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

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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.

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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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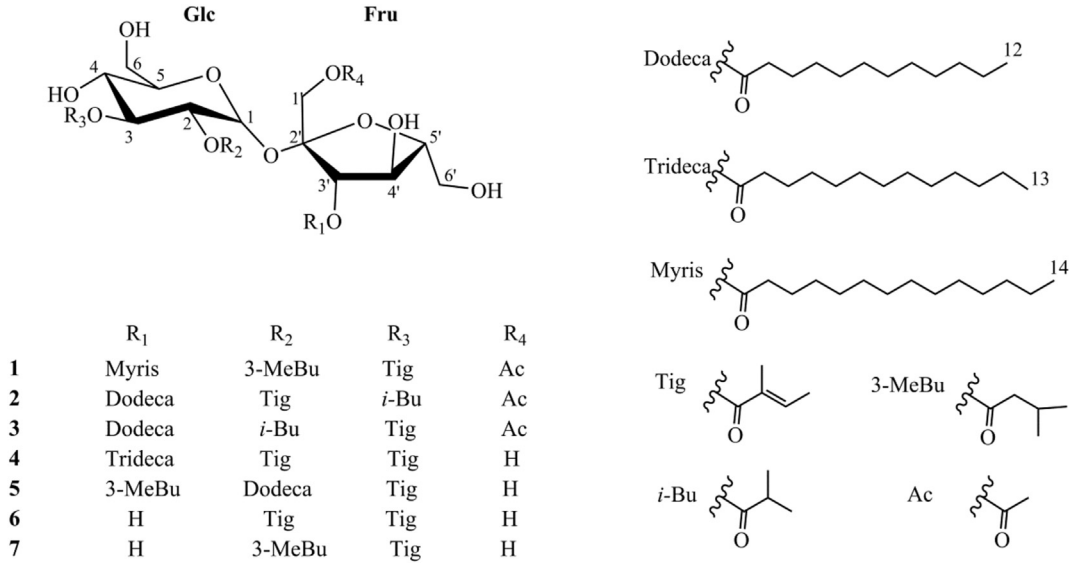

	R_1	R_2	R_3	R_4
1	Myris	3-MeBu	Tig	Ac
2	Dodeca	Tig	*i*-Bu	Ac
3	Dodeca	*i*-Bu	Tig	Ac
4	Trideca	Tig	Tig	H
5	3-MeBu	Dodeca	Tig	H
6	H	Tig	Tig	H
7	H	3-MeBu	Tig	H

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

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