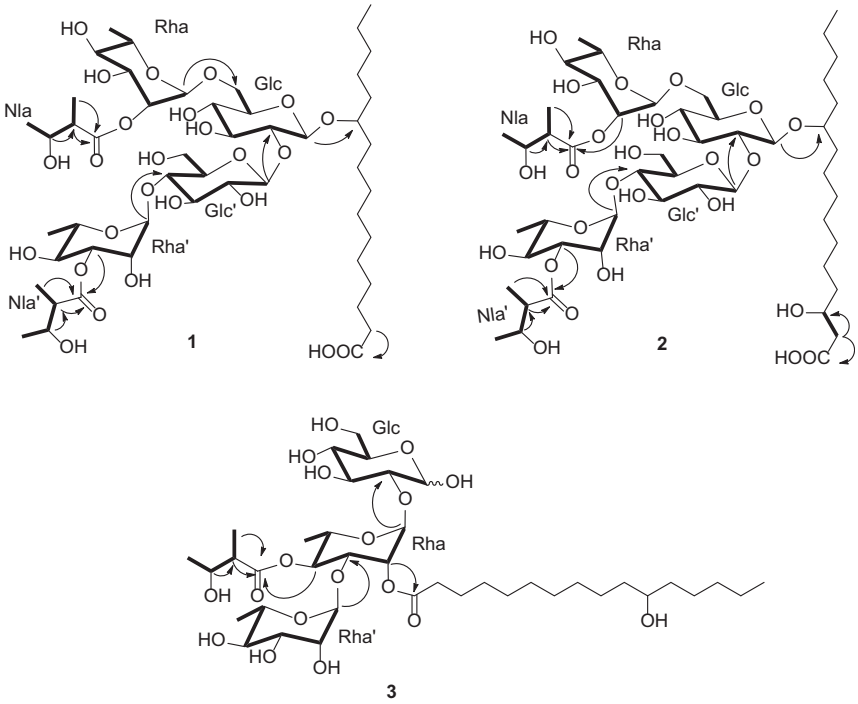

Figure 2. Key ^{1}H–^{1}H COSY (bold) and HMBC (arrow) of compounds **1**–**3**.

units (Fig. 2). The sugars obtained from the acid hydrolysates were further identified as L-rhamnose and D-glucose by the HPLC analysis and their corresponding optical rotations. The β-configuration of D-glucose was suggested by the coupling constants of the anomeric protons at δ_H 4.90 (d, J = 7.7 Hz, H-1 of β-Glc) and δ_H 5.24 (d, J = 7.8 Hz, H-1 of β-Glc′) in the ^{1}H NMR spectrum. The α-configuration of L-rhamnose was suggested by the chemical shift of C-5 of rhamnose (δ_C 68.5, 70.5) in the ^{13}C NMR spectrum.[12] Moreover, the long-range correlations from δ_H 4.90 (H-1 of β-Glc) to δ_C 80.3 (C-11 of Ag) indicated that β-Glc was the first hexose unit in the sugar moiety. Besides, the sequence of the sugar moiety was determined to be rhamnosyl-(1→4)-glucosyl-(1→2)-[rhamnosyl-(1→6)]-glucosyl by their long-range HMBC correlations: H-1 of β-Glc′ (δ_H 5.24) with C-2 of β-Glc (δ_C 83.6), H-1 of α-Rha′ (δ_H 5.82) with C-4 of β-Glc′ (δ_C 78.5), H-1 of α-Rha (δ_H 5.32) with C-6 of β-Glc (δ_C 68.8). In addition, the linkages between Nla and sugar moiety, that is, Nla located at OH-2 of α-Rha, Nla′ to H-3 of α-Rha′, were indicated by the HMBC correlations: δ_H 5.84 (H-2 of α-Rha) with δ_C 175.6 (C-1 of Nla), δ_H 5.66 (H-3 of α-Rha′) with δ_C 174.9 (C-1 of Nla′). Thus, the structure of compound **1** was defined as (11*S*)-dihydroxyhexadecanoic acid 11-*O*-(3-*O*-(2*R*,3*R*)-3-hydroxy-2-methybutyryl)-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranose-(1→2)-[(2-*O*-(2*R*,3*R*)-3-hydroxy-2-methybutyryl)-α-L-rhamnopyranosyl-(1→6)]-*O*-β-D-glucopyranose.

Dichondrin B (**2**) possessed the molecular formula $C_{50}H_{88}O_{26}$ based on the ^{13}C NMR data (Table 1) and the HRESIMS ion at m/z 1127.5464 $[M+Na]^+$ (calcd 1127.5460), which showed one more oxygen atoms than **1**. Furthermore, **2** contained the same complex sugar moiety and Nla groups as **1** according to their ^{1}H and ^{13}C spectra. However, the H_2/H_3 correlation in the ^{1}H–^{1}H COSY revealed long chain fatty acids in **2** had an additional 3-OH. The 3*S*, 11*S* configuration of 3,11-hydroxyhexadecanoic acid was determined by the chemical shift difference ($\Delta\delta = \delta_S - \delta_R$, $\Delta\delta_{16H}$ = −0.01, $\Delta\delta_{2Ha}$ = +0.02) according to the Mosher's method.[13] Thus, the structure of **2** was defined as (3*S*,11*S*)-dihydroxyhexadecanoic acid 11-*O*-(3-*O*-(2*R*,3*R*)-3-hydroxy-2-methybutyryl)-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranose-(1→2)-[(2-*O*-(2*R*,3*R*)-3-hydroxy-2-methybutyryl)-α-L-rhamnopyranosyl-(1→6)]-*O*-β-D-glucopyranose.

Dichondrin C (**3**) was obtained as white amorphous powder, and its molecular formula was determined as $C_{39}H_{70}O_{18}$ based on the HRESIMS peak at m/z 849.4472 $[M+Na]^+$ (calcd 849.4454) and ^{13}C NMR data (Table 1). The comparison of the ^{1}H and ^{13}C NMR data of **3** and cus-2, indicated that **3** lacked an acetyl at OH-6 of Glc. Furthermore, the anomeric protons at δ_H 5.19 (d, J = 3.5 Hz, H-1 of α-Glc), 4.57 (d, J = 7.7 Hz, H-1 of β-Glc) in the ^{1}H NMR spectrum suggested that **3** existed as an equilibrium mixture of α-and β-anomers, and the ratio (α:β) was identified as 8:5 by the integration of anomeric protons. Thus, the structure of **3** was defined as α-L-rhamnopyranosyl-(1→3)-[1-*O*-(11*S*)-11-hydroxyhexadecanoyl]-[4-*O*-(2*R*,3*R*)-3-hydroxy-2-methybutyryl]-α-L-rhamnopyranosyl-(1→2)-*O*-D-glucopyranose.

We then started the research on the MDR reversal activity of the isolated resin glycosides in KB/VCR cells by the SRB method,[14] and the results were presented in Table 2. All the isolated compounds

Table 2
Results of modulating MDR activities in KB/VCR cells of compounds **1**–**3**, cus-1, cus-2 and cuse 3[a]

Sample	Inhibition ratio% (25 μM)	Vincristine+sample[b]	
		IC_{50} value (μM)	RF[c] value
1	2.52	0.48	1.19
2	0.83	0.43	1.32
3	1.35	0.32	1.78
Cus-1	1.64	0.54	1.03
Cus-2	0.85	0.42	1.36
Cuse 3	−0.47	0.36	1.58
Verapamil[d]	0.45	0.02	28.5
Vincristine	—	0.57	—

[a] MDR: multidrug resistance.
[b] Serial dilutions ranging from 0.125 to 1 μM of vincristine in the presence or absence of 25 μM sample.
[c] RF: IC_{50} of VCR alone/IC_{50} of VCR in the presence of sample.
[d] The concentration of the positive control verapamil is 10 μM.

enhanced the cytotoxicity of vincristine by 1.03–1.78-fold when incorporated at the concentration of 25 μM, while the cytotoxicity assay showed the compounds **1**, **2**, **3**, cus-1, cus-2, and cuse 3 were not toxic at 25 μM as their inhibition ratios were less than 50%. In addition, the deacylated resin glycosides (cuse 3 and **3**) were more active than the acylated resin glycosides (cus-1 and cus-2), which demonstrated that the minor variations in the acylation pattern of oligosaccharide core could affect their MDR reversal activities.

In conclusion, we have made a phytochemical investigation on the crude extract of *Dichondra repens* resulting in six resin glycosides with an acyclic core, the three new resin glycosides dichondrins A–C (**1**–**3**), and three known resin glycosides cus-1, cus-2, and cuse 3. Moreover, the MDR reversal studies of these compounds showed that they could increase the cytotoxicity of vincristine by 1.03–1.78-fold when combined at a concentration of 25 μM.

Acknowledgments

The authors gratefully acknowledge grants from the National Science & Technology Major Project 'Key New Drug Creation and Manufacturing Program', China (Number: 2009ZX09301-001), the National Natural Sciences Foundation of China (No. 81473111), and the China Postdoctoral Science Foundation (No. Y412021031).

Supplementary data

Supplementary data (general experimental procedures, plant material, extraction and isolation, physical properties and NMR data for isolates, and procedures of MDR-reversal bioassay) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.083.

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