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Unusual ether-type resin glycoside dimers from the seeds of *Cuscuta chinensis*

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ABSTRACT

Two new resin glycosides, cuses 3 (**1**) and 4 (**2**), were initially obtained from the seeds of *Cuscuta chinensis*. Both of them contained a reducing glucose unit, and thus existed as a pair of isomers. In order to solve the existing problem of tautomerism, the remanent resin glycoside fraction was converted into aminoalditol derivatives with *p*-anisidine, and then another eight new resin glycosides cuses 5–12 (**3**–**10**) were further isolated. Cuses 7–12 (**6**–**10**) were considered to be generated via glycosidation of two acylated oligosaccharides, and thus characterized as ether-type resin glycoside dimers. Their structures including absolute stereochemistry were elucidated by a combination of spectroscopic and chemical methods. Compounds **3**–**10** exhibited cytotoxic activity toward MCF-7, SMMC-7721, and MG-63 cell lines with IC_{50} values ranging from 8.72 to 59.35 μg/mL.

1. Introduction

Resin glycosides, composed of glycosidic acids and characteristic organic acids, are important chemotaxonomic features of the family Convolvulaceae.[1] Their diversified structures and significant bioactivities have attracted more and more attentions in the past decades.[2–5] *Cuscuta chinensis* Lam., distributed from tropical to temperate zone, is a unique kind of parasite in the family Convolvulaceae. The seed of *C. chinensis* is a well-known traditional Chinese medicine (TCM), commonly used for improving sexual function, tonifying the livers and kidneys, and reducing urination.[6] Moreover, modern pharmacological studies showed that *C. chinensis* possessed antitumor, antiosteoporotic, and neuroprotective effects.[7–9] Previous chemical investigations on this plant have revealed several resin glycosides, cuses 1–2 and cuscutic acids A–D.[10,11] In our continuing endeavor to study on the novel active resin glycosides from plants in the Convolvulaceae,[12,13] we investigated the seeds of *C. chinensis* Lam. and initially isolated two new resin glycosides cus 3 (**1**) and cus 4 (**2**), along with two known resin glycosides cuses 1 and 2 from the MeOH extract, and obtained another eight new resin glycosides cuses 5–12 (**3**–**10**) from the aminoalditol derivatives of remanent resin glycoside fraction. Compounds **1** and **2** were elucidated to be trisaccharide resin glycosides with a reducing glucose unit, and thus both of them existed as an equilibrium mixture of α- and β-anomers. Compounds **3**–**10** were the aminoalditol derivatives of naturally occurring resin glycosides, the reducing sugars of which were protected by reductive amination with *p*-anisidine. Notably, compounds **6**–**10**, composed of two acylated oligosaccharides linked together by the glycosidic bond, represented the first example of the unusual ether-type resin glycoside dimeric structures. Herein, we reported the isolation, the structural elucidation, and the cytotoxic activity of compounds **1**–**10**, along with the plausible biogenetic pathway of the resin glycoside dimers **6**–**10**.

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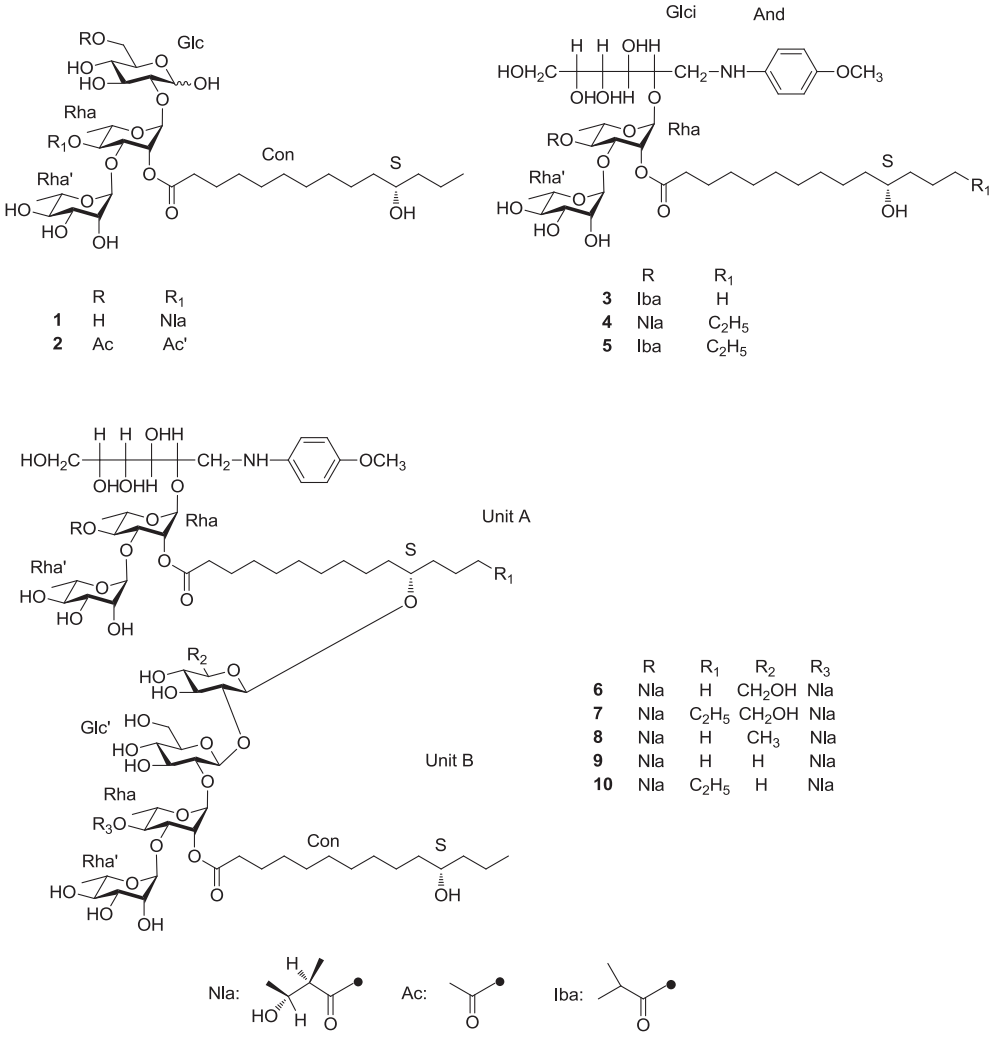


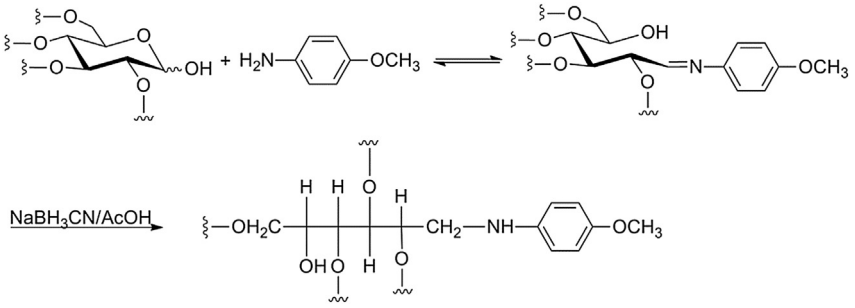
	R	R_1
1	H	Nla
2	Ac	Ac'

	R	R_1
3	Iba	H
4	Nla	C_2H_5
5	Iba	C_2H_5

	R	R_1	R_2	R_3
6	Nla	H	CH_2OH	Nla
7	Nla	C_2H_5	CH_2OH	Nla
8	Nla	H	CH_3	Nla
9	Nla	H	H	Nla
10	Nla	C_2H_5	H	Nla

2. Results and discussion

The MeOH-soluble fraction of the methanolic extract from the seeds of *C. chinensis* was chromatographied on a macroporous resin D101 column with the eluent of EtOH/H_2O. The 85% EtOH fraction was subjected to column chromatography over silica gel, ODS, and further purified by preparative HPLC, to afford two new resin glycosides **1** and **2**. Both of them existed as a pair of α- and β-anomers because of the lack of protection at anomeric carbon of the glucose unit. However, other resin glycoside peaks were seriously overlapped on the HPLC due to the tautomerism, and each attempt to isolate the pure compound was failed. Thus, in order to continue the phytochemical research, the resin glycoside fraction obtained from the 70% EtOH fraction was converted into amino-alditol derivatives by reductive amination with *p*-anisidine to protect the reducing sugar (Scheme 1).[10,14] The treated resin glycoside fraction was repeatedly purified to yield compounds **3**–**10**.



Scheme 1. The mechanism of reductive amination of glucose with *p*-anisidine.

2.1. Structural elucidation

Compound **1** was obtained as colorless gum. The molecular formula was deduced as $C_{37}H_{66}O_{18}$ by HRESIMS data at *m*/*z*

821.4135 $[M+Na]^+$ (calcd for $C_{37}H_{66}NaO_{18}$, 821.4125). It presented two peaks on the HPLC, which came to equilibrium in the ratio of 2:1 within a few hours after separation. In the NMR spectra of **1** (Table 1), six anomeric signals were observed, which were separated into two groups according to peak intensities, [δ_H 5.98 (d, J=3.5 Hz), 5.83 (br s), 5.44 (br s), δ_C 93.5, 100.8, 104.6] and [δ_H 5.31 (d, J=7.5 Hz), 6.33 (br s), 5.59 (br s), δ_C 97.4, 99.2, 104.7], indicating that compound **1** was a trisaccharide and existed as a pair of isomers.[10] The ^{1}H NMR spectrum also exhibited two groups of paramagnetically shifted non-anomeric ring proton signals, [δ 6.14, (dd, J=1.5, 3.5 Hz), 5.79 (dd, J=10.0, 10.0 Hz)] and [δ 6.17 (dd, J=1.5, 3.0 Hz), 5.88 (dd, J=10.0, 10.0 Hz)], and partially overlapped fatty acids signals in the region of δ 0.9–2.8, suggesting the existence of two fatty acyl groups. In addition, compound **1** was saponified with 3% K_2CO_3 to provide convolvulinolic acid (11-hydroxytetradecanoic acid, Con), nilic acid (3-hydroxy-2-methylbutyryl acid, Nla), and a trisaccharide compound **11** (Fig. 1).[11] The convolvulinolic acid was a characteristic component hydroxyl fatty acid of resin glycoside,[1] and its configuration was determined to be *S* by the advanced Mosher's method.[15] The nilic acid was concluded to be 2*R*,3*R* configuration by comparison of its ^{1}H NMR spectrum and specific rotation value ($[\alpha]_D^{27}$ −16.0) of the *p*-bromophenacyl ester with the literature data.[10,16] The structure of **11** was identified based on the spectroscopic data. And subsequent acidic hydrolysis of **11** also afforded D-glucose and L-rhamnose.[17] It could be seen that the structure of **11** was lack of protection on the anomeric carbon and had the tautomerism in solution. Therefore, it was deduced that compound **1** was a partially acylated trisaccharide resin glycoside, which had a reducing glucose unit and existed as an equilibrium mixture of α- and β-anomers.

A combination of HSQC, HMBC, and TOCSY experiments was employed for the assignment of the two groups of monosaccharides (Table 1). Each group comprised one glucopyranosyl unit and two rhamnopyranosyl units. The α- and β-configurations of the D-glucopyranosyl unit were distinguished by the coupling constants (J=3.5 Hz or 7.5 Hz, respectively) for the anomeric protons (δ_H 5.98 or 5.31, respectively) in the ^{1}H NMR spectrum, while the α-configuration for L-rhamnopyranosyl unit was determined by the chemical shift of C-5 of rhamnose in the ^{13}C NMR spectrum.[18] The interglycosidic connectivities were established from the correlations of H-3 (δ_H 4.83) of Rha with C-1 (δ_C 104.6) of Rha′, H-1 (δ_H 5.83) of Rha with C-2 (δ_C 82.5) of Glc of the α-anomer, and H-3 (δ_H 4.94) of Rha with C-1 (δ_C 104.7) of Rha′, H-1 (δ_H 6.33) of Rha with C-2 (δ_C 80.9) of Glc of the β-anomer in the HMBC spectrum. Specification of ester linkage sites was established by the key HMBC correlations between protons of sugars and acyl carbons of the fatty acids, i.e., δ_H 6.14/6.17 (H-2, Rha) with δ_C 173.5/173.6 (11*S*-convolvulinolic acid), δ_H 5.79/5.88 (H-4, Rha) with δ_C 175.9/175.9 (2*R*,3*R*-nilic acid) of the α-/β-anomer, respectively. Thus, the structure of **1** was elucidated as α-L-rhamnopyranosyl-(1→3)-*O*-[2-*O*-(11*S*)-11-hydroxytetradecanoyl]-[4-*O*-(2*R*,3*R*)-3-hydroxy-2-methylbutyryl]-α-L-rhamnopyranosyl-(1→2)-*O*-D-glucopyranose.

Compound **2**, colorless gum, and its molecular formula of $C_{36}H_{62}O_{18}$ was established by the positive HRESIMS ion at *m*/*z*

Table 1
^{1}H and ^{13}C NMR data of **1**and **2** (500 and 125 MHz, respectively)[a]

Position[b]	**1**				**2**			
	α-Anomer		β-Anomer		α-Anomer		β-Anomer	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Glc-1	5.98 d (3.5)	93.5	5.31 d (7.5)	97.4	5.96 d (3.0)	93.5	5.28 d (7.0)	97.4
2	4.17 dd (9.5, 3.5)	82.5	4.24[c]	80.9	4.17 dd (9.5, 3.5)	82.1	4.23[c]	80.6
3	4.87 dd (9.5, 9.5)	74.2	4.30 br t (9.0)	79.3	4.82 dd (9.5, 9.5)	74.0	4.27 br t (9.0)	79.0
4	4.25 dd (9.5, 9.0)	73.3	4.15 dd (9.5, 9.5)	72.8	4.09 dd (9.0, 9.0)	72.9	4.00 dd (9.5, 9.5)	72.3
5	4.79 m	73.8	3.99 m	78.8	4.80 m	70.9	4.02 m	75.5
6	4.44 dd (12.5, 6.0)	63.7	4.36 dd (12.5, 6.0)	63.4	4.80[c]	65.6	4.71[c]	65.3
	4.57 dd (12.5, 2.0)		4.55[c]		5.02 br d (11.5)		4.97[c]	
Rha-1	5.83 br s	100.8	6.33 br s	99.2	5.84 br s	100.8	6.32 br s	99.2
2	6.14 dd (3.5, 1.5)	73.2	6.17 dd (3.0, 1.5)	73.4	6.12 br d (3.0)	73.0	6.16 br s	73.2
3	4.83 dd (10.0, 3.5)	76.2	4.94 dd (10.0, 3.0)	76.0	4.70 dd (9.5, 3.5)	76.5	4.82[c]	76.3
4	5.79 dd (10.0, 10.0)	73.9	5.88 dd (10.0, 10.0)	74.3	5.64 dd (10.0, 10.0)	74.1	5.71 dd (10.0, 10.0)	74.5
5	4.75 dq (10.0, 6.0)	68.0	5.22 dq (9.5, 6.0)	67.9	4.60 dq (10.0, 6.0)	67.7	5.14 dq (9.5, 6.0)	67.5
6	1.50 d (6.0)	18.4	1.65 d (6.5)	18.6	1.31d (6.5)	18.2	1.46 d (6.5)	18.4
Rha′-1	5.44 br s	104.6	5.59 br s	104.7	5.37 br s	104.6	5.51 br s	104.8
2	4.55 br s	72.8	4.61 br s	72.9	4.47 br s	73.0	4.52 br s	73.0
3	4.46 dd (9.0, 3.0)	73.0	4.59 dd (9.0, 3.5)	73.1	4.45 dd (9.5, 3.0)	73.0	4.48 dd (9.0, 3.5)	73.0
4	4.23 dd (9.5, 9.5)	74.2	4.27 dd (9.5, 9.5)	74.2	4.23 dd (9.5, 9.5)	74.2	4.26 dd (9.5, 9.5)	74.1
5	4.33[c]	71.0	4.42 dq (10.0, 6.0)	71.0	4.33[c]	71.1	4.40 dq (10.0, 6.0)	71.1
6	1.55 d (6.5)	18.9	1.63 d (6.5)	19.0	1.58 d (6.0)	19.0	1.65 d (7.0)	19.0
Con-1		173.5		173.6		173.5		173.6
2	2.28[c]	34.9	2.28[c]	35.0	2.31[c]	34.9	2.31[c]	35.0
11	3.85 m	71.1	3.85 m	71.1	3.85 m	71.1	3.85 m	71.1
14	0.97 t (7.0)	15.0	0.97 t (7.0)	15.0	0.98 t (7.0)	15.0	0.98 t (7.0)	15.0
Nla-1		175.9		175.9				
2	2.72 m	49.4	2.72 m	49.3				
3	4.24[c]	69.8	4.24[c]	69.8				
4	1.32 d (6.0)	21.8	1.32 d (6.0)	21.7				
5	1.20 d (7.0)	14.5	1.22 d (7.0)	14.4				
Ac-1						171.3		171.3
2					1.98 s	21.2	1.94 s	21.2
Ac′-1						170.5		170.6
2					1.99 s	21.1	1.99 s	21.1

[a] Measured in pyridine-d_5. Chemical shifts (δ) are in parts per million relative to TMS. The spin coupling (J) is given in parentheses (Hz).
[b] Abbreviations: Glc=glucopyranosyl; Rha=rhamnopyranosyl; Con=11-hydroxytetradecanoyl; Nla=3-hydroxy-2-methylbutyryl; Ac=acetyl.
[c] Signal pattern unclear due to overlapping.

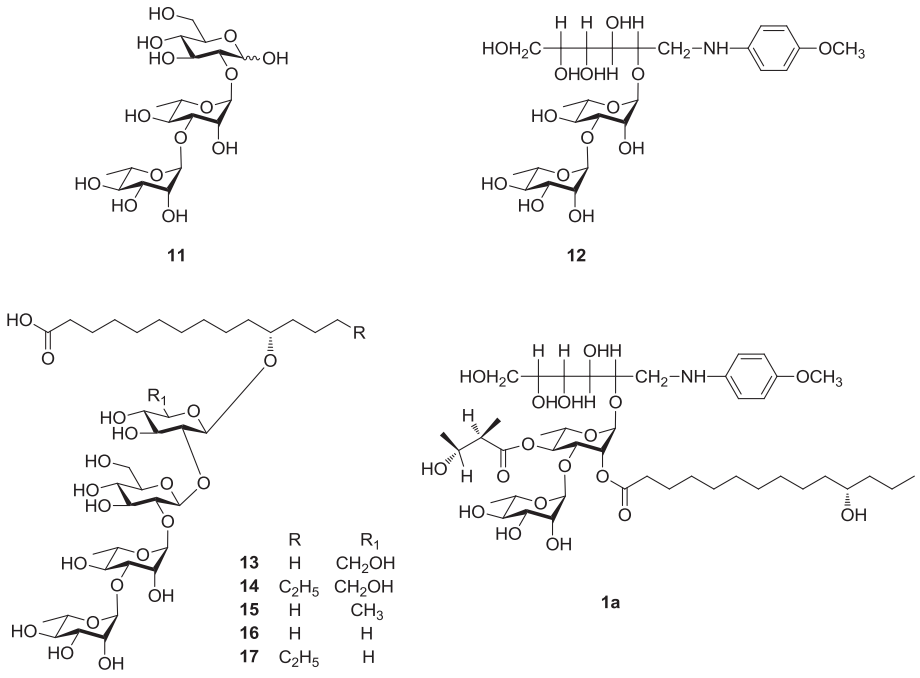


Fig. 1. Structures of compounds **11**–**17** and **1a**.

805.3826 $[M+Na]^+$ (calcd for $C_{36}H_{62}NaO_{18}$, 805.3828). It showed similar phenomenon as **1** on HPLC. The ^1H and ^{13}C NMR spectra (Table 1) of **2** were also similar to those of compound **1**, except for the absence of the signals of niloyl group. Additional two groups of methyl singlets at [δ_H 1.98 (s), 1.99 (s)] and [δ_H 1.94 (s), 1.99 (s)], revealed the appearance of two acetyl groups. Thus, it was deduced that compound **2** was analogue of **1** with acetyl groups rather than niloyl group. The locations of acyl groups were determined by the cross peaks between the signals of δ_H 6.12/6.16 (H-2, Rha) with δ_C 173.5/173.6 (C-1, 11*S*-convolvulinolic acid), δ_H 4.80/4.71 and 5.02/4.97 (H-6, Glc) with δ_C 171.3/171.3 (C-1, Ac), δ_H 5.64/5.71 (H-6, Rha) with δ_C 170.5/170.6 (C-1, Ac′) of the α-/β-anomer in the HMBC spectrum. Therefore, the structure of **2** was finally elucidated as α-L-rhamnopyranosyl-(1→3)-*O*-[2-*O*-(11*S*)-11-hydroxytetradecanoyl]-[4-*O*-acetyl]-α-L-rhamnopyranosyl-(1→2)-*O*-[6-*O*-acetyl]-D-glucopyranose.

Compound **3** was obtained as colorless gum. The molecular formula was deduced as $C_{43}H_{73}NO_{17}$ by HRESIMS data 898.4767 $[M+Na]^+$ (calcd for $C_{43}H_{73}NNaO_{17}$, 898.4771). The ^1H NMR spectrum (Table 2) of **3** exhibited two signals at δ_H 3.67 and 3.92 ascribed to be the nonequivalent 1-CH_2 of the aminoalditol sugar moiety,[10,14] along with two anomeric signals at δ_H 5.88 and 5.41, suggesting that compound **3** was an aminoalditol derivative of a trisaccharide. Basic hydrolysis afforded **12** (Fig. 1), 2-

Table 2
^1H and ^{13}C NMR data of **3**–**5** (500 and 125 MHz, respectively)[a]

Position[b]	**3**		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Glci-1	3.67 dd (14.0, 7.0)	47.0	3.63 dd (14.0, 7.5)	47.0	3.68 dd (14.0, 7.5)	46.9
	3.92 dd (14.0, 3.5)		3.89 dd (14.0, 3.0)		3.93 dd (14.0, 3.0)	
2	4.82 ddd (7.0, 7.0, 3.5)	80.3	4.78 m	80.5	4.85 ddd (7.0, 7.0, 3.5)	80.2
3	4.89 br d (7.0)	72.5	4.87 br d (6.5)	72.5	4.92 br d (7.0)	72.5
4	4.55[c]	73.8	4.53[c]	73.8	4.58[c]	73.7
5	4.43 m	73.1	4.42 m	73.0	4.46 m	73.1
6	4.49 dd (10.5, 3.5)	65.6	4.46 dd (11.0, 4.0)	65.6	4.51 dd (10.5, 3.5)	65.6
	4.39 dd (10.0, 6.0)		4.37 dd (11.0, 5.5)		4.41 dd (11.0, 5.5)	
Rha-1	5.88 br s	99.6	5.84 br s	99.7	5.91 br s	99.6
2	6.06 br s	73.4	6.06 br s	73.4	6.09 br s	73.4
3	4.69 dd (10.0, 3.5)	76.1	4.74 dd (10.0, 3.0)	75.8	4.71 dd (10.0, 3.5)	76.1
4	5.62 dd (10.0, 10.0)	73.9	5.72 dd (10.0, 10.0)	74.1	5.64 dd (10.0, 10.0)	73.8
5	4.56 dq (10.0, 6.5)	67.9	4.59 dq (10.0, 6.0)	68.3	4.58 dq (10.0, 6.5)	67.9
6	1.27 d (6.0)	18.4	1.44 d (6.0)	18.6	1.28 d (6.5)	18.4
Rha′-1	5.41 br s	104.6	5.48 br s	104.5	5.42 br s	104.6
2	4.45 br s	72.8	4.53 br s	72.9	4.47 br s	72.8
3	4.41 dd (10.0, 3.5)	73.0	4.44[c]	73.1	4.43[c]	73.0
4	4.21 dd (9.5, 9.5)	74.1	4.20 dd (9.5, 9.5)	74.2	4.24 dd (9.5, 9.5)	74.1
5	4.33 dq (10.0, 6.5)	71.1	4.34 dq (10.0, 6.0)	71.1	4.35 dq (10.0, 6.0)	71.1
6	1.59 d (6.0)	19.0	1.59 d (6.0)	19.0	1.62 d (6.0)	19.0
Con-1		173.5				
2	2.25 m	34.9				
11	3.83 m	71.2				
14	0.97 t (7.0)	15.0				

Table 2 (*continued*)

Position[b]	**3**		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Jal-1				173.5		173.5
2			2.23 m	35.0	2.25 m	34.9
11			3.83 m	71.2	3.84 m	71.5
16			0.85 t (7.0)	14.8	0.86 t (7.0)	14.8
Iba-1		176.6				176.6
2	2.55 m	35.0			2.55 m	34.9
2-Me	1.11 d (7.0)	19.6			1.12 d (7.0)	19.6
3	1.13 d (7.0)	19.6			1.13 d (7.0)	19.6
Nla-1				175.8		
2			2.72 m	49.4		
3			4.24[c]	69.7		
4			1.32 d (6.5)	21.8		
5			1.21 d (7.0)	14.4		
And-1		144.4		144.3		144.4
2, 6	6.92 d (9.0)	116.0	6.88 d (9.0)	116.0	6.92 d (9.0)	116.0
3, 5	6.90 d (9.5)	114.6	6.85 d (9.0)	114.7	6.90 d (9.0)	114.5
4		152.6		152.6		152.6
7-Me	3.66 s	56.2	3.63 s	56.2	3.66 s	56.1

[a] Measured in pyridine-d_5. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz).
[b] Abbreviations: Glci=glucitol residue; Rha=rhamnopyranosyl; Con=11-hydroxytetradecanoyl; Jal=11-hydroxyhexadecanoyl; Iba=2-methylpropanoyl; Nla=3-hydroxy-2-methylbutyryl; And=4-methoxyphenylamino.
[c] Signal pattern unclear due to overlapping.

methylpropanoic acid (Iba), and 11*S*-convolvulinolic acid. 2-Methylpropanoic acid was identified by the GC–MS analysis. Compound **12** was identified to be the aminoalditol derivative of **11**. Thus, it was deduced that compound **3** was derived from a natural resin glycoside, which had the same trisaccharide core as compounds **1** and **2**. The interglycosidic connectives were confirmed by the correlation peaks from H-1 (δ_H 5.88) of Rha to C-2 (δ_C 80.3) of Glci, from H-3 (δ_H 4.69) of Rha to C-1 (δ_C 104.6) of Rha′ in the HMBC spectrum. The esterification positions of the oligosaccharide core were elucidated via HMBC long-range couplings of δ_H 6.06 (H-2, HMBC spectra. From the above observations, C-2-OH of Rha was thus acylated by jalapinolic acid in **4** and **5**, while C-4-OH of Rha was acylated by (2*R*,3*R*)-3-hydroxy-2-methylbutyryl acid and 2-methylpropanoic acid in **4** and **5**, respectively. Therefore, the structures of **4** and **5** were determined as described.

Compound **6** was obtained as colorless gum, and its molecular formula of $C_{87}H_{149}NO_{40}$ was established by the HRESIMS ion at m/z 1848.9722 $[M+H]^+$ (calcd for $C_{87}H_{150}NO_{40}$, 1848.9729). In the positive ESIMS spectrum (Fig. 2), except for the quasimolecular ion ($[M+H]^+$, m/z 1849.4), the second highest peak at m/z 906.6 cor-

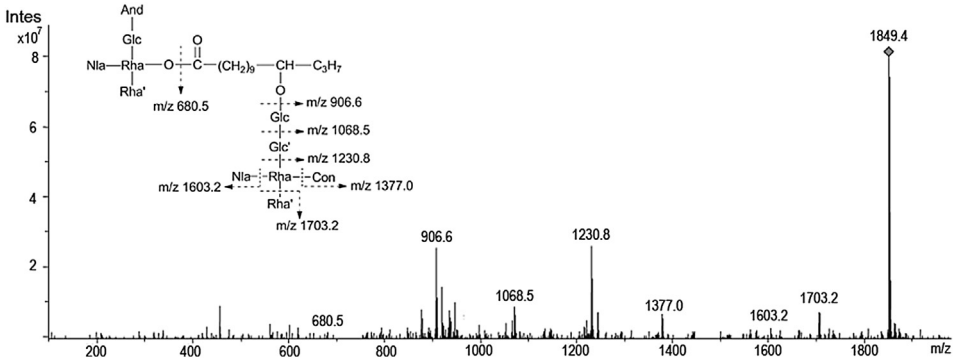


Fig. 2. Positive-ion ESIMS spectrum of compound **6**.

Rha) with δ_C 173.5 (11*S*-convolvulinolic acid), δ_H 5.62 (H-4, Rha) with δ_C 176.6 (2-methylpropanoic acid). Thus, the structure of **3** was established as shown.

Compounds **4** and **5**, colorless gum, gave quasimolecular ions of $[M+Na]^+$ at m/z 956.5191 ($C_{46}H_{79}NNaO_{18}$) and $[M+H]^+$ at m/z 904.5262 ($C_{45}H_{78}NO_{17}$), respectively. Their ^1H NMR and ^{13}C NMR spectra (Table 2) were similar to those of compound **3** except for the signals of fatty acids moieties. Compounds **4** and **5** were separately saponified with 3% K_2CO_3 to provide **12**, 11*S*-jalapinolic acid (11*S*-hydroxyhexadecanoic acid, Jal),[10,11] and an organic acid. The organic acids of **4** and **5** were determined to be (2*R*,3*R*)-nilic acid and 2-methylpropanoic acid, respectively. Moreover, the locations of acyl groups were established by the correlations observed in the

responded to the aminoalditol derivative of compound **1** (**1a**, Fig. 1), indicating that compound **6** may contain a moiety of **1a**. Additionally, apart from the common fragment ions produced by glycosidic cleavage of the sugar moieties, i.e., m/z 1703.2 [1849.4—$C_6H_{10}O_4$ (methylpentose)], 1230.8 [1377.0—$C_6H_{10}O_4$ (methylpentose)], 1068.5 [1230.8—$C_6H_{10}O_5$ (hexose)], 906.6 [1068.5—$C_6H_{10}O_5$ (hexose)], the presence of fragment ions at m/z 1603.2 [1703.2–100], 1377.0 [1603.2–226] and 680.5 [906.6–226], suggested that compound **6** may contain niloyl ($C_6H_8O_2$, 100) and convolvulinoloyl ($C_{14}H_{26}O_2$, 226) residues. These were confirmed by the alkaline hydrolysis of compound **6**.

On alkaline hydrolysis, compound **6** afforded **12**, 11*S*-convolvulinolic acid, (2*R*,3*R*)-nilic acid, and a water-soluble glycosidic

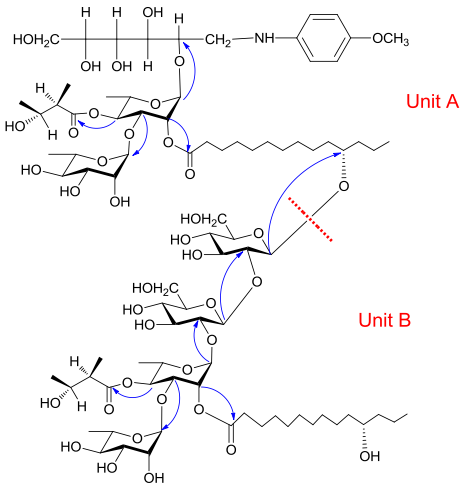


Fig. 3. Key HMBC correlations of compound **6**.

acid. The glycosidic acid was characterized as cuscutic acids A (**13**, Fig. 1), which has been previously obtained in the alkaline hydrolyzate of resin glycoside-like fraction of the seeds of *C. chinensis*.[11] The absolute configurations of the sugar units and convolvulinolic acid of cuscutic acids A (**13**) were determined by subsequent acidic hydrolysis to afford D-glucopyranose and L-rhamnopyranose in the aqueous layer,[18] and 11*S*-convolvulinolic acid in the ether layer.[15] All these evidences suggested that compound **6** was composed of two different acylated oligosaccharides, which were arbitrarily named as unit A and unit B, respectively (Fig. 3).

The ^{1}H and ^{13}C NMR spectra (Tables 3 and 5) of compound **6** exhibited six anomeric carbon signals at δ 99.7, 104.5, 102.9, 102.3, 98.6, 104.7 and one carbon signal at δ 47.0 due to C-1 of amino-alditol sugar moiety,[10] along with their corresponding protons signals at δ 5.85, 5.49, 4.96, 5.74, 6.28, 5.66, 3.65, and 3.91, which were subsequently assigned by the HSQC experiment. Detailed analysis of the TOCSY, HSQC, HMBC spectra led to the assignment of all the protons within each saccharide system. The sugar sequences were elucidated by the unambiguous correlations of H-1 (δ_H 5.85)

Table 3
^{1}H NMR data of **6**–**8** (500 MHz)[a]

Position[b]	**6**		**7**		**8**	
	Unit A	Unit B	Unit A	Unit B	Unit A	Unit B
Glci/Glc-1	3.65[c]	4.96 d (7.5)	3.66 dd (14.5, 7.5)	4.99 d (7.5)	3.66[c]	
	3.91[c]		3.91[c]		3.91 br d (13.0)	
2	4.80 ddd (7.0, 7.0, 3.5)	4.33 br d (8.5)	4.80 ddd (7.0, 7.0, 3.5)	4.34 br d (8.5)	4.82 ddd (7.0, 7.0, 3.5)	
3	4.89 br d (7.0)	4.52 dd (9.5, 9.5)	4.89 br d (7.0)	4.54 dd (9.5, 9.5)	4.91 br d (7.0)	
4	4.55[c]	4.16[c]	4.57[c]	4.18 dd (9.0, 9.0)	4.56[c]	
5	4.46[c]	4.01[c]	4.47[c]	4.04[c]	4.45[c]	
6	4.49 dd (11.0, 3.5)	4.51[c]	4.49 dd (11.0, 3.5)	4.52[c]	4.50 dd (11.0, 3.5)	
	4.38 dd (11.0, 5.5)	4.33[c]	4.40 dd (11.0, 5.5)	4.35[c]	4.39[c]	
Qui-1						4.87 d (7.5)
2						4.29 dd (9.5, 7.0)
3						4.42 dd (9.5, 9.5)
4						3.62 dd (9.5, 9.5)
5						3.84[c]
6						1.60 d (6.0)
Glc′-1		5.74 d (7.5)		5.77 d (7.5)		5.77 d (7.0)
2		4.21[c]		4.21[c]		4.21[c]
3		4.18[c]		4.18[c]		4.19[c]
4		4.03 dd (9.5, 9.5)		4.04 dd (9.5, 9.5)		4.03 dd (9.0, 9.0)
5		3.80[c]		3.82[c]		3.82[c]
6		4.45[c]		4.45[c]		4.45[c]
		4.29[c]		4.29[c]		4.29[c]
Rha-1	5.85 br s	6.28 br s	5.88 br s	6.31 br s	5.88 br s	6.30 br s
2	6.07 br s	6.09 br s	6.09 br s	6.11 br s	6.09 br s	6.10 br s
3	4.76 dd (10.0, 3.0)	5.10 dd (10.0, 3.5)	4.77 dd (10.0, 3.0)	5.12 dd (10.0, 3.0)	4.77 dd (10.0, 3.0)	5.10 dd (10.0, 3.0)
4	5.73 dd (10.0, 10.0)	5.83 dd (9.5, 9.5)	5.75 dd (10.0, 10.0)	5.86 dd (9.5, 9.5)	5.75 dd (10.0, 10.0)	5.84 dd (9.5, 9.5)
5	4.60[c]	5.33 dq (10.0, 6.0)	4.63 dq (10.0, 6.5)	5.33 dq (10.0, 6.0)	4.63 dq (10.0, 6.0)	5.33 dq (10.0, 6.0)
6	1.45 d (6.5)	1.78 d (6.0)	1.46 d (6.0)	1.81 d (6.0)	1.46 d (6.5)	1.78 d (6.0)
Rha′-1	5.49 br s	5.66 br s	5.50 br s	5.67 br s	5.50 br s	5.65 br s
2	4.55 br s	4.59 br s	4.57 br s	4.60 br s	4.56 br s	4.59 br s
3	4.48 br d (10.0)	4.48 br d (10.0)	4.49 br d (10.0)	4.49 br d (10.0)	4.48[c]	4.48[c]
4	4.21 dd (9.5, 9.5)	4.21 dd (9.5, 9.5)	4.23 dd (9.5, 9.5)	4.23 dd (9.5, 9.5)	4.23[c]	4.23[c]
5	4.37[c]	4.37[c]	4.38[c]	4.37[c]	4.37[c]	4.37[c]
6	1.58 d (6.0)	1.60 d (6.0)	1.60 d (6.0)	1.60 d (6.0)	1.60 d (6.0)	1.61 d (6.0)
Con-2	2.24[c]	2.29[c]		2.29[c]	2.22[c]	2.28[c]
11	3.91[c]	3.84[c]		3.84[c]	3.85[c]	3.84[c]
14	0.85 t (7.0)	0.96 t (7.0)		0.96 t (7.0)	0.92 t (7.0)	0.96 t (7.0)
Jal-2			2.22[c]			
11			3.91[c]			
16			0.82 t (7.0)			
Nla-2	2.74 dq (7.0, 7.0)	2.88 dq (7.0, 7.0)	2.75 dq (7.0, 7.0)	2.87 dq (7.0, 7.0)	2.75 dq (7.0, 7.0)	2.83 dq (7.0, 7.0)
3	4.24[c]	4.30[c]	4.25[c]	4.31[c]	4.24[c]	4.29[c]
4	1.31 d (6.5)	1.35 d (7.0)	1.31 d (6.0)	1.35 d (7.0)	1.31 d (6.5)	1.34 d (7.0)
5	1.22 d (6.5)	1.20 d (7.0)	1.22 d (7.0)	1.20 d (7.0)	1.22 d (7.0)	1.19 d (7.0)
And-2, 6	6.87 d (9.5)		6.88 d (9.5)		6.88 d (9.5)	
3, 5	6.87 d (9.0)		6.87 d (9.0)		6.85 d (9.5)	
7-Me	3.64 s		3.64 s		3.64 s	

[a] Measured in pyridine-d_5. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz).
[b] Abbreviations: Glci/Glc=glucitol residue for unit A and glucopyranosyl for unit B, respectively; Qui=quinovopranosyl; Rha=rhamnoyranosyl; Con=11-hydroxytetradecanoyl; Jal=11-hydroxyhexadecanoyl; Nla=3-hydroxy-2-methylbutyryl; And=4-methyoxyanilino.
[c] Signal pattern unclear due to overlapping.

Table 4
^{1}H NMR data of **9**, **10**, and **17** (500 MHz)[a]

Position[b]	**9**		**10**		**17**
	Unit A	Unit B	Unit A	Unit B	
Glci-1	3.65[c]		3.66[c]		
	3.91[c]		3.91[c]		
2	4.81 ddd (7.0, 7.0, 3.5)		4.82 ddd (7.0, 7.0, 3.5)		
3	4.90 br d (7.0)		4.90 br d (7.0)		
4	4.56[c]		4.56[c]		
5	4.46[c]		4.46[c]		
6	4.49 dd (11.0, 4.0)		4.50 dd (11.0, 4.0)		
	4.40 dd (11.0, 5.5)		4.40 dd (11.0, 6.0)		
Xyl-1		4.92 d (6.5)		4.94 d (6.5)	5.08 d (6.5)
2		4.28[c]		4.30 br d (7.0)	4.30 br d (7.0)
3		4.45 dd (8.5, 8.5)		4.45[c]	4.46 dd (8.5, 8.5)
4		4.16[c]		4.16[c]	4.20[c]
5		3.79 br d (11.0)		3.80[c]	3.77 dd (11.0, 9.5)
		4.32 dd (11.5, 6.5)		4.34 dd (11.5, 6.5)	4.33 dd (11.5, 6.5)
Glc′-1		5.69 d (6.5)		5.70 d (6.5)	5.64 d (7.0)
2		4.21[c]		4.21[c]	4.27[c]
3		4.18 dd (9.5, 9.5)		4.19 dd (9.5, 9.5)	4.24 dd (9.5, 9.5)
4		4.06 dd (9.0, 9.0)		4.07 dd (9.0, 9.0)	4.14 dd (9.0, 9.0)
5		3.80[c]		3.82[c]	3.84
6		4.44[c]		4.45[c]	4.45[c]
		4.29[c]		4.29[c]	4.31
Rha-1	5.87 br s	6.30 br s	5.87 br s	6.30 br s	6.34 br s
2	6.08 br s	6.10 br s	6.08 br s	6.11 br s	4.95 br s
3	4.76 dd (10.0, 3.0)	5.09 dd (10.0, 3.5)	4.77 dd (10.0, 3.0)	5.10 dd (10.0, 3.5)	4.84 dd (10.0, 3.5)
4	5.74 dd (10.0, 10.0)	5.83 dd (9.5, 9.5)	5.75 dd (10.0, 10.0)	5.84 dd (9.5, 9.5)	4.43[c]
5	4.61 dq (10.0, 6.0)	5.30 dq (10.0, 6.0)	4.62 dq (10.0, 6.0)	5.31 dq (10.0, 6.0)	5.01[c]
6	1.46 d (6.5)	1.77 d (6.0)	1.46 d (6.5)	1.78 d (6.0)	1.75 d (6.0)
Rha′-1	5.49 br s	5.65 br s	5.50 br s	5.65 br s	5.99 br s
2	4.56 br s	4.59 br s	4.56 br s	4.59 br s	4.71 br s
3	4.48 br d (10.0)	4.48 br d (10.0)	4.48 br d (10.0)	4.48 br d (10.0)	4.62 dd (10.0, 3.5)
4	4.22 dd (9.5, 9.5)	4.23 dd (9.5, 9.5)	4.23 dd (9.5, 9.5)	4.23 dd (9.5, 9.5)	4.22 dd (9.5, 9.5)
5	4.38[c]	4.38[c]	4.38[c]	4.38[c]	4.72[c]
6	1.60 d (6.0)	1.61 d (6.0)	1.60 d (6.0)	1.61 d (6.0)	1.58 d (6.5)
Con-2	2.24[c]	2.28[c]		2.30[c]	
11	3.85[c]	3.84[c]		3.84[c]	
14	0.89 t (7.0)	0.96 t (7.0)		0.97 t (7.0)	
Jal-2			2.23[c]		2.53 t (7.5)
11			3.85[c]		3.90[c]
16			0.83 t (7.0)		0.85 t (7.0)
Nla-2	2.74 dq (7.0, 7.0)	2.82 dq (7.0, 7.0)	2.75 dq (7.0, 7.0)	2.83 dq (7.0, 7.0)	
3	4.24[c]	4.30[c]	4.25[c]	4.31[c]	
4	1.31 d (6.5)	1.32 d (6.5)	1.31 d (6.0)	1.33 d (6.5)	
5	1.22 d (6.5)	1.18 d (7.0)	1.22 d (7.0)	1.19 d (7.0)	
And-2, 6	6.89 d (9.5)		6.87 d (9.5)		
3, 5	6.87 d (9.0)		6.87 d (9.0)		
7-Me	3.64 s		3.64 s		

[a] Measured in pyridine-d_5. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz).
[b] Abbreviations: Glci=glucitol residue; Xyl=xylopyranosyl; Rha=rhamnopyranosyl; Con=11-hydroxytetradecanoyl; Jal=11-hydroxyhexadecanoyl; Nla=3-hydroxy-2-methylbutyryl; And=4-methyoxyanilino.
[c] Signal pattern unclear due to overlapping.

Table 5
^{13}C NMR data of **6**–**10** and **17** (125 MHz)[a]

Position[b]	**6**		**7**		**8**		**9**		**10**		**17**
	Unit A	Unit B	Unit A	Unit B	Unit A	Unit B	Unit A	Unit B	Unit A	Unit B	
Glc/Glci-1	47.0	102.9	47.0	103.0	47.0		47.0		47.0		
2	80.4	80.6	80.4	80.6	80.4		80.4		80.4		
3	72.5	79.9	72.5	79.8	72.5		72.5		72.5		
4	73.8	72.5	73.7	72.4	73.8		73.7		73.7		
5	73.1	78.3	73.1	78.3	73.1		73.1		73.1		
6	65.6	63.2	65.5	63.2	65.6		65.6		65.6		
Qui-1						102.7					
2						80.7					
3						79.5					
4						77.6					
5						72.8					
6						19.0					
Xyl-1								103.2		103.2	103.1
2								80.6		80.9	81.6
3								79.2		79.2	78.6

(*continued on next page*)

Table 5 (*continued*)

Position[b]	**6**		**7**		**8**		**9**		**10**		**17**
	Unit A	Unit B	Unit A	Unit B	Unit A	Unit B	Unit A	Unit B	Unit A	Unit B	
4								71.6		71.6	71.3
5								67.1		67.1	67.0
Glc′-1		102.3		102.3		102.3		102.6		102.6	103.3
2		78.7		78.7		78.8		78.7		78.7	79.2
3		79.2		79.2		79.2		79.0		79.1	79.5
4		73.1		73.1		73.1		73.0		73.0	72.8
5		78.0		77.9		78.0		78.0		78.0	78.0
6		63.8		63.8		63.8		63.6		63.7	63.6
Rha-1	99.7	98.6	99.7	98.5	99.7	98.6	99.7	98.6	99.7	98.6	102.5
2	73.4	73.3	73.3	73.3	73.4	73.3	73.4	73.3	73.4	73.3	72.4
3	75.7	75.9	75.7	75.9	75.7	75.9	75.7	76.0	75.7	76.0	80.0
4	74.0	74.8	74.0	74.7	74.0	74.7	74.0	74.7	74.0	74.7	73.5
5	68.2	67.5	68.2	67.4	68.2	67.4	68.2	67.4	68.2	67.5	70.3
6	18.6	19.0	18.5	19.0	18.5	19.0	18.5	18.9	18.5	18.9	19.3
Rha′-1	104.5	104.7	104.4	104.7	104.5	104.7	104.4	104.7	104.4	104.7	104.5
2	72.9	72.8	72.9	72.8	72.9	72.9	72.9	72.8	72.9	72.8	72.9
3	73.0	73.0	73.0	73.0	73.0	73.0	73.0	73.0	73.0	73.0	73.2
4	74.2	74.2	74.1	74.1	74.1	74.1	74.2	74.2	74.2	74.2	74.7
5	71.0	71.0	71.0	71.0	71.0	71.0	71.0	71.0	71.0	71.0	71.3
6	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0
Con-1	173.6	173.7		173.7	173.5	173.6	173.5	173.7		173.7	
2	35.0	35.0		35.0	35.0	35.0	35.0	35.0		35.0	
11	81.1	71.2		71.1	80.8	71.1	80.9	71.2		71.2	
14	15.0	15.0		15.0	15.0	15.0	14.9	15.0		15.0	
Jal-1			173.6						173.5		178.0
2			35.0						35.0		35.6
11			81.3						81.0		80.5
16			14.7						14.7		14.7
Nla-1	175.8	176.1	175.8	176.1	175.8	176.1	175.8	176.0	175.8	176.0	
2	49.3	49.3	49.4	49.4	49.3	49.3	49.3	49.3	49.3	49.3	
3	69.7	70.1	69.7	70.0	69.7	70.0	69.7	70.1	69.7	70.1	
4	21.7	21.8	21.7	21.8	21.7	21.9	21.7	21.9	21.7	21.9	
5	14.4	14.5	14.4	14.5	14.4	14.5	14.4	14.5	14.4	14.5	
And-1	144.3		144.3		144.3		144.3		144.3		
2, 6	116.0		115.9		115.9		115.9		116.0		
3, 5	114.6		114.6		114.6		114.6		114.6		
7-Me	152.6		152.6		152.6		152.6		152.6		
	56.2		56.1		56.1		56.2		56.2		

[a] Measured in pyridine-d_5. Chemical shifts (δ) are in ppm relative to TMS.
[b] Abbreviations: Glci/Glc=glucitol residue for unit A and glucoypranosyl for unit B, respectively; Qui=quinovopranosyl; Xyl=xylopyranosyl; Rha=rhamnoyranosyl; Con=11-hydroxytetradecanoyl; Jal=11-hydroxyhexadecanoyl; Nla=3-hydroxy-2-methylbutyryl; And=4-methyoxyanilino.

of Rha with C-2 (δ_C 80.4) of Glci, H-3 (δ_H 4.76) of Rha with C-1 (δ_C 104.5) of Rha′ of unit A, and H-1 (δ_H 5.74) of Glc′ with C-2 (δ_C 80.6) of Glc, H-1 (δ_H 6.28) of Rha with C-2 (δ_C 78.7) of Glc′, H-3 (δ_H 5.10) of Rha with C-1 (δ_C 104.7) of Rha′ of unit B in the HMBC spectrum (Fig. 3). Four non-anomeric proton signals were found to be downfield-shifted to δ 6.07, 5.73, 6.09, and 5.83, respectively, demonstrating the presence of four sites of acylation. The acyl group at each site was revealed by the key HMBC correlations, i.e., from δ_H 6.07 (H-2, Rha) to δ_C 173.6 (11*S*-convolvulinolic acid), from δ_H 5.73 (H-4, Rha) to δ_C 175.8 (2*R*,3*R*-nilic acid) of the unit A, from δ_H 6.09 (H-2, Rha) to δ_C 173.7 (11*S*-convolvulinolic acid), from δ_H 5.83 (H-4, Rha) to δ_C 176.1 (2*R*, 3*R*-nilic acid) of the unit B. Then, the glycosidic linkage between the two units was determined by the cross peak between δ_H 4.96 (H-1, Glc of unit B) with δ_C 81.1 (11*S*-convolvulinolic acid of unit A) in the HMBC spectrum. On the basis of the above observations, compound **6** was established as depicted.

Compounds **7**–**9**, obtained as colorless gum, gave quasimolecular ion $[M+H]^+$ at *m*/*z* 1877.0024 ($C_{89}H_{154}NO_{40}$), 1832.9757 ($C_{87}H_{150}NO_{39}$), and 1818.9611 ($C_{86}H_{148}NO_{39}$), respectively. Their NMR spectra (Tables 3–5) were similar with those of compound **6**. The ^{13}C NMR spectra of **7**–**9** all showed carbon signals at δ_C 47.0 due to C-1 of aminoalditol sugar moiety,[10,14] suggesting that they were the aminoalditol derivatives of natural resin glycosides; and six anomeric carbon signals reflecting the presence of six sugar units. The ^1H NMR spectra of these compounds showed two methyl triplets resonated at δ_H 0.80–1.00 featuring two long-chain fatty acids; two groups of upfield protons, each containing two methyl doublets (δ_H 1.15–1.25 and 1.27–1.37, respectively) and one methylidyne (δ_H 2.70–2.90), suggesting the existence of two nilic acids; a group of four paramagnetically shifted non-anomeric ring protons signals (δ_H 5.70–6.10), indicating the occurring of four acylated sites. Alkaline hydrolysis of **7**–**9** with 3% K_2CO_3 all afforded **12**, 11*S*-convolvulinolic acid, (2*R*,3*R*)-nilic acid, and a glycosidic acid. The only difference of these compounds lay in the glycosidic acid moieties. Cuscutic acid D (**14**, Fig. 1) was obtained from **7**, Cuscutic acid C (**15**, Fig. 1) from **8**, Cuscutic acid B (**16**, Fig. 1) from **9**. The structures of these glycoside acids were established from a comparison of ESIMS, NMR spectra data, and acidic hydrolysis results with the reported data in the litetrature.[11] Therefore, it was deduced that compounds **7**–**9** were homologs of **6** with different sugar units or long-chain fatty acyl groups. Their acyl substituent patterns were determined to be the same with compound **6** by the correlations observed in the HMBC spectra: Thus, C-2-OH of Rha of unit A was acylated by the hydroxyl fatty acid aglycon of the glycosidic acid; C-2-OH of Rha of unit B was acylated by 11*S*-convolvulinolic acid; C-4-OH of Rha of unit A and B was acylated by (2*R*,3*R*)-nilic acid, respectively. Consequently, the structures of **7**–**9** were determined as described.

Compound **10**, colorless gum, exhibited a quasimolecular ion at *m*/*z* 1868.9716 $[M+Na]^+$ (calcd for $C_{88}H_{151}NNaO_{39}$, 1868.9755). Basic hydrolysis afforded **12**, (2*R*,3*R*)-nilic acid, 11*S*-convolvulinolic

acid, and a new tetrasaccharide glycosidic acid **17** (Fig. 1). The new glycosidic acid **17**, named as cuscutic acid E, was deduced as $C_{39}H_{70}O_{20}$ by HRESIMS data at *m/z* 881.4351 $[M+Na]^+$ (calcd for $C_{39}H_{70}NaO_{20}$ 881.4353). On acidic hydrolysis, **17** liberated 11*S*-jalapinolic acid, along with L-rhamnose, D-glucose, and D-xylose. The ^1H NMR spectrum of **17** showed four anomeric protons resonated at δ_H 5.08, 5.64, 6.34, and 5.99, which were correlated with δ_C 103.1, 103.3, 102.5, and 104.5 by the cross peaks in the HSQC spectrum, respectively. All the protons were assigned sequentially within each saccharide system by the TOCSY experiment (Table 4). The interglycosidic connectives and the position of the glycosidic bond were determined by the HMBC spectrum, which showed cross peaks from H-1 (δ_H 5.99) of Rha′ to C-3 (δ_C 80.0) of Rha, from H-1 (δ_H 6.34) of Rha to C-2 (δ_C 79.2) of Glc′, from H-1 (δ_H 5.64) of Glc′ to C-2 (δ_C 81.6) of Xyl, from H-1 (δ_H 5.08) of Xyl to C-11 (δ_C 80.5) of jalapinolic acid. Consequently, the structure of **17** was defined as (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-xylopyranoside. It could be seen from the structure of **17** that it had the same tetrasaccharide core with a different aglycon moiety 11*S*-jalapinolic acid rather than 11*S*-convolvulinolic acid compared with that of **15**. Therefore, it was deduced that compound **10** was also a resin glycoside dimer, as same as compounds **6**–**9**.

After assignment of all the proton and carbon signals of compound **10** by 1D and 2D NMR spectra (Tables 4 and 5), the locations of acyl groups were determined by the correlations between δ_H 6.08 (H-2, Rha) and δ_C 173.5 (11*S*-jalapinolic acid), δ_H 5.75 (H-4, Rha) and δ_C 175.8 (2*R*,3*R*-nilic acid) of the unit A, and between δ_H 6.11 (H-2, Rha) and δ_C 173.7 (11*S*-convolvulinolic acid), δ_H 5.84 (H-4, Rha) and δ_C 176.0 (2*R*,3*R*-nilic acid) of the unit B in the HMBC spectrum. Accordingly, the structure of compound **10** was elucidated as described.

As the isolation of compounds **3**–**10**, the UPLC-Q-TOF-MS experiment was carried out to check if there were MS signals for their corresponding prototypes (S63, Supplementary data) in the crude resin glycoside fraction. All the signals ascribable to the proposed prototypes of compounds **3**–**10** were detected (S64, Supplementary data). Therefore, it could be deduced that the prototypes of **3**–**10**, especially the dimeric structures of **6**–**10**, should be naturally existed in the *C. chinensis*. The discovery of **6**–**10** also confirmed the presumption proposed by Mikayaha et al. for the new types of resin glycosides in *C. chinensis*.[11]

Several ester-type resin glycoside dimers, such as purgins II–III,[19] tricolorins H–J,[20] batatins I–VI,[21] have been reported in the past few years. The structures of these dimers were considered as a macrocyclic resin glycoside esterified with its corresponding glycosidic acid. However, the dimeric structures of compounds **6**–**10** were probably generated from a different biosynthetic pathway. The biosynthetic origin of **6**–**10** was proposed to be the acylated trisaccharides isolated from this plant, and the plausible biosynthetic pathway of them was outlined in Scheme S1 of the Supplementary data. We postulated that the precursor acylated tetrasaccharide (unit B) was formed through glycosidation of a monosaccharide (glucose, quinovose or xylose) with the acylated trisaccharide **1** as a glycosidic donor. Then, the combination of unit B and another precursor, an acylated trisaccharide (**1** or the proposed prototype of **4**, unit A), via glycosidation afforded the dimeric structures, which were reacted with *p*-anisidine to yield compounds **6**–**10**. Therefore, the dimeric structures of compounds **6**–**10** were likely generated via glycosidation of two acylated oligosaccharides, and thus characterized as ether-type resin glycoside dimers.

The cytotoxic activity of compounds **1**–**10**, together with cuses 1 and 2, was evaluated toward the human breast cancer (MCF-7), human hepatoma (SMMC-7721), and human osteosarcoma (MG-63) cell lines. The screening results are summarized in Table 6.

Table 6
Cytotoxic activity of compounds **1**–**10**, cuses 1 and 2 (IC_{50} μg/mL)

Compound	MCF-7	SMMC-7721	MG-63
1	>100	>100	>100
2	>100	>100	>100
3	59.35±5.74	46.90±1.70	37.06±2.95
4	23.58±1.29	39.69±2.14	28.45±1.75
5	20.14±0.90	13.87±0.96	11.57±1.63
6	40.10±2.70	45.80±1.25	48.82±1.29
7	25.47±1.51	19.61±0.12	19.48±1.01
8	15.46±0.74	13.70±0.61	21.79±0.83
9	9.06±0.39	17.96±1.37	18.15±1.14
10	8.72±0.43	9.78±0.44	10.90±0.68
cus 1	>100	>100	>100
cus 2	>100	>100	>100
5-Fluorouracil	7.78±0.56	10.04±0.27	13.54±1.19

Each value represents the mean±SD from three independent experiments.

Compounds **1** and **2**, along with cuses 1 and 2, were considered inactive (IC_{50}>100 μg/mL), while compounds **3**–**10** exhibited weak to moderate cytotoxicity (IC_{50} 8.72–59.35 μg/mL) against these cell lines. The reductive amination seemed not to influence the cytotoxic activity since the effects of compound **1** and cus 1 on cell viabilities were comparable to those of their corresponding aminoalditol derivatives (Table S1, Supplementary data). Therefore, the potency of **3**–**10** could be attributed to their resin glycoside moieties.

Based on the results, the less polar compounds exhibited stronger cytotoxic activity than their polar analogues: compounds acylated with 11-hydroxyhexadecanoic acid possessed stronger activity than those with 11-hydroxytetradecanoic acid (**4** vs **1a**; **5** vs **3**; **7** vs **6**; **10** vs **9**); the 2-methylpropanoic acid promoted higher activity than the nilic acid (**3** vs **1a**; **5** vs **4**); compounds with xylose or quinovose unit were more potent than those with glucose unit (**8**, **9** vs **6**; **10** vs **7**). The possible reason might be that the existence of less polar groups could increase the lipophilicity of oligosaccharides and thus promote the amphipathic property, which was considered to play a key role in perturbing cell membranes through nonselective pore formation to exert the cytotoxicity and other activities.[22–24] In addition, it was noteworthy to mention that the dimers showed higher activity than their corresponding monomers (**6**, **8**, **9** vs **1a**; **7**, **10** vs **3**), which was probably caused by the ability of the dimers to form molecular aggregates of higher complexity.[22] In particular, compound **10** showed the highest cytotoxicity with IC_{50} values of 8.72, 9.78, and 10.90 μg/mL against MCF-7, SMMC-7721, and MG-63 cell lines, respectively, close to or better than those of the positive control.

2.2. Conclusions

In summary, 10 new compounds were isolated and identified from *C. chinensis* Lam, including two new naturally occurring resin glycosides cuses 3 (**1**) and 4 (**2**), and eight new resin glycosides cuses 5–12 (**3**–**10**), which were generated by the treatment of resin glycoside fraction with *p*-anisidine. Notably, compounds **6**–**10** were characterized as ether-type resin glycoside dimers, which were isolated from *C. chinensis* for the first time. With the discovery of compounds **6**–**10**, an extremely interesting thing was observed that the acylated oligosaccharides here, such as **1** and **4**, could form dimeric structures with another acylated oligosaccharide via intermolecular glycosidation, instead of macrocyclic ester via intramolecular glycosidation like other resin glycosides. Therefore, as Nohara et al. proposed, these compounds may represent a totally new group of resin glycosides.[10] And as it was exclusively found in *C. chinensis*, this group of resin glycosides might be of chemotaxonomic significance for the genus *Cuscuta*.

3. Experimental section

3.1. General experimental procedures

Optical rotations were determined with a JASCO P-1020 polarimeter. UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer. IR spectra were recorded in KBr-disc on a Bruker Tensor 27 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AV-500 NMR instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) using pyridine-d_5 as solvent. Mass spectra were obtained on an MS Agilent 1100 Series LC/MSD iron-trap mass spectrometer (ESIMS) and an Agilent 6520B Q-TOF spectrometer (HRESIMS), respectively. The GC–MS spectra were obtained using a Shimadzu GCMS-QP2010 Ultra. Absorbents for column chromatography (CC) were silica gel (Qingdao marine Chemical Co., Ltd., China), ODS (40–63 μm, FuJi, Japan), macroporous resin D101 (The Chemical Plant of Nankai University, Tianjin, China). Preparative HPLC was carried out using an Agilent 1100 series system equipped with an Agilent 1100 series multiple wavelength detector and a shim-pack RP-C_{18} column (20×200 mm). An Alltech ELSD 3300 evaporative light scattering detector (ELSD) was used for detection of compounds.

3.2. Plant material

The dried seeds of *C. chinensis* Lam. were purchased from Jiangsu province of China in July 2011, and authenticated by Professor Min-Jian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. 110728) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3. Extraction and isolation of 1 and 2

The dried and powdered seeds of *C. chinensis* Lam. (5 kg) were refluxed with MeOH (15 L×3) at 70 °C. After removal of the solvent in vacuo, the residue (260 g) was dissolved with MeOH (700 mL). The MeOH-soluble fraction (150 g) collected by filtration was chromatographied on a macroporous resin D101 column using a gradient of EtOH in H_2O (0:100, 60:40, 70:30, 85:15, 95:5, v/v) to yield five fractions (A–E).

Fraction D (16 g), brown syrup, was subjected to silica gel column chromatography eluting with CH_2Cl_2/MeOH (100:2, 100:30, 100:100, v/v) to afford three fractions (DA–DC). Fraction DB (3.3 g) was run on an ODS column using a step gradient of MeOH/H_2O (10:90 to 100:0, v/v), to afford six fractions (DB1–DB6). Fraction DB4 was performed on a silica gel column using a gradient of CH_2Cl_2/MeOH (12:1, 7:1, 1:1, v/v) to yield six subfractions (DB4A–DB4F) by TLC analysis. Subfraction DB4E was subjected to preparative HPLC with CH_3CN/H_2O (31:69, v/v) to produce **1** (80 mg). Subfraction DB4C was separated by preparative HPLC using CH_3CN/H_2O (37:63, v/v) to produce **2** (10 mg) and cus 1 (300 mg). Fraction DB5 was subjected to an open ODS column (CH_3CN/H_2O, 40:60 to 70:30, v/v) to give six subfractions (DB5A–DB5G). Subfraction DB5E was purified by a silica gel column using CH_2Cl_2/MeOH (12:1, v/v) to yield cus 2 (10 mg).

3.4. Preparation of aminoalditol derivative of resin glycoside fraction and isolation of 3–10

Fraction C (8 g), brown syrup, was subjected to silica gel column chromatography eluting with CH_2Cl_2/MeOH (100:2, 100:5, 100:30, 100:100, v/v) to afford five fractions (CA–CE). Fraction CD (4.2 g) collected from the elution with CH_2Cl_2/MeOH (100:30, v/v) was identified to be the target part containing resin glycosides by TLC and HPLC analysis.

The resin glycoside-like fraction (CD, 4.0 g) was dissolved in 10% AcOH/EtOH (15 mL), and *p*-anisidine (4.0 g) and $NaBH_3CN$ (1.5 g) were added to the solution. The mixture was allowed to stand at room temperature for 6 h. After removal of the solution, the derivative CDa was chromatographied over an MCI column with a continuous gradient of MeOH/H_2O (10:90 to 100:0, v/v) to afford six fractions (CDa1–CDa6). Fraction CDa5 eluted with MeOH/H_2O (90:10, v/v) was run on an ODS column using a step gradient of MeOH/H_2O (50:50 to 100:0, v/v) to yield five fractions (CDa5A–CDa5E). Separation of CDa5C was achieved by preparative HPLC with CH_3CN/H_2O (45:55, v/v), yielding **3** (35 mg) and **4** (42 mg). Fraction CDa5D was further subjected to passage over an open ODS column (CH_3CN/H_2O, 50:50 to 60:40, v/v) to give seven subfractions (CDa5DA–CDa5DG). Purification of CDa5DB, CDa5DD, and CDa5DF was carried out by preparative HPLC with CH_3CN/H_2O (42:58, 47:53, and 50:50, v/v, respectively) to give **6** (27 mg), **7** (40 mg), and **10** (6 mg), respectively. Subfraction CDa5DE was subjected to preparative HPLC using CH_3CN/H_2O (47:53, v/v) to yield **8** (12 mg), **9** (8 mg), and **5** (6 mg).

3.5. Characteristics of cuses 3–12 (1–10)

*3.5.1. Cus 3 (**1**).* Colorless gum; $[\alpha]_D^{27}$ −17.5 (*c* 0.27, MeOH); IR ν_{max} KBr cm^{-1}: 3453, 2932, 2854, 1729, 1642, 1384, 1075; ESIMS *m/z* 799.2 $[M+H]^+$ and 833.6 $[M+Cl]^-$; HRESIMS *m/z* 821.4135 $[M+Na]^+$ (calcd for $C_{37}H_{66}NaO_{18}$ 821.4125); ^{1}H and ^{13}C NMR spectral data: see Table 1.

*3.5.2. Cus 4 (**2**).* Colorless oil; $[\alpha]_D^{27}$ −6.5 (*c* 0.08, MeOH); IR ν_{max} KBr cm^{-1}: 3440, 2930, 2856, 1730, 1644, 1384, 1067; ESIMS *m/z* 805.7 $[M+Na]^+$ and 817.5 $[M+Cl]^-$; HRESIMS *m/z* 805.3826 $[M+Na]^+$ (calcd for $C_{36}H_{62}NaO_{18}$ 805.3828); ^{1}H and ^{13}C NMR spectral data: see Table 1.

*3.5.3. Cus 5 (**3**).* Colorless gum; $[\alpha]_D^{27}$ −34.2 (*c* 0.51, MeOH); IR ν_{max} KBr cm^{-1}: 3423, 2932, 2858, 1732, 1641, 1518, 1465, 1387, 1074, 822; UV (MeOH) λ_{max} (log ε) 312 (3.14), 245 (3.89), 204 (4.05) nm; ESIMS *m/z* 876.7 $[M+H]^+$ and 910.8 $[M+Cl]^-$; HRESIMS *m/z* 898.4767 $[M+Na]^+$ (calcd for $C_{43}H_{73}NNaO_{17}$ 898.4771); ^{1}H and ^{13}C NMR spectral data: see Table 2.

*3.5.4. Cus 6 (**4**).* Colorless gum; $[\alpha]_D^{27}$ −48.7 (*c* 0.13, MeOH); IR ν_{max} KBr cm^{-1}: 3442, 2931, 2855, 1729, 1642, 1516, 1461, 1384, 1069, 1045, 820; UV (MeOH) λ_{max} (log ε) 311 (3.24), 245 (3.98), 205 (4.10) nm; ESIMS *m/z* 934.8 $[M+H]^+$ and 968.9 $[M+Cl]^-$; HRESIMS *m/z* 956.5191 $[M+Na]^+$ (calcd for $C_{46}H_{79}NNaO_{18}$ 956.5189); ^{1}H and ^{13}C NMR spectral data: see Table 2.

*3.5.5. Cus 7 (**5**).* Colorless gum; $[\alpha]_D^{27}$ −46.3 (*c* 0.10, MeOH); IR ν_{max} KBr cm^{-1}: 3443, 2930, 2856, 1730, 1638, 1516, 1460, 1384, 1071, 1046, 820; UV (MeOH) λ_{max} (log ε) 311 (3.29), 245 (4.01), 205 (4.17) nm; ESIMS *m/z* 904.7 $[M+H]^+$ and 938.7 $[M+Cl]^-$; HRESIMS *m/z* 904.5262 $[M+H]^+$ (calcd for $C_{45}H_{78}NO_{17}$ 904.5264); ^{1}H and ^{13}C NMR spectral data: see Table 2.

*3.5.6. Cus 8 (**6**).* Colorless gum; $[\alpha]_D^{27}$ −34.2 (*c* 0.52, MeOH); IR ν_{max} KBr cm^{-1}: 3450, 2932, 2854, 1729, 1640, 1517, 1462, 1384, 1079, 818; UV (MeOH) λ_{max} (log ε) 309 (3.26), 245 (4.01), 204 (4.20) nm; ESIMS *m/z* 1849.4 $[M+H]^+$; HRESIMS *m/z* 1848.9722 $[M+H]^+$ (calcd for $C_{87}H_{150}NO_{40}$ 1848.9729); ^{1}H and ^{13}C NMR spectral data: see Tables 3 and 5.

*3.5.7. Cus 9 (**7**).* Colorless gum; $[\alpha]_D^{27}$ −44.5 (*c* 0.15, MeOH); IR ν_{max} KBr cm^{-1}: 3444, 2930, 2856, 1732, 1642, 1517, 1461, 1384, 1078, 1044, 819; UV (MeOH) λ_{max} (log ε) 309 (3.27), 245 (3.96), 204 (4.19) nm; ESIMS *m/z* 1877.4 $[M+H]^+$ and 1912.3 $[M+Cl]^-$;

HRESIMS *m/z* 1877.0024 $[M+H]^+$ (calcd for $C_{89}H_{154}NO_{40}$ 1877.0042); ^1H and ^{13}C NMR spectral data: see Tables 3 and 5.

*3.5.8. Cus 10 (**8**).* Colorless gum; $[\alpha]_D^{27}$ −42.9 (*c* 0.18, MeOH); IR ν_{max} KBr cm^{-1}: 3443, 2932, 2856, 1729, 1641, 1517, 1463, 1384, 1075, 1044, 818; UV (MeOH) λ_{max} (log ε) 311 (3.27), 244 (4.00), 204 (4.20) nm; ESIMS *m/z* 1833.6 $[M+H]^+$ and 1868.4 $[M+Cl]^-$; HRESIMS *m/z* 1832.9757 $[M+H]^+$ (calcd for $C_{87}H_{150}NO_{39}$ 1832.9780); ^1H and ^{13}C NMR spectral data: see Tables 3 and 5.

*3.5.9. Cus 11 (**9**).* Colorless gum; $[\alpha]_D^{27}$ −38.8 (*c* 0.57, MeOH); IR ν_{max} KBr cm^{-1}: 3445, 2932, 2854, 1729, 1643, 1516, 1461, 1384, 1078, 1044, 819; UV (MeOH) λ_{max} (log ε) 310 (3.20), 244 (4.04), 204 (4.29) nm; ESIMS *m/z* 1841.5 $[M+Na]^+$ and 1817.5 $[M-H]^-$; HRESIMS *m/z* 1818.9611 $[M+H]^+$ (calcd for $C_{86}H_{148}NO_{39}$ 1818.9623); ^1H and ^{13}C NMR spectral data: see Tables 4 and 5.

*3.5.10. Cus 12 (**10**).* Colorless gum; $[\alpha]_D^{27}$ −53.0 (*c* 0.14, MeOH); IR ν_{max} KBr cm^{-1}: 3451, 2932, 2854, 1728, 1645, 1516, 1462, 1384, 1078, 1046, 819; UV (MeOH) λ_{max} (log ε) 311 (3.29), 244 (4.03), 204 (4.24) nm; ESIMS *m/z* 1847.4 $[M+H]^+$; HRESIMS *m/z* 1868.9716 $[M+Na]^+$ (calcd for $C_{88}H_{151}NNaO_{39}$ 1868.9755); ^1H and ^{13}C NMR spectral data: see Tables 4 and 5.

3.6. Alkaline hydrolysis of 1–10

Compounds **1**–**10** (3.0 mg each) in 3% K_2CO_3/H_2O (3 mL) were refluxed at 95 °C for 1 h and acidified to pH 4.0 with 1 N HCl, respectively. The precipitates were filtered (the filtrate was saved) and methylated with MeOH, catalyzed with 0.5 N H_2SO_4 to yield 11-hydroxytetradecanoic acid methyl ester from **1**–**3** and **6**–**10**, and 11-hydroxyhexadecanoic acid methyl ester from **4** and **5**.

The filtrate was extracted with ether (3 mL×2). The ether layer was washed with H_2O, dried over anhydrous Na_2SO_4. The presence of acetic acid in **2**, 2-methylpropanoic acid in **3** and **5** was detected by GC–MS on a Shimadzu GCMS-QP2010 Ultra at 70 eV under the following conditions (30 m×0.25 mm×0.25 μm, RTX-5MS column; He, 0.8 mL/min; 35 °C, 3 min; 35–300 °C, Δ 10 °C/min): acetic acid (t_R 4.2 min) : *m/z* 60 $[M]^+$ (78), 45 (86), 43 (100); 2-methylpropanoic acid (t_R 7.8 min): *m/z* 88 $[M]^+$ (9), 73 (38), 71 (2), 60 (2), 55 (5), 45 (10), 43 (100), 41 (41), 29 (7), 27 (27). The presence of nilic acid in **1**, **4**, and **6**–**10** as well as its absolute configuration were identified by the preparation of 4-bromophenyacyl-(2*R*,3*R*)-nilate according to previously reported procedures.[10,11]

4-Bromophenyacyl-(2R,3R)-nilate: Colorless needles; $[\alpha]_D^{27}$ −16.0 (*c* 0.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.78 (2H, br d, *J*=8.5 Hz, ArH), 7.65 (2H, br d, *J*=8.5 Hz, ArH), 5.33, 5.43 (each 1H, d, *J*=16.5 Hz, CH_2COAr), 3.97 (1H, dq, *J*=7.0, 6.5 Hz, H-3), 2.62 (1H, dq, *J*=7.0, 7.0 Hz, H-2), 1.30 (1H, d, *J*=6.5 Hz, CH_3-4), 1.25 (1H, d, *J*=7.0 Hz, CH_3-5).

The residues obtained from aqueous phase of **1**–**5** were desalted on a Sephadex LH 20 column (MeOH), and subsequently purified by chromatography on silica gel column (CH_2Cl_2/MeOH) to give **11** from **1**–**2**, **12** from **3**–**5**.[11]

The aqueous phase of **6**–**10** was separately extracted with *n*-BuOH (3 mL×3). The lower layer was treated in the way as described for that of **1**–**5**, and yielded **12**. The *n*-BuOH layer was subjected to an open ODS column (MeOH/H_2O, 75:25, v/v) to obtain cuscutic acids A, D, C, B (**13**–**16**)[11] for **6**–**9**, respectively, and cuscutic acid E (**17**) for **10**.

Cuscutic acid E (**17**): Colorless gum; $[\alpha]_D^{27}$ −27.3 (*c* 0.24, MeOH); IR ν_{max} KBr cm^{-1}: 3453, 2929, 2854, 1638, 1401, 1075, 1042; ESIMS *m/z* 857.8 $[M-H]^-$; HRESIMS *m/z* 881.4351 $[M+Na]^+$ (calcd for $C_{39}H_{70}NaO_{20}$ 881.4353); ^1H and ^{13}C NMR spectral data: see Tables 4 and 5.

3.7. Preparation of Mosher's ester

The (*S*)- and (*R*)-MTPA ester derivatives of 11-hydroxytetradecanoic acid methyl ester and 11-hydroxyhexadecanoic acid methyl ester were prepared by using the advanced Mosher ester procedure.[15] Two aliquots of each compound (1 mg each in 50 μL pyridine-d_5) were transferred into two NMR tubes. Then, 5 μL of (*S*)- or (*R*)-MTPA chloride and 450 μL of pyridine-d_5 were added. The tubes were immediately sealed, shaken vigorously to ensure even mixing, and standed at room temperature in a desiccator in 14–16 h until the reaction was complete. The ^1H NMR spectra of the corresponding ester were obtained directly from the NMR tubes. The selected $\Delta\delta_H$ values [$\Delta\delta_H=\delta(S)-\delta(R)$] ($\Delta\delta_H$=+0.06, H-14 of 11-hydroxytetradecanoic acid methyl ester and $\Delta\delta_H$=+0.04, H-16 of 11-hydroxyhexadecanoic acid methyl ester) made it possible to conclude the 11*S* configurations of 11-hydroxytetradecanoic acid methyl ester and 11-hydroxyhexadecanoic acid methyl ester.

11S-Hydroxytetradecanoic acid methyl ester: Colorless oil; $[\alpha]_D^{27}$ +2.1 (*c* 0.11, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.67 (3H, s, OCH_3), 3.59 (1H, m, H-11), 2.30 (2H, t, *J*=7.5 Hz, H-2), 0.93 (3H, t, *J*=7.0 Hz, H-14); ESIMS *m/z* 259 $[M+Na]^+$.

^1H NMR data (500 MHz, pyridine-d_5) for (*S-MTPA*) *ester of* 11*S-hydroxytetradecanoic acid methyl ester*: δ_H 3.66 (3H, s, OCH_3), 5.29 (1H, m, H-11), 2.35 (2H, t, *J*=7.5 Hz, H-2), 0.88 (3H, t, *J*=7.0 Hz, H-14).

^1H NMR data (500 MHz, pyridine-d_5) for (*R-MTPA*) *ester of* 11*S-hydroxytetradecanoic acid methyl ester*: δ_H 3.65 (3H, s, OCH_3), 5.29 (1H, m, H-11), 2.35 (2H, t, *J*=7.5 Hz, H-2), 0.82 (3H, t, *J*=7.0 Hz, H-14).

11S-Hydroxyhexadecanoic acid methyl ester: Colorless oil; $[\alpha]_D^{27}$ +1.3 (*c* 0.14, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.65 (3H, s, OCH_3), 3.57 (1H, m, H-11), 2.28 (2H, t, *J*=7.5 Hz, H-2), 0.88 (3H, t, *J*=7.0 Hz, H-16); ESIMS *m/z* 309 $[M+Na]^+$.

^1H NMR data (500 MHz, pyridine-d_5) for (*S-MTPA*) *ester of* 11*S-hydroxyhexadecanoic acid methyl ester*: δ_H 3.66 (3H, s, OCH_3), 5.30 (1H, m, H-11), 2.36 (2H, t, *J*=7.5 Hz, H-2), 0.86 (3H, t, *J*=7.0 Hz, H-16).

^1H NMR data (500 MHz, pyridine-d_5) for (*R-MTPA*) *ester of* 11*S-hydroxyhexadecanoic acid methyl ester*: δ_H 3.65 (3H, s, OCH_3), 5.31 (1H, m, H-11), 2.35 (2H, t, *J*=7.5 Hz, H-2), 0.82 (3H, t, *J*=7.0 Hz, H-16).

3.8. Preparation of aminoalditol derivative of 11

A solution of **11** (5 mg) in 10% AcOH/EtOH (3 mL) was treated with *p*-anisidine (5 mg) and $NaBH_3CN$ (5 mg), and the reaction mixture was allowed to stand at room temperature for 4 h. After evaporation of the solvent in vacuo, the residue was desalted by chromatography over Sephadex LH 20 column (MeOH), and then purified by chromatography on silica gel column (CH_2Cl_2/MeOH) to give colorless oil. The ESIMS and NMR data of the colorless oil were superimposable on those of **12**.

3.9. Acid hydrolysis and sugar analysis of 11

Compound **11** (3 mg) was dissolved in 1 N H_2SO_4 (3 mL) and heated at 90 °C for 2 h. The mixture was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, and then concentrated to yield a residue of the sugar fraction. The residue was dissolved in 3 mL of dry pyridine and treated with 5 mg of L-cysteine methyl ester hydrochloride. After heated at 60 °C for 1 h, phenyl isothiocyanate (5 μL) was added, and the mixture was heated at 60 °C for another 1 h. The reaction mixture was analyzed by reversed-phase HPLC, which was performed on an Agilent 1200 HPLC system (Agilent Technologies Inc.) equipped with a photodiode array detector and an Agilent C18 column (particle size 5 μm, 250×4.6 mm) at 30 °C with isocratic elution of 25% CH_3CN in 0.1% HCOOH solution at a flow rate of 0.8 mL/min. Peaks were detected

at 250 nm. The existence of D-glucose and L-rhamnose was confirmed by comparison of the retention times with those of standard sugar derivatives prepared in the same way, which showed retention times of 18.4 min and 33.1 min, respectively.

3.10. Acid hydrolysis and sugar analysis of 13–17

Compounds **13**–**17** (2.0 mg each) were separately dissolved in 1 N H_2SO_4 (3 mL) and heated at 90 °C for 2 h. The reaction mixtures were cooled to room temperature and extracted three times with ether (3 mL×3). The ether layer was dried in vacuo and methylated with MeOH, catalyzed with 0.5 N H_2SO_4 to afford 11-hydroxy tetradecanoic acid methyl ester from **13**, **15**, and **16**, and 11-hydroxyhexadecanoic acid methyl ester from **14** and **17**, respectively.[10] Both of the absolute configurations of 11-hydroxytetra decanoic acid methyl ester and 11-hydroxyhexadecanoic acid methyl ester were determined to be *S* by the advanced Mosher's method described in Section 3.7.

Each aqueous layer of **13**–**17** was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column to afford a saccharide residue. The procedure applied to determine the absolute configurations of the sugars was same as described for the sugar analysis of **11**. The absolute configurations of the monosaccharides were determined as D-glucose and L-rhamnose in **13**–**17**, D-quinovose in **15**, and D-xylose in **16** and **17** by comparison of the retention times of the thiocarbamoyl-thiazolidine derivatives of the acid hydrolyzate of **13**–**17** with those of standard samples of D-glucose (18.4 min), L-rhamnose (33.1 min), D-quinovose (30.7 min), and D-xylose (22.1 min), respectively.

3.11. Cytotoxic activity assay

Cytotoxic activity toward human breast cancer (MCF-7), human hepatoma (SMMC-7721), and human osteosarcoma (MG-63) cell lines was performed according to the published method.[25] 5-Fluorouracil was used as the positive control with IC_{50} values at 7.78, 10.04, and 13.54 μg/mL, respectively.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.01.068. These data include MOL files and InChiKeys of the most important compounds described in this article.

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