Carbohydrate Research 449 (2017) 120–124

Contents lists available at ScienceDirect

Carbohydrate Research

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

Physakengoses K-Q, seven new sucrose esters from *Physalis alkekengi* var. *franchetii*

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ARTICLE INFO

Article history:
Received 28 March 2017
Received in revised form
12 June 2017
Accepted 25 July 2017
Available online 28 July 2017

Keywords:
Physalis alkekengi var. *franchetii*
Solanaceae
Sucrose ester
Antibacterial activity

ABSTRACT

Seven sucrose esters, physakengoses K-Q (**1**–**7**) were isolated from the aerial parts of *Physalis alkekengi* var. *franchetii*. Their structures were elucidated on the basis of extensive spectroscopic analyses and chemical methods. These new compounds were tested for their antimicrobial abilities against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Among the isolated sucrose esters, compounds **1**–**5** showed potent antibacterial activity with MIC values ranging from 2.16 to 12.76 μg/mL.

1. Introduction

Physalis alkekengi var. *franchetii* (Solanaceae) (Chinese name: "Jindenglong") [1], is widely distributed and cultivated in Europe and Asia [2]. The calyxes of *P. alkekengi* var. *franchetii* have been used as a traditional Chinese medicine for treatment of sore throat, cough, eczema, hepatitis, urinary problems and tumors [3]. Sucrose esters, structurally featured in sucrose and long fatty acid ester [4,5], have captured the attentions of many researchers due to their potent antibacterial and anti-inflammatory activities in recent years [6–8]. In continuing phytochemical studies of *P. alkekengi* var. *franchetii*, we have previously reported the isolation and structural determination of ten new sucrose esters physakengoses A-J [9], and most of them displayed potent antibacterial activities. This prompted us to further search for bioactive sucrose esters. As a result, 7 new sucrose esters, named physakengoses K-Q, were isolated from the aerial parts of *P. alkekengi* var. *franchetii* (Fig. 1). In this paper, we report the isolation, structure elucidation and antibacterial activity of the isolated sucrose esters from the aerial parts of *P. alkekengi* var. *franchetii*.

2. Results and discussion

2.1. Structural elucidation

Physakengose K (**1**, $C_{38}H_{64}O_{15}$) was obtained as an amorphous solid. The NMR data of **1** (Table 1) revealed that it contained signals for sucrose and long chain fatty acid ester moieties [10,11]. The presence of a sucrose unit was deduced from the analysis of the NMR spectra (Table 1), which showed the anomeric CH signals of the glucopyranose (δ_H 5.56, d, J = 3.5 Hz, H-1; δ_C 91.3, C-1) and that of the anomeric carbon of the fructofuranose (δ_C 103.4, C-2′). Alkaline hydrolysis also confirmed the existence of sucrose. Analysis of its NMR spectra (^{1}H, ^{13}C, HSQC and HMBC) and the comparison of NMR data between compound **1** and physakengose G [9] allowed the identification of the acyl groups as myristyl, tigloyl, 3-methylbutanoyl and acetyl. The positions of these groups were established by the HMBC correlations from H-3′ (δ_H 5.31) to C-1 (δ_C 175.1) of myristyl group, from H-2 (δ_H 4.86) to C-1 (δ_C 173.7) of 3-methylbutanoyl group, from H-3 (δ_H 5.41) to C-1 (δ_C 168.6) of tigloyl group and from H_2-1′ (δ_H 4.01, 4.09) to acetoxy carbonyl (δ_C 172.0) (Fig. 2). Thus, the structure of compound **1** was assigned as 1′-*O*-acetyl-2-*O*-(3-methylbutanoyl)-3′-*O*-myristyl-3-*O*-tigloylsucrose.

The NMR data (Table 1) of physakengose L (**2**, $C_{35}H_{58}O_{15}$) and physakengose M (**3**, $C_{35}H_{58}O_{15}$) also showed characteristic signals for sucrose and fatty acid ester. A comparison of the spectroscopic data of **2** with those of physakengose B [9] revealed an overall similarity except for the presence of signals attributable to an acetyl

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http://dx.doi.org/10.1016/j.carres.2017.07.010
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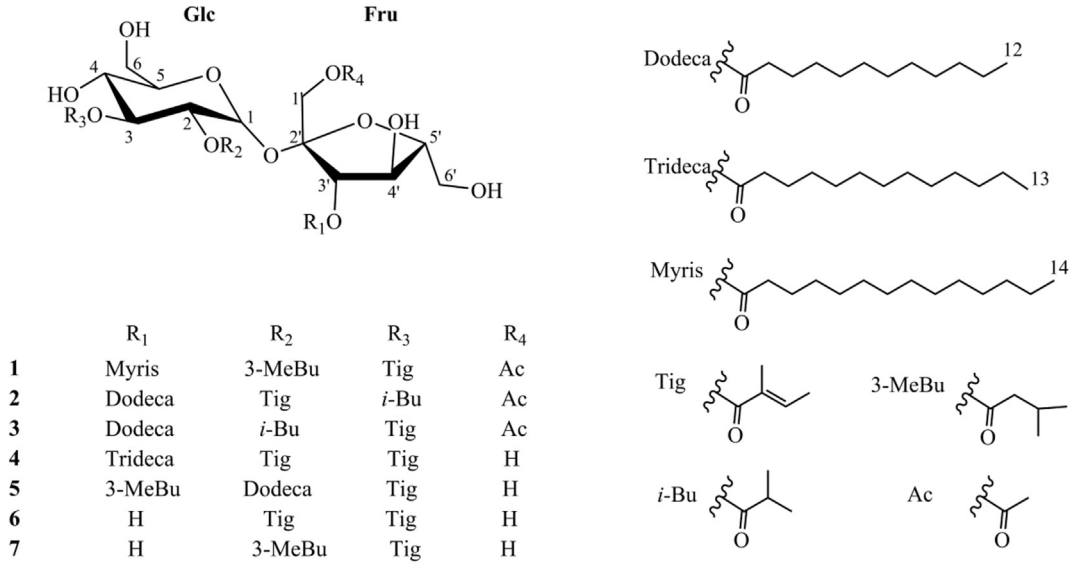

	R_1	R_2	R_3	R_4
1	Myris	3-MeBu	Tig	Ac
2	Dodeca	Tig	*i*-Bu	Ac
3	Dodeca	*i*-Bu	Tig	Ac
4	Trideca	Tig	Tig	H
5	3-MeBu	Dodeca	Tig	H
6	H	Tig	Tig	H
7	H	3-MeBu	Tig	H

Fig. 1. Structures of compounds **1**–**7**.

unit [δ_H 2.04 (3H, s); δ_C 171.8, 20.6], and the downfield shift of H_2-1′ (δ_H 3.92, 4.04) relative to those in physakengose B (δ_H 3.32, 3.46). The location of the acetyl unit was determined by analysis of HMBC spectrum. The ^{1}H and ^{13}C NMR data (Table 1) of compound **3** closely resemble those of **2**. The differences between them were determined by the HMBC spectrum; the correlations from H-2 (δ_H 4.84) to the carbonyl carbon of isobutyryl unit (δ_C 177.7) and from H-3 (δ_H 5.42) to the carbonyl carbon of tigloyl unit (δ_C 168.7) suggested that the isobutyryl and tigloyl units were attached to C-2 and C-3, respectively. Accordingly, the structures of compounds **2** and **3** were elucidated as 1′-*O*-acetyl-3′-*O*-dodecanoyl-3-*O*-isobutyryl-2-*O*-tigloylsucrose and 1′-*O*-acetyl-3′-*O*- dodecanoyl-2-*O*-isobutyryl-3-*O*-tigloylsucrose, respectively.

Physakengose N (**4**, $C_{35}H_{58}O_{14}$), isolated as an amorphous solid, contained one more CH_2 than physakengose F [9]. The ^{1}H and ^{13}C NMR data (Table 1) of **4** were almost superimposable with those of physakengose F. A comprehensive study on the 1D and 2D NMR spectra of compound **4** indicated that it had one more methylene group in its fatty acid chain. Thus, the structure of **4** was identified as 3′-*O*-tridecanoyl-2, 3-di-*O*-tigloylsucrose.

The 1D and 2D NMR spectroscopic data of physakengose O (**5**, $C_{34}H_{58}O_{14}$) showed highly similarity to those of physakengose E [9], except for the positions of dodecanoyl and 3-methylbutanoyl units. The HMBC correlations from H-2 (δ_H 4.84) to the carbonyl carbon of dodecanoyl (δ_C 174.4) and from H-3′ (δ_H 5.42) to the carbonyl carbon of 3-methylbutanoyl (δ_C 174.4) allowed us to formulate **5** as 2-*O*-dodecanoyl-3′-*O*-(3-methylbutanoyl)-3-*O*-tigloylsucrose.

Physakengoses P (**6**, $C_{22}H_{34}O_{13}$) and Q (**7**, $C_{22}H_{36}O_{13}$) were obtained as amorphous solids. Their ^{1}H and ^{13}C NMR spectra (Table 2) were similar to those of physakengoses F and E, except for the absence of signals for dodecanoyl unit attached to C-3′, which were further confirmed by their molecular formulas. The further analysis of their 2D NMR spectra allowed us to formulate **6** as 2, 3-di-*O*-tigloylsucrose and **7** as 2-*O*-(3-methylbutanoyl)-3-*O*-tigloylsucrose, respectively.

2.2. Antibacterial activity

The antibacterial activity of compounds **1**–**7** against *S. aureus, B. subtilis, P. aeruginosa and E. coli* was tested using disk diffusion assay with penicillin and streptomycin as positive controls for Gram-positive and Gram-negative bacteria, respectively. As shown in Table 3, compounds **1**–**5** had potent positive bacteriostatic effect both against Gram-positive and Gram-positive bacteria with MIC values ranging from 2.16 to 12.76 μg/mL but compounds **6** and **7** had no antibacterial activity. These results revealed that the long fatty acid chain attached to C-2 or C-3′ played an important role in the antibacterial activity.

3. Conclusion

Sucrose esters, characterized by containing long chain fatty acids attached to the disaccharide, are relatively rare compounds which have been isolated from the Solanaceae, Asteraceae, Cannaceae, and Polygalaceae families [11]. Regarding antibacterial activity, compounds **1**–**5** showed strong activity, but **6** and **7** were inactive. These results indicate that long chain fatty acid esters attached to sucrose are essential for the inhibitor of the strains tested.

4. Experimental

4.1. General experimental procedures

The optical rotation values were recorded on a Jasco P-1020 polarimeter and IR data were detected on a Bruker Tensor 27 spectrometer. 1D and 2D NMR experiments were carried out in methanol-d_4 on a Bruker Avance III NMR instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C), and TMS was set as the internal standard. Agilent UPLC-Q-TOF (6520B) was used to acquire HRESIMS data. HPLC analysis was performed on an Agilent 1260 Series instrument equipped with a DAD detector and a Shim-pack VP-ODS column (4.6 × 250 mm, i.d.). Silica gel (200–300 and 100–200 mesh, Qingdao Marine Chemical Co., Ltd.), MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and ODS (40–63 μm, Fuji) were used for column chromatography (CC). All chemical reagents used were analytical grade (Jiangsu Hanbon Science and Technology Co., Ltd., Nanjing, China).

4.2. Plant material

The aerial parts of *P. alkekengi* var. *franchetii* were collected in

Table 1
^{13}C NMR (125 MHz) and ^{1}H NMR data (500 MHz) of compounds **1**–**4** in CD_3OD.

Position	1		2		3		4	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	91.3	5.56, d (3.5)	91.3	5.61, d (3.5)	91.4	5.59 d (3.5)	91.9	5.59, d (3.3)
2	71.9	4.86, dd (10.4, 3.6)	72.4	4.79[a]	72.0	4.84, dd (10.4, 3.6)	72.7	4.79, dd (10.4, 3.5)
3	73.7	5.41, t (9.9)	73.2	5.41, t (9.9)	73.8	5.42, t (9.9)	73.8	5.47, t (9.9)
4	69.3	3.67, t (9.7)	69.0	3.68, t (9.7)	69.2	3.70, t (9.7)	69.2	3.72, t (9.7)
5	74.6	3.92, m	74.7	3.90, m	74.7	3.93, m	74.5	3.94, m
6a	61.9	3.84, dd (12.0, 1.8)	61.9	3.85, dd (11.8, 2.8)	61.9	3.83, dd (12.1, 2.0)	61.9	3.86, dd (12.1, 1.8)
6b		3.77, m		3.78, m		3.78, m		3.78, m
1a′	65.6	4.09, d (11.6)	65.9	4.04, d (11.6)	65.7	4.08, d (11.5)	64.1	3.47, d (11.7)
1b′		4.01, d (11.6)		3.92, d (11.6)		4.03, d (11.5)		3.34, d (11.7)
2′	103.4		103.3		103.3		105.1	
3′	79.0	5.31, d (8.2)	79.3	5.26, d (8.3)	79.1	5.31, d (8.3)	78.6	5.40, d (8.4)
4′	73.2	4.31, t (8.3)	73.2	4.30, t (8.3)	73.1	4.32, t (8.4)	73.5	4.31, t (8.5)
5′	84.3	3.88, m	84.2	3.86, m	84.2	3.87, m	84.1	3.88, m
6a′	63.0	3.77, m	63.0	3.78, m	63.0	3.78, m	63.1	3.78, m
6b′		3.77, m		3.78, m		3.78, m		3.78, m
3′-*O*-	Myris[b]		Dodeca[c]		Dodeca[c]		Trideca[d]	
1	175.1		175.1		175.1		175.1	
2a	34.8	2.57, m	34.8	2.58, m	34.8	2.54, m	34.9	2.56, m
2b		2.46, m		2.45, m		2.45, m		2.46, m
3	26.1	1.69, m	26.0	1.69, m	26.0	1.69, m	26.1	1.69, m
4–11	23.7–33.0	1.30[a]	23.7–33.1	1.30[a]	23.7–33.1	1.30[a]	23.7–33.1	1.30[a]
12	23.7–33.0	1.30[a]	14.4	0.90, t (6.9)	14.4	0.90, t (6.9)	23.7–33.1	1.30[a]
13	23.7–33.0	1.30[a]					14.4	0.90, t (6.8)
14	14.4	0.90, t (6.9)						
2-*O*-	3-MeBu[e]		Tig[f]		i-Bu[g]		Tig[f]	
1	173.7		168.4		177.7		168.6	
2a	44.3	2.17, d (6.9)	128.9		35.1	2.51, m	129.1	
2b		2.18, d (7.2)						
3	26.6	1.96, m	140.7	6.85, q (6.5)	19.5	1.04, d (7.1)	140.2	6.81, m
4	22.6	0.87, d (6.6)	14.6	1.80[a]	18.8	1.08, d (6.9)	14.3	1.78, d (7.2)
5	22.7	0.87, d (6.6)	12.0	1.79, br s			12.0	1.75, br s
3-O-	Tig[f]		i-Bu[g]		Tig[f]		Tig[f]	
1	168.6		177.9		168.7		168.8	
2	129.6		35.2	2.53, m	129.6		129.6	
3	138.9	6.87, q (6.7)	19.1	1.11, d (6.9)	138.9	6.85, dq like (1.3, 7.0)	138.7	6.81, m
4	14.4	1.79, d (7.1)	19.5	1.06, d (7.0)	14.3	1.79, d (7.1)	14.5	1.78, d (7.2)
5	12.2	1.82, br s			12.2	1.82, br s	12.2	1.79, br s
1′-*O*-	Ac		Ac		Ac			
1	172.0		171.8		172.0			
2	20.6	2.09, s	20.6	2.04, s	20.6	2.09, s		

[a] Overlapped signals.
[b] Myris = myristyl.
[c] Dodeca = dodecanoyl.
[d] Trideca = tridecanoyl.
[e] 3-MeBu = 3-methylbutanoyl.
[f] Tig = tigloyl.
[g] *i*-Bu = isobutyryl.

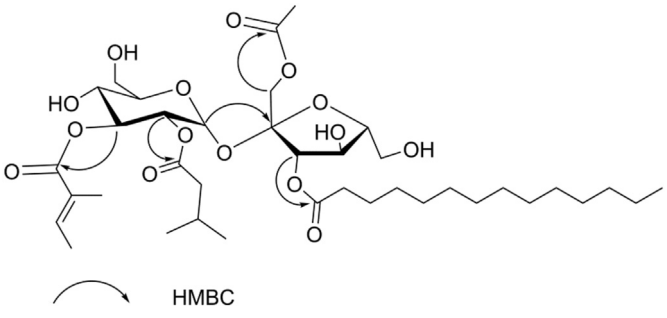

Fig. 2. Selected HMBC correlations of compound **1**.

October 2014 from Fuyang city, Anhui province, People's Republic of China, and identified by *Prof.* Mian Zhang. A voucher specimen (No.PAF-20150422) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

4.3. Extraction and isolation

The plant materials (3.0 kg) were extracted with 95% EtOH (3 × 10 L) under reflux. After removing the solvents under reduced pressure, the viscous residue (240.0 g) was obtained. The application of ^{1}H NMR spectroscopy-guided isolation prompted us to narrow the scope of targeted sucrose esters to the Fr. D of PE layer as reported earlier [9]. Thus, Fr. D (25.0 g) was further separated by an MCI gel column using MeOH-H_2O (30:10–90:10, v/v) as eluent to yield six major subfractions (Frs. D1-D6). Fr. D4 (8.0 g) was applied to an ODS column eluted with solvent system of MeOH-H_2O (70:30–90:10, v/v) to afford Frs. D4a-D4e. Fr. D4b was purified by preparative HPLC using MeOH-H_2O (85:15, v/v) as the mobile phase to give compound **1** (8.0 mg, t_R = 16.3 min). Compounds **2** (20.0 mg, t_R = 18.1 min) and **3** (15 mg, t_R = 17.3 min) were obtained from Fr. D4c by recycling-preparative HPLC eluted with MeOH-H_2O (80:20, v/v). The Fr. D4d was subjected to recycling-preparative HPLC with MeOH-H_2O (80:20, v/v) to furnish **4** (4.0 mg,

Table 2
^{13}C NMR (125 MHz) and ^{1}H NMR data (500 MHz) of compounds **5**–**7** in CD_3OD.

Position	5		Position	6		7	
	δ_C	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	91.1	5.57, d (3.6)	1	90.7	5.63, d (3.5)	90.9	5.59, d (3.6)
2	72.0	4.84[a]	2	72.8	4.80[a]	72.1	4.86[a]
3	73.9	5.40, t (9.8)	3	74.0	5.52, t (9.8)	74.0	5.46, t (9.8)
4	69.4	3.68, t (9.6)	4	69.3	3.70, t (9.6)	69.5	3.66, t (9.6)
5	74.4	3.93, m	5	74.3	3.99, m	74.1	3.98, m
6a	61.9	3.85, dd (12.3, 1.6)	6a	61.8	3.85, dd (11.9, 1.7)	61.8	3.84, dd (12.0, 2.1)
6b		3.78, m	6b		3.78, m		3.78, m
1a′	64.3	3.54, d (11.8)	1a′	63.1	3.47, d (11.9)	63.3	3.56, d (11.9)
1b′		3.43, d (11.8)	1b′		3.28, d (12.0)		3.41, d (11.9)
2′	105.0		2′	105.8		105.8	
3′	78.5	5.42, d (8.4)	3′	77.3	4.20, d (8.7)	77.6	4.19, d (8.7)
4′	73.4	4.31, t (8.5)	4′	75.3	4.02, t (8.5)	75.4	4.03, t (8.5)
5′	84.1	3.88, m	5′	83.9	3.75, m	83.9	3.77, m
6a′	63.1	3.78, m	6a′	63.5	3.78, m	63.6	3.78, m
6b′		3.78, m	6b′		3.78, m		3.78, m
2-*O*-	Dodeca[b]		2-*O*-	Tig[c]		3-MeBu[d]	
1	174.4		1	169.2		173.8	
2a	35.2	2.23, t (7.4)	2a	129.5		44.3	2.16, d (7.3)
2b		2.23, t (7.4)	2b				2.14, d (7.0)
3	25.9	1.50, m	3	140.2	6.84, dd (6.9, 1.1)	26.7	1.98, m
4–11	23.7–33.0	1.30[a]	4	14.5	1.79, s	22.6	0.88, d (6.7)
12	14.4	0.90, t (6.8)	5	12.1	1.79, s	22.7	0.88, d (6.7)
3-*O*-	Tig[c]		3-*O*-	Tig[c]		Tig[c]	
1	168.7		1	168.7		169.0	
2	129.6		2	129.1		129.5	
3	138.8	6.86, dq (7.1, 1.1)	3	139.0	6.84, dd (6.9, 1.1)	139.2	6.88, m
4	14.4	1.80, d (7.2)	4	14.4	1.79, s	14.4	1.79, d (7.1)
5	12.2	1.82, br s	5	12.0	1.79, s	12.1	1.82, s
3′-*O*-	3-MeBu[d]						
1	174.4						
2a	44.0	2.45, dd (15.1, 6.9)					
2b		2.32, dd (15.1, 7.4)					
3	26.9	2.18, m					
4	22.9	1.04, d (6.9)					
5	22.7	1.02, d (6.9)					

[a] Overlapped signals.
[b] Dodeca = Dodecanoyl.
[c] Tig = tigloyl.
[d] 3-MeBu = 3-methylbutanoyl.

Table 3
Minimum inhibitory concentration (MIC) of compounds **1**–**7** (μg/mL).[a]

Compounds	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
1	7.56 ± 0.33	4.25 ± 0.28	6.57 ± 0.23	5.63 ± 0.53
2	6.33 ± 0.34	8.58 ± 0.56	3.61 ± 0.43	2.16 ± 0.30
3	9.05 ± 0.54	4.11 ± 0.44	4.45 ± 0.34	10.42 ± 0.57
4	12.76 ± 0.87	6.14 ± 0.54	5.80 ± 0.32	4.02 ± 0.45
5	6.68 ± 0.51	6.29 ± 0.62	2.35 ± 0.25	2.91 ± 0.32
6	>50.00	>50.00	>50.00	>50.00
7	>50.00	>50.00	>50.00	>50.00
Penicillin[b]	0.06 ± 0.03	0.13 ± 0.05		
Streptomycin[b]			0.41 ± 0.15	0.45 ± 0.15

[a] Values are represented as the means ± SD based on three independent experiments.
[b] Penicillin and Streptomycin were used as positive controls.

t_R = 18.4 min) and **5** (17.0 mg, t_R = 19.4 min). Fr D6 (2.0 g) was chromatographed by an ODS column eluted with MeOH-H_2O (30:70–70:30, v/v) to get four major subfractions (Frs. D6a-D6d). Compounds **6** (10.0 mg, t_R = 11.7 min) and **7** (15.0 mg, t_R = 12.9 min) were obtained from Fr. D6b and Fr. D6c by preparative HPLC with MeOH-H_2O (45:55, v/v), respectively.

*4.3.1. Physakengose K (**1**)*

Amorphous solid; [α]25D+30.0 (*c* 0.1, MeOH); UV λ_{max} (log ε) 214 (3.82) nm; IR (KBr) ν_{max} 3430, 2926, 2854, 1746, 1650, 1465, 1384, 1262, 1167 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Table 1); HRESIMS *m/z* 783.4132 $[M+Na]^+$ (calcd for $C_{38}H_{64}NaO_{15}$, 783.4137).

*4.3.2. Physakengose L (**2**)*

Amorphous solid; [α]25D+38.0 (*c* 0.1, MeOH); UV λ_{max} (log ε) 217 (4.03) nm; IR (KBr) ν_{max} 3509, 2926, 2854, 1749, 1650, 1466, 1385, 1262, 1164 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Table 1); HRESIMS *m/z* 741.3666 $[M+Na]^+$ (calcd for $C_{35}H_{58}NaO_{15}$, 741.3668).

*4.3.3. Physakengose M (**3**)*

Amorphous solid; [α]25D+40.0 (*c* 0.1, MeOH); UV λ_{max} (log ε) 215 (4.04) nm; IR (KBr) ν_{max} 3435, 2926, 2855, 17496, 1650, 1467, 1385, 1265, 1161 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Table 1); HRESIMS *m/z* 741.3669 $[M+Na]^+$ (calcd for $C_{35}H_{58}NaO_{15}$, 741.3668).

*4.3.4. Physakengose N (**4**)*

Amorphous solid; [α]25D+35.0 (*c* 0.1, MeOH); UV λ_{max} (log ε) 217 (3.92) nm; IR (KBr) ν_{max} 3415, 2924, 2852, 1718, 1646, 1465, 1384, 1270, 1154 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Table 1); HRESIMS *m/z* 725.3716 $[M+Na]^+$ (calcd for $C_{35}H_{58}NaO_{14}$, 725.3719).

*4.3.5. Physakengose O (**5**)*

Amorphous solid; [α]25D+36.0 (*c* 0.1, MeOH); UV λ_{max} (log ε) 215 (3.99) nm; IR (KBr) ν_{max} 3421, 2926, 2855, 1724, 1651, 1465, 1383, 1265, 1153 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Table 2); HRESIMS *m/z* 713.3716 $[M+Na]^+$ (calcd for $C_{34}H_{58}NaO_{14}$, 713.3719).

4.3.6. Physakengose P (**6**)

Amorphous solid; [α]25D+74.0 (*c* 0.1, MeOH); UV λ_{max} (log ε) 215 (3.93) nm; IR (KBr) ν_{max} 3384, 2926, 2724, 1711, 1649, 1440 1385, 1273, 1138 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Table 2); HRESIMS *m/z* 529.1887 $[M+Na]^+$ (calcd for $C_{22}H_{34}NaO_{13}$, 529.1892).

4.3.7. Physakengose Q (**7**)

Amorphous solid; [α]25D+65.0 (*c* 0.1, MeOH); UV λ_{max} (log ε) 215 (3.97) nm; IR (KBr) ν_{max} 3413, 2922, 2851, 1707, 1649, 1384, 1265, 1052 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Table 2); HRESIMS *m/z* 531.2045 $[M+Na]^+$ (calcd for $C_{22}H_{36}NaO_{13}$, 531.2048).

4.4. Alkaline hydrolysis

Compounds **1**–**7** (2.0 mg each), dissolved in 2M aqueous NH_4OH (2 mL), were heated at 50 °C for 4 h. The reaction mixtures were neutralized with 2 M formic acid to pH 3 and extracted with EtOAc (3 × 3 mL). Then, the aqueous phase was repeatedly dried with the method reported in the literature [8]. Co-TLC analysis ($CHCl_3$: HAc: H_2O = 3: 3.5: 0.5, R_f = 0.41) in comparison with authentic sucrose, indicated the presence of sucrose.

4.5. Antimicrobial assay

Gram-positive *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus subtilis* ATCC 6633 (*B. subtilis*) and Gram-negative *Pseudomonas aeruginosa* ATCC 9027 (*P. aeruginosa*) and *Escherichia coli* ATCC 25922 (*E. coli*) were used to test antibacterial activity by disk diffusion assay [12]. Each paper disk (6 mm diameter) permeating with 10 μL of test sample (100 μg/mL methanol) was dried and placed on Mueller Hinton agar plate containing bacterial inoculum. Then the bacterial strains were incubated at 37 °C for 24 h. For evaluating antibacterial potential, diameter (in mm) of the growth inhibition zone was recorded. The broth microdilution method was used to measure the minimal inhibition concentration (MIC) according to the protocols of the National Committee for Clinical and Laboratory Standards [13]. Following inoculation of the test bacteria on nutrient agar for 18–24 h, a colony of approximately 1 mm in diameter was collected by using a sterile loop and was dissolved into Mueller Hinton broth. Each bacterial strain was diluted to 10^5 cfu/mL before antimicrobial assay. Stock solutions were firstly resolved in DMSO and diluted to varieties of concentrations (0–50.0 μg/mL, DMSO<1%) with broth afterwards. After incubation at 37 °C for 24 h, the optical density was recorded at 578 nm. MIC value was defined as the minimum concentration of compound at which the growth of microorganism was half inhibited. Streptomycin and penicillin were used as positive controls for Gram-positive bacteria and Gram-negative bacteria, respectively. All of the antimicrobial assays were performed in triplicate.

Acknowledgements

This research work was supported by Huahai Graduate Innovation Program, the Program for Changjiang Scholars, Innovative Research Team in University (IRT_15R63), and the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Conflict of interest

The authors declare no competing financial interests.

Appendix A. Supplementary data

HRESIMS, ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC spectra of **1**–**7** are available in Supporting information.

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.carres.2017.07.010.

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