Carbohydrate Research 344 (2009) 466–473

Contents lists available at ScienceDirect

Carbohydrate Research

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

# Novel acylated lipo-oligosaccharides from the tubers of *Ipomoea batatas*

Yong-Qin Yin, Jun-Song Wang, Jian-Guang Luo, Ling-Yi Kong *

*Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China*

ARTICLE INFO

*Article history:*
Received 29 September 2008
Received in revised form 23 December 2008
Accepted 24 December 2008
Available online 3 January 2009

*Keywords:*
Pentasaccharide
Lipo-oligosaccharides
Cytotoxic activity
*Ipomoea batatas*
Convolvulaceae

ABSTRACT

Nine new lipo-oligosaccharides, batatosides H–P, were isolated from the tubers of *Ipomoea batatas* (Convolvulaceae). Spectral and chemical methods allowed to characterize them as tetra- or penta-saccharides that form a macrolactone with the aglycone, (11*S*)-hydroxyhexadecanoic acid (jalapinolic acid), the absolute configuration of which was established by Mosher's method. Batatosides L and O showed a weak inhibitory effect on the growth of Hep-2 cells, while the others proved to be inactive.



## 1. Introduction

A group of glycosides of hydroxyaliphatic acids, such as jalapinolic acid, convolvulonic acid, and ipurolic acid, have already been characterized in the tubers of the genus *Ipomoea* (Convolvulaceae).[1–5] D-Glucose, D-fucose, L-rhamnose, and L-quinovose are the main monosaccharide constituents and are occasionally esterified with short chain fatty acids. The hydroxyacid aglycone usually cyclizes in the form of a macrocyclic lactone involving one hydroxyl of the oligosaccharide chain. Such type of fatty acyl glycoside has been characterized previously in various plant extracts, and was named as 'resin glycoside'.[6–10] Some 'resin glycosides' have been claimed to show antifungal,[11] antibacterial,[12] and cytotoxic[13] activities, as well as phytogrowth inhibition properties.[14] *Ipomoea batatas* L. is an important food produced throughout the world which is known under the common name of sweet potato, and is used independently in traditional medicine in China to eliminate abnormal secretions (apocenosis) and to promote hemostasis.[15] We reported previously on the isolation of several 'resin glycosides', batatosides A–G and simonin IV[16] and batatosides I–II[17] from *I. batatas*. From the HPLC–ESIMS profiles of extracts from this species, several other glycosides were further detected. We now report on the isolation of nine new ether-soluble 'resin glycosides' **1**–**9**, their structure elucidation, and some studies of their cytotoxic activity.

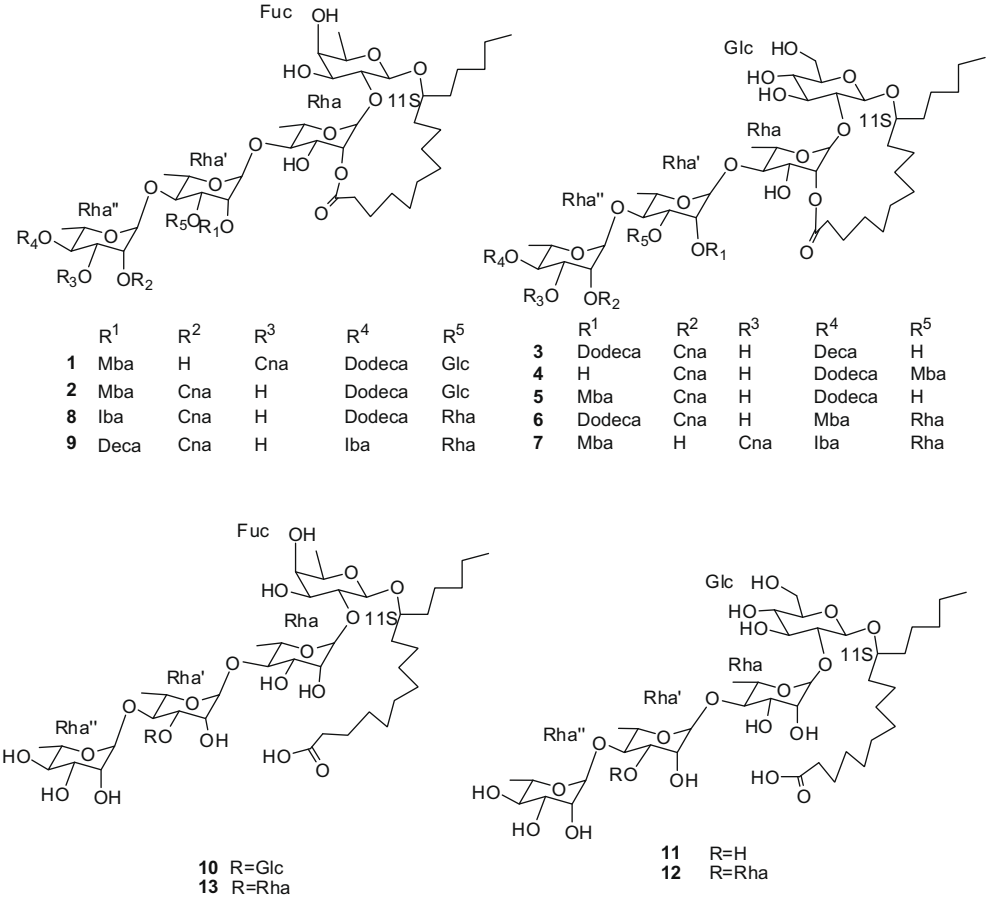
## 2. Results and discussion

A 95% ethanol extract of the dried tubers of *I. batatas* was suspended in water and partitioned with chloroform. The jalapin-like chloroform-soluble material was subjected to successive column chromatography over silica gel, Sephadex LH-20, MPLC, and HPLC to yield compounds **1**–**9**. The alkaline hydrolysis of each compound produced an organic acid fraction and a water-soluble aliphatic hydroxyacid glycoside. Peaks in the chromatograms of the organic acid fraction† were identified by comparison with authentic samples[16,17] as isobutyric acid methyl ester ($t_R$ 2.368 min, *m/z* 101 (32) $[M-H]^-$, 87 (48), 71 (50), 43 (100), in **7–9)**; (*S*)-2-methylbutyric acid methyl ester ($t_R$ 3.593 min, *m/z* 117 (5) $[M+H]^+$, 101 (23), 88 (87), 57 (100), 41 (57), in **1**, **2**, and **4–7)**; *n*-decanoyl acid methyl ester ($t_R$ 11.422 min, *m/z* 116 (10) $[M]^{+\cdot}$, 143 (45), 87 (64), 74 (100), 55 (55), 43 (80), in **3** and **9)**; *trans*-cinnamic acid methyl ester ($t_R$ 12.498 min, *m/z* 131 (100) $[M]^{+\cdot}$, 103 (76), 77 (35), in **1–9)**; and *n*-dodecanoyl methyl ester ($t_R$ 14.020 min, *m/z* 162 (45) $[M]^{+\cdot}$, 215 (23), 171 (31), 143 (55), 87 (85), 74 (100), 55 (86), 43 (96), 41 (56), in **1–6** and **8)**. The ether extract (1.1 mg) from the alkaline hydrolysis of **1** was purified by RP-C18 chromatography, eluted with 1:3 MeOH–$H_2O$, to give 2-methylbutyric acid (0.3 mg). This was proved to be in the *S*-configuration by comparing the optical rotation ($[\alpha]_D^{25}$ +19.0) with that of authentic (*S*)-2-methylbutyric acid. The structures of the glycosidic acids (Chart 1) were determined by comparison of their spectral data with those reported as operculinic acid A (**10**)[18] for **1** and **2**, operculinic acid E (**11**)[19] for **3–5,** simonic acid A (**12**)[18] for **6** and **7**, and simonic acid B (**13**)[20] for **8** and **9**. Subsequent acidic hydrolysis of glycosidic acids liberated the aglycone, 11-hydroxyhexadecanoic acid, which was converted to 11-hydroxyhexadecanoic acid methyl ester (**14**) and then to its methoxyphenylacetic acid (MPA) derivative (**15** and **16**) (Scheme 1). The chemical shift differences of **15** and **16** $\Delta\delta^{RS}_{H10} = 0.06$, $\Delta\delta^{RS}_{H12} = 0.13$,

* Corresponding author. Tel.: +86 25 85391289; fax: +86 25 85301528.
*E-mail address:* lykong@jlonline.com (L.-Y. Kong).

† Cna = *trans*-cinnamoyl; Deca = *n*-decanoyl; Dodeca = *n*-dodecanoyl; Iba = 2-methylbutanoyl; Mba = (2*S*)-methylbutanoyl.

0008-6215/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.12.022




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| **1** | Mba | H | Cna | Dodeca | Glc |
| **2** | Mba | Cna | H | Dodeca | Glc |
| **8** | Iba | Cna | H | Dodeca | Rha |
| **9** | Deca | Cna | H | Iba | Rha |

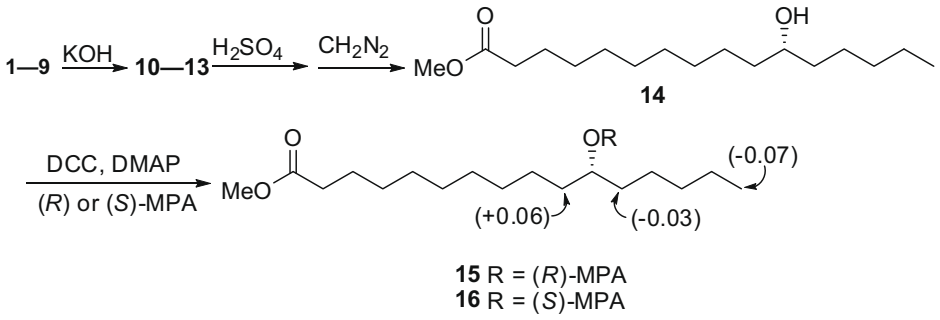
| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| **3** | Dodeca | Cna | H | Deca | H |
| **4** | H | Cna | H | Dodeca | Mba |
| **5** | Mba | Cna | H | Dodeca | H |
| **6** | Dodeca | Cna | H | Mba | Rha |
| **7** | Mba | H | Cna | Iba | Rha |




**10** R=Glc
**13** R=Rha

**11** R=H
**12** R=Rha

**Chart 1.** Structures of compounds **1–9** and the corresponding glycosidic acids **10–13**.




**Scheme 1.** Conversion of **1–9** to the aglycone methyl ester **14** and its MPA derivatives (**15** and **16**). $\Delta\delta(R–S)$ values are given in ppm.

$\Delta\delta^{RS}_{H16} = 0.07$ ppm)[29–33] made it possible to conclude that the chiral C-11 of the aglycone is in the *S*-configuration. GC–MS of the aqueous layers from all acidic hydrolyses allowed to determine the sugar constituents.

Batatoside H (**1**) and I (**2**), obtained as amorphous white powders, gave quasi-molecular ion at *m/z* 1419.7647 $[M+Na]^+$ and 1419.7675 $[M+Na]^+$, respectively, in the positive-ion HRESIMS, which suggested the same molecular formula $C_{72}H_{116}O_{26}$. Alkaline hydrolysis afforded operculinic acid A (**10**),[18] and dodecanoic, 2-methylbutyric, and *trans*-cinnamic acids. These organic acids were determined by GC–MS analysis. 2-Methylbutyric acid was found to have the *S*-configuration by comparison of its optical rotation value with that of an authentic sample. All these evidences suggested that **1** and **2** were isomeric. The $^1$H NMR data of **1** (Table 1) exhibited four doublet methyl signals for the four 6-deoxyhexose units, two trans-coupled olefinic protons at $\delta_H$ 6.63 (d, *J* 16.0 Hz, H-2 of Cna) and 7.88 (d, *J* 16.0 Hz, H-3 of Cna), a multiplet due to five protons at $\delta_H$ 7.27–7.45 (m, $C_6H_5$ of Cna), and at $\delta_H$ 0.79 (t, *J* 7.4 Hz, H-4 of Mba), 1.02 (d, *J* 7.0 Hz, $CH_3$-2 of Mba), and 2.38 (m, H-2 of Mba). Also a methyl triplet signal at 0.88 ppm and a triplet-like signal for a methylene group at C-2 (2.48 ppm) of a dodecanoyl group, and two signals at 2.27 (1H, m) and 2.44 (1H, m) ppm of the non-equivalent protons of the methylene group at C-2 in the aglycone moiety were observed, suggesting a macrocyclic lactone-type structure. A group of four non-anomeric ring proton signals was found to be paramagnetically shifted to $\delta$ 5.92, 6.33, 6.00, and 6.10 (Table 1), reflecting the presence of four sites of acylation. The HMQC spectrum of **1** indicated that anomeric carbons signals at 104.3, 98.5, 100.3, 103.3, and 105.5 ppm (Table 2) were correlated with the anomeric protons at 4.69 (d, *J* 7.5 Hz), 5.50 (br s), 5.77 (br s),

**Table 1**
$^1$H NMR [$\delta_H$ ($J$, Hz)] data of compounds **1**–**5** ($C_5D_5N$, 600 MHz)

| Position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Fuc-1 | 4.69 (d, 7.5) | 4.71 (d, 7.5) | | | |
| 2 | 4.16 (dd, 7.5, 9.4) | 4.14 (dd, 7.5, 9.8) | | | |
| 3 | 4.13 (dd, 9.4, 3.0) | 4.03 (dd, 9.8, 3.5) | | | |
| 4 | 3.95 (d, 3.0) | 3.95 (d, 3.5) | | | |
| 5 | 3.73 (br q, 7.0) | 3.75 (br q, 6.4) | | | |
| 6 | 1.49 (d, 6.4) | 1.50 (d, 6.4) | | | |
| Rha$^I$-1 | 5.50 (br s) | 5.50 (br s) | 5.60 (br s) | 5.60 (br s) | 5.60 (br s) |
| 2 | 5.92 (br s) | 5.93 (br s) | 6.06 (br s) | 6.04 (br s) | 6.06 (br s) |
| 3 | 5.00 (dd, 3.1, 9.4) | 5.00 (dd, 3.3, 9.3) | 5.08 (dd, 3.1, 9.6) | 5.08 (dd, 3.1, 9.5) | 5.08 (dd, 3.1, 9.3) |
| 4 | 4.24 (t, 9.4) | 4.16 (t, 9.5) | 4.24 (t, 9.6) | 4.25 (t, 9.5) | 4.24 (t, 9.3) |
| 5 | 4.31 (dq, 9.4, 6.2) | 4.48 (dq, 9.5, 6.2) | 4.43 (dq, 9.6, 7.0) | 4.41 (dq, 9.5, 6.2) | 4.42 (dq, 9.3, 6.2) |
| 6 | 1.64 (d, 6.2) | 1.63 (d, 6.2) | 1.63 (d, 7.0) | 1.66 (d, 6.2) | 1.63 (d, 6.2) |
| Rha$^{II}$-1 | 5.77 (br s) | 5.86 (d, 1.6) | 6.10 (br s) | 6.18 (br s) | 6.06 (br s) |
| 2 | 6.33 (br s) | 6.30 (br s) | 6.06 (br s) | 4.95 (br s) | 6.01 (br s) |
| 3 | 4.75 (dd, 3.4, 9.2) | 4.50[a] | 4.72 (dd, 3.1, 9.5) | 5.77 (dd, 3.1, 9.5) | 4.73 (dd, 3.1, 9.5) |
| 4 | 4.32 (t, 9.2) | 4.79 (dd, 3.4,9.8) | 4.32 (t, 9.5) | 4.60 (t, 9.5) | 4.27 (t, 9.5) |
| 5 | 4.36 (dq, 9.2, 6.1) | 4.00[a] | 4.41 (dq, 9.5, 6.1) | 4.41 (dq, 9.5, 6.1) | 4.38 (dq, 9.5, 6.0) |
| 6 | 1.66 (d, 6.1) | 1.56 (d, 6.2) | 1.69 (d, 6.1) | 1.64 (d, 6.1) | 1.70 (d, 6.0) |
| Rha$^{III}$-1 | 6.27 (br s) | 6.35 (br s) | 6.19 (br s) | 5.71 (br s) | 6.20 (br s) |
| 2 | 5.25 (br s) | 6.31 (br s) | 6.24 (br s) | 5.95 (br s) | 6.23 (br s) |
| 3 | 6.00 (dd 3.1, 10.0) | 4.82 (dd, 3.5, 9.9) | 4.79 (dd, 2.5, 10.0) | 4.72 (dd, 3.3, 9.8) | 4.80 (dd, 3.2, 9.8) |
| 4 | 6.10 (t, 10.0) | 5.84 (t, 9.8) | 5.84 (t, 10.0) | 5.81 (t, 9.8) | 5.84 (t, 9.8) |
| 5 | 4.52 (dq, 10.0, 6.2) | 4.48 (dq, 9.5, 6.2) | 4.50 (dq, 10.0, 6.2) | 4.41 (dq, 9.8, 6.2) | 4.50 (dq, 9.8, 6.2) |
| 6 | 1.47 (d, 6.2) | 1.68 (d, 5.7) | 1.56 (d, 6.2) | 1.49 (d, 6.2) | 1.56 (d, 6.2) |
| Glc-1 | 5.05 (d, 7.7) | 5.06 (d, 7.7) | 4.87 (d, 7.6) | 4.93 (d, 7.6) | 4.87 (d, 7.6) |
| 2 | 3.92 (dd, 7.7, 9.0) | 3.93 (dd, 7.7, 8.9) | 3.88 (m) | 3.87[a] | 3.88[a] |
| 3 | 4.17 (t, 9.0) | 4.09 (t, 8.9) | 4.14 (m) | 4.17 (t, 8.9) | 4.14 (t, 8.9) |
| 4 | 3.80 (t, 9.0) | 3.88 (t, 8.9) | 4.12 (m) | 4.11 (t, 8.9) | 4.12 (t, 8.9) |
| 5 | 3.79 (ddd, 2.6, 5.8, 9.0) | 3.79 (ddd, 2.2, 5.9, 8.9) | 3.84 (m) | 3.85[a] | 3.84[a] |
| 6 | 4.07 (dd, 5.8, 11.7) | 4.39 (dd, 2.2, 12.0) | 4.31 (dd, 5.1, 11.5) | 4.32 (dd, 5.1, 11.5) | 4.31(dd, 5.1, 11.5) |
| | 4.38 (dd, 2.6, 11.7) | 4.05 (dd, 5.9, 12.0) | 4.44 (dd, 2.2, 11.5) | 4.42 (dd, 2.2, 11.5) | 4.44 (dd, 2.2, 11.5) |
| Jal-2 | 2.27 (m) | 2.28 (m) | 2.27 (m) | 2.25 (m) | 2.25 (m) |
| | 2.44 (m) | 2.43 (m) | 2.42 (m) | 2.37 (m) | 2.39 (m) |
| 11 | 3.85 (m) | 3.85 (m) | 3.88 (m) | 3.88 (m) | 3.88 (m) |
| 16 | 0.79 (t, 7.4) | 0.87 (t, 7.1) | 0.83 (t, 7.2) | 0.83 | 0.85 (t, 7.0) |
| Cna-2 | 6.63 (d, 16.0) | 6.63 (d, 16.0) | 6.46 (d, 15.9) | 6.60 (d, 15.9) | 6.46 (d, 15.9) |
| 3 | 7.88 (d, 16.0) | 7.88 (d, 16.0) | 7.74 (d, 15.9) | 7.88 (d, 15.9) | 7.74 (d, 15.9) |
| Deca-2 | | | 2.50 (m) | | |
| 10 | | | 0.83 (t, 6.9) | | |
| Dodeca-2 | 2.48 (t, 7.3) | 2.48 (t, 7.3) | 2.33 (t, 7.0) | 2.48 (m) | 2.46 (m) |
| 12 | 0.88 (t, 6.9) | 0.88 (t, 6.9) | 0.87 (t, 7.2) | 0.87 (t, 7.1) | 0.87 (t, 6.9) |
| Mba-2 | 2.38 (m) | 2.38 (m) | | 2.78 (m) | 2.46 (m) |
| 2-$CH_3$ | 1.02 (d, 7.0) | 1.02 (d, 7.0) | | 1.01 (d, 7.0) | 0.82 (d, 7.4) |
| 4 | 0.79 (t, 7.4) | 0.79 (t, 7.4) | | 1.21[a] | 1.08 (d, 7.0) |

[a] Signal pattern unclear due to overlapping.

6.27 (br s), and 5.05 (d, $J$ 7.7 Hz) ppm, respectively. All protons were assigned sequentially within each saccharide system by a combination of 2D NMR COSY and TOCSY experiments, leading to the identification of one glucopyranosyl unit, one fucopyranosyl unit, and three rhamnopyranosyl units as the monosaccharides present in **1**. The anomeric configurations for the sugar moieties were assigned as $\beta$ for the fucopyranosyl and glucopyranosyl moieties, and $\alpha$ for the rhamnopyranosyl unit by comparison with values reported for the corresponding residues in oligosaccharides[10,19–21] The interglycosidic connectivities were determined from the following HMBCs (Fig. 1): C-2 (80.0 ppm) of fucose with H-1 (5.50 ppm) of rhamnose$^I$; C-4 (82.1 ppm) of rhamose$^I$ with H-1 (5.77 ppm) of rhamnose$^{II}$; C-4 (80.8 ppm) of rhamnose$^{II}$ with H-1 (6.27 ppm) of rhamnose$^{III}$; and C-3 (70.6 ppm) of rhamnose$^{II}$ with H-1 (5.05 ppm) of glucose. Specification of the sites of ester linkages of the respective organic acids and jalapinolic acid was also obtained by HMBC experiments (Fig. 2). Thus, HMBCs were detected between H-2 of rhamnose$^{II}$ ($\delta_H$ 6.33) and $\delta_C$ 176.0 (C-1 of Mba); H-3 of rhamnose$^{III}$ ($\delta_H$ 6.00) and $\delta_C$ 166.4 (C-1 of Cna); H-4 of rhamnose$^{III}$ ($\delta_H$ 6.10) and $\delta_C$ 173.4 (C-1 of Dodeca); and H-2 of rhamnose$^I$ ($\delta_H$ 5.92) and $\delta_C$ 173.3 (C-1 of aglycone), respectively. The position of the jalapinolic acid unit was finally determined by HMBC between jalapinolic acid H-11 (3.85 ppm) and fucose C-1 (104.3 ppm). These correlations established the structure of **1** (Chart 1).

The NMR spectra (Tables 1 and 2) of **2** were similar to those of **1**, with the only difference being the location of the *trans*-cinnamoyl group. In the HMBC spectrum of **2**, the H-2 proton of rhamnose$^{III}$ at $\delta$ 6.31 showed HMBCs to the carbonyl group at $\delta$ 166.6 (C-1 of *trans*-cinnamoyl), which suggested that the *trans*-cinnamoyl group was located at C-2 of rhamnose$^{III}$ in **2** rather than at C-3 of rhamnose$^{III}$ as in **1**. Accordingly, the structure of **2** was established as shown in Chart 1.

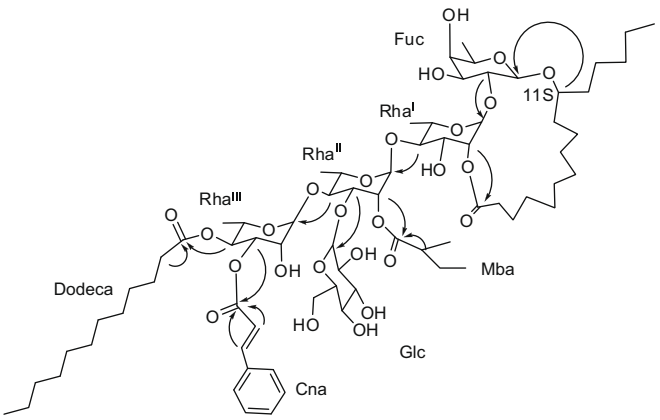
Batatoside J (**3**), amorphous white powder, gave quasi-molecular ion at $m/z$ 1343.7816 $[M+Na]^+$ ($C_{71}H_{116}O_{22}Na$), which was determined by positive-ion HRESIMS. Basic hydrolysis of it afforded operculinic acid E (**11**) as the glycosidic acid, and dodecanoic, decanoic, and *trans*-cinnamic acids as organic acids. The position of the jalapinolic acid moiety in the oligosaccharide was determined by correlation between jalapinolic acid H-11 (3.88 ppm) and glu-

**Table 2**
$^{13}C$ NMR ($\delta_C$) data of compounds **1–9** ($C_5D_5N$, 150 MHz)

| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Fuc-1 | 104.3 | 104.1 | | | | | | 104.3 | 104.2 |
| 2 | 80.0 | 81.0 | | | | | | 80.3 | 80.1 |
| 3 | 73.4 | 73.1 | | | | | | 73.4 | 73.2 |
| 4 | 72.9 | 72.6 | | | | | | 73.0 | 72.9 |
| 5 | 70.8 | 70.6 | | | | | | 70.8 | 70.8 |
| 6 | 17.3 | 17.1 | | | | | | 17.4 | 17.3 |
| $Rha^{I}$-1 | 98.5 | 98.3 | 98.6 | 98.7 | 98.6 | 98.7 | 98.7 | 98.8 | 98.7 |
| 2 | 73.6 | 73.4 | 73.6 | 73.7 | 73.6 | 73.5 | 73.5 | 73.9 | 74.0 |
| 3 | 70.6 | 69.2 | 70.6 | 70.3 | 70.6 | 69.6 | 69.6 | 69.9 | 69.6 |
| 4 | 82.1 | 79.8 | 80.1 | 81.1 | 80.1 | 80.4 | 80.5 | 80.3 | 80.1 |
| 5 | 68.4 | 68.2 | 68.2 | 68.3 | 68.2 | 70.6 | 70.6 | 68.2 | 68.0 |
| 6 | 19.1 | 18.9 | 18.9 | 18.8 | 18.9 | 19.3 | 19.3 | 19.5 | 19.4 |
| $Rha^{II}$-1 | 100.3 | 99.6 | 100.2 | 103.4 | 100.2 | 99.0 | 99.3 | 99.1 | 98.9 |
| 2 | 73.9 | 72.6 | 73.9 | 69.6 | 73.9 | 73.0 | 73.0 | 73.1 | 73.1 |
| 3 | 70.6 | 77.1 | 70.6 | 79.5 | 70.6 | 79.0 | 79.0 | 79.9 | 79.4 |
| 4 | 80.8 | 79.8 | 80.8 | 75.6 | 80.8 | 80.3 | 80.2 | 80.3 | 79.4 |
| 5 | 68.4 | 68.5 | 68.3 | 68.6 | 68.3 | 68.3 | 68.3 | 68.6 | 68.6 |
| 6 | 19.0 | 18.9 | 19.3 | 19.3 | 19.3 | 18.2 | 18.2 | 18.9 | 18.9 |
| $Rha^{III}$-1 | 103.3 | 99.4 | 100.2 | 100.1 | 100.2 | 100.1 | 103.6 | 100.3 | 100.1 |
| 2 | 70.0 | 73.8 | 74.0 | 74.1 | 74.0 | 71.1 | 70.2 | 74.1 | 73.9 |
| 3 | 72.0 | 68.0 | 68.2 | 68.0 | 68.2 | 73.7 | 73.8 | 68.2 | 68.0 |
| 4 | 71.6 | 74.8 | 75.2 | 75.0 | 75.2 | 73.5 | 71.6 | 75.2 | 74.8 |
| 5 | 68.2 | 68.5 | 68.8 | 68.8 | 68.8 | 68.6 | 68.4 | 68.5 | 68.6 |
| 6 | 18.1 | 17.8 | 18.1 | 17.9 | 18.1 | 18.7 | 17.7 | 18.0 | 18.0 |
| $Rha^{IV}$-1 | | | | | | 104.6 | 104.8 | 104.8 | 104.2 |
| 2 | | | | | | 73.5 | 73.4 | 72.2 | 72.2 |
| 3 | | | | | | 72.5 | 72.6 | 72.6 | 72.6 |
| 4 | | | | | | 72.2 | 72.5 | 73.5 | 73.5 |
| 5 | | | | | | 68.6 | 68.7 | 68.6 | 68.6 |
| 6 | | | | | | 18.5 | 18.5 | 18.6 | 18.6 |
| Glc-1 | 105.5 | 104.9 | 104.5 | 104.5 | 104.5 | 104.4 | 104.5 | | |
| 2 | 75.2 | 74.8 | 81.8 | 82.0 | 81.8 | 81.9 | 81.9 | | |
| 3 | 79.0 | 77.9 | 76.6 | 76.5 | 76.6 | 76.4 | 76.5 | | |
| 4 | 71.6 | 68.6 | 71.9 | 72.0 | 71.9 | 71.8 | 72.0 | | |
| 5 | 77.9 | 77.6 | 78.0 | 77.9 | 78.0 | 77.9 | 77.9 | | |
| 6 | 63.0 | 62.9 | 62.9 | 62.9 | 62.8 | 62.7 | 62.9 | | |
| Jal-1 | 173.3 | 172.8 | 173.4 | 173.4 | 173.3 | 173.2 | 173.2 | 173.0 | 173.0 |
| 2 | 33.4 | 33.0 | 34.5 | 33.4 | 33.4 | 34.1 | 34.3 | 34.3 | 34.1 |
| 11 | 82.4 | 82.1 | 82.8 | 82.1 | 82.8 | 82.7 | 82.7 | 82.4 | 82.2 |
| 16 | 14.3 | 14.0 | 14.3 | 14.0 | 14.3 | 14.2 | 14.2 | 14.3 | 14.2 |
| Cna-1 | 166.4 | 166.6 | 166.4 | 166.5 | 166.4 | 166.5 | 166.4 | 166.8 | 166.7 |
| 2 | 118.7 | 118.7 | 118.7 | 118.5 | 118.7 | 118.1 | 118.3 | 118.5 | 118.3 |
| 3 | 145.2 | 145.2 | 145.2 | 145.5 | 145.2 | 146.0 | 145.6 | 145.6 | 145.5 |
| Deca-1 | | | 173.6 | | | | | | 172.8 |
| 2 | | | 34.3 | | | | | | 34.2 |
| 10 | | | 14.3 | | | | | | 14.2 |
| Dodeca-1 | 173.4 | 173.4 | 173.3 | 173.3 | 173.4 | 173.5 | | 173.5 | |
| 2 | 34.6 | 34.6 | 34.6 | 34.3 | 34.6 | 33.1 | | 34.3 | |
| 12 | 14.2 | 14.2 | 14.3 | 14.3 | 14.2 | 14.2 | | 14.3 | |
| Iba-1 | | | | | | | 176.4 | 175.9 | 176.6 |
| 2 | | | | | | | 34.4 | 34.3 | 34.2 |
| 3 | | | | | | | 19.4 | 19.1 | 18.9 |
| 3′ | | | | | | | 18.9 | 19.0 | 19.1 |
| Mba-1 | 176.0 | 176.2 | | 176.5 | 176.5 | 175.3 | 175.3 | | |
| 2 | 41.2 | 41.2 | | 41.1 | 41.1 | 41.3 | 41.5 | | |
| 2-$CH_3$ | 16.6 | 16.6 | | 16.9 | 16.9 | 16. 7 | 16.8 | | |
| 4 | 11.4 | 11.4 | | 11.8 | 11.8 | 11.7 | 11.8 | | |

cose C-1 (104.5 ppm) in the HMBC spectrum. The esterification positions of the oligosaccharide core were also revealed by HMBC long-range correlations. Thus, an *n*-dodecanoyl unit was attached to C-2 of rhamnose$^{II}$, a *trans*-cinnamoyl group was attached to C-2 of rhamnose$^{III}$, and a decanoyl substituent was located at C-4 of rhamnose$^{III}$. The structure of batatoside J was hence assigned as **3** (Chart 1).

Batatosides K (**4**) and L (**5**), obtained as amorphous white powder, displayed quasi-molecular ion at *m/z* 1273.7097 $[M+Na]^+$ from positive HRESIMS, corresponding to a molecular formula of $C_{66}H_{106}O_{22}$, 146 mass unit less than **2**. Their proton and carbon signals in the $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) were similar to those of **2**, except for the loss of one unit of 6-deoxyhexose in **4** and **5**, which was confirmed by alkaline hydrolysis, providing operculinic acid E and the same organic acids mixture as for **2**. The HMBC spectrum allowed the esterification sites to be established through the correlations between carbonyl and proton signals of the monosaccharides: thus for **4** and **5**, a *trans*-cinnamoyl substituent was located at C-2 of rhamnose$^{III}$, an *n*-dodecanoyl residue was assigned at C-4 of rhamnose$^{III}$, and the jalapinolic acid unit was ester-




1

**Figure 1.** Key HMBCs from H to C for batatoside H (**1**).

ified at C-1 of glucose. HMBCs also revealed the differences between the two compounds: thus for **4**, a 2-methylbutanoyl was located at C-3 of rhamnose$^{II}$, whereas for **5**, it was at C-2 of rhamnose$^{II}$. A long-range HMBC cross-peak between jalapinolic acid H-11 (3.88 ppm) and glucose C-1 (104.5 ppm) was observed, allowing the position assignment of the jalapinolic acid moiety in the oligosaccharide. Thus, the structures of batatosides K and L were assigned to **4** and **5** (Chart 1).

Batatosides M–P (**6–9**), obtained as amorphous white powder, gave quasi-molecular ion at *m/z* 1419.7537 $[M+Na]^+$ ($C_{72}H_{116}O_{26}Na$), 1307.6406 $[M+Na]^+$ ($C_{64}H_{100}O_{26}Na$), 1389.7557 $[M+Na]^+$ ($C_{71}H_{114}O_{25}Na$), and 1361.7169 $[M+Na]^+$ ($C_{69}H_{110}O_{25}Na$), respectively. Alkaline hydrolysis afforded simonic acid A (**12**) as the glycosidic acid present in **6** and **7**, and simonic acid B (**13**) in **8** and **9**. The location of the jalapinolic acid moiety in the oligosaccharide core was determined by the observed HMBC cross-peaks from jalapinolic acid H-11 to glucose C-1 in **6** and **7**, and to fucose C-1 in **8** and **9**. The esterification positions were determined by three-bond correlations between carbonyl and proton signals of the monosaccharides using HMBC: thus C-2-OH of rhamnose$^{II}$ was acylated by an *n*-dodecanoyl substituent in **6**, 2-methylbutanoyl in **7**, isobutanoyl in **8**, and *n*-decanoyl in **9**; C-2-OH of rhamnose$^{III}$ was acylated by a *trans*-cinnamoyl substituent in **6**, **8**, and **9**; C-3-OH of rhamnose$^{III}$ was acylated by a 2-methylbutanoyl substituent in **6** and by a *trans*-cinnamoyl in **7**; C-4-OH of rhamnose$^{III}$ was acylated by an *n*-dodecanoyl substituent in **8**; and C-2-OH of rhamnose$^{I}$ was esterified by jalapinolic acid in **6–9**. Consequently, the structures of **6–9** were determined as shown in Chart 1.

Compounds **1–9** were subjected to a cytotoxic assay using laryngeal carcinoma (Hep-2) cells. Compounds **5** and **8** showed cytotoxic activities against Hep-2 cells with $ED_{50}$ value at 3.5 μg/mL and 2.0 μg/mL, respectively. Other compounds with $ED_{50}$ over 20 μg/mL were considered to be inactive. Compound **4**, an isomer of **5**, differed from **5** only in the esterification position of 2-methylbutanoyl; compounds **8** and **9** share simonic acid B as core with the same esterification sites, two of which were acylated by different organic acids. Thus, these compounds showed surprisingly large differences in cytotoxicity in spite of minor structural differences. Similar to cyclic dextrins, the coexistence of both hydrophobic and hydrophilic sections and the macrolactone ring in 'resin glycosides' were suggested to help the delivery of compounds or iron across the cell membrane,[22–25] leading to various biological activities, which, however, could hardly explain the sensitivity to structure of this type of compounds. Certainly, additional investigations on the mechanisms of action of these compounds would be of value.

Sweet potato varied greatly in the content of periderm 'resin glycosides', showing inhibitory effect on the growth of pathogenic

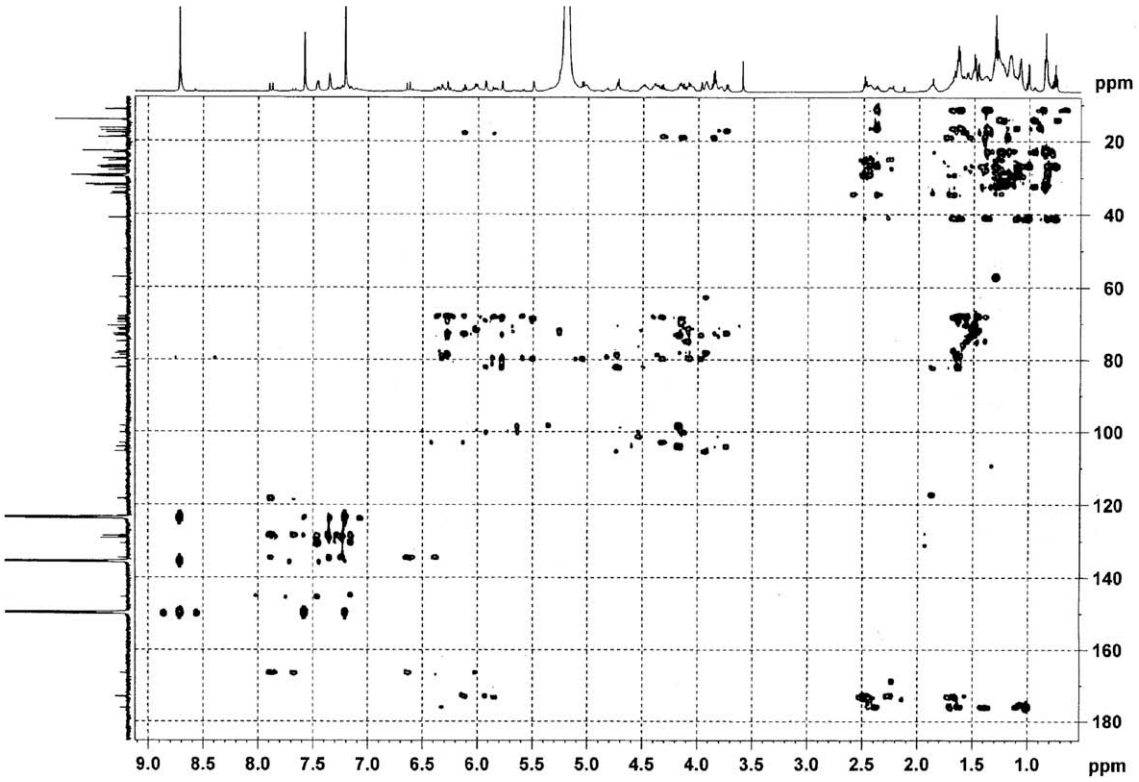



**Figure 2.** HMBC spectrum in $C_5D_5N$ of **1**.

fungi of roots.[26] Levels ranged from 0.05% to 10.02% of the periderm dry weight, which could be partly due to stress of the environment, such as weed, insects, and disease.

'Resin glycosides' content was positively correlated with the relative inhibition of yellow nutsedge (*Cyperus esculentum* L.)[27] and the developmental time of the diamondback moth larvae,[28] while it was negatively correlated with insect damage ratings of sweet potato breeding clones[29] and lifetime fecundity of diamondback moth.[28]

Therefore, these 'resin glycosides' may be stored as allelochemicals, contributing to disease resistance and allelopathy of sweet potato.

## 3. Experimental

### 3.1. General procedures

$^1$H, $^{13}$C, and 2D NMR spectra were recorded on a Bruker ACF-600 spectrometer (600 MHz and 150 MHz, respectively) with TMS as internal standard. Mass spectra were obtained on a MS Agilent 1100 series LC/MSD ion trap mass spectrometer (ESIMS), an Agilent TOF MSD 1946D spectrometer, and positive-ion HR-ESIMS were recorded with an Agilent TOF MSD 1946D spectrometer. UV spectra were obtained on a Shimadzu UV-2501PC spectrophotometer. The IR (KBr) spectra were obtained on a Nicolet Impact 410 spectrometer. HPLC separations were performed on an Agilent 1100 series instrument with a Shim-park RP-$C_{18}$ column (200 × 20 mm i.d.) and UV detector at 280 nm. GC-MS was run on a Varian CP-3800 instrument. Optical rotations were measured with a JASCO P-1020 polarimeter in MeOH solution. TLC was performed on pre-coated Silica Gel 60 $F_{254}$ (Qingdao Marine Chemical Co. Ltd) and detected by spraying with 10% $H_2SO_4$–EtOH. Column chromatography was carried out with Silica Gel H (Qingdao Marine Chemical Co. Ltd), Sephadex LH-20 (20–100 μm, Pharmacia), and ODS-$C_{18}$ (100–200 μm, Waters).

### 3.2. Plant material

The tubers of *I. batatas* were collected in Yanlin County, Hunan, China, in September 2004. The plant material was identified by Prof. Min-Jian Qin, and a voucher specimen (No. 040912) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

### 3.3. Isolation of resin glycosides

To obtain more resin glycosides, we collected 50 kg of *I. batatas*, which was extracted with 95% EtOH (3 × 20 L × 2 h) at 80 °C, the extract soln was concentrated under diminished pressure, and allowed to stand overnight so as to fully precipitate the starch. The supernatant soln was further concentrated to produce a residue, which was partitioned between chloroform (5 × 0.5 L) and water (0.5 L) to give 130 g and 50 g of extracts from these two layers, respectively. The chloroform extract was chromatographed over silica gel and eluted with a gradient $CHCl_3$–MeOH, and then submitted to RP-$C_{18}$ column chromatography to afford three fractions. Further purified by successive RP-18 preparative HPLC eluted with 90% MeOH, Fraction 1 gave 12.7 mg **6** at $t_R$ 6.44 min, 16.5 mg 7 at $t_R$ 8.91 min, and 15.6 mg **9** at $t_R$ 5.91 min; with 96% MeOH, Fraction 2 afforded 8.7 mg **4** at $t_R$ 5.36 min and 26.0 mg **5** at $t_R$ 7.91 min; with 98% MeOH, Fraction 3 gave 38.5 mg **1** at $t_R$ 26.5 min, 9.24 mg **2** at $t_R$ 27.7 min, 10.4 mg **3** at $t_R$ 34.5 min, and 20.3 mg **8** at $t_R$ 21.4 min.

#### 3.3.1. Batatoside H (1)

White amorphous powder, $[\alpha]_D^{25}$ −13.2 (*c* 0.3, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 280 (4.21), 217 (4.06), 205 (4.04) nm; IR $\nu_{max}$ (KBr): 3444, 2928, 1727, 1636, 1137, 1073 cm$^{-1}$; $^1$H and $^{13}$C NMR data ($C_5D_5N$): see Tables 1 and 2; ESIMS: *m/z* 1431 $[M+Cl]^-$; HRESIMS: calcd for $C_{72}H_{116}O_{26}Na$ $[M+Na]^+$: *m/z* 1419.7645; found: *m/z* 1419.7647).

#### 3.3.2. Batatoside I (2)

White amorphous powder, $[\alpha]_D^{25}$ −33.6 (*c* 0.5, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 280(4.33), 217 (4.18), 205 (4.16) nm; IR $\nu_{max}$ (KBr): 3450, 2929, 1724, 1636, 1136, 1070 cm$^{-1}$; $^1$H and $^{13}$C NMR data ($C_5D_5N$): see Tables 1 and 2; ESIMS: *m/z* 1431 $[M+Cl]^-$; HRESIMS: calcd for $C_{72}H_{116}O_{26}Na$ $[M+Na]^+$: *m/z* 1419.7647; found: *m/z* 1419.7675.

#### 3.3.3. Batatoside J (3)

White amorphous powder, $[\alpha]_D^{25}$ −20.8 (*c* 0.4, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 279 (4.17), 217 (3.99), 205 (4.07) nm; IR $\nu_{max}$ (KBr): 3444, 2927, 1736, 1638, 1134, 1056 cm$^{-1}$; $^1$H and $^{13}$C NMR data ($C_5D_5N$): see Tables 1 and 2; ESIMS: *m/z* 1343 $[M+Na]^+$; HRESIMS: calcd for $C_{71}H_{116}O_{22}Na$ $[M+Na]^+$: *m/z* 1343.7850; found: *m/z* 1343.7816.

#### 3.3.4. Batatoside K (4)

White amorphous powder, $[\alpha]_D^{25}$ −22.3 (*c* 0.4, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 281 (4.15), 217 (3.99), 204 (3.95) nm; IR $\nu_{max}$ (KBr): 3444, 2927, 1736, 1638, 1134, 1056 cm$^{-1}$; $^1$H and $^{13}$C NMR data ($C_5D_5N$): see Tables 1 and 2; ESIMS: *m/z* 1285 $[M+Cl]^-$; HRESIMS: calcd for $C_{66}H_{106}O_{22}Na$ $[M+Na]^+$: *m/z* 1273.7067; found: *m/z* 1273.7097.

#### 3.3.5. Batatoside L (5)

White amorphous powder, $[\alpha]_D^{25}$ −29.6 (*c* 0.4, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 281 (3.94), 217 (3.79), 204 (3.77) nm; IR $\nu_{max}$ (KBr): 3444, 2927, 1725, 1637, 1136, 1055 cm$^{-1}$; $^1$H and $^{13}$C NMR data ($C_5D_5N$): see Tables 1 and 2; ESIMS: *m/z* 1249 $[M-H]^-$; HRESIMS: calcd for $C_{66}H_{106}O_{22}Na$ $[M+Na]^+$: *m/z* 1273.7067; found: *m/z* 1273.7097.

#### 3.3.6. Batatoside M (6)

White amorphous powder, $[\alpha]_D^{25}$ −36.4 (*c* 0.4, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 280 (4.28), 217 (4.38), 204 (4.28) nm; IR $\nu_{max}$ (KBr): 3450, 2932, 1736, 1638, 1139, 1060 cm$^{-1}$; $^{13}$C and $^1$H NMR data ($C_5D_5N$): see Tables 2 and 3; ESIMS: *m/z* 1395 $[M-H]^-$; HRESIMS: calcd for $C_{72}H_{116}O_{26}Na$ $[M+Na]^+$: *m/z* 1419.7647; found: *m/z* 1419.7537.

#### 3.3.7. Batatoside N (7)

White amorphous powder, $[\alpha]_D^{25}$ −32.5 (*c* 0.5, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 280 (4.16), 217 (4.00), 205 (3.98) nm; IR $\nu_{max}$ (KBr): 3444, 2932, 1730, 1635, 1135, 1053 cm$^{-1}$; $^{13}$C and $^1$H NMR data ($C_5D_5N$): see Tables 2 and 3; ESIMS: *m/z* 1283 $[M-H]^-$; HRESIMS: calcd for $C_{65}H_{102}O_{25}Na$ $[M+Na]^+$: *m/z* 1307.6395; found: *m/z* 1307.6406.

#### 3.3.8. Batatoside O (8)

White amorphous powder, $[\alpha]_D^{25}$ −26.4 (*c* 0.6, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 280 (4.25), 217 (4.09), 206 (4.12) nm; IR $\nu_{max}$ (KBr): 3446, 2930, 1724, 1635, 1132, 1070 cm$^{-1}$; $^{13}$C and $^1$H NMR data ($C_5D_5N$): see Tables 2 and 3; ESIMS: *m/z* 1365 $[M-H]^-$; HRESIMS: calcd for $C_{71}H_{114}O_{25}Na$ $[M+Na]^+$: *m/z* 1389.7541; found: *m/z* 1389.7557.

#### 3.3.9. Batatoside P (9)

White amorphous powder, $[\alpha]_D^{25}$ −30.6 (*c* 0.4, MeOH); UV (MeOH): $\lambda_{max}$ (log ε): 280 (4.10), 217 (4.02), 205 (4.08) nm; IR $\nu_{max}$ (KBr): 3452, 2929, 1739, 1638, 1135, 1051 cm$^{-1}$; $^{13}$C and $^1$H NMR data ($C_5D_5N$): see Tables 2 and 3; ESIMS: *m/z* 1373 $[M+Cl]^-$; HRE-

**Table 3**
$^1$H [$\delta_H$ (*J*, Hz)] data of compounds **6–9** ($C_5D_5N$, 600 MHz)

| Position[b] | 6 | 7 | 8 | 9 |
|---|---|---|---|---|
| Glc-1 | 4.94 (d, 7.6) | 4.76 (d, 7.6) | | |
| 2 | 3.88[a] | 3.88[a] | | |
| 3 | 4.18 (t, 8.9) | 4.18 (t, 8.9) | | |
| 4 | 4.13 (t, 8.9) | 4.13 (t, 8.9) | | |
| 5 | 3.85[a] | 3.85[a] | | |
| 6 | 4.35 (dd, 5.1, 11.5) | 4.35 (dd, 5.1, 11.5) | | |
| | 4.41 (dd, 2.8, 11.5) | 4.41 (dd, 2.2, 11.5) | | |
| Fuc-1 | | | 4.75 (d, 7.5) | 4.72 (d, 7.5) |
| 2 | | | 4.16 (dd, 7.5, 9.4) | 4.15 (dd, 7.5, 9.4) |
| 3 | | | 4.08 (dd, 9.4, 3.5) | 4.07 (dd, 9.4, 3.5) |
| 4 | | | 3.98 (d, 3.5) | 3.98 (d, 3.5) |
| 5 | | | 3.77 (q, 6.4) | 3.76 (q, 6.4) |
| 6 | | | 1.50 (d, 6.4) | 1.49 (d, 6.4) |
| Rha$^I$-1 | 5.60 (br s) | 5.49 (br s) | 5.47 (br s) | 5.47 (br s) |
| 2 | 6.06 (br s) | 6.06 (br s) | 5.96 (br s) | 5.94 (br s) |
| 3 | 5.07 (dd, 3.2, 9.0) | 5.07 (dd, 3.2, 9.0) | 5.01(dd, 3.2, 9.5) | 5.01 (dd, 3.2, 9.5) |
| 4 | 4.20 (t, 9.0) | 4.24 (t, 9.0) | 4.21(t, 9.5) | 4.21 (t, 9.5) |
| 5 | 4.42 (dq, 9.0, 6.0) | 4.36 (dq, 9.0, 6.2) | 4.44 (m) | 4.44 (dq, 9.5, 6.1) |
| 6 | 1.57 (d, 6.0) | 1.57 (d, 6.2) | 1.62 (d, 6.1) | 1.62 (d, 6.1) |
| Rha$^{II}$-1 | 6.06 (br s) | 6.09 (br s) | 6.11 (br s) | 6.14 (br s) |
| 2 | 6.00 (br s) | 5.97 (br s) | 5.99 (br s) | 6.01 (br s) |
| 3 | 4.63 (dd, 3.2, 9.2) | 4.66 (dd, 3.2, 9.2) | 4.65 (dd, 3.0, 9.1) | 4.61 (dd, 3.0, 9.3) |
| 4 | 4.35 (t, 9.2) | 4.28 (t, 9.2) | 4.32 (t, 9.1) | 4.34 (t, 9.3) |
| 5 | 4.49 (dq, 9.2, 6.2) | 4.49 (dq, 9.2, 6.2) | 4.37 (dq, 9.1, 5.8) | 4.44 (dq, 9.3, 5.8) |
| 6 | 1.73 (d, 6.2) | 1.63 (d, 6.2) | 1.67 (d, 5.8) | 1.65 (d, 5.8) |
| Rha$^{III}$-1 | 5.91 (br s) | 5.96 (br s) | 5.94 (br s) | 5.81 (br s) |
| 2 | 6.17 (br s) | 4.94 (br s) | 6.04 (br s) | 6.01 (br s) |
| 3 | 5.94 (dd, 3.2, 9.3) | 5.90 (dd, 3.2, 9.3) | 4.68 (dd, 3.0, 9.8) | 4.68 (dd, 3.0, 9.4) |
| 4 | 4.35 (t, 9.3) | 6.02 (t, 9.3) | 5.78 (t, 9.8) | 5.72 (t, 9.4) |
| 5 | 4.45 (dq, 9.3, 6.0) | 4.39 (dq, 9.3, 6.3) | 4.40 (dq, 9.8, 6.1) | 4.34 (dq, 9.4, 6.1) |
| 6 | 1.57 (d, 6.0) | 1.40 (d, 6.3) | 1.65 (d, 6.1) | 1.65 (d, 6.1) |
| Rha$^{IV}$-1 | 5.60 (br s) | 5.71 (br s) | 5.85 (br s) | 5.67 (br s) |
| 2 | 4.72 (br s) | 4.77 (br s) | 4.78 (br s) | 4.78 (br s) |
| 3 | 4.44[a] | 4.44[a] | 4.39 (dd, 3.3, 9.0) | 4.39 (dd, 3.3, 9.0) |
| 4 | 4.20 (t, 9.3) | 4.20 (t, 9.3) | 4.19 (t, 9.0) | 4.19 (t, 9.0) |
| 5 | 4.30 (dq, 9.3, 6.0) | 4.39 (dq, 9.3, 6.0) | 4.26 (dq, 9.0, 5.9) | 4.26 (dq, 9.0, 6.4) |
| 6 | 1.60 (d, 6.0) | 1.40 (d, 6.0) | 1.54 (d, 5.9) | 1.48 (d, 6.4) |
| Ag-2 | 2.25 (m) | 2.23 (m) | 1.91 (m) | 2.24 (m) |
| | 2.38 (m) | 2.37 (m) | 2.23 (m) | 2.38 (m) |
| 11 | 3.87 (m) | 3.87 (m) | 3.85 (m) | 3.85 (m) |
| 16 | 0.84 (t, 7.0) | 0.80 (t, 7.0) | 0.89 (t, 6.7) | 0.84 (t, 6.7) |
| Cna-2 | 6.83 (d, 16.0) | 6.51 (d, 16.0) | 6.55 (d, 15.9) | 6.52 (d, 15.9) |
| 3 | 7.92 (d, 16.0) | 7.79 (d, 16.0) | 7.76 (d, 15.9) | 7.74 (d, 15.9) |
| Deca-2 | | | | 2.31 (t, 7.0) |
| 10 | | | | 0.82 (t, 7.2) |
| Dodeca-2 | 2.42 (m) | | 2.34 (t, 7.0) | |
| 12 | 0.84 (t, 7.0) | | 1.11 (t, 7.2) | |
| Iba-2 | | 2.60 (m) | 2.40[a] | 2.62 (m) |
| 3 | | 1.10 (d, 5.9) | 1.07 (d, 7.0) | 1.16 (d, 7.0) |
| 3′ | | 1.09 (d, 7.5) | 1.16 (d, 7.0) | 1.19 (d, 7.0) |
| Mba-2 | 2.35 (m) | 2.34 (m) | | |
| 2-$CH_3$ | 1.09 (d, 7.0) | 1.05 (d, 7.0) | | |
| 4 | 0.85 (t, 7.0) | 0.83 (t, 7.0) | | |

[a] Signal pattern unclear due to overlapping.

SIMS: calcd for $C_{69}H_{110}O_{25}Na$ $[M+Na]^+$: *m/z* 1361.7228; found: *m/z* 1361.7169.

### 3.4. Hydrolysis

#### 3.4.1. Alkaline hydrolysis and determination of organic acids

Compounds **1–9** (3 mg each) in 5% KOH (3 mL) were refluxed at 90 °C for 2 h separately. The reaction mixtures were acidified to pH 4 and extracted with ether (30 mL). The ether layer was washed with water, dried over anhyd $Na_2SO_4$, and esterified with MeOH using 0.5 N $H_2SO_4$ as catalyst. The methyl esters were analyzed by GC–MS on a model 3800 gas chromatograph interfaced with a model 2200 mass spectrometer (Varian) in EI at 70 eV under the following conditions: 30 m × 0.25 mm i.d., 0.25 m, VF-5 ms capillary column (Varian); column temperature, 160–240 °C temperature programmed at 10 °C/min; carrier gas, $N_2$ (30 mL/min).

#### 3.4.2. Acid hydrolysis and sugar analysis

After extraction with ether, the alkaline hydrolysate of **1–9** was further partitioned with *n*-BuOH (30.0 mL) to afford glycosidic acids (**10–13**), which were evaporated to dryness and hydrolyzed by 1 N $H_2SO_4$ in 4 mL THF under reflux for 4 h. The hydrolysate was cooled to room temperature and extracted three times with ether and then reacted with an excess of diazomethane to furnish 11-hydroxyhexa-

decanoic acid methyl ester (**14**). The aq layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column and was extracted with *n*-BuOH, and then concentrated to yield a saccharide residue. An aliquot of this residue was subjected to silica gel chromatography (6:4:1 $CHCl_3$–MeOH–$H_2O$) to give D-fucose: $[\alpha]_D^{25}$ +66.4 (*c* 0.8, water); L-rhamnose: $[\alpha]_D^{25}$ −9.7 (*c* 1.0, water); and D-glucose: $[\alpha]_D^{25}$ +100.0 (*c* 1.0, water). Another aliquot was treated with water (0.05 mL) and pyridine (0.03 mL) at 60 °C for 1 h with stirring. After the solvent was evaporated and the reaction mixture was dried, pyridine (0.5 mL), hexamethyldisilazane (0.8 mL), and trimethylsilyl chloride (0.4 mL) were added to the residue. The reaction mixture was heated at 60 °C for 30 min. Under the same conditions as mentioned in Section 3.4.1, the supernatant was identified by GC–MS; the presence of D-fucose, L-rhamnose, and D-glucose was confirmed by comparison of the retention times of their TMSi derivatives with those of standard sugar derivatives prepared in a similar way, which showed retention times of 4.57 min, 5.09 min, and 8.03 min, respectively.

### 3.5. Preparation of Mosher's esters

To compound **14** (2.0 mg, in 1.5 mL of $CH_2Cl_2$) was added a soln of (*R*)-methoxyphenylacetic acid (*R*-MPA, 12.0 mg) and 4-dimethylaminopyridine (DMAP, 10.0 mg) in $CH_2Cl_2$ (1.0 mL), followed by *N,N*-dicyclohexylcarbodiimide (DCC, 10.0 mg), and the resultant mixture was stirred for 17.0 h at 25.0 °C. EtOAc (30.0 mL) was added to quench the reaction mixture that was filtered. The filtrate was concentrated and purified by silica gel chromatography eluting with 19:1 cyclohexane–EtOAc to give **15** (2.6 mg, 94%). The (*S*)-MPA ester **16** (1.2 mg 85%) was prepared in the same way. For selected $\Delta\delta$ values [$\delta(R) - \delta(S)$], see Scheme 1.

#### 3.5.1. 11*S*-hydroxyhexadecanoic acid methyl ester (14)

Colorless oil ($CHCl_3$), $[\alpha]_D^{25}$ +1.1 (*c* 0.2, $CHCl_3$); IR $\nu_{max}$ (KBr): 3333, 2920, 2850, 1207 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$): $\delta_H$ 3.67 (s, 3, $OCH_3$), 3.58 (m, 1H, OCH-11), 2.30 (t, 2H, *J* 7.5 Hz, $OCOCH_2$-2), 1.62 (t, 2H, *J* 7.0, $CH_2$-10), 1.44 (m, 2H, $CH_2$-12), 0.89 (t, 3H, *J* 6.9 Hz, $CH_3$-16); ESITOF-MS: *m/z* 309 $[M+Na]^+$.

#### 3.5.2. 11-(*R*-MPA)-hydroxyhexadecanoic acid methyl ester (15)

Colorless oil ($CHCl_3$), $[\alpha]_D^{25}$ −2.0 (*c* 0.1, $CHCl_3$); IR $\nu_{max}$ (KBr): 3442, 2927, 2855, 1743, 1261, 802 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$): $\delta_H$ 7.44 (m, 2H, $C_6H_2$), 7.34 (m, 3H, $C_6H_3$), 4.90 (m, 1H, OCH-11), 4.73 (s, 1H, OCH), 3.67 (s, 3H, $OCH_3$), 3.41 (s, 3H, $OCH_3$), 2.30 (t, 2H, *J* 7.4 Hz, $OCOCH_2$-2), 1.67 (m, 2H, $CH_2$-10), 1.41 (m, 2H, $CH_2$-12), 0.77 (t, 3H, *J* 7.1 Hz, $CH_3$-16); ESIMS: *m/z* 457 $[M+Na]^+$; 435 $[M+H]^+$.

#### 3.5.3. 11-(*S*-MPA)-hydroxyhexadecanoic acid methyl ester (16)

Colorless oil ($CHCl_3$), $[\alpha]_D^{25}$ +1.4 (*c* 0.20, $CHCl_3$); IR $\nu_{max}$ (KBr): 3453, 2961, 2926, 2852, 1742, 1261 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$): $\delta_H$ 7.44 (m, 2H, $C_6H_2$), 7.34 (m, 3H, $C_6H_3$), 4.90 (m, 1H, OCH-11), 4.73 (s, 1H, OCH), 3.67 (s, 3H, $OCH_3$), 3.41 (s, 3H, $OCH_3$), 2.30 (t, 2H, *J* 7.6 Hz, $OCOCH_2$-2), 1.61 (m, 2H, $CH_2$-10), 1.54 (m, 2H, $CH_2$-12), 0.84 (t, 3H, *J* 7.1 Hz, $CH_3$-16); ESIMS: *m/z* 457 $[M + Na]^+$.

### 3.6. Cytotoxic activity assays

The assay of cytotoxic activity against laryngeal carcinoma (Hep-2) cells of compounds **1–9** was performed according to the published method[34] using vinblastine as positive control with an $ED_{50}$ value at 0.004 μg/mL.


## Acknowledgments

This research work was supported by the National Natural Science Foundation of China (No. 30472144) and the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (707033). We acknowledge Dr. Bing Ma, Shandong University, for measuring the TOCSY, HMQC, and HMBC NMR spectra.


## Supplementary data

Supplementary data (HRESIMS, $^1H$- and $^{13}C$ NMR spectra) of compounds **1–9**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.12.022.

## References


1. León, I.; Enríquez, R. G.; Nieto, D. A.; Alonso, D.; Reynolds, W. F.; Aranda, E.; Villa, J. *J. Nat. Prod.* **2005**, *68*, 1141–1146.
2. Noda, N.; Takahashi, N.; Kawasaki, T.; Miyahara, K.; Yang, C. R. *Phytochemistry* **1994**, *36*, 365–371.
3. Ono, M.; Fujimoto, K.; Kawata, M.; Fukunaga, T.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1992**, *40*, 1400–1403.
4. Escalante-Sánchez, E.; Rosas-Ramírez, D.; Linares, E.; Bye, R.; Pereda-Miranda, R. *J. Agric. Food Chem.* **2008**, *56*, 9423–9428.
5. Escalante-Sánchez, E.; Pereda-Miranda, R. *J. Nat. Prod.* **2007**, *70*, 1029–1034.
6. Noda, N.; Ono, M.; Miyahara, K.; Kawasaki, T.; Okabe, M. *Tetrahedron* **1987**, *43*, 3889–3902.
7. Noda, N.; Kobayashi, H.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1988**, *36*, 627–633.
8. Noda, N.; Kobayashi, H.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1988**, *36*, 920–929.
9. Noda, N.; Nishi, M.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1988**, *36*, 1707–1713.
10. Ono, M.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1989**, *37*, 3209–3213.
11. Barnes, C. C.; Smalley, M. K.; Manfredi, K. P.; Kindscher, K.; Loring, H.; Sheeley, D. M. *J. Nat. Prod.* **2003**, *66*, 1457–1462.
12. Chérigo, L.; Pereda-Miranda, R.; Fragoso-Serrano, M.; Jacobo-Herrera, N.; Kaatz, G. W.; Gibbons, S. *J. Nat. Prod.* **2008**, *71*, 1037–1045.
13. Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997**, *53*, 9007–9022.
14. León, I.; Enríquez, R. G.; Gnecco, D.; Villarreal, M. L.; Cortés, D. A.; Reynolds, W. F.; Yu, M. *J. Nat. Prod.* **2004**, *67*, 1552–1556.
15. Li, S. Z.. In *(Min Dynasty) Compendium of Materia Medica*; People's Medical Publishing House: Beijing, China, 1999; Vol. 2.
16. Yin, Y. Q.; Li, Y.; Kong, L. Y. *J. Agric. Food Chem.* **2008**, *56*, 2363–2368.
17. Yin, Y. Q.; Kong, L. Y. *J. Asian Nat. Prod. Res.* **2008**, *10*, 233–238.
18. Noda, N.; Yoda, S.; Kawasaki, T.; Miyahata, K. *Chem. Pharm. Bull.* **1992**, *40*, 3163–3168.
19. Ono, M.; Fukunaga, T.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1990**, *38*, 2650–2655.
20. Pereda-Miranda, R.; Mata, R.; Anaya, A. L.; Wickramaratne, D. B. M.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1993**, *56*, 571–582.
21. Noda, N.; Kogetsu, H.; Kawasaki, T.; Miyahara, K. *Phytochemistry* **1992**, *31*, 2761–2766.
22. Lu, S. F.; Guo, Z. W.; Ouyang, Q. Q.; Hui, Y. Z. *Youji Huaxue* **1997**, *17*, 488–497.
23. Rencurosi, A.; Mitchell, E. P.; Cioci, G.; Perez, S.; Pereda-Miranda, R.; Imberty, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5918–5922.
24. Kitagawa, I.; Ohashi, K.; Koyama, W.; Kawanishi, H.; Yamamoto, T.; Nishino, T.; Shibuya, H. *Chem. Pharm. Bull.* **1989**, *37*, 1416–1418.
25. Kitagawa, I.; Ohashi, K.; Kawanishi, H.; Shibuya, H.; Shinkai, K.; Akedo, H. *Chem. Pharm. Bull.* **1989**, *37*, 1679–1681.
26. Harrison, H. F.; Peterson, J. K.; Jackson, D. M.; Snook, M. E. *Allelopathy J.* **2003**, *12*, 53–60.
27. Peterson, J. K.; Harrison, H. F.; Snook, M. E. *Allelopathy J.* **1999**, *6*, 201–208.
28. Jackson, D. M.; Peterson, J. K. *J. Econ. Entomol.* **2000**, *93*, 388–393.
29. Cao, S. G.; Guza, R. C.; Wisse, J. H.; Miller, J. S.; Evans, R.; Kingston, D. G. I. *J. Nat. Prod.* **2005**, *68*, 487–492.
30. Enríquez, R. G.; León, I.; Perez, F.; Walls, F.; Carpenter, K. A.; Puzzuoli, F. V.; Reynolds, W. F. *Can. J. Chem.* **1992**, *70*, 1000–1008.
31. Seco, J. M.; Quinoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–117.
32. Ono, M.; Yamada, F.; Noda, N.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1993**, *41*, 1023–1026.
33. Ono, M.; Kubo, K.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1989**, *37*, 241–244.
34. Chérigo, L.; Pereda-Miranda, R. *J. Nat. Prod.* **2006**, *69*, 595–599.