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Update in Bioinformatics

Potent α-glucosidase inhibitors from the roots of *Panax japonicus* C. A. Meyer var. *major*

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ABSTRACT

Bioassay-guided fractionation of the $CHCl_3$ soluble portion of the roots of *Panax japonicus* C. A. Meyer var. *major* afforded an active fraction with inhibitory activity against baker's yeast α-glucosidase with an IC_{50} value 1.02 mg/mL. Furthermore, the active fraction isolated contained three previously unreported polyacetylenes, designated panaxjapynes A–C, together with 11 other compounds, including four polyacetylenes, five phenolic compounds, a sesquiterpenoid, and a sterol glucoside. The structures of the compounds were elucidated by spectroscopic and chemical methods. Compared with the control acarbose (IC_{50} 677.97 μM), six compounds were shown to be more potent α-glucosidase inhibitors with IC_{50} values in the range 22.21–217.68 μM.

1. Introduction

The roots of *Panax* species (Araliaceae) are widely used either in Chinese herbal medicine or in food in Asian regions, primarily because it is well-known for its medicinal properties. Specifically, the active agents in the roots of *Panax* species include polyacetylenes and dammarane saponins. Research in the field of pharmacology of dammarane saponins has been investigated for decades, and ample knowledge regarding compounds such as ginsenoside-Re, -Rh1, -Rb1, and -Rg1 has been obtained (Cho et al., 2004, 2006; Lee et al., 2003a,b; Leung et al., 2007). In traditional Chinese medicine, the roots of *Panax japonicus* C. A. Meyer var. *major* (Chinese name: Zu-Tziseng or Zhuershen) are prescribed as an expectorant, an antitussive, a hemostatic, a mediate sedative, an analgesic and an antitussive (State Administration of Traditional Chinese Medicine, 1999). Generally speaking, the roots have been reported in pharmacological studies on ginseng saponins (Morita et al., 1982). In contrast with earlier investigations, research interests have increasingly shifted towards to polyacetylenes, and it has been reported that the Araliaceae family is notable as a rich source of C17 polyacetylenes (Satoh et al., 2007; Bernart et al., 1996). Polyacetylenes have been reported to demonstrate various biological activities, such as cytotoxicity and antiplatelet effects (Jung et al., 2002; Bernart et al., 1996; Teng et al., 1989). In addition, Wang et al. reported that falcarindiol, which is a C17 polyacetylene isolated from *Aegiceras corniculatum*, showed inhibitory activity towards PTP1B (Wang et al., 2006). PTP1B is a key element in the negative regulation of the insulin signaling pathway and plays an important role in diabetes. α-Glucosidase, an hydrolytic enzyme, is essential during digestion of carbohydrates and in biosynthesis of glycoproteins. Inhibitors of α-glucosidase may potentially reduce progression of diabetes by decreasing digestion and absorption of carbohydrates (Ma et al., 2008). Interestingly, this finding indicates that polyacetylenes have a potential positive effect on diabetes. Thus, we report the isolation and structure elucidation of three polyacetylenes, panaxjapyne A–C (**1**–**3**), together with 11 known compounds (**4**–**14**) from the active $CHCl_3$-soluble portion (PJC) of the roots of *P. japonicus* C. A. Meyer var. *major*. We also present an evaluation of the α-glucosidase inhibitory activities of the isolated compounds.

2. Results and discussion

The ethanolic extract of the roots of *P. japonicus* var. *major* exhibited α-glucosidase inhibitory activity with an IC_{50} of 64.68 mg/mL. The crude ethanol extract was partitioned with $CHCl_3$ (PJC), *n*-BuOH (PJB) and H_2O (PJW), in successive extractions. The $CHCl_3$ fraction (PJC) showed α-glucosidase inhibitory activity with an IC_{50} value of 1.02 mg/mL, while the PJB and the PJW extracts gave IC_{50} >11.75 and >11.76 mg/mL. The active $CHCl_3$

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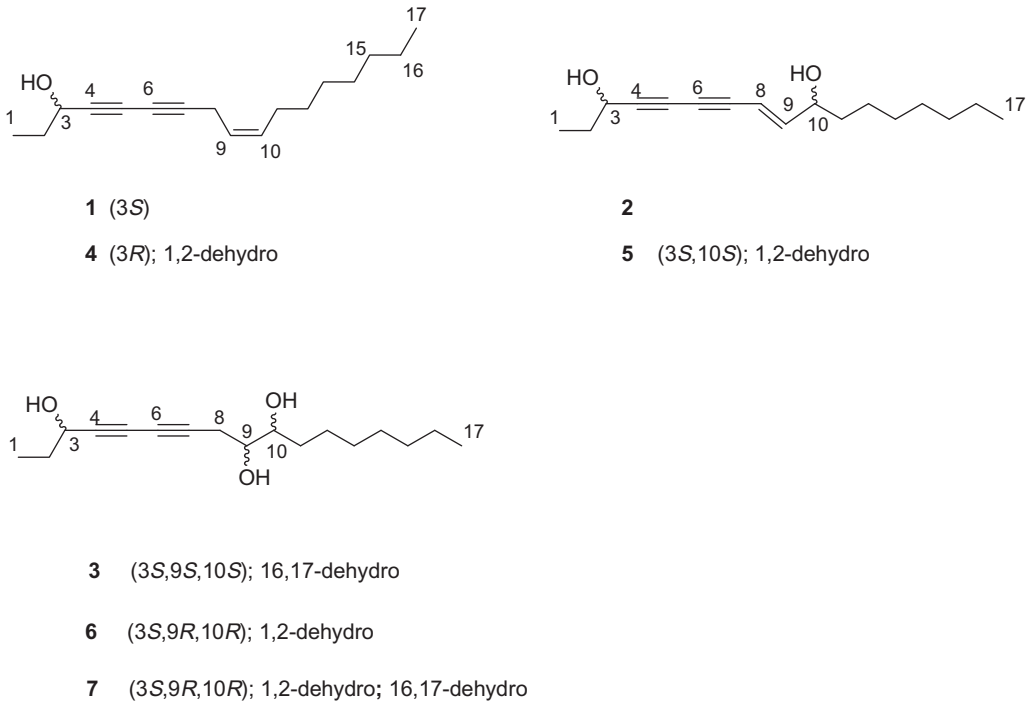

1 (3*S*)

2

3 (3*S*,9*S*,10*S*); 16,17-dehydro

4 (3*R*); 1,2-dehydro

5 (3*S*,10*S*); 1,2-dehydro

6 (3*S*,9*R*,10*R*); 1,2-dehydro

7 (3*S*,9*R*,10*R*); 1,2-dehydro; 16,17-dehydro

Fig. 1. Structures of compounds **1**–**7** isolated from the roots of *P. japonicus* var. *major*.

fraction was purified by column chromatography and HPLC using different solvent combinations, affording three panaxjapyne A–C (**1–3**) (Fig. 1) and 11 known compounds identified as (3*R*)-(−)-falcarinol (**4**) (Zheng et al., 1999), (3*S*,10*S*)-panaxydiol (**5**) (Hirakura et al., 1992), (3*S*,9*R*,10*R*)-panaxytriol (**6**) (Kobayashi et al., 1999), (3*S*,9*R*,10*R*)-gensenoyne C (**7**) (Hirakura et al., 1991; Satoh et al., 2002), docosyl *trans*-ferulate (**8**) (Kuo and Chen, 1999), vanillin (**9**) (Ito et al., 2001), syring-aldehyde (**10**) (Chen et al., 1997), 3,4,5-trimethoxy benzoic acid (**11**) (Chen et al., 1997), 2,6-dimethoxyphenol (**12**) (Goda et al., 1987), 1β,6α-dihydroxy-4(14)-eudesmene (**13**) (Hu et al., 1996) and β-sitosteryl β-D-glucoside (**14**) (Basent et al., 1993) by comparing their spectroscopic data with those reported in the literature.

Panaxjapyne A, compound (**1**), was obtained as a yellowish oil with a positive optical rotation ($[\alpha]_D$ +50.0). The HRESIMS of **1** exhibited a sodiated molecular ion peak at *m/z* 269.1880 $[M+Na]^+$, consistent with the molecular formula $C_{17}H_{26}O$. This was supported by the presence of 17 carbon signals in its ^{13}C NMR spectrum. UV absorptions at 269 and 254 nm suggested the presence of a conjugated diyne in **1** (Hirakura et al., 1992), and an IR absorption for a triple bond was noted at 2234 cm^{-1}, and other bands were assigned to hydroxyl (3422 cm^{-1}) and olefinic (1679 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed two olefinic proton doublets at δ 5.49 and 5.36, with a coupling constant of 10.4 Hz attributed to a double bond in the *cis* configuration. An oxymethine proton appeared at δ 4.34. The ^{13}C NMR spectrum (Table 1) combined with HMQC experiments indicated four non-protonated alkyne carbons (δ 79.5, 76.7, 69.9 and 64.1), an oxygen-bearing carbon (δ 64.0), two olefinic carbon resonances (δ 133.0 and 122.0), two methyl groups (δ 9.3 and 14.1) and eight methylenes (δ 30.7, 31.8, 29.2 (C × 3), 27.2, 22.6 and 17.6). A terminal methyl group at δ 1.00 (H-1) showed a ^3J HMBC correlation with C-3 (δ 64.0) and ^1H–^1H COSY crosspeaks with the H-2 (δ 1.72) methylene protons. Furthermore, the HMBC spectrum showed ^2J and ^3J correlations of H-3 (δ 4.34) with C-4 (δ 76.7) and C-5 (δ 69.9), and H-8 (δ 3.01) with C-6 (δ 79.5) and C-7 (δ 64.1), indicating a conjugated diyne system between C-3 and C-8. This finding was further confirmed by HMBC correlations of H-8 to C-9/C-10, H-11 (δ 2.01) to C-9/C-10/C-12/C-13, of the olefinic proton H-9 to C-7/C-8 and of the second terminal methyl group H-17 (δ 0.99) to C-15/C-16. The stereochemistry at C-3 was determined using a modified Mosher's method for elucidation of the absolute configuration of a secondary alcohol by application of ^1H NMR spectroscopy (Ohtani et al., 1991). Compound **1** was treated with (*S*)-(+)- and (*R*)-(−)-α-methoxyl-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) to afford the (*R*)- and (*S*)-MTPA esters **1a** and **1b**, respectively. Analysis of the difference in chemical shifts of the neighboring protons H-1 (−0.026 ppm), H-2 (−0.068 ppm) and H-8 (+0.005 ppm) enabled assignment of the *S* configuration at C-3. On the basis of the above analysis, the structure of **1** was established as (*Z*)-heptadeca-9-en-4,6-diyn-(3*S*)-ol, and this compound has been named panaxjapyne A.

Panaxjapyne B, compound (**2**), was isolated as a yellowish oil with a negative optical rotation ($[\alpha]_D$ −120.0). Its HRESIMS showed a sodiated molecular ion peak at *m/z* 285.1832 $[M+Na]^+$, corresponding to the molecular formula $C_{17}H_{26}O_2$. The UV spectrum displayed typical absorption bands for a conjugated ene-yne-yne chromophore at λ_{max} 284, 268 and 254 nm (Hirakura et al., 1992), with IR absorption bands at 3391, 2230 and 1605 cm^{-1} due to hydroxyl, triple bond and olefinic functionalities, respectively. The ^1H NMR spectrum showed a pair of *trans* olefinic protons at δ 6.32 (dt, J = 15.0, 6.0 Hz) and 5.76 (d, J = 15.0 Hz) and two oxymethine protons at δ 4.42 (t, J = 6.5 Hz) and 4.19 (q, J = 6.0 Hz). The ^{13}C NMR spectrum of **2** displayed 17 carbon resonances, including four non-protonated acetylenic carbons (δ 82.9, 76.7, 73.7 and 69.6), two oxygen-bearing carbons (δ 64.2 and 72.1), two olefinic carbons (δ 149.7 and 108.1), two methyl groups (δ 14.1 and 9.3), and seven methylenes (δ 36.9, 31.8, 30.7, 29.4, 29.2, 25.2 and 22.6). The downfield shift of the olefinic protons in **2** compared with those in **1** indicated that the double bond was in conjugation with the diyne, this being further supported by long range ^3J and ^4J correlations of H-8 (δ 5.76) with C-5 (δ 69.6) and C-6 (δ 73.7) in the HMBC spectrum. In addition, the oxymethine H-10 (δ 4.19, d, J = 6.0 Hz) displayed crosspeaks with H-9

Table 1
^{13}C (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data for compounds **1**–**3** in $CDCl_3$.

No.	**1**		**2**		**3**	
	δ_C (*m*)	δ_H, *m* (*J*, Hz)	δ_C (*m*)	δ_H, *m* (*J*, Hz)	δ_C (*m*)	δ_H, *m* (*J*, Hz)
1	9.3 (*q*)	1.00, *t* (7.4)	9.3 (*q*)	1.03, *t* (8.0)	9.3 (*q*)	1.01, *t* (7.0)
2	30.7 (*t*)	1.72, *m*	30.7 (*t*)	1.77, *m*	30.7 (*d*)	1.75, *m*
3	64.0 (*d*)	4.34, *t* (6.0)	64.2 (*d*)	4.42, *t* (6.5)	64.1 (*d*)	4.36, *t* (6.5)
4	76.7 (*s*)	–	82.9 (*s*)	–	77.3 (*s*)	–
5	69.9 (*s*)	–	69.6 (*s*)	–	69.5 (*s*)	–
6	79.5 (*s*)	–	73.7 (*s*)	–	66.7 (*s*)	–
7	64.1 (*s*)	–	76.7 (*s*)	–	77.3 (*s*)	–
8	17.6 (*t*)	3.01, *d* (6.8)	108.1 (*d*)	5.76, *d* (15.0)	25.0 (*t*)	2.60, dd (17.0, 5.5) 2.55, dd (17.0, 5.5)
9	122.0 (*d*)	5.49, *dt* (6.8)	149.7 (*d*)	6.32, dd (15.0, 6.0)	72.1 (*d*)	3.64, *m*
10	133.0 (*d*)	5.36, *dt* (10.4, 7.0)	72.1 (*d*)	4.19, *q* (6.0)	73.0 (*d*)	3.60, *m*
11	27.2 (*t*)	2.01, *q* (7.0)	36.9 (*t*)	1.52, *m*	33.5 (*t*)	1.51, *m*
12	29.2 (*t*)	1.35, *m*	25.2 (*t*)	1.38, *m*	25.4 (*t*)	1.41, *m*
13	29.2 (*t*)	1.27, *m*	29.4 (*t*)	1.28, *m*	28.8 (*t*)	1.41, *m*
14	29.2 (*t*)	1.27, *m*	29.2 (*t*)	1.28, *m*	29.0 (*t*)	1.41, *m*
15	31.8 (*t*)	1.27, *m*	31.8 (*t*)	1.28, *m*	33.7 (*t*)	2.05, *m*
16	22.6 (*t*)	1.27, *m*	22.6 (*t*)	1.28, *m*	139.0 (*t*)	5.80, ddt (15.0, 10.4, 6.4)
17	14.1 (*q*)	0.99, *t* (6.6)	14.1 (*q*)	0.88, *t* (5.0)	114.3 (*d*)	4.99, dd (15.0, 1.5) 4.94, dd (10.4, 1.5)

(δ 6.32, dd, J = 15.0, 6.0 Hz) and H-11 (δ 1.52, m) in the COSY spectrum. The HMBC experiment showed correlations of H-10 to C-8/C-9/C-11/C-12, and the C12–17 moieties were confirmed by analysis of COSY and HMBC spectra. Based on the above results, the structure of **2** was established as (*E*)-heptadeca-8-en-4,6-diyne-3,10-diol, and this compound has been named panaxjapyne B. The absolute configuration of **2** was not elucidated because of a lack of material.

Panaxjapyne C (**3**) was obtained as a yellowish oil with a positive optical rotation ($[\alpha]_D$ +20.6). The HRESIMS exhibited a sodiated molecular ion peak at *m/z* 301.1778 $[M+Na]^+$ corresponding to the molecular formula $C_{17}H_{26}O_3$. The UV absorption maxima at 269 and 256 nm suggested the presence of a conjugated diyne (Hirakura et al., 1992), whereas the IR spectrum contained hydroxyl (3399 cm^{-1}), triple bond (2234 cm^{-1}) and double bond (1655 cm^{-1}) functionalities, respectively. The ^1H NMR spectrum showed terminal vinyl protons at δ 5.80 (ddt, J = 15.0, 10.4, 6.4 Hz), δ 4.99 (dd, J = 15.0, 1.5 Hz) and δ 4.94 (dd, J = 10.4, 1.5 Hz) as well as three oxymethine protons (δ 4.36, 3.64 and 3.60). The ^{13}C NMR spectrum, aided by HMQC analysis, exhibited four non-protonated acetylene carbons (δ 77.3, 77.3, 69.5 and 66.7), three oxygen-bearing carbons (δ 73.0, 72.1 and 64.1), two olefinic carbons (δ 139.0 and 114.3), a methyl group (δ 9.3) and seven methylenes (δ 30.7, 29.0, 28.8, 25.4 and 25.0). The NMR spectroscopic data of **3** was similar to that of **1** except for the presence of signals corresponding to two oxymethine protons at δ 3.64 (m) and 3.60 (m) and terminal vinyl protons at δ 5.80, 4.99 and 4.94 in **3** instead of two *cis* coupled olefinic protons (δ 5.49 and 5.36) and a terminal methyl group (δ 0.99) in **1**. This finding suggested that the olefinic double bond and the methyl group in **3** were hydroxylated and dehydrogenated, respectively. Key ^1H–^1H COSY correlations were also observed between H-9 (δ 3.64) and H-10 (δ 3.60), H-15 (δ 2.05, m) and H-16 (δ 5.80), and H-16 to H-17 (δ 4.99, 4.94) in the COSY spectrum. This assignment was further supported by HMBC correlations of H-8 (δ 2.60 and 2.55) to C-6 (δ 66.7)/C-7 (δ 77.3)/C-9 (δ 72.1)/C-10 (δ 73.0), H-10 (δ 3.60) to C-8/C-9/C-11/C-12 (δ 25.4) and H-17 (δ 4.99 and 4.94) to C-15 (δ 33.7). Thus, the structure of **3** was elucidated as heptadeca-16-en-4,6-diyne-3,9,10-triol, and this compound was named panaxjapyne C.

Because the 9,10-diol in panaxytriol (**6**) was presumed to be obtained from hydrolysis of its corresponding expoxy moiety, two stereochemical isomers, 9*R*,10*R* and 9*S*,10*S*, are possible. Recently, Satoh et al. synthesized four panaxytriol isomers [(3*R*,9*R*,10*R*), (3*S*,9*R*,10*R*), (3*R*,9*S*,10*S*), (3*S*,9*S*,10*S*)] and reported their optical rotation values of −18.6, +47.3, −47.3 and +17.6, respectively (Satoh et al., 2002). We also isolated panaxytriol (**6**) and recorded an optical rotation of $[\alpha]_D$ +46.7, and thus concluded that the absolute configuration of **6** was 3*S*, 9*R*, 10*R*. Furthermore, the optical rotation of **3** was $[\alpha]_D$ +20.6 and showed similar structural features to those of **6**, thus the stereochemistry at C-3, C-9 and C-10 was assigned as *S*, *S*, *S* in compound **3**.

Using the same method as applied to compound **7**, $[\alpha]_D$ +46.0, the absolute configurations were assigned as 3*S*,9*R*,10*R*. In addition, the absolute configuration of 3*R* in the known compound **4** ($[\alpha]_D$ −30.6) was determined by Mosher's method, using the difference in the chemical shifts of H-1 (+0.066 ppm), H-2 (+0.087 ppm) and H-8 (−0.009 ppm). The stereochemical configurations 3*S*,10*S* in compound **5** were obtained by comparison of the optical rotation ($[\alpha]_D$ −36.1) with the literature value of −33.8 (Kwon et al., 1997).

Compounds **1**–**14**, excluding **2** and **11**, were tested for their inhibitory effect on yeast α-glucosidase activity. Among them, **1**, **3**, **4**, **5**, **9** and **14** exhibited significant inhibition compared with the control drug acarbose (IC_{50} 677.97 μM), having IC_{50} values of 71.82, 175.42, 67.78, 22.21, 217.68 and 75.00 μM, respectively (Table 2). Compound **1** (IC_{50} 71.82 μM) gave more potent inhibition values than **3** (IC_{50} 175.42 μM), indicating that the diols at C-9 and C-10 and the terminal vinylic group are responsible for decreasing α-glucosidase inhibitory activity. Furthermore, compound **5** (IC_{50} 22.21 μM) exhibits a strong inhibitory effects.

Table 2
α-Glucosidase inhibitory effects of compounds **1**, **3**–**5**, **9** and **14**.

Compounds	IC_{50} (μM)
1	71.82 ± 1.22
3	175.42 ± 5.84
4	67.78 ± 0.01
5	22.21 ± 1.73
9	217.68 ± 5.32
14	75.00 ± 17.11
Acarbose	677.97

Results are presented as means ± SEM (n = 2–3).
Compounds **2** and **11** were not tested, and compounds **6**–**8**, **10**, **12** and **13** were inactive (IC_{50} > 1 mM).

3. Concluding remarks

Three polyacetylene compounds, (**1**–**3**), previously unreported, and 11 other compounds (**11**–**14**) were isolated by bioassay-guided fractionation of extracts of the roots of *P. japonicus* C. A. Meyer var. *major*. To the best of our knowledge, polyacetylenes isolated from *Panax* species have not been studied for their inhibitory effect on α-glucosidase activity, although a polyacetylenic acid isolated from the marine sponge *Callyspongia truncata* has been reported as an α-glucosidase inhibitor (Nakao et al., 2002). These findings show that there is an opportunity to develop polyacetylenes as possible antidiabetic agents or as dietary adjuncts to existing therapies.

4. Experimental

4.1. General procedures

IR spectra were recorded on a Shimadzu FTIR Prestige-21 spectrometer. Optical rotations were measured using a Jasco DIP-370 Polarimeter, whereas UV spectra were obtained on a Hitachi UV-3210 spectrophotometer. ESI and HRESI mass spectra were recorded on a Bruker APEX II mass spectrometer. NMR spectra, including ^{1}H, ^{13}C, COSY, NOESY, HMBC and HMQC experiments, were acquired on Bruker AVANCE-500 and AMX-400 instruments, using tetramethylsilane (TMS) as an internal standard; all chemical shifts are reported in parts per million (ppm, δ). Silica gel (E. Merck 70–230, 230–400 mesh) was used for column chromatography. RP-HPLC was carried out using a Shimadzu LC-8A pump and a SPD-10AV UV–Vis detector.

4.2. Plant material

The dried roots of *P. japonicus* var. *major* were collected in Yunnan province of China by Prof. H.D. Sun (State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences) in 2006. The material was identified by Prof. C.S. Kuoh (Department of Life Science, National Cheng Kung University), and a voucher specimen (TSWu 2006010) has been deposited in the Department of Chemistry, National Cheng Kung University, Tainan, Taiwan.

4.3. Extraction and isolation

The dried roots of *P. japonicus* var. *major* (4.6 kg) were pulverized and extracted with EtOH (5 × 10 L) for 6 h under conditions of reflux. The filtrate was concentrated under reduced pressure to obtain a crude ethanol extract (PJ, 1212 g), which was suspended in H_2O (1.5 L), and then partitioned with $CHCl_3$ (5 × 1 L) and *n*-BuOH (6 × 1 L) to obtain $CHCl_3$- (PJC, 77 g), *n*-BuOH- (PJB, 482 g) and H_2O-soluble portions (PJW), respectively. The ethanol extract, $CHCl_3$-, *n*-BuOH- and H_2O-soluble portions of *P. japonicus* var. *major* were found to inhibit α-glucosidase activity with IC_{50} values of 64.68, 1.02, >11.75 and >11.76 mg/mL, respectively. The active $CHCl_3$ extract was subjected to silica gel CC eluted with *n*-hexane–EtOAc (6:1) in a step gradient, gradually increasing the polarity with EtOAc, to afford eight fractions (Fr. 1–8). Purification of Fr. 2 was accomplished by using silica gel CC and eluted with *n*-hexane–EtOAc (9:1), followed by purification using reversed-phase HPLC and elution with MeOH–H_2O (82:18, v/v) to yield **1** (8.1 mg), **2** (1.9 mg), **4** (232 mg) and **5** (5.2 mg). Fr. 3 was purified by silica gel CC eluted with *n*-hexane–EtOAc (6:1) to obtain **4** (54 mg) and **8** (3.3 mg), whereas Fr. 4 was subjected to silica gel CC eluted with *n*-hexane–$CHCl_3$ (1:2) to afford **12** (2.3 mg). Fr. 5 was separated using silica gel CC eluted with *n*-hexane–$CHCl_3$ (1:2) and further purified by reversed phase HPLC eluted with MeOH–H_2O (72:28, v/v) to afford **3** (8.2 mg), **6** (1.6 mg), **7** (8.0 mg), **9** (4.1 mg), **10** (7.8 mg), **11** (2.1 mg) and **13** (2.1 mg). Fr. 7 was purified over a silica gel CC eluted with EtOAc–MeOH (29:1) to obtain **14** (137.1 mg).

4.4. Panaxjapyne A (*1*)

Yellow oil; $[\alpha]_D$ +50.0 (*c* 0.02, MeOH); UV (MeOH) (log ε) λ_{max} nm: 269 (1.4), 254 (1.2), 216 (2.9), 206 (1.0); IR (KBr)ν_{max} cm^{-1}: 3422, 3055, 3024, 2234, 1679, 1285, 1227, 1015, 968; for ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data, see Table 1; ESI-MS *m/z*: 269 [M+Na]$^+$, 231; HRESI-MS *m/z*: 269.1880 [M+Na]$^+$ (calc. 269.1881).

4.5. Panaxjapyne B (*2*)

Yellow oil; $[\alpha]_D$ −120.0 (*c* 0.01, MeOH); UV (MeOH) (log ε) λ_{max} nm: 284 (3.6), 268 (3.7), 254 (3.6), 215 (4.0), 206 (4.4); IR(KBr)ν_{max} cm^{-1}: 3391, 2928, 2855, 2230, 1713, 1605, 1462, 1286, 922; for ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data, see Table 1; ESI-MS *m/z*: 285 [M+Na]$^+$; HRESI-MS *m/z*: 285.1832 [M+Na]$^+$ (calc. 285.1830).

4.6. Panaxjapyne C (*3*)

Yellow oil; $[\alpha]_D$ +20.6 (*c* 0.02, MeOH); UV (MeOH) (log ε) λ_{max} nm: 269 (3.1), 256 (3.2), 243 (3.2), 207 (3.6); IR(KBr)ν_{max} cm^{-1}: 3399, 2882, 2854, 2234, 1740, 1655, 1231, 1045, 1022, 980; for ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data, see Table 1; ESI-MS *m/z*: 301 [M+Na]$^+$; HRESI-MS *m/z*: 301.1778 [M+Na]$^+$ (calc. 301.1780).

4.7. α-Glucosidase inhibitory assay

α-Glucosidase inhibitory activity was determined by modification of the procedure previously reported by Matsui et al. (1996). Briefly, each well in 96-well plates contained 150 μL of 2 mM 4-nitrophenyl α-D-glucopyranoside (PNP-G) in 100 mM potassium phosphate buffer (pH 7.0) and 20 μL of the sample in DMSO. The reaction was initiated by the addition of 150 μL of the enzyme solution (32 mU/ml from baker's yeast purchased from Sigma Chemical Co.). The plates were incubated at 37 °C for 20 min. The absorbance of 4-nitrophenol released from PNP-G at 400 nm was measured by a μ Quant universal microplate spectrophotometer. The increase in absorbance (ΔA) was compared with that of the control (DMSO in place of sample solution) to calculate the inhibition.

$$\text{Inhibition } (\%) = (\Delta A_{\text{control}} - \Delta A_{\text{sample}})/\Delta A_{\text{control}} \times 100\%$$

The concentration of inhibition required for 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.04.013.

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