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Novel highly oxygenated and B-ring-seco-*ent*-diterpene glucosides from the seeds of *Prinsepia utilis*

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ABSTRACT

Two novel highly oxygenated and one rare B-ring cleave *ent*-kaurane diterpene glucosides, named prinsoside A (**1**), B (**2**) and C (**3**), respectively, were isolated from an ethanolic extract of the seeds of *Prinsepia utilis*. To our knowledge, prinsoside B (**2**) was the most highly oxygenated *ent*-kaurane diterpene glucoside discovered in nature. Their structures were elucidated by various spectroscopic methods including 1D, 2D NMR and HR-ESI-MS analyses, with the absolute configurations clarified by CD measurements on their corresponding diterpenoid aglycones obtained by enzymatic hydrolysis with β-glucosidase. Compounds **1**–**3** exhibited weak α-glucosidase inhibitory activities.

1. Introduction

Genus *Prinsepia* (Rosaceae) encompasses five species, which are mainly distributed in the Himalayan mountain district with four species growing in China,[1] among which *Prinsepia utilis* Royle and *Prinsepia sinensis* Oliver ex Bean are used, both as traditional Chinese herbal medicines[2] to treat a variety of disorders such as skin problems, fracture, rheumatism and inflammation.[3] A few phytochemical studies have been done on the stems and leaves of *Prinsepia* which revealed the presence of triterpenoids, hemiterpenes, flavonoids and hydroxynitrile glucosides.[4–7] However, little is known on the chemical constituents of the fruits besides essential oils. Thus, we conducted an extensive phytochemical investigation on the seeds of *P. utilis*, which led to the isolation of two new highly oxygenated *ent*-kaurane diterpene glucosides (**1** and **2**), and one rarely B ring-cleaved kauranoid diterpene glucoside (**3**). Hydrolysis of the kaurane diterpene glucosides gave rise to three new kaurane diterpenoid aglycones. This report describes the structural characterization of the new compounds and their α-glucosidase inhibitory activities.

2. Results and discussion

Prinsoside A (**1**) was obtained as a white needle crystal. The molecular formula was established to be $C_{27}H_{44}O_{12}$, based on the sodiated molecular ion $[M+Na]^+$ determined at *m/z* 583.2706 (calcd for $C_{27}H_{44}O_{12}Na$, 583.2833) in the high-resolution electrospray ionization mass spectroscopy (HR-ESI-MS). In the ^1H NMR spectrum, a typical anomeric proton was observed at δ_H 4.33 (1H, d, J=7.8 Hz), in tandem with a distinct signal set assignable to a hexose unit (δ_C 105.1, 78.0, 77.8, 75.2, 71.6 and 62.7) observed in the ^{13}C NMR spectrum, implying the presence of a glucopyranosyl moiety by a comparison with the corresponding data reported in the literature.[8] Moreover, a β-D-configuration was also suggested based on the relatively large coupling constant of the anomeric proton (J=7.8 Hz), the chemical shift of the anomeric carbon (δ_C 105.1), and biogenesis consideration.

In the ^{13}C NMR data (Table 1), a total of 27 carbon signals, besides the signals arising from the glucopyranosyl moiety, were observed, including three methyls (δ_C 51.9, 32.6, 17.0), seven methylenes (δ_C 74.0, 41.6, 40.9, 34.7, 27.1, 20.0, 19.2), six methines (δ_C 82.7, 79.1, 72.1, 52.5, 49.3, 45.0), four quaternary carbons (δ_C 80.7, 52.3, 45.5, 41.8), and one carbonyl carbon (δ_C 180.2). A closer inspection revealed that the ^{13}C NMR data was similar to that reported for the known diterpene glucoside *ent*-6α,7α,16β,17-tetrahydroxykauran-19-oate 16-*O*-β-D-glucopyranoside.[9] The only difference is that compound **1** has one more hydroxylated ^{13}C signal, which also accounts for its mass spectral data.

The five hydroxyl groups were ascribed to C-6, 7, 15, 16 and 17 (Fig. 1), respectively, through interpretation of the COSY and HMBC spectral data. Briefly, the analysis of ^1H–^1H COSY correlations of **1** established three coupling segments as of C-1/C-2/C-3 (**a**), C-5/C-6/

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Table 1
^{1}H and ^{13}C NMR data of **1**–**3**

Position	**1**		**2**		**3**	
	δ_C[a]	δ_H[b] (J in Hz)	δ_C[a]	δ_H (J in Hz)[b]	δ_C[a]	δ_H (J in Hz)[b]
1a	41.6	1.84, m	82.7	3.39, m	35.0	1.75, m
1b		0.93, m				1.59, m
2a	19.2	1.63, m	30.5	1.85, m	19.0	1.47, m
2b		1.47, m		1.57, m		
3a	40.9	2.30, m	38.3	2.13, m	32.1	2.05, m
3b		1.13, m		1.27, m		1.31, m
4	45.5		44.9		42.4	
5	52.5	1.95, d, (11.1)	50.6	1.91, d (11.3)	55.8	1.93, m
6	72.1	4.23, dd, (11.1, 2.2)	71.4	4.28, d, (11.3, 1.9)	100.8	5.67, s
7	79.1	3.80, m, (2.2)	79.1	3.77, d, (1.9)	207.3	9.80, s
8	52.3		52.7		59.6	
9	49.3	1.59, m	49.5	1.87, m	49.0	2.09, m
10	41.8		46.8		40.6	
11a	20.0	1.80, m	20.8	2.96, m	20.7	1.84, m
11b		1.43, m		1.42, m		1.77, m
12a	27.1	1.70, m	27.4	1.60, m	26.3	1.78, m
12b		1.49, m		1.52, m		1.66, m
13	45.0	2.18, m	45.1	2.16, m	46.5	2.30, m
14a	34.7	1.80, m	35.6	1.80, m	32.1	2.41, m
14b		1.64, m		1.60, m		1.71, m
15a	82.7	3.65, s	82.5	3.70, m	47.5	1.86, m
15b						1.60, m
16	80.7		81.0		81.2	
17a	74.0	4.14, d, (10.3)	74.0	4.12, d, (10.3)	74.1	4.24, d, (10.3)
17b		3.63, d, (10.3)		3.64, d, (10.3)		3.60, d, (10.3)
18	32.6	1.46, s	32.3	1.45, s	30.6	1.40, s
19	180.2		180.0		184.3	
20	17.0	0.87, s	13.2	0.98, s	22.6	0.73, s
OMe	51.9	3.72, s	51.2	3.60, s		
Glc-1′	105.1	4.33, d, (7.8)	105.1	4.33, d, (7.8)	105.2	4.32, d, (7.8)
Glc-2′	77.8	3.40, m	77.9	3.39, m	77.9	3.38, m
Glc-3′	75.2	3.26, m	75.2	3.25, m	75.3	3.24, m
Glc-4′	71.6	3.30, m	71.6	3.31, m	71.7	3.29, m
Glc-5′	78.0	3.30, m	78.0	3.30, m	78.1	3.30, m
Glc-6′a	62.7	3.89, br d, (11.1)	62.7	3.89, br d, (11.1)	62.8	3.89, dd, (1.3, 11.7)
Glc-6′b		3.67, dd, (1.3, 11.1)		3.67, dd, (1.3, 11.1)		3.68, br d, (11.7)

[a] Measured in CD_3OD at 125 MHz.
[b] In CD_3OD at 500 MHz.

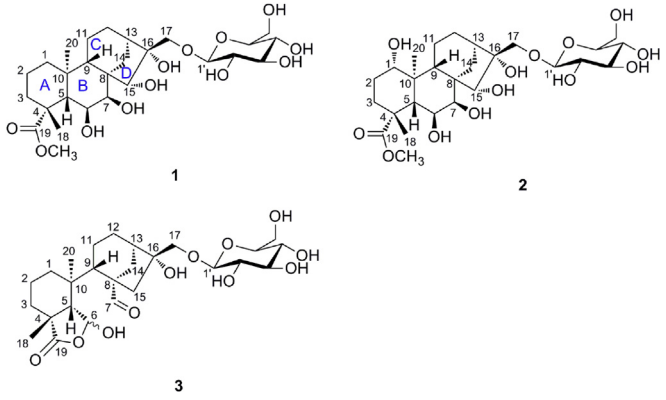


Fig. 1. Structures of compounds **1**–**3**.

C-7 (**b**) and C-12/C-13/C-14 (**c**) (Fig. 2). In the HMBC spectrum, the correlations between H-14 and C-9, C-12, C-15, C-16; H-11 and C-8, C-13; H-13 and C-11, revealed the presence of a bicyclo [3.2.1] octane system (rings C and D). Rings A and B were assigned based on the HMBC correlations between H-1 and C-20, C-3; H-6 and C-8, C-10; H-7 and C-15; Me-18 and C-3, C-4, C-5; as well as Me-20 and C-1, C-5, C-9, C-10. Furthermore, the conclusive evidence supporting the presence of hydroxyl groups at C-6, C-7, C-15 and C-16 was derived from the HMBC correlations between H-6 and C-4, C-8, C-10; H-7 and C-15; H-15 and C-7, C-17. In addition, the observation of HMBC correlation between H-1′ and C-17 verified the location of the glucopyranosyl moiety at C-17. The relative configuration of **1** was deduced by the analysis of ROESY correlations (Fig. 3). The key ROESY correlations between H-6 and H-7 indicated that the two protons were cofacial and arbitrarily assigned as α-orientation, while H-5 was assigned as β-orientation. The correlation between H-6 and H-7, H-12b, Me-20 and between H-7 and H-6, H-12b, H-14b confirmed Me-20 to be α-oriented. In addition, the ROESY correlation between H-5, H-1 and H-15 and between H-15 with H-17b revealed that the OH group at C-15 and C-16 was of α-orientation. Thus, **1** was shown to be a glucoside with methyl *ent*-6α,7α,15β,16β,17-pentahydroxykauran-19-oate as the aglycone, which linked to a glucosyl unit by a glycosidic bond via the hydroxyl at C-17.

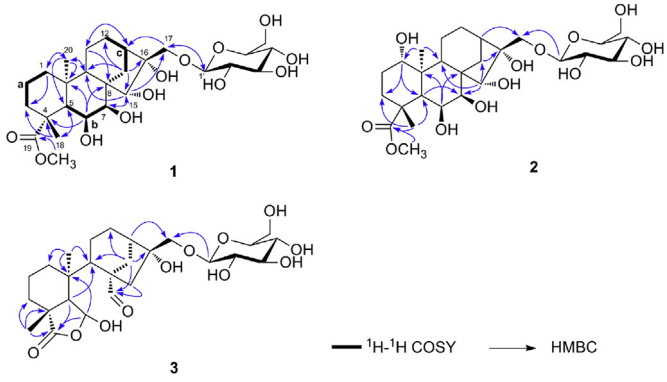


Fig. 2. Key 2D NMR correlations of **1**–**3**.

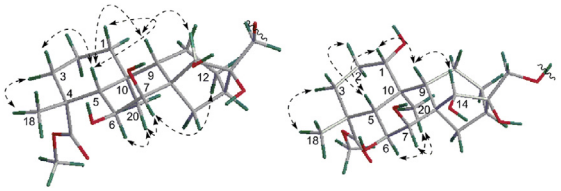


Fig. 3. Key ROESY correlations of **1**and **2**.

To determine the absolute configuration of the glucosyl linkage, efforts were made with an aim to obtain the free aglycone and glycone moieties. Unexpectedly, **1** (as well as **2** and **3**) was found to be resistant to acidic hydrolysis with mineral acids even at high concentration and temperature (refluxed in 6 N HCl for 6 h), presumably due to the steric hindrances of this rigid molecule. However, the enzymatic hydrolysis underwent readily by soluble β-glucosidase to give D-glucose as determined by TLC comparison with an authentic sample, which further confirmed the β-D-configuration of glucosyl moiety.

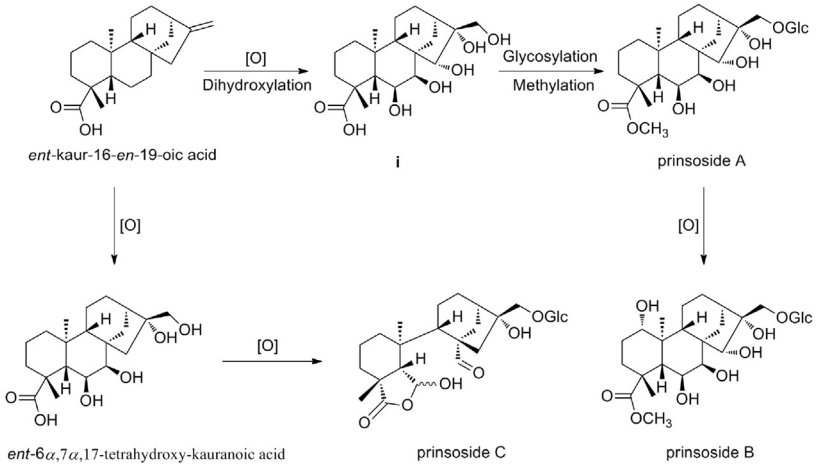
Furthermore, the enzymatic hydrolysate was subjected to chromatographic purification to afford an aglycone (**1a**), which was characterized as methyl-6β,7β,15α,16α-tetrahydroxykauran-19-oate by spectroscopic methods (NMR and ESI-MS). The CD spectrum of both **1** and **1a** displayed a similar negative (λ_{max} 223 nm) Cotton effect to that of *ent*-kaur-16-*en*-19-oic acid which also exhibited a negative (λ_{max} 216 nm) Cotton effect,[10] suggesting **1** possessed an *ent*-kaurane molecular skeleton. Taken together, **1** was concluded as methyl *ent*-6α,7α,15β,16β,17-pentahydroxykauran-19-oate 17-*O*-β D-glucopyranoside.

Prinsoside B (**2**) was obtained as a yellow gum with the molecular formula $C_{27}H_{44}O_{13}$ based on the quasi-molecular ion $[M+Na]^+$ measured at *m/z* 599.2651 (calcd for $C_{27}H_{44}O_{13}Na$, 599.2782) by positive mode HR-ESI-MS. The high similarity between the ^1H and ^{13}C NMR spectra of **1** and **2** (Table 1) indicated that **2** is also an *ent*-kaurane diterpenoid glucoside, with a structure very similarly to **1** except for the presence of an extra hydroxyl group. A closer inspection of the ^{13}C NMR spectra of **2** in comparison to **1** revealed that the downfield shifts were observed at C-1 (from δ_C 41.6 to 82.7), C-2 (from δ_C 18.4 to 30.5) and C-10 (from δ_C 40.6 to 46.8 ppm), suggesting that the hydroxyl group of compound **2** was located at C-1. The HMBC spectrum provided further evidence for the conclusion above, based on several key correlations (Fig. 2.) such as H-1 to C-3 and to C-5, and Me-20 to C-1. The relative configuration of the hydroxyl at C-1 was deduced to be α-oriented based on ROESY spectrum wherein conclusive correlation perks were observed between H-1 and H-5, H-9.

In analogy with **1**, compound **2** was subjected to enzymatic hydrolysis with β-glucosidase to afford D-glucose and the aglycone **2a**. All of the NMR data was accountable with the assigned structure and, as expected, **2a** showed a negative Cotton effect (λ_{max} 226 nm), confirming that **2** (**2a**) had an *ent*-kaurane backbone. Therefore, **2** was established as methyl *ent*-1β,6α,7α,15β,16β,17-hexahydroxykauran-19-oate 17-*O*-β-D-glucopyranoside.

Prinsoside C (**3**) was obtained as a yellow gum, and its molecular formula, $C_{26}H_{40}O_{11}$, was assigned based on negative mode HR-ESI-MS spectrum wherein a pseudo-molecular ion $[M-H]^-$ was measured at *m/z* 527.2500 (calcd for $C_{26}H_{39}O_{11}$, 527.2571), which indicated seven degrees of unsaturation. The ^1H NMR data displayed signals indicative of two methyl groups (δ_H 0.73, s and 1.40, s), a hemiacetal proton (δ_H 5.67, br s), a glucopyranosyl moiety (δ_H 4.32, d, *J*=7.8 Hz), and an aldehyde proton (δ_H 9.80, s). This, in tandem with its ^{13}C NMR spectrum composed of 26 carbon signals consisting of two methyl groups (δ_C 22.6 and 30.6), eight methylene groups (δ_C 19.0, 20.7, 26.3, 32.1, 32.1, 35.0, 47.5 and 74.1), three methine carbons (δ_C 46.5, 49.0 and 55.8), one hemiacetal carbon (δ_C 100.8), four quaternary carbons (δ_C 40.6, 42.4, 59.6 and 81.2), and two carbonyl carbons (δ_C 184.3 and 207.3), in addition to a glucopyranosyl moiety (δ_C 105.1, 75.3, 78.1, 71.7, 77.9, 62.8), indicated that **3** was similar in structure to coccinin, namely *ent*-6β,16α,17-trihydroxy-7,19-dioxo-6,19-epoxy-6,7-seco-kaurane 6-*O*-β-D-glucopyranoside, which was previously isolated from *Phaseolus coccineus*.[11] The only difference between **3** and coccinin was observed on the NMR signals at C-6 [δ_H 5.67, br s/δ_C 100.8 in **3**; while δ_H 6.30, br s/δ_C 104.7 in coccinin], as well as on C-17 [δ_H 4.24, d, *J*=10.3 Hz, 3.60, d, *J*=10.3 Hz/δ_C 74.1 in **3**; while δ_H 6.30, 2H, br s/δ_C 65.7 in coccinin], suggesting that the glucosyl moiety in **3** was linked to C-17, rather than to C-6 as in coccinin, which was further confirmed by the conclusive correlation between anomeric proton (δ_H 4.32, d) and the methylene signal (δ_C 74.1) in the HMBC of **3** (Fig. 2). Thus, the structure of **3** should be a diterpenoid glucoside composed of an unusual B-ring *seco*-kaurane framework. Similarly to compounds **1** and **2**, the absolute configuration of the kaurane diterpene and the glucosyl moiety was determined by means of enzymatic hydrolysis and CD measurements. Enzymatic hydrolysis of **3** with β-glucosidase afforded D-glucose and the aglycone **3a**, which was shown to be *ent*-6β,16α,17-trihydroxy-7,19-dioxo-6,19-epoxy-6,7-*seco*-kaurane based on NMR and HR-ESI-MS spectral analysis as well as the negative Cotton effect (−3.74, λ_{max} 233 nm) observed in the CD spectrum. Taken together, **3** was elucidated as *ent*-6β,16α,17-trihydroxy-7-oxokauran-19,6-olide 17-*O*-β D-glucopyranoside.

A plausible biogenetic pathway was proposed as shown in Scheme 1 to account for the biosynthesis of prinsoside A–C in this plant. Briefly, *ent*-kaur-16-*en*-19-oic acid was assumed to be the biogenetic precursors for prinsoside A and B. They undergo selective allylic oxidation, methylene oxidation and dihydroxylation to yield the key intermediate i, which in turn transform into prinsoside A upon glycosylation and methylation. The further oxidation of prinsoside A would generate prinsoside B. Prinsoside C ought to be also generated from *ent*-kaur-16-*en*-19-oic acid. The selective oxidation and dihydroxylation would induce the hydroxyl groups and give rise to the natural diterpenoid *ent*-6α,7α,16β,17-tetrahydroxy-kauranoic acid.[9] Then, the further B ring-cleavage followed by intramolecular hemiacetalation would transform it to prinsoside C.



Scheme 1. Proposed biogenetic pathway of compounds **1**–**3**.

Compounds **1**–**3** and **1a**–**3a** exhibited weak α-glucosidase inhibitory (IC_{50}>1.0 mM), inferior to the reference compound acarbose with an IC_{50} value of 0.371 mM.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. UV spectra were recorded in MeOH on a Perkin–Elmer Lambda 35 UV–vis spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker Advance-500 spectrometer with TMS as internal standard. HR-ESI-MS data were obtained on a Bruker Bio TOF IIIQ mass spectrometer. All solvents were of analytical grade

(Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (200–300 mesh) was used for column chromatography, and precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC. C18 reversed-phase silica gel (150–200 mesh, Merck), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) was also used for column chromatography. TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in alcohol followed by heating.

3.2. Plant material

The mature fruits of *P. utilis* were collected in 2012 in Lijiang City, Yunnan Province of China. The identity of the plant material was verified by Dr. Fa-Guo Wang of South China Botanical Garden (SCBG) and a voucher specimen was deposited at the Laboratory of Natural Product Chemistry Biology, SCBG.

3.3. Extraction and isolation

The air-dried powder of *P. utilis* fruits (20 kg) was exhaustively extracted with EtOH (3×20 L). The resulting EtOH extract (1.15 kg) was suspended in H_2O and further partitioned into three fractions as follows: petroleum ether (A, 800 g), EtOAc (B, 100 g), and H_2O (C, 270 g). Subsequently, fraction (Fr.) C was subjected to resin D101 eluting with a gradient of increasing EtOH in H_2O (0%–95%) to afford Fr. A_1–A_4. Fr. A_3 (8 g, EtOH/H_2O, 50:50) was subjected to column chromatography (silica gel; $CHCl_3$/MeOH, 10:1→1:1, v/v) to afford Fr. A_{31}–A_{34}. Fr. A_{33} purified by Sephadex LH-20, ($CHCl_3$/MeOH, 1:3), RP-18 (MeOH/H_2O, 40:60→90:10, v/v), and repeated silica gel column afforded **2** (1.5 g). Fr. A_4 (12 g, EtOH/H_2O, 90:10) was subjected to column chromatography (silica gel; $CHCl_3$/MeOH, 15:1→2:1, v/v) to afford Fr. A_{41}–A_{45}. Fr. A_{43} was chromatographed on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) and RP-18 (MeOH/H_2O, 60:40→90:10, v/v) then purified by repeated silica gel column to afford **3** (1.5 g). Fr. A_{44} was subsequently purified by Sephadex LH-20 ($CHCl_3$/MeOH, 1:3) and repeated silica gel column to afford **1** (170 mg).

3.4. Prinsoside A (1)

White amorphous solid; $[\alpha]_D^{20}$=−55.9 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ε) 311.3 (2.00) nm; CD $\Delta\varepsilon$ (*c* 0.051, MeOH) −4.26 (223); ^{1}H and ^{13}C NMR, see Table 1; ESI-MS *m/z* 583 $[M+Na]^+$; HR-ESI-MS *m/z* 583.2706 ($[M+Na]^+$, calcd for $C_{27}H_{44}O_{12}Na$, 583.2833).

3.5. Prinsoside B (2)

Yellow gum; $[\alpha]_D^{20}$=−30.0 (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ε) 291.0 (3.48) nm; CD $\Delta\varepsilon$ (*c* 0.01, MeOH) −9.58 (204); ^{1}H and ^{13}C NMR, see Table 1; ESI-MS *m/z* 577 $[M+H]^+$; HR-ESI-MS *m/z* 577.2839 $[M+H]^+$, calcd for $C_{27}H_{44}O_{13}$, 577.2782.

3.6. Prinsoside C (3)

Yellow gum; $[\alpha]_D^{20}$=−34.7 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 228.5 (3.34), 280.3 (3.05) nm; CD $\Delta\varepsilon$ (*c* 0.013, MeOH) −1.18 (226); ^{1}H and ^{13}C NMR, see Table 1; ESI-MS *m/z* 527 $[M-H]^-$; HR-ESI-MS *m/z* 527.2500 $[M-H]^-$, calcd for $C_{26}H_{40}O_{11}$, 527.2571.

3.7. Enzymatic hydrolysis of 1–3

A solution of each sample in H_2O (6 mL) was individually hydrolysed with crude β-Glucosidase (30 mg, from almonds, Solabio) at 37 °C for 72 h. Reaction mixtures of **1** and **2** were concentrated in vacuo and purified with flash column chromatography (silica gel, $CHCl_3$/MeOH, 8:1, v/v) to afford aglycones **1a** (5.92 mg), **2a** (6.80 mg), respectively. The reaction mixtures of **3** were concentrated in vacuo and purified with flash column chromatography (silica gel, $CHCl_3$/MeOH, 12:1→10:1, v/v) to afford aglycone **3a** (8.0 mg). The aglycone of each compound was identified by NMR data analysis, including 2D NMR data (see Supplementary data), because no spectroscopic data had been reported in literature.

3.8. 1a

White amorphous solid; $[\alpha]_D^{20}$=−37.1 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ε) 206.4 (3.15) nm; CD $\Delta\varepsilon$ (*c* 0.005, MeOH) −3.84 (224); ^{1}H and ^{13}C NMR, see Table 2; ESI-MS *m/z* 421 $[M+Na]^+$; HR-ESI-MS *m/z* 421.2199 $[M+Na]^+$, calcd for $C_{21}H_{34}O_7Na$, 421.2305.

Table 2
^{1}H and ^{13}C NMR data of **1a**–**3a**

Position	**1a**		**2a**		**3a**	
	δ_C[a]	δ_H[b] (*J* in Hz)	δ_C[c]	δ_H (*J* in Hz)[d]	δ_C[c]	δ_H (*J* in Hz)[d]
1a	41.6	1.76, m	81.4	3.27, m	35.0	1.75, m
1b		0.91, m				1.69, m
2a	18.4	1.50, m	29.1	1.74, m	19.0	1.44, m
2b				1.43, m		
3a	40.1	2.30, m	43.1	2.02, m	32.1	2.07, m
3b		1.13, m		1.15, m		1.32, m
4	44.6		43.7		42.5	
5	51.7	2.29, d, (11.1)	49.2	1.79, d, (11.3)	55.8	1.94, m
6	70.8	4.63, dd, (11.1, 2.2)	70.0	4.15, d, (10.8, 1.9)	100.9	5.67, s
7	78.6	4.30, m, (2.2)	78.1	3.63, d, (1.9)	207.3	9.80, s
8	51.5		51.2		59.7	
9	48.2	1.81, m	48.0	1.74, m	49.0	2.09, m
10	40.6		45.4		40.6	
11a	19.3	1.93, m	19.4	2.85, m	20.8	1.83, m
11b		1.39, m		1.28, m		
12a	26.5	1.81, m	26.1	1.43, m	26.4	1.70, m
12b		1.50, m				
13	43.9	2.52, m	43.1	2.01, m	46.2	2.26, m
14a	34.2	2.09, m	34.3	1.68, m	32.2	2.42, m
14b		1.69, m		1.48, m		1.70, m
15a	82.0	4.02, s	81.0	3.53, m	47.8	1.83, m
15b						1.60, m
16	80.7		80.3		82.0	
17a	65.7	4.09, m	64.5	3.55, m	66.1	3.75, d, (11.4)
17b						3.67, d, (11.4)
18	32.2	1.73, s	30.9	1.32, s	30.6	1.44, s
19	178.4		178.6		184.3	
20	16.6	0.90, s	11.8	0.86, s	22.6	0.73, s
OMe	51.0	3.67, s	50.5	3.67, s		

[a] Measured in pyridine-d_5 at 125 MHz.
[b] In pyridine-d_5 at 500 MHz.
[c] In CD_3OD at 125 MHz.
[d] In CD_3OD at 500 MHz.

3.9. 2a

White amorphous solid; $[\alpha]_D^{20}$=−27.1 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 279.0 (2.69), 221.2 (3.07) nm; CD $\Delta\varepsilon$ (*c* 0.016, MeOH) −9.3 (226); ^{1}H and ^{13}C NMR, see Table 2; ESI-MS *m/z* 437 $[M+Na]^+$; HR-ESI-MS *m/z* 437.2152 $[M+Na]^+$, calcd for $C_{21}H_{34}O_8Na$, 437.2254.

3.10. 3a

White amorphous solid; $[\alpha]_D^{20}$=−9.8 (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ε) 274.4 (2.89) nm; CD $\Delta\varepsilon$ (*c* 0.017, MeOH) −3.74 (233); ^{1}H and ^{13}C NMR, see Table 2; ESI-MS *m/z* 367 $[M+H]^+$; HR-ESI-MS *m/z* 367.2097 $[M+H]^+$, calcd for $C_{20}H_{31}O_6$, 367.2042.

3.11. α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity of six compounds (**1**–**3** and **1a**–**3a**) was determined spectrophotometrically in a 96-well microtiter plate based on *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as a substrate following the method described in the literature with slight modifications.[12,13] In brief, α-glucosidase (20 μL, 0.5 U/mL) and various concentrations (20, 10, 5, 2.5, 1.25 mM, 8 μL) of tested compounds in DMSO were mixed with 67 mM phosphate buffer (112 μL, pH 6.8) at room temperature for 10 min. The test blank was set by adding phosphate buffer instead of the α-glucosidase using the same method. Reactions were initiated by the addition of 5.0 mM PNPG (20 μL). The reaction mixture was incubated for 15 min at 37 °C in a final volume of 160 μL. Then, 0.2 M Na_2CO_3 (80 μL) was added to the incubation solution to stop the reaction. The activities were detected in a 96-well plate, and the absorbance was determined at 405 nm (for *p*-nitrophenol). The negative control and negative blank was set by adding neat DMSO instead of the sample via the same way as the test and test blank. Acarbose was utilized as positive control. Inhibition rate (%)=$[(OD_{negative\ control}-OD_{blank})-(OD_{test}-OD_{test\ blank})]/(OD_{negative\ blank}-OD_{blank})\times 100\%$. IC_{50} values of the samples were calculated using the Microsoft Office Excel 2007 and SPSS Statistics 17.0.

The α-glucosidase inhibitory activity of six compounds (**1**–**3** and **1a**–**3a**) was determined spectrophotometrically in a 96-well microtiter plate based on *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as a substrate following the method described in the literature with slight modifications.[12,13] In brief, α-glucosidase (20 μL, 0.5 U/mL) and various concentrations (20, 10, 5,2.5, 1.25 mM) of tested compounds (120 μL) in 67 mM phosphate buffer (pH 6.8) were mixed at room temperature for 10 min. Reactions were initiated by the addition of 5.0 mM PNPG (20 μL). The reaction mixture was incubated for 15 min at 37 °C in a final volume of 160 μL. Then, 0.2 M Na_2CO_3 (80 μL) was added to the incubation solution to stop the reaction. The activities were detected in a 96-well plate, and the absorbance was determined at 405 nm (for *p*-nitrophenol). The negative blank was set by adding phosphate buffer instead of the sample via the same way as the test. Acarbose was utilized as positive control. The blank was set by adding phosphate buffer instead of the α-glucosidase using the same method. Inhibition rate (%)=$[(OD_{negative\ control}-OD_{blank})-(OD_{test}-OD_{test\ blank})]/(OD_{negative\ blank}-OD_{blank})\times 100\%$. IC_{50} values of the samples were calculated using the Microsoft Office Excel 2007 and SPSS Statistics 17.0.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.10.055.

References and notes

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