Phytochemistry 71 (2010) 693–700

Contents lists available at ScienceDirect

Phytochemistry

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

# Gynostemosides A–E, megastigmane glycosides from *Gynostemma pentaphyllum*

Zhen Zhang [a], Wei Zhang [b], Yan-Ping Ji [a], Yun Zhao [a], Chuan-Gui Wang [b], Jin-Feng Hu [a,*]

[a] Department of Natural Products for Chemical Genetic Research, Key Laboratory of Brain Functional Genomics, Ministry of Education and Shanghai Key Laboratory of Brain Functional Genomics (MOE&SBFG), East China Normal University, Shanghai 200062, PR China
[b] Institute of Biomedical Sciences, School of Life Science, East China Normal University, Shanghai 200241, PR China

ARTICLE INFO

*Article history:*
Received 5 August 2009
Received in revised form 12 October 2009
Available online 25 January 2010

*Keywords:*
*Gynostemma pentaphyllum*
Cucurbitaceae
Megastigmane glycosides
Absolute configuration

ABSTRACT

Megastigmane glycosides (**1**–**5**) together with seven (**6**–**12**) related known compounds were isolated from the whole plants of *Gynostemma pentaphyllum*. The structures were elucidated by means of spectroscopic methods, including 2D NMR, HR-ESIMS, and circular dichroism (CD), as well as chemical transformations to be (3*R*, 4*R*, 5*S*, 6*S*, 7*E*)-3,4,6-trihydroxymegastigmane-7-en-9-one-3-*O*-*β*-D-glucopyranoside (gynostemoside A, **1**), (3*S*, 4*S*, 5*R*, 6*R*, 7*E*, 9*R*)-3,4,6,9-tetrahydroxymegastigmane-7-en-3-*O*-*β*-D-glucopyranoside (gynostemoside B, **2**), (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3,4,9-trihydroxymegastigmane-7-en-9-*O*-*β*-D-glucopyranoside (gynostemoside C, **3**), (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3,4,9-trihydroxymegastigmane-7-en-3-*O*-*β*-D-glucopyranoside (gynostemoside D, **4**), and (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3,4,9-trihydroxymegastigmane-7-en-4-*O*-*β*-D-glucopyranoside (gynostemoside E, **5**), respectively.



## 1. Introduction

The whole plant of *Gynostemma pentaphyllum* (Thurb.) Makino (Cucurbitaceae), named "Jiao-Gu-Lan" in Chinese, is used for a popular traditional Chinese medicine (TCM) as a cough suppressant, diuretic, antipyretic and tonic (Jiangsu New Medicine College, 1977). The plant is widely distributed in Southern China in warm and humid environments. Earlier phytochemical investigations established that it contains dammarane-type glycosides, including the protopanaxadiol-type ginsenosides (i.e., ginsenosides $Rb_1$, $Rb_3$, Rc, Rd, $F_2$, $Rg_3$) previously isolated from *Panax ginseng* (Araliaceae) (Razmovski-Naumovski et al., 2005; Hu et al., 1996). Modern pharmacological studies also showed that *G. pentaphyllum* has a variety of bioactivities similar to *P. ginseng* (Rujjanawate et al., 2004; Norberg et al., 2004). Therefore, "Jiao-Gu-Lan" earned its favorable name of "Southern Ginseng" (Liu et al., 2004a). During a reinvestigation focusing on the constituents rather than dammarane-type saponins from the ethanol extract of *G. pentaphyllum*, and in a continuation of our work with the discovery of novel anticancer agents from plants (Wu et al., 2009, 2010), five new (**1**–**5**) and two known (**6**, **7**) megastigmane glycosides, together with five known megastigmanes (**8**–**12**) were obtained (Fig. 1). The cytotoxicity of the isolated compounds against a small panel of human tumor cell lines (A549, H460, U251, U2OS, and MCF-7) was investigated. In this paper, we report the isolation and absolute structure elucidation of the new compounds and the cytotoxicity assay results.

## 2. Results and discussion

### 2.1. Extraction and separation of megastigmane metabolites

The dried whole plants of *G. pentaphyllum* were extracted with 95% EtOH at room temperature. After evaporation of the solvent, the aqueous residue was diluted with $H_2O$ and exhaustively extracted with *n*-BuOH. Evaporation of the solvent from the *n*-BuOH layer under reduced pressure yielded a brown residue (77.8 g), which was successively subjected to column chromatography (CC) over silica gel, MCI gel and Sephadex LH-20, respectively. Finally, semi-preparative HPLC afforded compounds **1**–**12** (Fig. 1). Based on the spectroscopic analysis and comparison with the literature, the known compounds were identified as citroside A (**6**) (Umehara et al., 1988; Osorio et al., 1999), citroside B (**7**) (Umehara et al., 1988), (3*S*, 4*S*, 5*S*, 6*S*, 9*R*)-3,4-dihydroxy-5,6-dihydro-*β*-ionol (**8**) (Rodríguez et al., 1992; Pérez et al., 1996a), (3*S*, 5*R*, 6*R*, 7*E*, 9*S*)-3,5,6,9-tetrahydroxy-7-en-megastigmane (**9**) (Takeda et al., 2000), (3*S*, 4*S*, 5*R*, 6*R*)*-3,4,6-trihydroxy-5,6-dihydro-*β*-ionol (**10**) (Pérez et al., 1996b), (*E*)-4-(*r*-1′,*t*-2′,*c*-4′-trihydroxy-2′,6′,6′-trimethylcyclohexyl)but-3-en-2-one (**11**) (Tan et al., 1989), and 4′-dihydrophaseic acid (**12**) (Walton et al., 1973; Zaharia et al., 2005).

### 2.2. Structure elucidation of compounds *1*–*5*

The molecular formula of compound **1** was determined to be $C_{19}H_{32}O_9$ based on a *pseudo*-molecular ion peak at *m*/*z* 427.1974 $[M+Na]^+$ in its positive mode HR-ESIMS. A strong absorption ($\nu_{max}$) at 1641 $cm^{-1}$ in its IR spectrum together with the

* Corresponding author. Tel.: +86 21 62237510; fax: +86 21 62606791.
*E-mail address:* jfhu@brain.ecnu.edu.cn (J.-F. Hu).

0031-9422/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2009.12.017

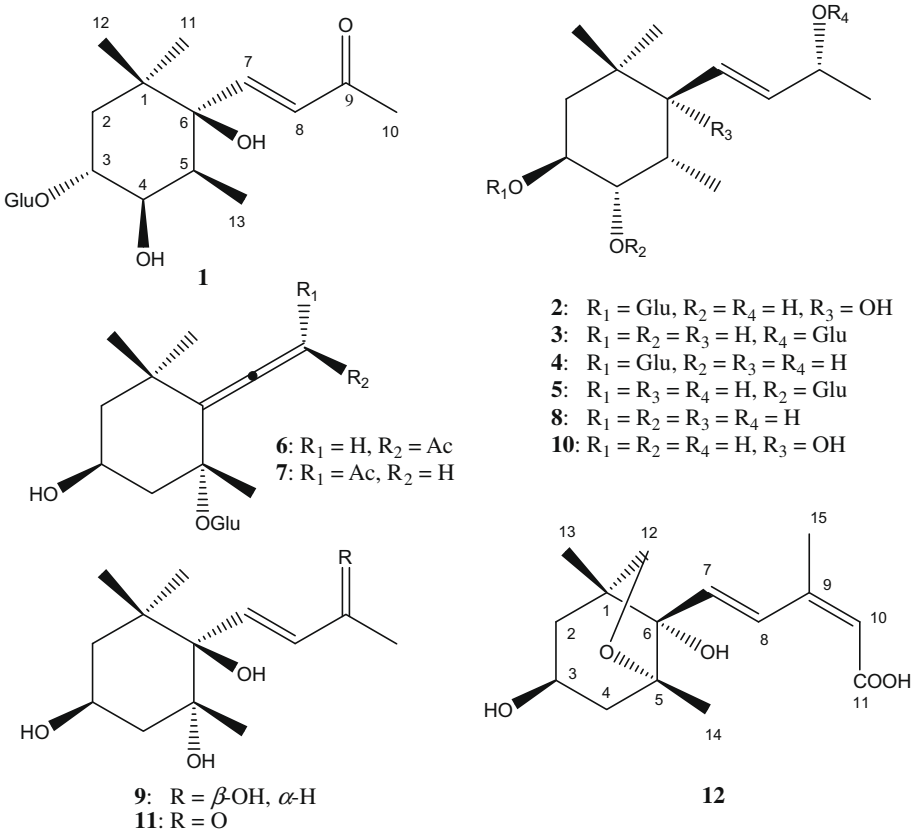



**Fig. 1.** Isolated compounds **1**–**12**.

maximum absorption ($\lambda_{max}$) at 232 nm in its UV (MeOH) spectrum indicated a conjugated enone moiety in the structure of **1**. The $^{13}C$ and DEPT NMR spectra (Table 1) of **1** showed, in addition to the six signals attributed to a glucopyranosyl moiety, 13 carbon signals classified as four methyls, one methylene, five methines, and three quaternary carbons. Comparison of these data with those of the known compounds **6**–**11** suggested that **1** was a megastigmane glucoside.

The $^1H$ NMR spectrum (Table 2) of **1** possessed signals of three methyl groups singlet at $\delta$ 2.26 (3H, *s*, Me-10), 1.37 (3H, *s*, Me-11), and 1.04 (3H, *s*, Me-12), one methyl group doublet at $\delta$ 1.21 (3H, *d*, $J$ = 7.0 Hz, Me-13), two oxymethines at $\delta$ 4.57 (1H, *br d*, $J$ = 2.2 Hz, H-3) and 4.45 (1H, *br s*, H-4), an anomeric proton at $\delta$ 4.96 (1H, *d*, $J$ = 7.8 Hz, H-1′), and a pair of protons at $\delta$ 6.87 (1H, *d*, $J$ = 15.7 Hz, H-7) and 6.82 (1H, *d*, $J$ = 15.7 Hz, H-8) in a *trans*-disubstituted double bond conjugated with a ketone in the side-chain.

**Table 1**
$^{13}C$ (125 MHz) NMR spectroscopic data of **1**–**5**.[a]

| | **1** (DEPT)[b] | **2** (DEPT)[b] | **3** (DEPT)[b] | **3** (DEPT)[c] | **4** (DEPT)[b] | **5** (DEPT)[b] |
|---|---|---|---|---|---|---|
| 1 | 38.8 (C) | 38.5 (C) | 33.5 (C) | 34.1 (C) | 33.3 (C) | 33.1 (C) |
| 2 | 36.5 ($CH_2$) | 36.8 ($CH_2$) | 41.9 ($CH_2$) | 42.0 ($CH_2$) | 40.5 ($CH_2$) | 41.9 ($CH_2$) |
| 3 | 77.5 (CH) | 78.1 (CH) | 71.6 (CH) | 71.4 (CH) | 78.3 (CH) | 67.3 (CH) |
| 4 | 72.2 (CH) | 72.9 (CH) | 74.7 (CH) | 72.4 (CH) | 70.8 (CH) | 81.3 (CH) |
| 5 | 31.6 (CH) | 32.1 (CH) | 31.0 (CH) | 31.6 (CH) | 31.1 (CH) | 30.1 (CH) |
| 6 | 80.0 (C) | 79.7 (C) | 50.4 (CH) | 51.2 (CH) | 50.1 (CH) | 50.8 (CH) |
| 7 | 150.8 (CH) | 131.3 (CH) | 132.6 (CH) | 133.7 (CH) | 129.7 (CH) | 129.6 (CH) |
| 8 | 130.6 (CH) | 135.5 (CH) | 134.8 (CH) | 135.7 (CH) | 138.1 (CH) | 138.2 (CH) |
| 9 | 197.3 (C) | 67.7 (CH) | 76.1 (CH) | 77.9 (CH) | 67.8 (CH) | 67.9 (CH) |
| 10 | 27.1 ($CH_3$) | 24.6 ($CH_3$) | 21.1 ($CH_3$) | 21.4 ($CH_3$) | 24.5 ($CH_3$) | 24.5 ($CH_3$) |
| 11 | 26.6 ($CH_3$) | 26.4 ($CH_3$) | 32.6 ($CH_3$) | 32.9 ($CH_3$) | 32.1 ($CH_3$) | 31.9 ($CH_3$) |
| 12 | 26.3 ($CH_3$) | 26.7 ($CH_3$) | 23.9 ($CH_3$) | 24.0 ($CH_3$) | 23.3 ($CH_3$) | 23.5 ($CH_3$) |
| 13 | 13.2 ($CH_3$) | 13.3 ($CH_3$) | 17.6 ($CH_3$) | 17.4 ($CH_3$) | 17.5 ($CH_3$) | 17.3 ($CH_3$) |
| 1′ | 103.3 (CH) | 103.4 (CH) | 102.2 (CH) | 102.2 (CH) | 103.3 (CH) | 102.9 (CH) |
| 2′ | 75.0 (CH) | 75.0 (CH) | 75.2 (CH) | 75.4 (CH) | 75.1 (CH) | 74.8 (CH) |
| 3′ | 78.4 (CH) | 78.5 (CH) | 78.3 (CH) | 78.1 (CH) | 78.5 (CH) | 78.4 (CH) |
| 4′ | 71.3 (CH) | 71.4 (CH) | 71.2 (CH) | 71.3 (CH) | 71.4 (CH) | 71.7 (CH) |
| 5′ | 78.3 (CH) | 78.2 (CH) | 78.0 (CH) | 78.0 (CH) | 78.1 (CH) | 78.0 (CH) |
| 6′ | 62.5 ($CH_2$) | 62.5 ($CH_2$) | 62.3 ($CH_2$) | 62.5 ($CH_2$) | 62.5 ($CH_2$) | 62.7 ($CH_2$) |

[a] Assignments were made by a combination of 1D and 2D NMR techniques (COSY, HSQC and HMBC).
[b] Recorded in $C_5D_5N$.
[c] Recorded in $CD_3OD$.

**Table 2**
$^1$H (500 MHz) NMR spectroscopic data of **1–5** (in $C_5D_5N$).[a]

| | **1** $\delta$ (mult, *J*, Hz) | **2** $\delta$ (mult, *J*, Hz) | **3** $\delta$ (mult, *J*, Hz) | **4** $\delta$ (mult, *J*, Hz) | **5** $\delta$ (mult, *J*, Hz) |
|---|---|---|---|---|---|
| H-$2_{ax}$ | 2.41 (1H, *dd*, 14.0, 2.2) | 2.49 (1H, *dd*, 14.1. 2.0) | 2.20 (1H, *dd*, 14.0, 2.5) | 2.16 (1H, *dd*, 14.0, 2.5) | 2.24 (1H, *br d*, 13.8) |
| H-$2_{eq}$ | 1.76 (1H, *br d*, 14.0) | 1.80 (1H, *br d*, 14.1) | 1.80 (1H, *br d*, 14.0) | 1.87 (1H, *br d*, 14.0) | 1.78 (1H, *br d*, 13.8) |
| H-$3_{eq}$ | 4.57 (1H, *br d*, 2.2) | 4.59 (1H, *br d*, 2.0) | 4.48 (1H, *br d*, 2.5) | 4.53 (1H, *br d*, 2.5) | 4.68 (1H, *br s*) |
| H-$4_{eq}$ | 4.45 (1H, *br s*) | 4.45 (1H, *br s*) | 4.18 (1H, *br s*) | 4.36 (1H, *br s*) | 4.32 (1H, *br s*) |
| H-$5_{ax}$ | 2.73 (1H, *dq*, 7.0, 2.0) | 2.69 (1H, *br q*, 6.8) | 2.45 (1H, *m*) | 2.42 (1H, *m*) | 2.51 (1H, *m*) |
| H-6 | | | 2.43 (1H, *dd*, 11.3, 9.3) | 2.39 (1H, *dd*, 11.5, 9.0) | 2.27 (1H, *dd*, 11.5, 9.1) |
| H-7 | 6.87 (1H, *d*, 15.7) | 5.80 (1H, *d*, 15.5) | 5.63 (1H, *dd*, 15.7, 9.3) | 5.56 (1H, *dd*, 15.6, 9.0) | 5.64 (1H, *dd*, 15.5, 9.1) |
| H-8 | 6.82 (1H, *d*, 15.7) | 6.36 (1H, *dd*, 15.5, 6.1) | 5.83 (1H, *dd*, 15.7, 6.7) | 5.80 (1H, *dd*, 15.6, 6.2) | 5.71 (1H, *dd*, 15.5, 6.0) |
| H-9 | | 4.69 (1H, *dq*, 7.0, 6.1) | 4.71 (1H, *dq*, 6.7, 6.5) | 4.59 (1H, *dq*, 6.5, 6.2) | 4.58 (1H, *dq*, 6.5, 6.0) |
| H-10 | 2.26 (3H, *s*) | 1.48 (3H, *d*, 7.0) | 1.44 (3H, *d*, 6.5) | 1.46 (3H, *d*, 6.5) | 1.45 (3H, *d*, 6.5) |
| H-11 | 1.37 (3H, *s*) | 1.14 (3H, *s*) | 1.05 (3H, *s*) | 0.94 (3H, *s*) | 0.82 (3H, *s*) |
| H-12 | 1.04 (3H, *s*) | 1.38 (3H, *s*) | 1.39 (3H, *s*) | 1.24 (3H, *s*) | 1.35 (3H, *s*) |
| H-13 | 1.21 (3H, *d*, 7.0) | 1.31 (3H, *d*, 6.8) | 1.21 (3H, *d*, 5.5) | 1.19 (3H, *d*, 6.2) | 1.24 (3H, *d*, 6.5) |
| 1′ | 4.96 (1H, *d*, 7.8) | 4.97 (1H, *d*, 7.8) | 5.01 (1H, *d*, 7.5) | 4.98 (1H, *d*, 7.8) | 4.92 (1H, *d*, 7.5) |
| 2′ | 4.07 (1H, *dd*, 8.9, 7.8) | 4.03 (1H, *dd*, 9.0, 7.8) | 4.04 (1H, *dd*, 9.1, 7.5) | 4.04 (1H, *dd*, 8.9, 7.8) | 4.04 (1H, *dd*, 8.9, 7.5) |
| 3′ | 4.21 (1H, *dd*, 9.2, 8.9) | 4.21 (1H, *dd*, 9.0, 8.8) | 4.23 (1H, *dd*, 9.1, 8.9) | 4.21 (1H, *dd*, 9.2, 8.9) | 4.19 (1H, *dd*, 9.1, 8.9) |
| 4′ | 4.26 (1H, *dd*, 9.2, 9.0) | 4.25 (1H, *dd*, 9.1, 8.8) | 4.26 (1H, *dd*, 9.2, 8.9) | 4.23 (1H, *dd*, 9.2, 9.0) | 4.22 (1H, *dd*, 9.1, 9.0) |
| 5′ | 3.89 (1H, *m*) | 3.88 (1H, *m*) | 3.93 (1H, *m*) | 3.86 (1H, *m*) | 3.84 (1H, *m*) |
| 6′a | 4.54 (1H, *br d*, 11.4) | 4.52 (1H, *br d*, 11.5) | 4.50 (1H, *dd*, 11.5, 1.8) | 4.50 (1H, *dd*, 11.3, 1.8) | 4.52 (1H, *br d*, 11.2) |
| 6′b | 4.40 (1H, *dd*, 11.4, 5.5) | 4.39 (1H, *dd*, 11.5, 5.6) | 4.38 (1H, *dd*, 11.5, 5.8) | 4.39 (1H, *dd*, 11.3, 5.8) | 4.38 (1H, *dd*, 11.2, 5.5) |
| OH-6 | 5.61 (*s*) | 5.60 (*s*) | | | |

[a] Assignments were made by a combination of 1D and 2D NMR techniques (COSY, NOESY, HSQC and HMBC).

These data demonstrated that **1** has features very similar to those of lasianthionoside A, a megastigmane glucoside previously isolated from the leaves of *Lasianthus fordii* (Rubiaceae) (Takeda et al., 2004). Its aglycone as 3,4,6-trihydroxy-megastigmane-7-en-9-one was determined by detailed 1D and 2D NMR (COSY, HSQC and HMBC) spectroscopic analyses. In the COSY NMR spectrum of **1**, a spin system of $-CH_2-CH(O)-CH(O)-CH(CH_3)-$ was found between: $H_2$-2 and H-3; H-3 and H-4; H-4 and H-5; and H-5 and Me-13. The chemical shifts of the corresponding carbons were subsequently deduced through an HSQC NMR experiment to be $\delta$ 36.5 (C-2), 77.5 (C-3), 72.2 (C-4), 31.6 (C-5), and 13.2 (C-13), respectively. In the HMBC spectrum, the anomeric proton at $\delta$ 4.96 was found to have a clear $^3J$ correlation with the oxymethine carbon at $\delta$ 77.5, which enabled us to assign C-3 as the glycosidic linkage position, implying a free secondary hydroxyl group located at C-4 and a free tertiary hydroxyl group bonded to C-6 ($\delta$ 80.0). The $\beta$-glycosidic linkage was determined based on the observed coupling constant (7.8 Hz) of the anomeric proton. Notably, the proton chemical shift of the tertiary hydroxyl group at C-6 can be unambiguously assigned at $\delta$ 5.61 (1H, *s*) through the HMBC correlations ($^2J$, $^3J$) between this exchangeable proton (OH-6) and C-5 ($\delta$ 31.6), C-6 ($\delta$ 80.0), and C-7 ($\delta$ 150.8).

The relative stereochemistry at C-3, C-4, C-5, and C-6 was characterized through extensive analyses of the coupling patterns of the protons bonded to the cyclohexane ring and the NOE correlations in the NOESY NMR experiment. The small coupling constants (Table 2) found for H-3 and H-4 indicated both protons have an equatorial orientation. In the NOESY NMR spectrum, clear NOE correlations (Fig. 2) were observed between: H-$2_{ax}$ at $\delta$ 2.41 and Me-12 at $\delta$ 1.04; H-$2_{eq}$ at $\delta$ 1.76 and Me-11 at $\delta$ 1.37; Me-11 and H-5 at $\delta$ 2.73; H-$2_{ax}$ and OH-6 at $\delta$ 5.61; as well as Me-13 at $\delta$ 1.21 and OH-6. Similar to the known compound (3*S*, 5*R*, 6*S*, 7*E*)-3,5,6-trihydroxy-7-megastigmane-9-one (Sun et al., 2007), the *S* configuration at C-6 in **1** was determined from a negative Cotton effect ($\Delta\varepsilon_{238nm}$ −8.69) in its CD spectrum. In addition, enzymatic hydrolysis of **1** with $\beta$-glucosidase gave only D-glucose as the sugar moiety, which was identified by direct comparison with an authentic sample. Therefore, compound **1** was elucidated to be (3*R*, 4*R*, 5*S*, 6*S*, 7*E*)-3,4,6-trihydroxymegastigmane-7-en-9-one-3-*O*-$\beta$-D-glucopyranoside.

The molecular weight of compound **2** and its chemical formula of $C_{19}H_{34}O_9$ were deduced from its positive mode HR-ESIMS, which resulted in a $[M+Na]^+$ ion peak at *m*/*z* 429.2064. Its $^1$H and $^{13}$C NMR spectroscopic data (Tables 1 and 2) showed that **2** is an analogue of **1**, with an additional oxymethine at $\delta$ 4.69 (1H, *dq*, *J* = 7.0, 6.1 Hz, H-9, $\delta_C$: 67.7) in place of the ketone carbonyl at C-9 ($\delta_C$: 197.3) in the side-chain of **1**. This was supported by the absence of the IR absorption band of the $\alpha,\beta$-unsaturated ketone moiety in **2**. Therefore, **2** was the C-9 ketone reduction product of **1**. As for **1**, the glycosidic linkage position at C-3 and the proton chemical shift [$\delta$ 5.60 (1H, *s*)] of the exchangeable tertiary hydroxyl group at C-6 were determined by analysis of the HMBC correlations. In the HMBC NMR spectrum, the anomeric proton at $\delta$ 4.97 was found to have a clear $^3J$ correlation with C-3 at $\delta$ 78.1 (CH), while the exchangeable proton at $\delta$ 5.60 exhibited $^2J$ or $^3J$ correlations with C-5 ($\delta$ 32.1, CH), C-6 ($\delta$ 79.7, C) and C-7 ($\delta$ 131.3, CH). Similar to compound **1**, the coupling constant (7.8 Hz) of the anomeric proton in **2** also indicated a $\beta$-glycosidic linkage.

The relative stereochemistry at the chiral centers in the cyclohexane of **2** was judged from the NOE correlations (Fig. 2) in its NOESY NMR spectrum and the observed coupling patterns (Table 2) of the protons linked to this ring. To further determine the absolute configuration, compound **2** was subjected to enzymatic hydrolysis with $\beta$-glucosidase to yield D-glucose and 3,4,6,9-tetrahydroxy-7-en-megastigmane (**2a**) as its aglycone. Compound **2a** was then subjected to a modified Mosher's method (Takeda et al., 2000; Ohtani et al., 1991) by treatment with (*S*)- and (*R*)-$\alpha$-methoxy-$\alpha$-trifluoromethylphenyl acetic acid (MTPA) in the presence of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP) to give its 3,9-di-(*S*)-MTPA ester (**2b**) and 3,9-di-(*R*)-MTPA ester (**2c**). In their $^1$H NMR spectra, although the signals of H-7, H-8 and H-9 of **2c** overlapped around $\delta$ 5.56–5.63, the chemical shifts of H-7 and H-8 in **2b** could be observed at higher fields ($\Delta\delta$: negative, <−0.03 at least), while its Me-10 resonated at a lower field ($\Delta\delta$: positive) (Fig. 3) when compared with those of **2c**, indicating an *R* configuration at C-9 in **2a**. The *S* configuration at C-3 was confirmed by the observed difference of the chemical shifts of the protons bonded to C-2, C-4, C-5 and C-13. In **2b**, H-4, H-5, and Me-13 were at lower fields ($\Delta\delta$: positive) and the two protons at C-2 were

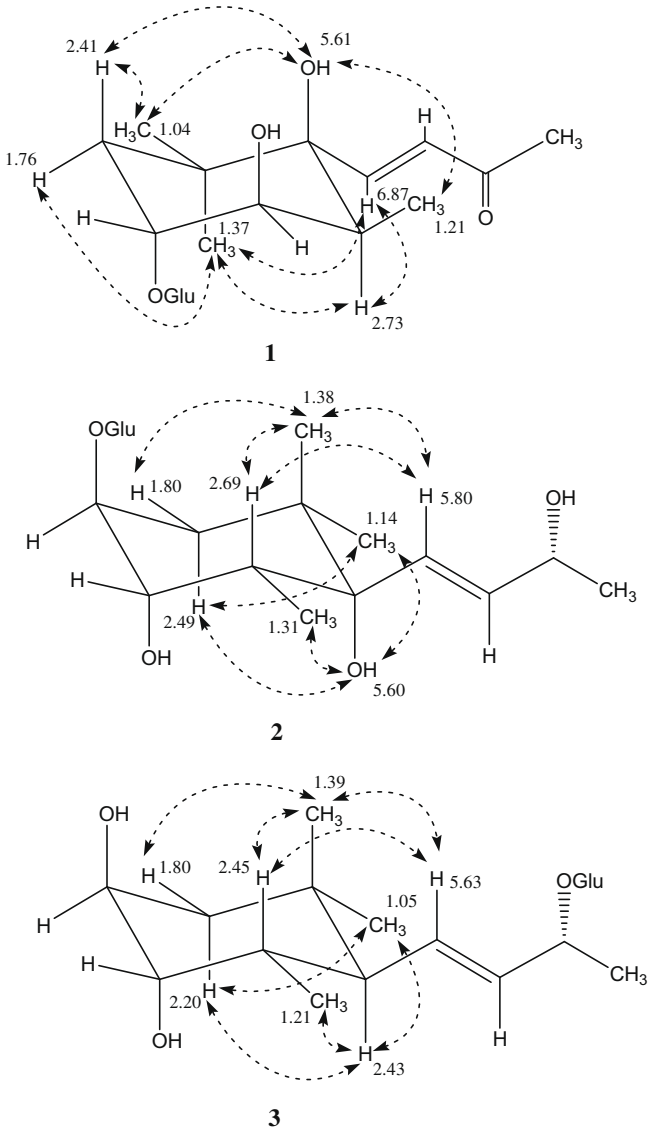
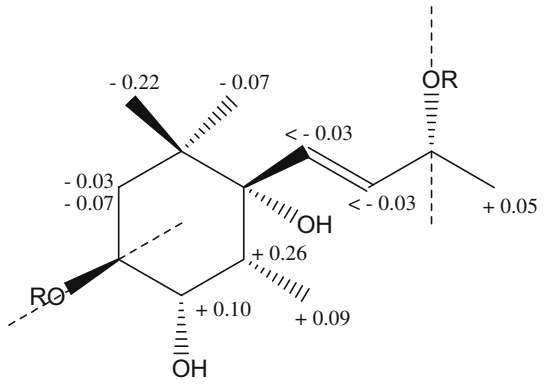



**Fig. 2.** Key NOE correlations in NOESY NMR spectra of compounds **1**–**3**.




**2b:** R = (*S*)-MTPA
**2c:** R = (*R*)-MTPA

**Fig. 3.** Results with the modified Mosher's method ($\Delta\delta_H = \delta_S - \delta_R$).

shifted to higher fields ($\Delta\delta$: negative) (Fig. 3) when compared with those of **2c**. Therefore, structure **2** was elucidated as (3*S*, 4*S*, 5*R*, 6*R*, 7*E*, 9*R*)-3,4,6,9-tetrahydroxy-megastigmane-7-en-3-*O*-*β*-D-glucopyranoside. Meanwhile, **2a** was found to be identical to **10** [whose absolute configuration at C-9 was previously unknown (Pérez et al., 1996b)] by comparing their spectroscopic data and physical properties. Thus, the structure of compound **10** can be now updated as (3*S*, 4*S*, 5*R*, 6*R*, 9*R*)-3,4,6-trihydroxyl-5,6-dihydro-*β*-ionol.

The $^1$H and $^{13}$C NMR spectra (Tables 1 and 2) of compound **3** showed general features similar to those of **2**. The most obvious difference between these two compounds was that the oxygen-bearing quaternary carbon signal at $\delta$ 79.7 (C-6) in **2** was replaced by a methine carbon signal at $\delta$ 50.4 in **3**, and hence an additional proton was observed at $\delta$ 2.40 (1H, *dd*, *J* = 11.3, 9.3 Hz, H-6) in **3**. In the COSY spectrum of **3**, a spin system of –$CH_2$–CH(O)–CH(O)–CH($CH_3$)–CH–CH=CH–CH(O)–$CH_3$ was found, indicating that **3** is a 6-deoxy analogue of **2**. This was further supported by its chemical formula $C_{19}H_{34}O_8$ deduced from a *pseudo*-molecular ion peak at *m*/*z* = 413.2143 $[M+Na]^+$ in its positive mode HR-ESIMS. In the HMBC NMR spectrum of **3**, a $^3J$ correlation was observed between the anomeric proton at $\delta$ 5.01 (1H, *d*, *J* = 7.5 Hz) and C-9 at $\delta$ 76.1, demonstrating C-9 as the glycosidic linkage position. Compound **3** has a structure identical to the known compound **C-1** (Ibraheim and Abdallah, 1994) previously isolated from the Egyptian plant *Maerua crassifolia* Forssk. However, their NMR spectroscopic data recorded in $C_5D_5N$ are quite different, indicating that the two compounds are diastereomers. Enzymatic hydrolysis of **3** with *β*-glucosidase gave D-glucose and an aglycone, which was identical to compound **8** (Pérez et al., 1996a; Rodríguez et al., 1992) based on the analyses of their HPLC chromatograms and $^1$H NMR spectra. In addition, a $^{13}$C NMR spectrum in $CD_3OD$ (Table 1) of **3** showed that the chemical shift ($\delta$ 77.9) of C-9 was almost consistent with that of in (9*R*)- rather than in (9*S*)-3-oxo-*α*-ionol-9-*O*-*β*-D-glucopyranoside [9*R*: C-9 ($\delta$ 77.0), 9*S*: C-9 ($\delta$ 74.7)] (Pabst et al., 1992) previously isolated from raspberry fruits. Thus, **3** was deduced to be (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3,4,9-trihydroxymegastigmane-7-en-9-*O*-*β*-D-glucopyranoside, which was in full agreement with the NOE correlations (Fig. 2).

Based on their HR-ESIMS, the chemical formulae of compounds **4** and **5** were both determined to be the same as compound **3**. The NMR spectroscopic features (Tables 1 and 2) of compounds **3**–**5** were also very similar. Like compounds **1**–**3**, the homonuclear proton connectivities in compounds **4** and **5** were determined by analysis of their COSY spectra, and their heteronuclear proton and carbon connectivities were deduced by HSQC and HMBC NMR experiments. In their HMBC spectra, clear $^3J$ correlations were observed between the anomeric proton at $\delta$ 4.98 (1H, *d*, *J* = 7.8 Hz) and C-3 at $\delta$ 78.3 in **4**, and between the anomeric proton at $\delta$ 4.92 (1H, *d*, *J* = 7.5 Hz) and C-4 at $\delta$ 81.3 in **5**, indicating the only difference between **4** and **5** was the glycosidic linkage position. The enzymatic hydrolysis of **4** and **5** with *β*-glucosidase both gave D-glucose and the same aglycone, whose structure was also identical to compound **8** (Pérez et al., 1996a; Rodríguez et al., 1992). Consequently, the structures of **4** and **5** were elucidated to be (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3,4,9-trihydroxymegastigmane-7-en-3-*O*-*β*-D-glucopyranoside, and (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3,4,9-trihydroxymegastigmane-7-en-4-*O*-*β*-D-glucopyranoside, respectively.

### 2.3. Other known megastigmane metabolites

All the previously known compounds (**6**–**12**) from other plant sources were isolated from *G. pentaphyllum* for the first time. Their $^1$H NMR spectroscopic data recorded either in $CD_3OD$ (**7**, **11**, **12**) or in $C_5D_5N$ (**8**, **9**) (Table 3), and their $^{13}$C NMR data recorded either in $CD_3OD$ (**6**, **7**, **12**) or in $C_5D_5N$ (**9**) (Table 4) were reported for the first time. Among them, the chemical shifts of 4′-dihydrophaseic

**Table 3**
$^1$H (500 MHz) NMR spectroscopic data of **7**–**9**, **11** and **12**.

| | **7**[a] $\delta$ (mult, *J*, Hz) | **8**[b] $\delta_H$ (mult, *J*, Hz) | **9**[b] $\delta$ (mult, *J*, Hz) | **11**[a] $\delta$ (mult, *J*, Hz) | **12**[a,c] $\delta$ (mult, *J*, Hz) |
|---|---|---|---|---|---|
| 2 | 1.90 (1H, overlapped)<br>1.25 (1H, *m*) | 2.24 (1H, *dd*, 14.2, 2.4)<br>1.82 (1H, *br d*, 14.2) | 2.59 (1H, *t*, 12.2, 11.8)<br>2.05 (1H, *dd*, 12.2, 2.3) | 1.42 (2H, *m*) | 1.85 (1H, *dd*, 12.2, 7.0)<br>1.66 (1H, *dd*, 12.2, 2.0) |
| 3 | 4.36 (1H, *m*) | 4.50 (1H, *br d*, 2.4) | 4.91 (1H, *m*) | 4.12 (1H, *m*) | 4.11 (1H, *m*) |
| 4 | 2.48 (1H, *br d*, 13.2)<br>1.90 (1H, overlapped) | 4.22 (1H, *br s*) | 2.51 (1H, overlapped)<br>2.47 (1H, overlapped) | 1.46 (2H, *m*) | 2.03 (1H, *dd*, 13.5, 7.5)<br>1.74 (1H, *dd*, 13.5, 3.0) |
| 5 | | 2.49 (1H, overlapped) | | | |
| 6 | | 2.48 (1H, overlapped) | | | |
| 7 | | 5.74 (1H, *dd*, 15.5, 9.0) | 6.83 (1H, *d*, 15.9) | 6.36 (1H, *d*, 15.2) | 6.50 (1H, *d*, 15.8) |
| 8 | 5.92 (1H, *s*) | 5.85 (1H, *dd*, 15.5, 6.5) | 6.51 (1H, *dd*, 15.9, 6.1) | 7.40 (1H, *d*, 15.2) | 7.96 (1H, *d*, 15.8) |
| 9 | | 4.63 (1H, *dq*, 6.5, 6.2) | 4.78 (1H, *qd*, 6.3, 6.1) | | |
| 10 | 2.28 (3H, *s*) | 1.49 (1H, *d*, 6.2) | 1.51 (3H, *d*, 6.3) | 2.31 (3H, *s*) | 5.77 (1H, *br s*) |
| 11 | 1.13 (3H, *s*) | 1.02 (3H, *s*) | 1.66 (3H, *s*) | 1.11 (3H, *s*) | |
| 12 | 1.45 (3H, *s*) | 1.42 (3H, *s*) | 1.27 (3H, *s*) | 0.85 (3H, *s*) | 3.80 (1H, *d*, 7.5)<br>3.71 (1H, *d*, 7.5) |
| 13 | 1.45 (3H, *s*) | 1.26 (3H, *d*, 6.5) | 1.64 (3H, *s*) | 1.25 (3H, *s*) | 0.93 (3H, *s*) |
| 14 | | | | | 1.15 (3H, *s*) |
| 15 | | | | | 2.08 (3H, *br s*) |
| 1′ | 4.55 (1H, *d*, 7.9) | | | | |
| 2′ | 3.16 (1H, *dd*, 9.0, 7.9) | | | | |
| 3′ | 3.35 (1H, *dd*, 9.0, 8.9) | | | | |
| 4′ | 3.24 (1H, *dd*, 8.9, 8.8) | | | | |
| 5′ | 3.20 (1H, *m*) | | | | |
| 6′ | 3.80 (1H, *br d*, 11.8)<br>3.62 (1H, *dd*, 11.8, 4.8) | | | | |

[a] Recorded in $CD_3OD$.
[b] Recorded in $C_5D_5N$.
[c] Assignments were made by a combination of 1D and 2D NMR techniques (COSY, HSQC and HMBC).

**Table 4**
$^{13}$C (125 MHz) NMR spectroscopic data of **6**, **7**, **9** and **12**.

| | **6** (DEPT)[a] | **7** (DEPT)[a] | **9** (DEPT)[b] | **12** (DEPT)[a,c] |
|---|---|---|---|---|
| 1 | 37.0 (C) | 36.9 (C) | 40.2 (C) | 49.4 (C) |
| 2 | 49.9 ($CH_2$) | 49.9 ($CH_2$) | 47.0 ($CH_2$) | 44.5 ($CH_2$) |
| 3 | 62.9 (CH) | 62.8 (CH) | 64.1 (CH) | 66.1 (CH) |
| 4 | 48.1 ($CH_2$) | 46.8 ($CH_2$) | 46.7 ($CH_2$) | 46.0 ($CH_2$) |
| 5 | 77.8 (C) | 77.7 (C) | 76.9 (C) | 87.9 (C) |
| 6 | 119.1 (C) | 119.5 (C) | 78.3 (C) | 83.4 (C) |
| 7 | 200.7 (C) | 202.1 (C) | 130.4 (CH) | 135.1 (CH) |
| 8 | 101.4 (CH) | 101.5 (CH) | 136.2 (CH) | 132.2 (CH) |
| 9 | 212.9 (C) | 212.9 (C) | 68.2 (CH) | 151.0 (C) |
| 10 | 26.6 ($CH_3$) | 27.3 ($CH_3$) | 24.8 ($CH_3$) | 120.3 (CH) |
| 11 | 26.7 ($CH_3$) | 27.5 ($CH_3$) | 27.6 ($CH_3$) | 170.6 (C) |
| 12 | 30.1 ($CH_3$) | 29.8 ($CH_3$) | 26.2 ($CH_3$) | 77.4 ($CH_2$) |
| 13 | 32.5 ($CH_3$) | 32.8 ($CH_3$) | 27.6 ($CH_3$) | 16.2 ($CH_3$) |
| 14 | | | | 19.5 ($CH_3$) |
| 15 | | | | 21.1 ($CH_3$) |
| 1′ | 98.7 (CH) | 98.5 (CH) | | |
| 2′ | 75.3 (CH) | 75.4 (CH) | | |
| 3′ | 78.7 (CH) | 79.4 (CH) | | |
| 4′ | 71.8 (CH) | 71.6 (CH) | | |
| 5′ | 78.6 (CH) | 78.8 (CH) | | |
| 6′ | 63.8 ($CH_2$) | 63.8 ($CH_2$) | | |

[a] Recorded in $CD_3OD$.
[b] Recorded in $C_5D_5N$.
[c] Assignments were made by a combination of 1D and 2D NMR techniques (COSY, HSQC and HMBC).

acid (**12**) (Walton et al., 1973; Zaharia et al., 2005) were unambiguously assigned here for the first time by detailed 1D and 2D NMR spectroscopic analyses, with the relative stereochemistry was further investigated using a NOESY NMR experiment.

### 2.4. Biogenetic consideration and cytotoxic assay

Based on a biogenetic considerations, the isolated megastigmane derivatives **1**–**12** are degraded carotenoid-like compounds probably formed by initial cleavage of cartenoids and subsequently interconverted by either distinct transferase or synthase enzymes (Enzell, 1985; Baumes et al., 2002; Setha et al., 2004). In fact, various carotenoids have been detected in *G. pentaphyllum* (Liu et al., 2004b). Naturally occurring megastigmane derivatives were previously found to have anti-proliferative (Zhou et al., 2009; Liu et al., 2008; Janakiram et al., 2008; Chen et al., 2006; Liu et al., 2004c; Liu et al., 2004d; Duncan et al., 2004; Yu et al., 1995), anticancer (Ito et al., 2002; Jung et al., 1998), and cytotoxic effects (Kuang et al., 2008; Alija et al., 2006; Perrone et al., 2005; Kubo and Morimitsu, 1995). Our isolated compounds, however, were tested against human lung adenocarcinoma (A549, H460), human glioma (U251), human osteosarcoma (U2OS), and human breast (MCF-7) cancer cells using the MTT method (Wu et al., 2009, 2010), but were inactive.

## 3. Concluding remarks

Through a combination of silica gel, MCI gel and Sephadex LH-20 column chromatography, as well as semi-preparative HPLC, five new megastigmane glycosides (**1**–**5**) and several related known compounds (**6**–**12**) were isolated from the well-known traditional Chinese medicine "Jiao-Gu-Lan" (*G. pentaphyllum*). Based on extensive spectroscopic methods and a few chemical transformations, the absolute configuration of the new megastigmane glycosides was completely elucidated. Megastigmane derivatives have, to date, never been reported from this plant. This class of naturally occurring compounds could stimulate future phyochemical genomics studies.

## 4. Experimental

### 4.1. General procedures

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. CD spectra were obtained on a JASCO 810 spectrometer. IR and UV spectra were recorded on Thermo Nicolet NEXUS 670 FT-IR and Libra S35 spectrometer, respectively. NMR spectra

were measured on Bruker AM 500 Avance DRX-500 spectrometer. Chemical shifts ($\delta$ in ppm) were referenced to the residual solvent signals [$CD_3OD$ ($\delta_H/\delta_C$: 3.30/49.0), $C_5D_5N$ ($\delta_H$: 7.20, 7.57, 8.72; $\delta_C$: 149.8, 135.5, 123.4)]. Low-resolution electrospray ionization mass spectrometry (LR-ESIMS) was carried out on a Bruker Esquire 3000plus instrument. HR-ESIMS was measured on a Bruker Daltonics micrOTOF mass spectrometer. Semi-preparative HPLC was performed on a Beckman System consisting of a Beckman Coulter System Gold 508 autosampler, Gold 126 gradient HPLC pumps with a Beckman System Gold 166 single wavelength UV detector (254 nm), a Sedex 80 (SEDERE, France) evaporative light-scattering detector (ELSD), and a Beckman Coulter Ultrasphare ODS column (250 × 10 mm, 5 μm). The solvents for chromatography were analytical grade (Shanghai Chemical Reagents Co. Ltd., China) and those for HPLC were HPLC grade (Jiangsu Hanbon Science and Technology Co. Ltd., China). $\beta$-Glucosidase (emulsion), (*R*)- and (*S*)-MTPAs, 4-DMAP and EDC·HCl were all purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Ji-Yi-Da Silysia Chemical Ltd., China), MCI gel CHP20P (75–120$\mu$, Mitsubishi Chemical Industries, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Silica gel-precoated plates ($GF_{254}$, 0.25 mm, Yantai Kang-Bi-Nuo Silysia Chemical Ltd., China) were used for TLC. TLC was performed with EtOAc–AcOH–MeOH–$H_2O$, $CH_2Cl_2$–MeOH–$H_2O$ or $CH_2Cl_2$–MeOH as mobile phases. Spots were visualized by spraying with 5% vanillin in EtOH (containing 1% $H_2SO_4$) followed by heating to 150 °C.

### 4.2. Plant material

The dried whole plants of *G. pentaphyllum* were purchased from Shanghai Kang-Qiao TCM Materials Co. Ltd., and were originally collected from Anhui province of China in December 2004. The plant was identified by Prof. Jian-Wei Chen (College of Pharmacy, Nanjing University of Traditional Chinese Medicine). A voucher specimen (No. 060416) was deposited at the Herbarium of the Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University.

### 4.3. Extraction and isolation

The air-dried and ground material (10 kg) was extracted with EtOH–$H_2O$ (10 L × 4, 95:5, v/v) at room temperature (each process lasting 24 h). After removal of the solvent by evaporation, the residue (520 g) was suspended in $H_2O$ (750 mL), and then extracted with *n*-BuOH (5 × 750 mL). The combined *n*-BuOH fractions were concentrated *in vacuo* to give a residue (77.8 g), which was subjected to silica gel CC (8.0 × 82 cm) using a $CH_2Cl_2$–MeOH gradient (10:1–1:1–MeOH neat, v/v) to yield 10 fractions (Fr.1–Fr.10). Fr.2 ($CH_2Cl_2$–MeOH 10:1, 3.0 g) was applied to an MCI gel (column: 5.0 × 45 cm), this being eluted using a stepwise gradient elution with MeOH/$H_2O$ (from 1:4 to MeOH neat, v/v) to afford four subfractions (Fr.2A–Fr.2D). Compounds **10** (8.2 mg) and **11** (2.1 mg) were isolated from Fr.2B (MeOH/$H_2O$ 3:7, 510 mg) through silica gel CC (column: 3.0 × 45 cm) with $CH_2Cl_2$–MeOH–$H_2O$ (20:1:0.05) as eluant, with both further purified by gel permeation chromatography (GPC) on Sephadex LH-20 (column: 3.0 × 98 cm) in MeOH. Fr.2C (MeOH/$H_2O$ 2:3, 360 mg) was subjected to silica gel CC (3.0 × 45 cm) with a $CH_2Cl_2$–MeOH gradient (15:1–1:1–MeOH neat, v/v) to yield compound **12** (10.0 mg). Compound **8** (3.2 mg) was isolated from Fr.2D (MeOH/$H_2O$ 1:1, 74.0 mg) first via silica gel CC (3.0 × 45 cm) with $CH_2Cl_2$–MeOH–$H_2O$ (15:1:0.05, v/v), and then was further purified by using semi-preparative HPLC. The method was developed in a way that resulted in an isocratic gradient of $CH_3CN$–$H_2O$ containing 0.1% (v/v) HAc (13:87, v/v) for 32 min, followed by $CH_3CN$–$H_2O$ (95:5, v/v) for 5 min (flow rate: 3 mL/min; **8**: $t_R$ = 27.5 min).

Fr.4 ($CH_2Cl_2$–MeOH 8:1, 1.0 g) was subjected to MCI gel CC (5.0 × 45 cm) using a stepwise gradient elution with MeOH/$H_2O$ (from 3:7 to MeOH neat, v/v) to afford compound **9** (30.2 mg), which was further purified by GPC on Sephadex LH-20 (column: 4.0 × 130 cm) in MeOH. Compounds **1** (3.0 mg), **6** (150.2 mg), and **7** (2.0 mg) were obtained from Fr.5 ($CH_2Cl_2$–MeOH 6:1, 9.6 g) through silica gel CC (column: 3.0 × 70 cm) with EtOAc–MeOH–HAc–$H_2O$ (80:3.5:3:1, v/v)]. Compound **6** was further purified by GPC on Sephadex LH-20 (column: 4.0 × 130 cm) in MeOH. Both compounds **1** and **7** were then re-purified by using semi-preparative HPLC. The method employed an isocratic gradient of $CH_3CN$–$H_2O$ containing 0.1% (v/v) HAc (1:9, v/v) for 36 min, followed by $CH_3CN$–$H_2O$ (95:5, v/v) for 5 min (flow rate: 3 mL/min; **1**: $t_R$ = 24.3 min, **7**: $t_R$ = 29.4 min).

Fr.6 ($CH_2Cl_2$–MeOH 4:1, 15 g) was subjected to silica gel CC (5.0 × 70 cm) with a $CH_2Cl_2$–MeOH gradient (9:1–1:1–MeOH neat, v/v) to afford four subfractions (Fr.6A–Fr.6D). Compounds **3** (3.1 mg), **4** (13.0 mg) and **5** (5.6 mg) were isolated from Fr.6B ($CH_2Cl_2$–MeOH 9:1, 2100 mg) via silica gel CC (4.0 × 70 cm) with EtOAc–MeOH–HAc–$H_2O$ (80:3.5:3:1, v/v) and were further purified by using semi-preparative HPLC. The method was developed in a way that resulted in an isocratic gradient of MeOH in $H_2O$ containing 0.1% (v/v) HAc (24:76, v/v) for 36 min, and followed by MeOH–$H_2O$ (95:5, v/v) for 5 min (flow rate: 3 mL/min; **3**: $t_R$ = 29.6 min, **4**: $t_R$ = 23.9 min, **5**: $t_R$ = 19.9 min). Fr.6C ($CH_2Cl_2$–MeOH 6:1, 1500 mg) was subjected to silica gel CC (4.0 × 70 cm) with EtOAc–MeOH–HAc–$H_2O$ (80:3.5:3:1, v/v) as the eluent to furnish compound **2** (14.1 mg), which was further purified by Sephadex LH-20 GPC (column: 3.0 × 98 cm) in MeOH.

### 4.4. Gynostemoside A (**1**)

White amorphous power; $[\alpha]_D^{22}$ −26.1 (*c* 0.13, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 232 (3.71); CD (MeOH) $\Delta\varepsilon_{238nm}$ −8.69, $\Delta\varepsilon_{208nm}$ +7.77; IR (film, MeOH) $\nu_{max}$ 3359 (*br*), 2922, 2850, 1641, 1560, 1465, 1426, 1360, 1274, 1168, 1138 $cm^{-1}$; for $^{13}C$ and $^1H$ NMR spectroscopic data, see Tables 1 and 2; ESIMS *m*/*z* = 427 $[M+Na]^+$, 831 $[2M+Na]^+$, 449 $[M+HCOO^-]^-$, 807 $[2M-H]^-$; HR-ESIMS *m*/*z* = 427.1974 $[M+Na]^+$ (calcd for $C_{19}H_{32}O_9Na$, 427.1944, $\Delta_m$ = +7.0 ppm).

### 4.5. Gynostemoside B (**2**)

White amorphous power; $[\alpha]_D^{22}$ −17.9 (*c* 0.34, MeOH); IR (film, MeOH) $\nu_{max}$ 3392 (*br*), 2971, 2926, 2900, 1407, 1393, 1251, 1077, 1040, 1027, 973, 891 $cm^{-1}$; for $^{13}C$ and $^1H$ NMR spectroscopic data, see Tables 1 and 2; ESIMS *m*/*z* = 429 $[M+Na]^+$, 451 $[M+HCOO^-]^-$, 811 $[2M-H]^-$; HR-ESIMS *m*/*z* = 429.2064 $[M+Na]^+$ (calcd for $C_{19}H_{34}O_9Na$, 429.2101, $\Delta_m$ = −8.5 ppm).

### 4.6. Gynostemoside C (**3**)

White amorphous power; $[\alpha]_D^{22}$ −9.9 (*c* 0.15, MeOH); IR (film, MeOH) $\nu_{max}$ 3416 (*br*), 2987, 2971, 2901, 1407, 1394, 1382, 1250, 1230, 1076, 1066, 1056, 1028, 892 $cm^{-1}$; for $^{13}C$ and $^1H$ NMR spectroscopic data, see Tables 1 and 2; ESIMS *m*/*z* = 413 $[M+Na]^+$, 803 $[2M+Na]^+$, 435 $[M+HCOO^-]^-$, 779 $[2M-H]^-$; HR-ESIMS *m*/*z* = 413.2143 $[M+Na]^+$ (calcd for $C_{19}H_{34}O_8Na$, 413.2151, $\Delta_m$ = −2.0 ppm).

### 4.7. Gynostemoside D (**4**)

White amorphous powder; $[\alpha]_D^{22}$ −27.3 (*c* 0.64, MeOH); IR (film, MeOH) $\nu_{max}$ 3379 (*br*), 2922, 2866, 1455, 1346, 1055, 1033,

1014 $cm^{-1}$; for $^{13}C$ and $^{1}H$ NMR spectroscopic data, see Tables 1 and 2; ESIMS $m/z$ = 413 $[M+Na]^+$, 803 $[2M+Na]^+$, 435 $[M+HCOO^-]^-$, 779 $[2M-H]^-$; HR-ESIMS $m/z$ = 413.2182 $[M+Na]^+$ (calcd for $C_{19}H_{34}O_8Na$, 413.2151, $\Delta_m$ = +7.4 ppm).

### 4.8. Gynostemoside E (**5**)

White amorphous powder; $[\alpha]_D^{22}$ −30.9 (*c* 0.23, MeOH); IR (film, MeOH) $\nu_{max}$ 3385 (*br*), 2962, 2924, 1367, 1167, 1074, 1039 $cm^{-1}$; for $^{13}C$ and $^{1}H$ NMR spectroscopic data, see Tables 1 and 2; ESIMS: $m/z$ = 413 $[M+Na]^+$, 435 $[M+HCOO^-]^-$, 779 $[2M-H]^-$; HR-ESIMS: $m/z$ = 413.2113 $[M+Na]^+$ (calcd for $C_{19}H_{34}O_8Na$, 413.2151, $\Delta_m$ = −9.3 ppm).

### 4.9. Enzymatic hydrolysis of compounds **1**–**5**

A solution of compounds **1**–**5** (1.4, 13.0, 1.6, 5.5, 1.5 mg, respectively) in 0.2 M acetic acid and sodium acetate buffer (pH 3.8, 1.5 mL) was treated with lyophilized β-glucosidase (13.2, 41.2, 15.0, 36.3, 14.9 mg, respectively) from almonds, and the solution was stirred at 40 °C for 48 h. After cooling, the reaction mixture of compound **1** was extracted with EtOAc (2 mL × 3). D-glucose in the aqueous residue was detected by comparison with an authentic sample. For compound **2**: after cooling, the reaction mixture was extracted with EtOAc (2 mL × 3). The EtOAc extract was then concentrated under reduced pressure, and the residue was purified by semi-preparative HPLC [ACN–$H_2O$–HAc (16:84:0.1, v/v/v)] to give **2a** (4.9 mg, 62.7%). Similarly, each EtOAc extract from the enzymatic hydrolysis product of **3**–**5** was concentrated and then subjected to silica gel CC [2.0 × 25 cm, EtOAc–MeOH–HAc–$H_2O$ (36:3.5:3:1, v/v/v/v)] to afford **8** with a yield of 0.7 (74.8%), 2.0 (62.2%), 0.7 (79.8%) mg, respectively.

### 4.10. Preparation of 3,9-di-O-(S)-MTPA ester (**2b**) and 3,9-di-O-(R)-MTPA ester (**2c**) of **2a**

A solution of **2a** (2.4 mg) in dry $CH_2Cl_2$ (1.5 mL) was treated with (*S*)-MTPA (37.7 mg) in the presence of EDC·HCl (31.2 mg) and 4-DMAP (19.7 mg), and the mixture was stirred at room temperature for 90 min. The reaction mixture was added to $CH_2Cl_2$ (1.5 mL) and successively washed with $H_2O$ (1.5 mL), 2 M HCl (1.5 mL), $NaHCO_3$-saturated $H_2O$ (1.5 mL), and brine (1.5 mL), respectively. The organic layer was combined and dried ($Na_2SO_4$) and evaporated under reduced pressure. The residue was purified by semi-preparative HPLC [ACN–$H_2O$–HAc (72:28:0.1, v/v/v)] to furnish **2b** (2.6 mg, 39.1%). Through a similar procedure, **2c** (2.5 mg, 37.6%) was generated from 2.4 mg of **2a** using (*R*)-MTPA (35.6 mg) in a $CH_2Cl_2$ solution containing EDC·HCl (30.1 mg) and 4-DMAP (18.6 mg). (3*S*, 4*S*, 5*R*, 6*R*)-3,4,6-Trihydroxyl-5,6-dihydro-β-ionol-3,9-di-*O*-(*S*)-MTPA ester (**2b**): $^{1}H$ NMR ($CDCl_3$, 500 MHz) δ 0.58 (3H, *s*, $CH_3$-12), 0.71 (3H, *s*, $CH_3$-11), 0.94 (3H, *d*, *J* = 7.0 Hz, $CH_3$-13), 1.41 (3H, *d*, *J* = 6.5 Hz, $CH_3$-10), 1.42 (1H, overlapped, H-$2_{eq}$), 1.93 (1H, *m*, H-5), 2.13 (1H, *dd*, *J* = 16.0, 3.5 Hz, H-$2_{ax}$), 3.54 (3H, *s*, $OCH_3$), 3.55 (3H, *s*, $OCH_3$), 3.68 (1H, *br s*, H-4), 5.26 (1H, *d*, *J* = 3.5 Hz, H-3), 5.50 (1H, *d*, *J* = 15.6 Hz, H-7), 5.53 (1H, *dd*, *J* = 15.6, 6.2 Hz, H-8), 5.59 (1H, *m*, H-9), 7.33–7.37 (3H, *m*), 7.41–7.43 (3H, *m*), and 7.50–7.53 (4H, *m*). (3*S*, 4*S*, 5*R*, 6*R*)-3,4,6-Trihydroxyl-5,6-dihydro-β-ionol-3,9-di-*O*-(*R*)-MTPA ester (**2c**): $^{1}H$ NMR ($CDCl_3$, 500 MHz) δ 0.78 (3H, *s*, $CH_3$-11), 0.80 (3H, *s*, $CH_3$-12), 0.85 (3H, *d*, *J* = 7.1 Hz, $CH_3$-13), 1.36 (3H, *d*, *J* = 6.2 Hz, $CH_3$-10), 1.45 (1H, *d*, *J* = 16.1 Hz, H-$2_{eq}$), 1.67 (1H, *m*, H-5), 2.20 (1H, *dd*, *J* = 16.1, 3.0 Hz, H-$2_{ax}$), 3.51 (3H, *s*, $OCH_3$), 3.54 (3H, *s*, $OCH_3$), 3.58 (1H, *br s*, H-4), 5.27 (1H, *d*, *J* = 3.0 Hz, H-3), 5.56–5.63 (3H, H-7, H-8 and H-9), 7.35–7.40 (6H, *m*), and 7.50–7.51 (4H, *m*).

## Acknowledgments

The authors gratefully acknowledge Prof. Jian-Wei Chen (Nanjing University of TCM) for the plant identification. This work was supported by NSFC Grants (90713040, 30640068), NCET Grant (NCET-06-0422), and STCSM Grants (07DZ22006, 06DZ19002, 06PJ14033).

## Appendix A. Supplementary data

Pivotal NMR, CD and LR-/HR-ESIMS spectra of isolated compounds (**1**–**9**, **11**, **12**) and semisynthetics (**2b**, **2c**) are available as Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2009.12.017.

## References

Alija, A.J., Bresgen, N., Sommerburg, O., Langhans, C.D., Siems, W., Eckl, P.M., 2006. β-Carotene breakdown products enhance genotoxic effects of oxidation stress in primary rat hepatocytes. Carcinogenesis 27, 1128–1133.

Baumes, R., Wirth, J., Bureau, S., Gunata, Y., Razungles, A., 2002. Biogeneration of $C_{13}$-norisoprenoid compounds: experiments supportive for an apo-carotenoid pathway in grapevines. Anal. Chim. Acta 458, 3–14.

Chen, W.L., Liu, Q.F., Wang, J., Zou, J., Meng, D.H., Zuo, J.P., Zhu, X.Z., Zhao, W.M., 2006. New guaiane, megastigmane and eudesmane-type sesquiterpenoids and anti-inflammatory constituents from *Youngia japonica*. Planta Med. 72, 143–150.

Duncan, R.E., Lau, D., El-Sohemy, A., Archer, M.C., 2004. Geraniol and β-ionone inhibit proliferation, cell cycle progression, and cyclin-dependent kinase 2 activity in MCF-7 breast cancer cells independent of effects on HMG-CoA reductase activity. Biochem. Pharmacol. 68, 1739–1747.

Enzell, C., 1985. Biodegradation of carotenoids – an important route to aroma compounds. Pure Appl. Chem. 57, 693–700.

Hu, L.H., Chen, Z.L., Xie, Y.Y., 1996. New triterpenoid saponins from *Gynostemma pentaphyllum*. J. Nat. Prod. 59, 1143–1145.

Ibraheim, Z.Z., Abdallah, O.M., 1994. Miner constituents of *Maerua crassifolia* Forssk growing in Egypt. Bull. Fac. Pharm. Cairo Univ. 32, 407–410.

Ito, H., Kobayashi, E., Li, S.H., Hatano, T., Sugita, D., Kubo, N., Shimura, S., Itoh, Y., Tokuda, H., Nishino, H., 2002. Antitumor activity of compounds isolated from leaves of *Eriobotrya japonica*. J. Agric. Food Chem. 50, 2400–2403.

Janakiram, N.B., Cooma, I., Mohammed, A., Steele, V.E., Rao, C.V., 2008. β-Ionone inhibits colonic aberrant crypt foci formation in rats, suppresses cell growth, and induces retinoid × receptor-α in human colon cancer cells. Mol. Cancer Ther. 7, 181–190.

Jiangsu New Medicine College, 1977. Dictionary of Chinese Medicinal Materials. Shanghai Scientific and Technological Publisher, Shanghai. p. 16–17.

Jung, M., Mo, H.B., Elson, C.E., 1998. Synthesis and biological activity of β-ionone-derived alcohols for cancer chemoprevention. Anticancer Res. 18, 189–192.

Kuang, H.X., Xia, Y.G., Yang, B.Y., Wang, Q.H., Lü, S.W., 2008. Sesquiterpene glucosides from *Chloranthus japonicus* Sieb. Chem. Biodivers. 5, 1736–1742.

Kubo, I., Morimitsu, Y., 1995. Cytotoxicity of green tea flavor compounds against two solid tumor cells. J. Agric. Food Chem. 43, 1626–1628.

Liu, X., Ye, W.C., Mo, Z.Y., Yu, B., Zhao, S.X., Wu, H.M., Che, C.T., Jiang, R.W., Mak, T.C.W., Hsiao, W.L.W., 2004a. Five new ocotillone-type saponins from *Gynostemma pentaphyllum*. J. Nat. Prod. 67, 1147–1151.

Liu, H.L., Kao, T.H., Chen, B.H., 2004b. Determination of carotenoids in the Chinese medicine herb Jiao-Gu-Lan (*Gynostemma pentaphyllum* Makino) by liquid chromatography. Chromatographia 60, 411–417.

Liu, J.R., Yang, B.F., Chen, B.Q., Yang, Y.M., Dong, H.W., Song, Y.Q., 2004c. Inhibition of β-ionone on SGC-7901 cell proliferation and upregulation of metalloproteinases-1 and -2 expression. World J. Gastroenterol. 10, 167–171.

Liu, J.R., Chen, B.Q., Yang, B.F., Dong, H.W., Sun, C.H., Wang, Q., Song, G., Song, Y.Q., 2004d. Apoptosis of human gastric adenocarcinoma cells induced by β-ionone. World J. Gastroenterol. 10, 348–351.

Liu, J.R., Sun, X.R., Dong, H.W., Sun, C.H., Sun, W.G., Chen, B.Q., Song, Y.Q., Yang, B.F., 2008. β-Ionone suppresses mammary carcinogenesis, proliferative activity and induces apoptosis in the mammary gland of the Sprague-Dawley rat. Int. J. Cancer 122, 268–2698.

Norberg, Å., Hoa, N.K., Liepinsh, E., Phan, D.V., Thuan, N.D., Jörnvall, H., Sillard, R., Östenson, C.G., 2004. A novel insulin-releasing substance, phanoside, from the plant *Gynostemma pentaphyllum*. J. Biol. Chem. 279, 41361–41367.

Ohtani, I., Kusumi, T., Kashman, Y., Kakisawa, H., 1991. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. J. Am. Chem. Soc. 113, 4092–4096.

Osorio, C., Duque, C., Fujimoto, Y., 1999. $C_{13}$-norisoprenoid glucoconjugates from Lulo (*Solanum quitoense* L.) leaves. J. Agric. Food Chem. 47, 1641–1645.

Pabst, A., Barron, D., Sémon, E., Schreier, P., 1992. Two diastereomeric 3-oxo-α-ionol β-D-glucosides from raspberry fruit. Phytochemistry 31, 1649–1652.

Pérez, C., Trujillo, J., Almonacid, L.N., Trujillo, J., Navarro, E., Alonso, S.J., 1996a. Absolute structures of two new $C_{13}$-norisoprenoids from *Apollonias barbujana*. J. Nat. Prod. 59, 69–72.

Pérez, C., Trujillo, J.M., Almonacid, L.N., Navarro, E., Alonso, S.J., 1996b. New $C_{13}$-norisoprenoids from *Apollonias barbujana*. Nat. Prod. Lett. 8, 1–6.

Perrone, A., Plaza, A., Bloise, E., Nigro, P., Hamed, A.I., Belisario, M.A., Pizza, C., Piacente, S., 2005. Cytotoxic furostanol saponins and a megastigmane glucoside from *Tribulus parvispinus*. J. Nat. Prod. 68, 1549–1553.

Razmovski-Naumovski, V., Huang, T.H.W., Tran, V.H., Li, G.Q., Duke, C.C., Roufogalis, B.D., 2005. Chemistry and pharmacology of *Gynostemma pentaphyllum*. Phytochem. Rev. 4, 197–219.

Rodríguez, M.L., Brito, B.I., González, A.G., Almonacid, L.N., Pérez, C., Trujillo, J.M., 1992. Structure of a new $C_{13}$-norisoprenoid. Acta Cryst. C48, 2192–2194.

Rujjanawate, C., Kanjanapothi, D., Amornlerdpison, D., 2004. The anti-gastric ulcer effect of *Gynostemma pentaphyllum* Makino. Phytomedicine 11, 431–435.

Setha, S., Kondo, S., Hirai, N., Ohigashi, H., 2004. Xanthoxin, abscisic acid and its metabolite levels associated with apple fruit development. Plant Sci. 166, 493–499.

Sun, Y., Zhan, Y.C., Sha, Y., Pei, Y.H., 2007. Norisoprenoids from *Ulva lactuca*. J. Asian Nat. Prod. Res. 9, 321–325.

Takeda, Y., Okada, Y., Masuda, T., Hirata, E., Shinzato, T., Takushi, A., Yu, Q., Otsuka, H., 2000. New megastigmane and tetraketide from the leaves of *Euscaphis japonica*. Chem. Pharm. Bull. 48, 752–754.

Takeda, Y., Shimizu, H., Masuda, T., Hirata, E., Shinzato, T., Bando, M., Otsuka, H., 2004. Lasianthionosides A–C, megastigmane glucosides from leaves of *Lasianthus fordii*. Phytochemistry 65, 485–489.

Tan, S.T., Wilkins, A.L., Holland, P.T., 1989. Isolation and X-ray crystal structure of (*E*)-4-(r-1′,t-2′,c-4′-trihydroxy-2′,6′,6′-trimethyl-cyclohexyl)but-3-en-2-one, a constituent of New Zealand thyme honey. Aust. J. Chem. 42, 1799–1804.

Umehara, K., Hattori, I., Miyase, T., Ueno, A., Hara, S., Kageyama, C., 1988. Studies on the constituents of leaves of *Citrus unshiu* Marcov. Chem. Pharm. Bull. 36, 5004–5008.

Walton, D.C., Dorn, B., Fey, J., 1973. The isolation of an abscisic-acid metabolite 4′-dihydrophaseic acid, from non-imbibed *Phaseolus vulgaris* seed. Planta (Berl.) 112, 87–90.

Wu, S.B., Ji, Y.P., Zhu, J.J., Zhao, Y., Xia, G., Hu, Y.H., Hu, J.-F., 2009. Steroids from the leaves of Chinese *Melia azedarach* and their cytotoxic effects on human cancer cell lines. Steroids 74, 761–765.

Wu, S.B., Pang, F., Wen, Y., Zhang, H.-F., Zhao, Z., Hu, J.-F., 2010. Antiproliferative and apoptotic activities of linear furocoumarins from *Notopterygium incisum* on cancer cell lines. Planta Med. 76, 82–85.

Yu, S.G., Anderson, P.J., Elson, C.E., 1995. Efficacy of $\beta$-ionone in the chemoprevention of rat mammary carcinogenesis. J. Agric. Food Chem. 43, 2144–2147.

Zaharia, L.I., Galka, M.M., Ambrose, S.J., Abrams, S.R., 2005. Preparation of deuterated abscisic acid metabolites for use in mass spectrometry and feeding studies. J. Labelled Compd. Radiopharm. 48, 435–445.

Zhou, J.M., Geng, G.Y., Batist, G., Wu, J.H., 2009. Syntheses and potential anti-prostate cancer activities of ionone-based chalcones. Bioorg. Med. Chem. Lett. 19, 1183–1186.