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Chemical constituents of *Solanum coagulans* and their antimicrobial activities

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[ABSTRACT] The present study aimed at determining the chemical constituents of *Solanum coagulans* and their antimicrobial activities. The compounds were isolated by various chromatographic techniques and their structures were elucidated on the basis of extensive spectroscopic analysis, chemical methods, and comparison with reported spectroscopic data. One new phenolic glycoside, methyl salicylate 2-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**1**), together with 12 known compounds (**2**–**13**), were isolated from the aerial parts of *Solanum coagulans*. Compound **1** was a new phenolic glycoside, and **2**–**6** were isolated from *Solanum* genus for the first time. The antimicrobial activities of the isolated compounds were also evaluated. Compound **7** showed remarkable antifungal activity against *T. mentagrophytes*, *M. gypseum* and *E. floccosum* with MIC values being 3.13, 1.56 and 3.13 μg·mL^{-1}, respectively.

[KEY WORDS] *Solanum coagulans*; chemical constituents; antimicrobial activity

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Introduction

Solanum is an important genus of Solanaceae family, which is widely distributed in the regions from temperate zone to the tropics [1]. Previous phytochemical investigations on the genus *Solanum* have demonstrated that it is a fertile source of steroidal glycosides [2–4] and steroidal alkaloid glycosides [5–7]. *Solanum coagulans* Forsk is an herb or shrub and often used in folk medicine for the treatment of edema, rheumatic arthritis, and toothache. However, the chemical constituents and biological activities of this species have not been reported so far. In the course of searching for new and bioactive chemical constituents from Chinese folk medicines [8–10], the phytochemical and antimicrobial investigation on the aerial parts of the species was performed. In the present study, one new phenolic glycoside, methyl salicylate 2-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**1**), together with 12 known ones (**2**–**13**), was isolated. Herein, we report the isolation, structural elucidation, and evaluation for the antimicrobial activity of these compounds.

Results and Discussion

The MeOH extract of the aerial parts of *S. coagulans* was suspended in water and then partitioned with EtOAc and *n*-BuOH. The *n*-BuOH soluble material was subjected to repeated column chromatography (CC) on silica gel and reversed phase silica gel column to afford compounds **1**–**13** (Fig. 1).

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{20}H_{28}O_{13}$ on the base of the quasi-molecular ion at *m*/*z* $[M]^+$ 476.153 6 (calcd. for $C_{20}H_{28}O_{13}$, 476.153 0) in HR-EI-MS and ^{13}C NMR data, representing seven indices of hydrogen deficiency. The UV (MeOH) spectrum of compound **1** exhibited maximal absorpt-

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These authors have no conflict of interest to declare.

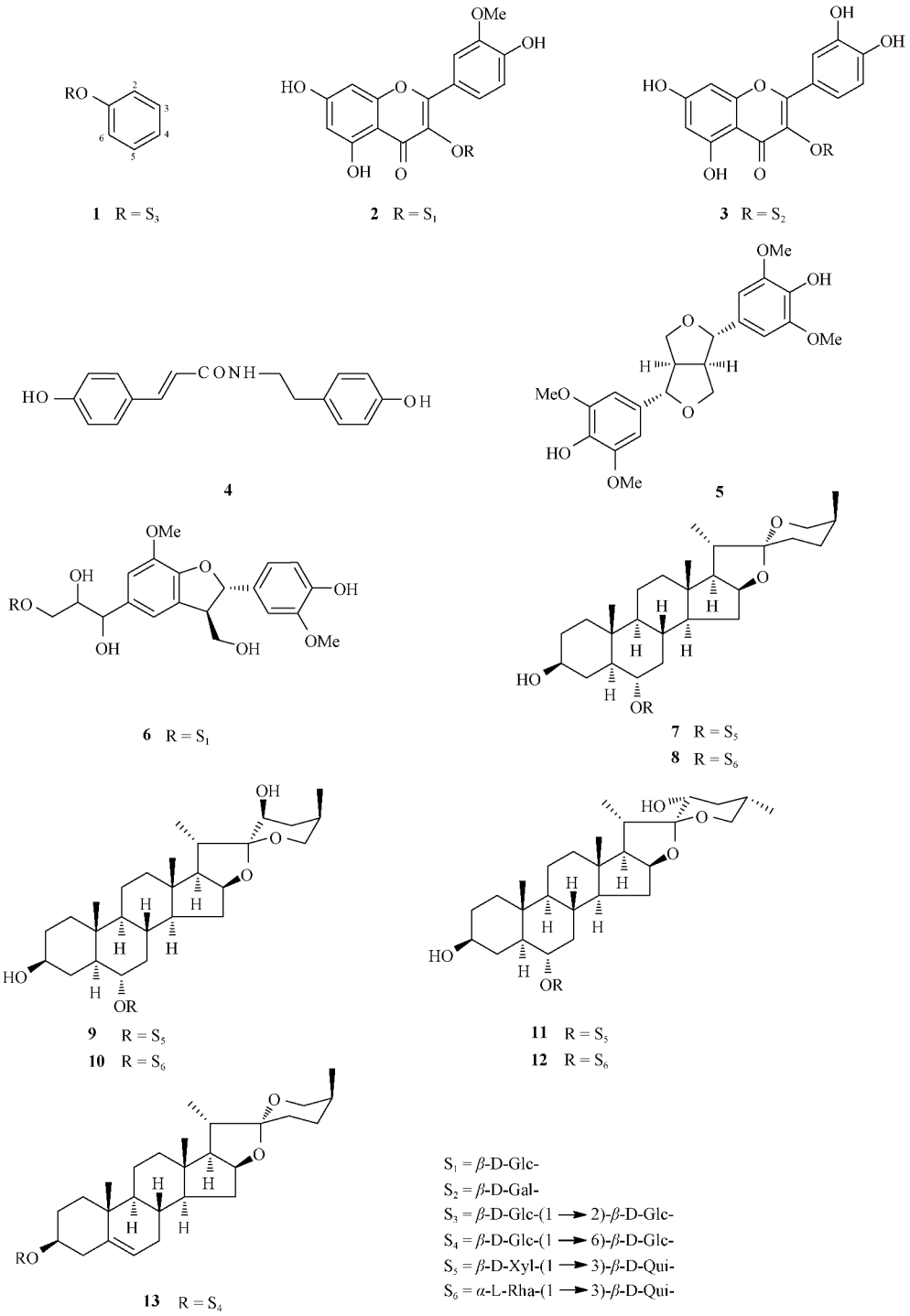

Fig. 1 Structures of compounds 1–13

ion bands at 204, 228, and 290 nm, suggesting the presence of an aromatic ring. Its IR spectrum revealed the presence of hydroxyl (3 429 cm^{-1}), an ester carbonyl (1 716 cm^{-1}) and aromatic (1 631, 1 491 and 1 453 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) displayed signals for four aromatic protons at δ_H 7.77 (1H, d, J = 7.7 Hz, H-3), 7.51 (1H, t, J = 7.4 Hz, H-5), 7.25 (1H, d, J = 8.5 Hz, H-6), and 7.06 (1H, t, J = 7.5 Hz, H-4), one methoxyl protons at δ_H 3.87 (3H, s), and two anomeric protons at δ_H 5.34 (1H, d, J = 7.4 Hz) and 4.82 (1H, d, J = 7.8 Hz). The ^{13}C NMR and DEPT spectra of compound **1** showed 20 carbon signals, 8 of which were assigned to the aglycone moiety, including those corresponding to one methoxyl carbon at δ_C 52.6, one carbonyl carbon at δ_C 167.9 (C-7), one oxy-bearing aromatic carbon at δ_C 157.6 (C-1), four aromatic methine at δ_C 134.9 (C-5), 132.4 (C-3), 122.5 (C-4), and 116.2 (C-6), and one

aromatic quaternary carbon at δ_C 121.3 (C-2), while the remaining were due to two hexose units. The aforementioned NMR signals suggested that compound **1** belonged to a phenolic glycoside with a skeleton of methyl salicylate [11], which was supported by the HMBC and ^{1}H–^{1}H COSY correlations (Fig. 2). The sugar moiety of compound **1** consisted of two residues as evidenced by ^{1}H and ^{13}C NMR spectra, which displayed two anomeric protons at δ_H 5.34 (d, J = 7.4 Hz, H-1′) and 4.82 (d, J = 7.8 Hz, H-1″), attached to anomeric carbons at δ_C 99.6 and 104.3. Acid hydrolysis of compound **1** yielded D-glucose, which was determined by GC analysis of their corresponding trimethylsilated L-cysteine adducts. The *β*-configuration of the two glucose units was determined on the large coupling constant ($J_{1,2}$ > 7.0 Hz) of the anomeric protons. In addition, the HMBC correlations unambiguously established the connectivity of the sugars: H-1′ (δ_H 5.34) with C-1 (δ_C 157.6) and H-1″ (δ_H 4.82) with C-2′ (δ_C 83.0). Consequently, compound **1** was elucidated to be methyl salicylate 2-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-gucopyranoside.

Table 1 The NMR data of compound 1 in CD_3OD[a]

No.	δ_H (mult., Hz)	δ_C
1		157.6 (s)
2		121.3 (s)
3	7.77 (d, J = 7.8 Hz)	132.4 (d)
4	7.06 (t, J = 7.5 Hz)	122.5 (d)
5	7.51 (t, J = 7.4 Hz)	134.9 (d)
6	7.25 (d, J = 8.5 Hz)	116.2 (d)
7		167.9 (s)
-OMe	3.87 (s)	52.6 (q)
	1-*O*-*β*-D-Glc	
1′	5.34 (d, J = 7.4 Hz)	99.6 (d)
2′	3.80 (m)	83.0 (d)
3′	3.70 (m)	77.6 (d)
4′	3.48 (m)	70.8 (d)
5′	3.47 (m)	77.8 (d)
6′a	3.86 (m)	62.3 (t)
6′b	3.68 (m)	
	2′-*O*-*β*-D-Glc	
1″	4.82 (d, J = 7.8 Hz)	104.3 (d)
2″	3.20 (m)	76.0 (d)
3″	3.37 (m)	77.6 (d)
4″	3.36 (m)	70.6 (d)
5″	3.11 (m)	77.3 (d)
6″a	3.46 (m)	61.4 (t)
6″b	3.13 (m)	

[a] ^{1}H and ^{13}C NMR were recorded at 500 MHz and 100 MHz, respectively.

The known compounds were identified as isorhamnetin 3-*O*-*β*-D-glucopyranoside (**2**) [12], quercetin 3-*O*-*β*-D-galactopyranoside (**3**) [13], *N*-*p*-coumaroyltyramine (**4**) [14], syringaresinol (**5**) [15], radulignan (**6**) [16], neochlorogenin 6-*O*-*β*-D-xylopyranosyl (1→3)-*β*-D-quinovopyranoside (**7**) [17], neochlorogein 6-*O*-*α*-L-rhamnpyranosyl(1→3)-*β*-D- quinovopyranoside (**8**) [17], (22*R*, 23*S*, 25*R*)-3*β*, 6*α*, 23-trihydroxy-5*α*-spirostane 6-*O*-*β*-D- xylopyranosyl (1→3)-*β*-D-quinovopyranoside (**9**) [18], torvoside K (**10**) [3], (22*R*, 23*R*, 25*S*)-3*β*,6*α*, 23-trihydroxy-5*α*-spirostane 6-*O*-*β*-D-xylopyranosyl (1→3)-*O*-*β*-D-quinovopyranoside (**11**) [18], torvoside L (**12**) [3], and torvoside M (**13**) [19] by comparing their physical and spectroscopic data with reported values in literature.

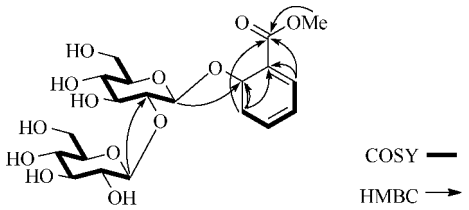

Fig. 2 Key 2D correlations of compound 1

All the compounds except **4** and **5** were screened for their antimicrobial activities against five bacteria and three fungi. Compound **7** showed more potent antifungal activities against *T. mentagrophytes*, *M. gypseum*, and *E. floccosum*, with the MIC values being 3.13, 1.56, and 3.13 μg·mL^{-1}, respectively, compared with the positive control griseofulvin (the MIC values, 3.13, 3.13, and 6.25 μg·mL^{-1}, respectively).

Experimental

General experimental procedures

Optical rotations were measured with a JASCO P-1020 digital polarimeter (JASCO, Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2401 PC spectraphotometer (Shimadzu Corp., Kyoto, Japam). IR spectra were recorded on a Bruker Tensor-27 infrared spectrophotometer (Bruker BioSpin GmBH, Rheinstetten, Germany) using KBr pellets. 1D and 2D NMR spectra were performed on Bruker AM-400 and DRX-500 spectrometers (Bruker BioSpin GmBH, Rheinstetten, Germany) with TMS (Tetramethylsilane) as internal standard. HR-EI-MS was recorded on a Waters AutoSpec Premier P776 spectrometer (Waters, Milford, MA, USA). Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd., Tokyo, Japan). Medium pressure liquid chromatography was employed using a Büchi pump system. Fractions were monitored by TLC (GF_{254}, Qingdao Marine Chemical Ltd., Qingdao, China), with the heating silica gel plates being sprayed with 10% H_2SO_4 in ethanol. GC analysis was performed on a HP5890 gas chromatograph (Agilent Technologies Co. Ltd., Palo Alto, USA) equipped with a H_2 flame ionization detector.

Table 2 Antimicrobial activities of the tested compounds (MIC, μg·mL^{-1})

Compound	Bacteria					Fungi		
	A[a]	B[b]	C[c]	D[d]	E[e]	F[f]	G[g]	H[h]
1	100	50	25	100	100	NT[k]	NT	NT
2	12.5	12.5	50	25	25	100	100	100
3	6.25	6.25	6.25	12.5	6.25	NA	200	NA
6	100	25	50	100	100	200	100	NA
7	50	NA[l]	NA	NA	NA	3.13	1.56	3.13
8	NA	NA	NA	NA	NA	6.25	6.25	6.25
9	NA	NA	NA	NA	NA	NA	NA	NA
10	100	50	25	100	100	200	50	NA
11	50	50	50	100	100	200	50	200
12	50	50	50	100	100	200	50	200
13	NA	100	NA	NA	50	NA	12.5	200
RD[m]	0.20	0.78	1.56	0.20	1.56	3.13	3.13	6.25

[a]A: *Staphylococcus aureus* (ATCC 25922). [b]B: *Pseudomonas aeruginosa* (ATCC 27853). [c]C: *Enterococcus faecalis* (ATCC 10541). [d]D: *Providencia smaitii* (ATCC 29916). [e]E: *Escherichia coli* (ATCC 8739). [f]F: *Trichophytom mentagrophytes* (ATCC 4439). [g]G: *Microsporum gypseum* (CBS 118893). [h]H: *Epidermophyton floccosum* (CBS 566.94). [k]NT: Not tested. [l]NA: Not active. [m]RD: Reference drug (Gentamycin for bacteria and griseofulvin for fungi)

Plant materials

The aerial parts of *Solanum coagulans* were obtained in March 2008 from Xishuangbanna, Yunnan province, China. The plants materials were identified by Mr. Jing-Yun Cui, Xishuangbanna Tropic Botanical Garden, Chinese Academy of Sciences. A voucher specimen (Cui 20080309) has been deposited in Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered aerial parts of *S. coagulans* (11.5 kg) were extracted with MeOH (30 L × 3, 24 h each) at room temperature. Then, MeOH was removed by evaporation under reduced pressure. The total extract was suspended in water and successively partitioned with EtOAc and *n*-BuOH, respectively. The *n*-BuOH fraction (580 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (20 : 1 to 1 : 0), to give five fractions (1–5). Fraction 2 (78 g) was chromatographed over C_{18} reverse- phased silica and eluted with MeOH–H_2O (60 : 40 to 100 : 0) mixtures to give compounds **7** (2.2 g) and **8** (3.0 g). Fraction 3 (85.8 g) was subjected to C_{18} reverse-phased silica, eluted with MeOH–H_2O (50 : 40 to 90 : 10) mixtures, to afford compounds **4** (32 mg), **9** (600 mg), **10** (1.3 g), **11** (135 mg), and **12** (3.2 g). Similarly, Fraction 4 (92 g) was separated by C_{18} reverse-phased silica and eluted with MeOH–H_2O (25 : 75 to 80 : 20) to give 4 fractions (4A–4D). Compounds **1** (12 mg), **5** (16 mg), and **6** (132 mg) were purified from fraction 4A (5.8 g) by silica gel column chromatography eluted with $CHCl_3$–MeOH (6 : 1 to 2 : 1). Using the same procedure, fraction 4B (18 g) was subjected to column chromatography on silica gel and eluted with $CHCl_3$–MeOH (12 : 1 to 5 : 1) to obtain compounds **2** (400 mg), **3** (123 mg), and **13** (320 mg).

*Methyl salicylate 2-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**)*

White amorphous powder; $[\alpha]_D^{25}$ – 69.2 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.41), 228 (3.92), 290 (3.41) nm; IR (KBr) ν_{max}: 3 429, 2 923, 1 715, 1 602, 1 491, 1 453, 1246, 1 077 cm^{-1}; ^{1}H (500 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectral data, see Tables 1; ESI-MS *m/z* 499 $[M + Na]^+$; HR-EI-MS *m/z* 476.1536 $[M]^+$ (calcd. for $C_{20}H_{28}O_{13}$, 476.153 0).

*Acid hydrolysis of compound **1** and GC analysis*

Acid hydrolysis and sugar analysis were carried out as described in the literature [20]. The absolute configuration of the sugar residues was determined to be D-glucose (t_R 19.01 min).

Antibacterial assay

The antimicrobial assay was performed using the technique of reference [21]. The compounds were evaluated at the concentration range of 100–0.78 μg·mL^{-1} for bacteria and 200–0.78 μg·mL^{-1} for fungi, respectively. Gentamycin (for bacteria) and griseofulvin (for fungi) were used as positive controls. MIC was defined as the lowest concentration that inhibited visible growth. The MIC > 100 mg·mL^{-1} for bacteria or > 200 mg·mL^{-1} for fungi was considered to be inactive.

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