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Pentasaccharide resin glycosides from *Ipomoea cairica* and their cytotoxic activities

Bangwei Yu [a,b], Jianguang Luo [a], Junsong Wang [a], Dongming Zhang [b], Shishan Yu [b], Lingyi Kong [a,*]

[a] State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China

[b] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xian Nong Tan Street, Beijing 100050, People's Republic of China

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ABSTRACT

Six partially acylated pentasaccharide resin glycosides, cairicosides A–F, were isolated from the aerial parts of *Ipomoea cairica*. These compounds were characterized as a group of macrolactones of simonic acid A, partially acylated with different organic acids. The lactonization site of 11*S*-hydroxyhexadecanoic acid (jalapinolic acid) was bound to the second saccharide moiety at C-3 in cairicosides A–E, while at C-2 in cairicoside F. Structures were established by spectroscopic and chemical methods. Compounds cairicosides A–E exhibited moderate cytotoxicity against a small panel of human tumor cell lines with IC_{50} values in the range of 4.28–14.31 μM.

1. Introduction

Ipomoea cairica (L.) Sweet (Convolvulaceae) is a perennial climbing herb, known as mile-a-minute vine. It is widely distributed from tropical to subtropical regions and is used as folk medicine all over the world (Song et al., 2009; Ferreira et al., 2006; Thomas et al., 2004). In mainland China, *I. cairica*, as an invasive species, is harmful to the ecosystem (Li and Xie, 2001), and its aerial parts are orally used to cure sores (Flora, 1979). Lignans, benzenoids, coumarins, flavonoids, steroids, and fatty acids (Lin et al., 2008), as well as cyanidins, have been found in this species (Pomilio and Sproviero, 1972). Among them, some lignans showed interesting biological properties, such as inhibiting replication of human immunodeficiency virus type I (Schroder et al., 1990), cytotoxic activity (Lin et al., 2008), significant anti-tumor, and Ca^{+}-antagonist activities (Pàska et al., 1999). Resin glycosides were shown to be rich in many species of the family Convolvulaceae, as described in a recent review (Pereda-Miranda et al., 2010). However, so far, no resin glycoside has been reported from *I. cairica*. As a part of our ongoing chemical studies on the resin glycosides with biological activity from *Ipomoea* species (Yin et al., 2008a,b, 2009; Yin and Kong, 2008; Yu et al., 2011), a chemical investigation of *I. cairica* was undertaken.

In the present study, six new partially acylated pentasaccharide resin glycosides, designated as cairicosides A–F (**1**–**6**) (Fig. 1), were isolated from the aerial parts of *I. cairica*. These new compounds are macrolactones of simonic acid A, partially esterified with different fatty acids. The lactonization site of the aglycone, jalpinolic acid, was attached to the second saccharide at C-3 in **1**–**5**, while at C-2 in **6**. Their structures were elucidated on the basis of extensive spectroscopic data interpretation and chemical degradation. Herein, we reported an isolation and structure elucidation of these compounds, and their cytotoxic activity against a series of human tumor cell lines.

2. Results and discussion

The EtOH extract of the aerial parts of *I. cairica* was suspended in H_2O to afford H_2O-soluble and H_2O-insoluble fractions. The H_2O-insoluble fraction was resuspended in MeOH–H_2O (4:1, v/v), and allowed to stand overnight so as to precipitate chlorophyll. The supernatant was subjected to chromatography on D101 macroporous resin followed by repeated chromatography on silica gel, Sephadex LH-20, and ODS columns, as well as preparative HPLC to yield six new glycoresins, cairicosides A–F (**1**–**6**) (Fig. 1). The ^{1}H and ^{13}C NMR spectra of these new compounds with the resulting signals were useful to confirm the nature of resin glycoside.

Cairicoside A (**1**), obtained as a white, amorphous powder, was found to have the molecular formula $C_{70}H_{112}O_{26}$ as determined

* Corresponding author. Tel./fax: +86 25 83271405.
E-mail addresses: cpu_lykong@126.com, lykong@cpu.edu.cn (L. Kong).

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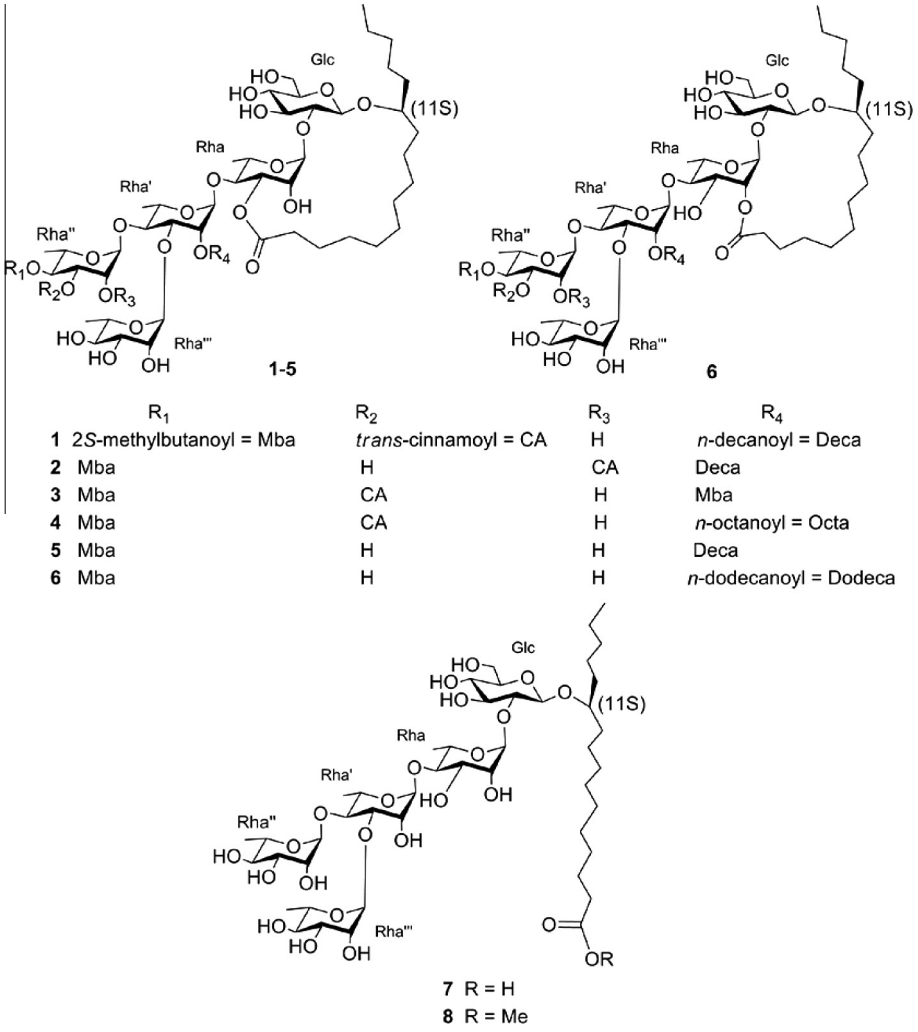

	R_1	R_2	R_3	R_4
1	2*S*-methylbutanoyl = Mba	*trans*-cinnamoyl = CA	H	*n*-decanoyl = Deca
2	Mba	H	CA	Deca
3	Mba	CA	H	Mba
4	Mba	CA	H	*n*-octanoyl = Octa
5	Mba	H	H	Deca
6	Mba	H	H	*n*-dodecanoyl = Dodeca

Fig. 1. Structures of compounds **1**–**6** and their derivatives compounds **7** and **8**.

through HRESIMS (negative mode, $[M-H]^-$ peak at *m/z* 1367.7356, calcd for $C_{70}H_{111}O_{26}$, 1367.7369). Its IR spectrum exhibited absorptions of hydroxyl (3450 cm^{-1}), carbonyl (1737 cm^{-1}), and aromatic (1638 cm^{-1}) groups. Alkaline hydrolysis of **1** afforded a glycosidic acid and ether soluble organic acids. These organic acids were identified as *n*-decanoic acid, 2-methylbutanoic acid, and *trans*-cinnamic acid, on the basis of the GC–MS experiments. The glycosidic acid, which gave key fragments at *m/z* 1017 $[M-H]^-$, 871 $[M-H-C_6H_{10}O_4]^-$, 725 $[871-C_6H_{10}O_4]^-$, 579 $[725-C_6H_{10}O_4]^-$, 433 $[579-C_6H_{10}O_4]^-$, and 271 $[433-C_6H_{10}O_5]^-$ in negative ion ESIMS (S25, Supporting Information), was indicative of a pentasaccharide resin glycoside composed of hexose and deoxyhexose (1:4), and the hexose linked directly with aglycone (Noda et al., 1992). A combination of NMR spectroscopic data and key fragments in ESIMS spectrum led to identification of the glycosidic acid as simonic acid A (**7**) (Chart 1), previously obtained from *I. batatas* (Yin et al., 2009; Noda et al., 1992), and *I. murucoides* (Chérigo et al., 2008). 2-Methylbutanoic acid purified from the alkaline hydrolysates was proved to be *S*-configuration by comparing the specific rotation value with that of authentic 2*S*-mehtylbutanoic acid. The sugars obtained from the acidic hydrolysates of simonic acid A methyl ether (**8**) were identified as L-rhamnopyranose and D-glucopyranose by GC–MS analysis of their chiral derivatives (Luo et al., 2008). The 11*S*-configuration was determined on the basis of Mosher's method (Yin et al., 2008a,b, 2009; Yin and Kong, 2008; Yu et al., 2011).

The ^1H NMR spectrum (Table 1) of **1** showed the presence of four methyl doublets at δ_H 1.74, 1.64, 1.45, 1.70 featuring four 6-deoxyhexose units, as soon as many overlapped methylene signals in the range of δ_H 1.2–2.0 assignable to the long chain fatty acids. Further features were resonances observed in the range of δ_H 5.0–6.5 due to anomeric protons and acylated protons, and the two nonequivalent protons (δ_H 2.99 and 2.28) of the methylene group at C-2 in the aglycone contributable to its macrocyclic lactone-type structure (Yin et al., 2008a,b, 2009; Yin and Kong, 2008; Yu et al., 2011; Escobedo-Martínez and Pereda-Miranda, 2007). A pair of distinctive *trans*-coupled olefinic protons (δ_H 6.55 and 7.83, each J = 16.0 Hz) and five phenyl protons (δ_H 7.34, m, 3H, and 7.46, m, 2H) were present in the ^1H NMR spectrum, suggesting the presence of a *trans*-cinnamoyl moiety. The protons at δ_H 0.82 (t, J = 7.5 Hz), 1.14 (d, J = 7.0 Hz), and 2.45 (tq, J = 7.0, 7.0 Hz) were assignable to a 2-methylbutanoyl group. The ^{13}C NMR spectrum of **1** (Table 2) exhibited five signals at δ_C 99.5, 100.2, 101.5, 103.7, and 104.3 assigned to anomeric carbons of five sugar units, and δ_C 175.9, 174.9, 173.0, and 166.1 for four ester carbonyl carbons. On the basis of these data, compound **1** was determined to be a partially acylated pentasaccharide resin glycoside. All proton and carbon resonances were assigned by a combination of ^1H and ^{13}C NMR and 2D NMR experiments (HSQC, HMBC, and TOCSY) (Tables 1 and 2). These procedures allowed the identification of one glucopyranosyl, and four rhamnopyranosyl units in **1**. The β-configuration of the D-glucose was suggested by a large coupling constant

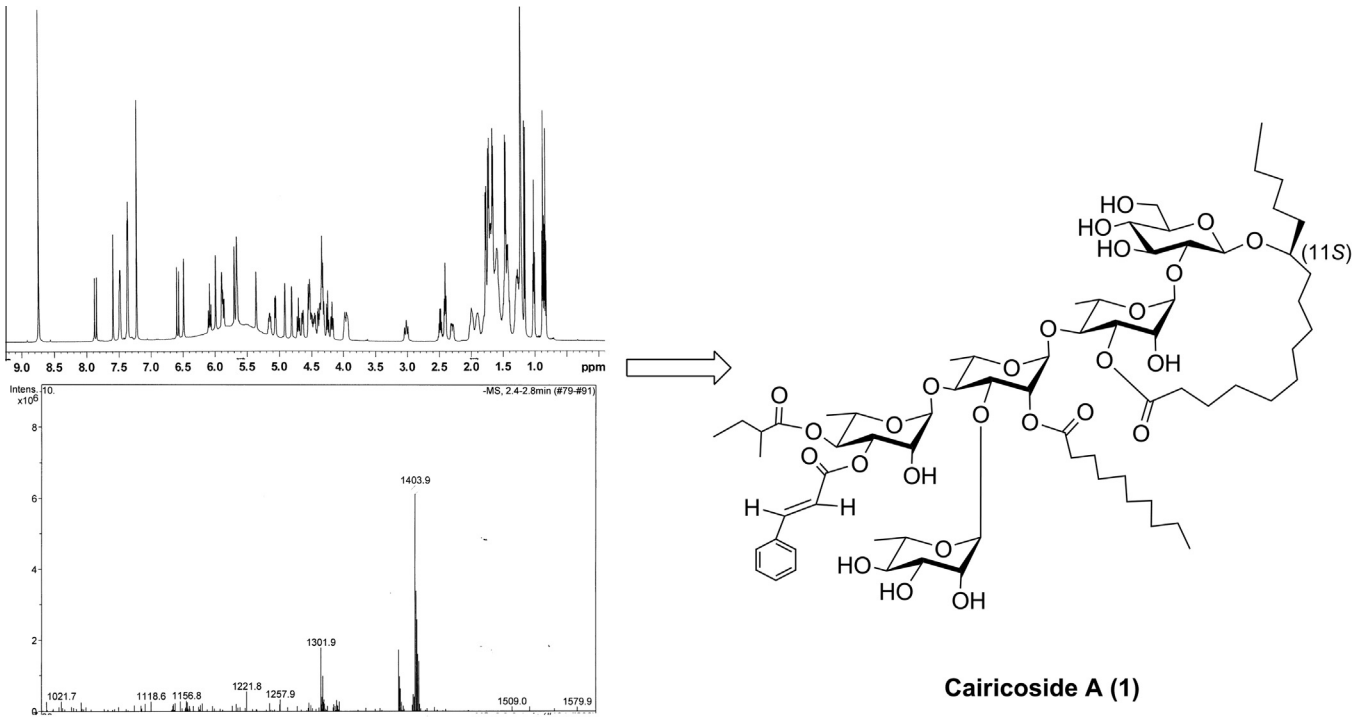

Chart 1. ^{1}H NMR, negative ions, ESI-MS spectrum and structure of the isolated compound **1**, cairicoside A.

(J = 7.0 Hz) for the anomeric proton (δ_H 5.04) in the ^{1}H NMR spectrum, while the α-configuration for L-rhamnose was deduced from the chemical shift of C-5 of rhamnose in the ^{13}C NMR spectrum (Yin et al., 2008a,b, 2009; Yin and Kong, 2008; Yu et al., 2011; Sang et al., 2000). The interglycosidic connectivities were confirmed by HMBC correlations: H-1 (δ_H 6.47) of Rha with C-2 (δ_C 75.5) of Glc, H-1 (δ_H 5.68) of Rha′ with C-4 (δ_C 78.7) of Rha, H-1 (δ_H 5.97) of Rha″ with C-4 (δ_C 79.7) of Rha′, and H-1 (δ_H 5.64) of Rha‴ with C-3 (δ_C 80.1) of Rha′. The position of the aglycone, 11-hydroxyhexadecanoic acid, in the oligosaccharide core was determined by the correlation between δ_H 3.96 (H-11 of Ag) and δ_C 101.3 (C-1 of Glc) in the HMBC spectrum. The observed $^{3}J_{CH}$ coupling between the carbonyl carbon of the lactone (δ_C 174.9) and H-3 of Rha (δ_H 5.64) indicated that the lactonization site of the aglycone was corroborated as C-3 of Rha. The positions of acyl residues were finally established by the key HMBC correlations from protons of sugars to acyl carbons of the fatty acids, i.e., δ_H 5.87 (H-2 of Rha′) to δ_C 173.0 (C-1 of *n*-decanoyl), δ_H 5.84 (H-3 of Rha″) to δ_C 166.1 (C-1 of *trans*-cinnamoyl), and δ_H 6.06 (H-4 of Rha″) to δ_C 175.9 (C-1 of 2*S*-methylbutanoyl). From these observations, the structure of cairicoside A (**1**) was elucidated as (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-decanoyl)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside-(1,3″-lactone).

Cairicoside B (**2**) exhibited the same molecular formula, $C_{70}H_{112}O_{26}$, as **1**, inferred by HRESIMS at *m/z* 1367.7356 $[M-H]^-$ (calcd for $C_{70}H_{111}O_{26}$, 1367.7369). Basic hydrolysis also gave simonic acid A (**7**), and *n*-decanoic, 2-methylbutanoic, and *trans*-cinnamic acids. The ^{1}H NMR spectrum (Table 1) of **2** was similar to that of **1** except that the aromatic proton signals appeared as one multiplet (δ_H 7.26) in **2** rather than two multiplets (δ_H 7.46 and δ_H 7.34) observed for **1**. These data suggested that they were positional isomers (Yu et al., 2011). The acyl residues in the oligosaccharide core were determined by HMBC correlations between δ_H 5.87 (H-2 of Rha′), 5.98 (H-2 of Rha″), 5.74 (H-4 of Rha″) and δ_C 172.6 (C-1 of *n*-decanoyl), 166.5 (C-1 of *trans*-cinnamoyl), 176.0 (C-1 of 2*S*-methylbutanoyl), respectively. Therefore, the structure of cairicoside B (**2**) was identified as (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl-(1→4)]-*O*-(2-*O*-*n*-decanoyl)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside-(1,3″-lactone).

Cairicosides C–F (**3**–**6**), white amorphous powder, were assigned molecular formulas of $C_{65}H_{102}O_{26}$, $C_{68}H_{108}O_{26}$, $C_{61}H_{106}O_{25}$, and $C_{63}H_{110}O_{25}$, respectively, by HRESIMS. Compounds **3**–**6** were identified as analogues of **1** on the basis of their NMR spectroscopic data. Interpretation of the ^{1}H and ^{13}C NMR data for **3**–**6** (**3**–**5** in Tables 1 and 2, **6** in Table 3) indicated that they share the same pentasaccharide skeleton. The nonequivalent protons of the methylene group at C-2 of the jalapinolic moiety suggested the presence of a macrocyclic lactone-type structure. Independent alkaline hydrolysis of **3**–**6** afforded a mixture of organic acids and a glycosidic acid, respectively. Using GC–MS experiments, a 2*S*-methylbutanoic acid was found in **3**–**6**, and a *trans*-cinnamoic acid was detected in **3** and **4**. Additionally, *n*-octanoic acid, *n*-decanoic acid, and *n*-dodecanoic acid were found in compounds **4**–**6**, respectively. The glycosidic acid obtained was also proved to be simonic acid A (**8**) from analysis of the NMR and MS data. The key HMBC correlations confirmed the esterification positions of acyl residues in the oligosaccharide core. Thus a 2*S*-mehtylbutanoyl group was located at C-4 of Rha″ in **3**–**6**, and an additional 2*S*-mehtylbutanoyl group was located at C-2 of Rha′ in **3**; a *trans*-cinnamoyl group was located at C-3 of Rha″ in**3** and **4**; *n*-octanoyl, *n*-decanoyl, and *n*-dodecanoyl groups were located at C-2 of Rha′, respectively, in **4**–**6**. The lactonization position of the aglycone was bonded at C-3 of the second monosaccharide for **3**–**5**, while at C-2 of the second monosaccharide for **6**, on the basis of corresponding HMBC correlation. Accordingly, the structures of **3**–**6** were depicted as shown.

The isolated compounds **1**–**5** were evaluated for their cytotoxic activities against the MCF-7 (human breast cancer cell line), Hela (human cervical cancer cell line), SGC-7901 (human gastric cancer cell line), HepG-2 (human hepatocellar carcinoma cell line), and

Table 1
^{1}H NMR spectroscopic data of compounds **1**–**5** (500 MHz, in pyridine-d_5).[a]

Position[b]	1	2	3	4	5
Glc-1	5.04, d (7.0)	5.03, d (7.0)	5.02, d (7.5)	5.03, d (7.0)	5.02, d (7.5)
2	4.31*	4.30*	4.27–4.29*	4.28–4.30*	4.27–4.29*
3	4.35*	4.35*	4.29–4.31*	4.30–4.32*	4.29–4.31*
4	4.16, t (8.5)	4.15, t (8.5)	4.15, t (9.0)	4.15, t (8.5)	4.15,d (9.0)
5	3.93*	3.92*	3.92*	3.92*	3.90*
6a	4.38, dd (12.0, 5.0)	4.37*	4.35, dd (11.5, 5.0)	4.37, dd (11.5, 5.0)	4.35–4.37*
6b	4.51*	4.50*	4.49, dd (11.5, 3.5)	4.50*	4.49, dd (9.5, 3.0)
Rha-1	6.47, br s	6.47, br s	6.45, br s	6.46, br s	6.45, br s
2	5.34, br s	5.35, br s	5.31, br s	5.33, br s	5.31, br s
3	5.64*	5.62*	5.63*	5.63*	5.61, dd (9.5, 2.5)
4	4.68, t (9.5)	4.67*	4.71, t (9.5)	4.67, t (9.5)	4.67, t (9.5)
5	5.13, dq (9.5, 6.0)	5.10, dq (9.5, 6.0)	5.10, dq (9.5, 6.0)	5.11, dq (9.5, 6.5)	508, dq (9.5, 6.0)
6	1.74 d (6.0)	1.72 d (6.0)	1.72 d (6.0)	1.74, d (6.0)	1.71, d (6.0)
Rha′-1	5.68, br s	5.69, br s	5.65, br s	5.67, br s	5.67, br s
2	5.87, br s	5.87, br s	5.82, br s	5.86, br s	5.82, br s
3	4.61, br.d (9.5)	4.56, br.d (9.0)	4.62, dd (9.5, 2.5)	4.61, br.d (9.5)	4.51, dd (10.0, 3.0)
4	4.32*	4.34*	4.27, dd (9.5, 9.5)	4.32*	4.30, dd (10.0, 10.0)
5	4.43, dq (9.5, 6.0)	4.41*	4.43, dq (9.5, 6.0)	4.42, dq (9.5, 6.0)	4.35, dq (10.0, 6.0)
6	1.64 d (6.0)	1.64 d (6.0)	1.63, d (6.0)	1.63, d (6.0)	1.61, d (6.0)
Rha″-1	5.97, br s	5.82, br s	5.92, br s	5.96, br s	5.91, br s
2	4.89, br s	5.98, br s	4.90, br s	4.88, br s	4.62, br s
3	5.84, dd (10.0, 2.0)	4.66*	5.85, dd (10.0, 2.5)	5.84, dd (10.0, 2.0)	4.42, dd (10.0, 3.0)
4	6.06, t (10.0)	5.74, t (9.0)	6.05, t (10.0)	6.05, t (10.0)	5.77, t (9.5)
5	4.47*	4.38*	4.48*	4.47*	4.31–4.35*
6	1.45, d (6.0)	1.50, d (6.0)	1.43, d (6.0)	1.43, d (6.0)	1.38, d (6.5)
Rha‴-1	5.64*, br s	5.59, br s	5.65*, br s	5.63*, br s	5.55, br s
2	4.79, br s	4.92, br s	4.77, br s	4.78, br s	4.77, br s
3	4.53, br.d (9.0)	4.45, br.d (9.0)	4.42*, dd (9.0, 2.5)	4.52, br.d (9.5)	4.49, dd (9.5, 3.0)
4	4.23, t (9.0)	4.23, t (9.0)	4.21, t (9.0)	4.22, t (9.0)	4.22, t (9.5)
5	4.31*	4.33*	4.27*	4.30*	4.27*
6	1.70, d (6.0)	1.69, d (6.0)	1.70, d (6.0)	1.70, d (6.0)	1.70, d (6.0)
Ag-2	2.99, m; 2.28, m	2.99, m; 2.28, m	2.83, m; 2.25, m	2.99, m; 2.28, m	2.94, m; 2.25, m
Ag-11	3.96*	3.92*	3.94*	3.95*	3.90*
Ag-16	1.00, t (6.5)	0.98, t (7.0)	0.98, t (7.0)	0.99, t (6.5)	0.93, t (7.0)
CA-2	6.55, d (16.0)	6.47, d (16.0)	6.53, d (16.0)	6.55, d (16.0)	
CA-3	7.83, d (16.0)	7.75, d (16.0)	7.82, d (16.0)	7.83, d (16.0)	
CA-2′/6′	7.46, 2H, m	7.26, m	7.44, 2H, m	7.45, 2H, m	
CA-3′/5′	7.34, 2H, m	7.26, m	7.33, 2H, m	7.33, 2H, m	
CA-4′	7.34, m	7.26, m	7.33, m	7.33, m	
Deca-2	2.39, t (7.0)	2.40, t (7.0)			2.35, t (7.0)
Deca-10	0.86, t (7.0)	0.83, t (7.0)			0.86, t (7.0)
Octa-2				2.38, t (7.0)	
Octa-8				0.83, t (7.5)	
Mba^I-2			2.40, tq (7.0, 7.0)		
Mba^I-4			1.14, d (7.0)		
Mba^I-2-Me			0.89, t (7.5)		
Mba^{II}-2	2.45, tq (7.0, 7.0)	2.51, tq (6.5, 6.5)	2.44, tq (7.0, 7.0)	2.46, tq (7.0, 7.0)	2.48, tq (7.0, 7.0)
Mba^{II}-4	1.14, d (7.0)	1.21, d (6.5)	1.13, d (7.0)	1.13, d (6.5)	1.19, d (6.5)
Mba^{II}-2-Me	0.82, t (7.5)	0.91, t (7.5)	0.80, t (7.0)	0.81, t (7.5)	0.91, t (7.5)

[a] Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, br.s = broad singlet, d = doublet, t = triplet, m = multiplet, q = quartet. All assignments are based on ^{1}H–^{1}H TOCSY experiments.

[b] Abbreviations:Glc = glucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Mba = 2*S*-methylbutanoyl; CA = *trans*-cinnamoyl; Deca = *n*-decanoyl; Octa = *n*-octanoyl; Me = methyl.

A549 (human lung adencarcinoma epithelial cell line), using doxorubicin as positive control. Compounds **1**–**5** showed moderate cytotoxicity against these human tumor cell lines, with IC_{50} values in the range 4.28–14.31 μM (Table 4), and compounds **1**–**4** exhibited more potent cytotoxcity than that of compound **5**. It is notable that the trans-cinnamoyl units were present in **1**–**4** rather than in **5**, which suggested that the presence of a *trans*-cinnamoyl group enhanced such cytotoxic activities of these compounds.

3. Concluding remarks

In this paper, a phytochemical investigation of the aerial parts of I. cairica is described. Six partially acylated pentasaccharides resin glycosides, cairicosides A–F, are isolated from this species for the first time. These compounds are a group of macrolactones of simonic acid A, partially acylated with different organic acids. In the morning glory family (Convolvulaceae), macrolactones of simonic acid A with lactonization site of the second saccharide moiety at C-3 have been reported only one time, i.e., as simonin II from *I. batatas* (Noda et al., 1992). Cairicosides A–D, showed more potent cytotoxcity than that of cairicoside E. The susceptibility of a panel human tumor cell lines to these compounds seems to correlate to the acylation degree of the oligosaccharide core.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were determined on a Shimadzu UV-2450 spectrophotometer. IR spectra were measured on a Bruker Tensor-27 spectrophotometer. 1D and 2D NMR experiments were

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**5** (125 MHz, in pyridine-d_5).[a]

Position[b]	1	2	3	4	5
Glc-1	101.5	101.2	101.3	101.4	101.2
2	75.5	75.1	75.4	75.4	75.1
3	79.6	79.3	79.4	79.6	79.4
4	72.3	71.9	72.3	72.1	71.9
5	78.1	77.8	77.9	78.0	77.9
6	63.0	62.7	62.8	62.9	62.6
Rha-1	100.2	100.0	100.1	100.1	100.0
2	69.8	69.5	69.7	69.7	69.5
3	77.7	77.4	77.7	77.6	77.6
4	78.7	78.3	77.3	78.6	77.9
5	68.1	67.8	67.9	68.0	67.8
6	19.4	19.0	19.2	19.2	19.1
Rha′-1	99.5	99.0	98.9	99.3	99.0
2	73.1	72.8	72.7	72.9	72.7
3	80.1	79.4	79.5	80.1	80.0
4	79.7	79.3	80.1	79.5	79.0
5	68.3	68.1	68.3	68.2	68.1
6	18.8	18.5	18.5	18.7	18.5
Rha″-1	103.7	100.2	103.6	103.6	103.5
2	70.1	73.8	69.9	69.9	72.5
3	73.3	67.9	73.1	73.2	70.0
4	71.5	74.6	71.3	71.4	74.6
5	68.2	68.1	68.1	68.1	67.9
6	17.8	17.9	17.6	17.7	17.6
Rha‴-1	104.3	104.0	104.3	104.1	104.1
2	72.7	72.0	72.5	72.6	72.4
3	72.5	72.3	72.3	72.4	72.3
4	73.8	73.4	73.4	73.7	73.5
5	70.9	70.7	70.5	70.7	70.6
6	18.7	18.5	18.6	18.6	18.5
Ag-1	174.9	174.6	174.6	174.8	174.7
Ag-2	33.5	33.4	33.7	33.6	33.4
Ag-11	79.5	79.1	79.4	79.4	79.1
Ag-16	14.5	14.1	14.3	14.3	14.1
CA-1	166.1	166.5	165.9	166.0	
CA-2	118.5	118.1	118.3	118.3	
CA-3	145.3	145.3	145.2	145.2	
CA-1′	134.8	134.5	134.6	134.6	
CA-2′/6′	128.5	128.3	128.3	128.3	
CA-3′/5′	129.3	128.7	129.1	129.1	
CA-4′	130.7	130.3	130.5	130.5	
Deca-1	173.0	172.6			172.7
Deca-2	34.5	34.2			34.2
Deca-10	14.3	13.9			14.0
Octa-1				172.8	
Octa-2				34.3	
Octa-8				14.1	
Dodeca-1					
Dodeca-2					
Dodeca-12					
Mba^{I}-1			175.7		
Mba^{I}-2			41.4		
Mba^{I}-4			16.6		
Mba^{I}-2-Me			11.5		
Mba^{II}-1	175.9	176.0	175.2	175.8	176.1
Mba^{II}-2	41.6	41.3	41.3	41.5	41.3
Mba^{II}-4	16.9	16.6	16.7	16.7	16.8
Mba^{II}-2-Me	11.8	11.4	11.6	11.7	11.5

[a] Chemical shifts (δ) are in ppm relative to TMS. All assignments are based on HSQC and HMBC experiments.
[b] Abbreviations: Glc = glucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Mba = 2*S*-methylbutanoyl; CA = *trans*-cinnamoyl; Octa = *n*-octanoyl; Deca = *n*-decanoyl; Dodeca = *n*-dodecanoyl, Me = methyl.

Table 3
^{1}H and ^{13}C NMR data for compound **6** (500 MHz, in pyridine-d_5).[a]

Position[b]	δ_C, mult.	δ_H (J in Hz)
6		
Glc-1	104.3, CH	4.89, d (7.5)
2	81.8, CH	3.87*
3	76.3, CH	4.13, dd (9.0, 9.0)
4	71.7, CH	4.09, dd (9.0, 9.0)
5	77.7, CH	3.83*
6a	62.6, CH_2	4.44–4.46*
6b		4.29*
Rha-1	98.5, CH	5.57, br s
2	73.4, CH	6.06, br s
3	69.7, CH	5.07, dd (9.5, 3.0)
4	79.7, CH	4.21, dd (9.5, 9.5)
5	68.8, CH	4.36–4.38*
6	19.2, CH_3	1.56, d (6.0)
Rha′-1	98.8, CH	6.18, br s
2	72.9, CH	6.02, br s
3	79.7, CH	4.56, dd (10.0, 3.0)
4	79.2, CH	4.26, dd (10.0, 10.0)
5	68.2, CH	4.36–4.38*
6	18.4, CH_3	1.62, d (6.0)
Rha″-1	103.5, CH	5.91, br s
2	72.3, CH	4.81, br s
3	70.0, CH	4.44*
4	74.6, CH	5.79, t (10.0)
5	68, CH	4.29*
6	17.6, CH_3	1.36, d (6.5)
Rha‴-1	104.4, CH	5.59, br s
2	72.6, CH	4.66, br s
3	72.5, CH	4.48*
4	73.4, CH	4.22*
5	70.5, CH	4.25*
6	18.6, CH_3	1.58, d (6.0)
Ag-1	173.7, qC	
Ag-2	34.1, CH	2.37, m; 2.28, m
Ag-11	82.6, CH	3.86*
Ag-16	14.0, CH_3	0.83, t (7.0)
CA-1		
CA-2		
CA-3		
CA-1′		
CA-2′/6′		
CA-3′/5′		
CA-4′		
5-Oodeca-1		
5-Oxodeca-2		
5-Oxodeca-3		
5-Oxodeca-4		
5-Oxodeca-5		
5-Oxodeca-6		
5-Oxodeca-7		
5-Oxodeca-8		
5-Oxodeca-9		
5-Oxodeca-10		
Dodeca-1	172.7, qC	
Dodeca-2	34.2, CH_2	2.31*
Dodeca-12	14.0, CH	0.78, t (7.0)
Mba-1	176.1, qC	
Mba-2	41.3, CH	2.47, tq (7.0, 7.0)
Mba-4	16.8, CH_3	1.16, d (7.0)
Mba-2-Me	11.5, CH_3	0.88, t (7.0)

[a] Chemical shifts (δ) are in ppm relative to TMS. All assignments are based on HSQC and HMBC experiments.
[b] Abbreviations: Glc = glucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Mba = 2*S*-methylbutanoyl; CA = *trans*-cinnamoyl; Dodeca = *n*-dodecanoyl; 5-Oxodeca = 5-oxodecanoyl, Me = methyl.

conducted on a Bruker AV-500 NMR instrument using pyridine-d_5 as solvent with TMS as internal standards, and chemical shifts were recorded as δ values. ESIMS experiment was performed on an Agilent 1100 Series LC/MSD ion-trap mass spectrometer [sample was solved in MeOH (10 ppm of NaCl added)]. HRESIMS data were acquired using an Agilent TOF MSD G1969A and Agilent 6520B Q-TOF mass spectrometer. GC–MS experiment was performed on an Agilent 6890 instrument coupled to an Agilent 5975 mass spectrometer. Absorbents for column chromatography (CC) were silica gel (200–300 μm, Qingdao Marine Chemical Co., Ltd., China), Sephadex LH-20 (75–150 μm, Pharmacia, Sweden), ODS (40–63 μm, FuJi, Japan), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd., Japan), and macroporous resin D101 (Qingdao Marine Chemical Co., Ltd., China). Preparative HPLC was performed using an Agilent 1100 series instrument equipped with a UV detector at 210 and 280 nm and Shim-Pack RP-C_{18}

Table 4
Cytotoxicity data for compounds **1–5** from *I. cairica* in selected human lines.[a]

Compound	Cell lines				
	MCF-7	Hela	SGC-7901	Hep-G2	A549
1	7.63	7.17	5.72	6.99	4.28
2	6.96	6.32	5.94	6.85	4.91
3	6.01	6.49	6.34	6.24	5.55
4	6.68	5.78	4.69	6.80	4.46
5	8.67	14.31	9.29	8.58	6.53
Doxorubicin	0.52	1.62	0.85	0.22	0.16

[a] Results are expressed as IC_{50} values in μM.

column (20 × 200 mm i.d.). Thin-layer chromatography was performed on pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd., China) and detected by spraying with 10% H_2SO_4–EtOH.

4.2. Plant material

The dried aerial parts of *I. cairica* were collected from Xishuangbanna, Yunnan Province, People's Republic of China, in November 2009. The botanical identification was made by Prof. Min-Jian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. 20091100) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

4.3. Extraction and isolation of compounds **1–6**

The dried aerial parts (7.0 kg) of *I. cairica* were powdered and extracted with EtOH–H_2O (3 × 60 L × 3 h, 95:5, v/v) at 80 °C. Then the solvent was concentrated in vacuo, to produce a residue (1.1 kg), which was suspended in H_2O (8 L) to afford H_2O-soluble and H_2O-insoluble fractions. The H_2O-insoluble fraction (600 g) was resuspended in MeOH–H_2O (4:1, v/v) and allowed to stand overnight so as to precipitate chlorophyll. The supernatant solution was further concentrated to give a residue (350 g), which was applied to a D101 macroporous resin column using a gradient of EtOH–H_2O (45%, 60%, 70%, 85%, and 95% EtOH in H_2O) to yield five fractions (A–E).

Fraction D (8 g) was subjected to silica gel CC eluting with a gradient of CH_2Cl_2–MeOH (20:1, 10:1, 4:1, 1:0, v/v) to afford four subfractions (D1–D4). Subfraction D2 (0.8 g) obtained from elution with CH_2Cl_2–MeOH (100:10, v/v), and subjected to Sephadex LH-20 CC to give a resinous syrup D2a, which was purified by preparative HPLC (Shim-Pack RP-C_{18}, 200 × 20 mm, 5 μm, detection at 280 nm) using MeOH–H_2O (80:20, v/v) to afford compound **3** (27 mg, t_R = 13.3 min) [Shimadzu VP-ODS, 4.6 × 150 mm, 5 μm, MeOH–H_2O (80:20, v/v), 1 mL/min]. Fraction D3 (1.5 g) was purified on Sephadex LH-20 CC eluting with MeOH, and further subjected to open ODS CC eluting with MeOH–H_2O (7:3, 8:2, 9:1 and 10:0, v/v) to give four fraction (D3a–D3d). Fraction D3d was applied to Sephadex LH-20 CC eluting with MeOH to afford compound **5** (315 mg, t_R = 11.3 min) and compound **6** (27 mg, t_R = 17.5 min) [Shimadzu VP-ODS, 4.6 × 150 mm, 5 μm, MeOH–H_2O (95:5, v/v), 1 mL/min].

Fraction E (11 g) was subjected to silica gel CC eluting with gradient of CH_2Cl_2–MeOH (100:2, 100:5, 100:10, 100:0, v/v) to afford four subfractions (E1–E4). Subfraction E3 obtained from elution with CH_2Cl_2–MeOH (100:10, v/v), was subjected to Sephadex LH-20 CC eluting with $CHCl_3$–MeOH (1:1 v/v) to yield a light yellow resinous syrup, E3a (1.5 g). E3a was further separated on open ODS CC eluted with a gradient of MeOH–H_2O (7:3, 8:2, 85:15 and 10:0, v/v) to give four fractions (E3aa–E3da). Fraction E3ab was further separated by preparative HPLC (Shim-Pack RP-C_{18}, 200 × 20 mm, 5 μm, detection at 280 nm) eluting with MeOH–H_2O (85:15, v/v) at a flow rate of 10 mL/min at 30 °C, and yielded compound **4** [27 mg, t_R = 9.3 min, Shimadzu VP-ODS, 4.6 × 150 mm, 5 μm, MeOH–H_2O (85:15, v/v), 1 mL/min. Fraction E3ac was subjected to preparative HPLC eluted with MeOH–H_2O (88:12, v/v, 10 mL/min), to afford compounds **1** (147 mg, t_R = 8.0 - min) and **2** (10 mg, t_R = 10.5 min) [Shimadzu VP-ODS, 4.6 × 150 mm, 5 μm, MeOH–H_2O (88:12, v/v), 1 mL/min].

4.4. Compound characterization

4.4.1. Cairicoside A (**1**)

White, amorphous powder; $[\alpha]_D^{21}$ −62.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.3), 217 (4.3), 280 (4.5) nm; IR ν_{max} (KBr) cm^{-1}: 3450, 2932, 2857, 1737, 1638, 1041; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative ESIMS *m/z* 1403 $[M+Cl]^-$; negative HRESIMS *m/z* 1367.7356 $[M-H]^-$ (calcd for $C_{70}H_{111}O_{26}$, 1367.7369).

4.4.2. Cairicoside B (**2**)

White, amorphous powder; $[\alpha]_D^{21}$ −57.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.3), 217 (4.3), 280 (4.5) nm; IR ν_{max} (KBr) cm^{-1}: 3450, 2932, 2857, 1736, 1638, 1041; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative ESIMS *m/z* 1403 $[M+Cl]^-$; negative HRESIMS *m/z* 1367.7356 $[M-H]^-$ (calcd for $C_{70}H_{111}O_{26}$, 1367.7369).

4.4.3. Cairicoside C (**3**)

White, amorphous powder; $[\alpha]_D^{21}$ −55 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.2), 217 (4.2), 280 (4.4) nm; IR ν_{max} (KBr) cm^{-1}: 3444, 2933, 2859, 1737, 1637, 1045; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative ESIMS *m/z* 1333 $[M+Cl]^-$; negative HRESIMS *m/z* 1297.6580 $[M-H]^-$ (calcd for $C_{65}H_{101}O_{26}$, 1297.6586).

4.4.4. Cairicoside D (**4**)

White, amorphous powder; $[\alpha]_D^{21}$ −50.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.3), 217 (4.2), 280 (4.4) nm; IR ν_{max} (KBr) cm^{-1}: 3445, 2933, 2859, 1738, 1637, 1054; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative ESIMS *m/z* 1375 $[M+Cl]^-$; negative HRESIMS *m/z* 1375.6812 [M+Cl]− (calcd for $C_{68}H_{108}$ ClO_{26}, 1375.6823).

4.4.5. Cairicoside E (**5**)

White, amorphous powder; $[\alpha]_D^{21}$ −55.8 (*c* 0.21, MeOH); IR ν_{max} (KBr) cm^{-1}: 3433, 2931, 2857, 1738, 1054; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; negative ESIMS *m/z* 1273 $[M+Cl]^-$; negative HRESIMS *m/z* 1237.6931 $[M-H]^-$ (calcd for $C_{61}H_{105}O_{25}$, 1237.6950).

4.4.6. Cairicoside F (**6**)

White, amorphous powder; $[\alpha]_D^{33}$ −32.4 (*c* 0.17, MeOH); IR ν_{max} (KBr) cm^{-1}: 3444, 2928, 2856, 1735, 1136, 1056 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 3; negative ESIMS *m/z* 1301 $[M+Cl]^-$; negative HRESIMS *m/z* 1301.7013 $[M+Cl]^-$ (calcd for $C_{63}H_{110}Cl$ $O_{25,}$ 1301.7030).

4.5. Alkaline hydrolysis of isolates 1–6

Compounds **1–6** (2.0 mg each) in 5% KOH (3 mL) were individually heated at 90 °C until reflux began, this being maintained for 2 h, respectively. The end reaction mixture was acidified to pH 4.0 with 2 N HCl and extracted with CH_2Cl_2 (3 mL × 2) and *n*-BuOH (3 mL × 2), respectively. The organic layers were combined, then washed with H_2O, and dried over anhydrous Na_2SO_4, these were

then directly analyzed by GC–MS on a model 6890 GC intrument equipped with a model 5975 MS (Agilent) under the following conditions: 30 m × 0.32 mm × 0.25 μm, DB-5 MS column; He, 0.8 mL/min; 50 °C, 3 min; 50–300 °C, Δ10 °C/min, 70 eV. From the GC–MS spectrum and by comparison with authentic samples of 2-methylbutanoic acid (t_R 4.1 min): *m/z* 87 (24), 74 (100), 73 (17), 57 (72), 55 (12), 45 (21), 41 (60), 39 (38), 29 (44), 27 (22), *trans*-cinnamic acid (t_R 10.5 min): *m/z* 148 $[M]^+$ (76), 147 (100), 131 (21), 120 (7), 103 (48), 102 (23), 91 (24), 77 (35), 74(7), 63 (6), 51 (35), 50 (10), 45 (15), *n*-decanoic acid (t_R 9.8 min): *m/z* 172 $[M]^+$ (4), 155 (2), 143 (10), 129 (50), 115 (13), 101 (7), 87 (15), 73 (80), 60 (100), 57 (48), 55 (45), 43 (52), 41 (50), 29 (21), 27 (13), *n*-dodecanoic acid (t_R 11.4 min): *m/z* 200 $[M]^+$ (8), 183 (2), 171 (7), 157 (28), 143 (10), 129 (36), 115 (17), 101 (13), 87 (15), 85 (26), 83 (15), 73 (90), 71 (26), 60 (100), 57 (54), 55 (60), 43 (77), 41 (67), 29 (26), 27 (14), and *n*-octanoic acid (t_R 8.2 min): *m/z* $[144]^+$ (1), 115 (8), 101 (23), 85 (18), 73 (58), 69 (11), 60 (100), 55 (32), 45 (13), 43 (48), 41 (36), 39 (14), 29 (16), 27 (14) were identified. The *n*-BuOH layer was subjected to an open ODS column (MeOH–H_2O, 75:25, v/v) to obtain the glycosidic acid, which gave key fragments at *m/z* 1017 $[M-H]^-$, 871 $[M-H-C_6H_{10}O_4]^-$, 725 $[871-C_6H_{10}O_4]^-$, 579 $[725-C_6H_{10}O_4]^-$, 433 $[579-C_6H_{10}O_4]^-$, 271 $[433-C_6H_{10}O_5]^-$ (S29, Supporting Information) in the negative ESIMS, and was identified as simonic acid A (Yin et al., 2009; Noda et al., 1992; Chérigo et al., 2008).

By the same procedure, the organic acids fraction (4.0 mg) from the alkaline hydrolysis of compound **5** was purified on ODS CC eluting with MeOH–H_2O (25:75), to give 2-methylbutanoic acid (0.7 mg). This was proved to have *S* configuration by comparing the specific rotation ($[\alpha]_D^{25}$ +19.1) with that of authentic 2*S*-methylbutanoic acid (Yin et al., 2008a,b, 2009; Yin and Kong, 2008; Yu et al., 2011).

4.6. Acid hydrolysis and sugar analysis

The glycosidic acid (**7**, 20 mg, from alkaline hydrolysis) was methylated with MeOH and catalyzed with 0.5 N H_2SO_4 to give simonic acid A methyl ester (**8**). Compound **8** was hydrolyzed with 1 N H_2SO_4 and extracted with Et_2O to obtain 11-hydroxyhexadecanoic acid methyl ester (Yin et al., 2008b). The aqueous layer of acidic hydrolysis was concentrated under reduced pressure to give a residue of the sugars. The protocols applied to determinate the stereochemistry of sugars were the same as our previous research, which allowed the identification of the mixture sugars of L-rhamnose and D-glucose by comparison their derivatives with those of authentic samples (Luo et al., 2008).

4.7. Preparation of Mosher's Esters

The procedures for preparation of Mosher's esters to determination of absolute configuration of 11*S* of the aglycone were same as described previously from *I. batatas* (Yin et al., 2008b) and *I. pes-caprae* (Yu et al., 2011).

5. Determination of cytotoxic activity

The following human tumor cell lines were used: MCF-7, Hela, SGC-7901, Hep-G2, and A549. All cells were maintained in RPMI-1640 or DMEM medium (Hyclone Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) and harvested with trypsin and suspended in a final concentration of 1×10^5 cells/mL. Aliquots (0.1 mL) of cells suspension were seeded evenly into 96-well culture multi-plates and incubated in a 37 °C incubator containing 5% CO_2 for 24 h before testing compound addition. A series of concentrations for pure compounds were added to designated wells in triplicate, with the doxorubicin (Sigma, St. Louis, MO) was used as positive control. After 48 h, MTT assay was performed as described previously (Lu et al., 2009).

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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.phytochem.2013.07.006. It includes ^1H and ^{13}C NMR, ESIMS, and HRESIMS spectra of cairicosides A–F (**1**–**6**).

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