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Enzymatic Degradation of Parvifloside

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Abstract: Parvifloside (**1**), a new furostanol pentaglycoside, was isolated from the fresh rhizomes of *Dioscorea parviflora* C. T. Ting. On the basis of spectroscopic and chemical methods, its structure was elucidated as (25*R*)_26_O_β_glucopyranosyl_furost_5_en_3β, 22ξ, 26_triol 3_O_β_*D*_glucopyranosyl (1→3)_β_*D*_glucopyranosyl (1→4)_[α_*L*_rhamnopyranosyl (1→2)]_β_*D*_glucopyranoside. Six prosapogenins (**2**- **7**) were obtained from the enzymatic degradation of **1** by cellulase, but only **3** and **4** were obtained by β_glucosidase. The structures of all compounds were determined by spectroscopic data. The activity of the isolated compounds on deformation of mycelia germinated from *Pyricularia oaryzae* P_2b conidia was evaluated.

Key words: *Dioscorea parviflora*; parvifloside; steroidal glycoside; enzymatic degradation

It is well known that the furostanol saponin, a kind of C_27 steroidal glycoside which is widely distributed in higher plants, especially in Monocotyledoneae[1], is easily transformed to its spirostanol counterpart by β_glucosidase or cellulase[2, 3]. We have reported a series of steroidal saponins with different aglycone and sugar linkage patterns from liliaceous plants[3- 6]. The phylogenetic pathway of these glycosides in plant secondary metabolism is still ambiguous. *Dioscorea parviflora*, an endemic species of family Dioscoreaceae in Yunnan Province, is used as a material to produce diosgenin which is a precursor material for industrial synthesis of steroidal drugs in China[7]. Previous studies have reported the isolation of four steroidal saponins from this plant[8]. In this study, we report the isolation and structural elucidation of a new furostanol pentaglycoside from the fresh rhizomes of *D. parviflora*. The enzymatic degradation of the new furostanol saponin and the activity of the isolated compounds on deformation of mycelia germinated from *Pyricularia oaryzae* P_2b conidia were carried out as well.

1 Results and Discussion

The 70% EtOH extract of the fresh rhizomes of *D. parviflora* was partitioned by *n*_butanol and water, the aqueous soluble phase was chromatographed successively on Diaion HP_20, silica gel and RP_8 to afford **1** as white amorphous powder. Compound **1** showed red color reaction to Ehrlich reagent (p_dimethylaminobenzaldehyde (1 g) and 20% hydrochloric acid (100 mL)) in TLC. After the normal procedure with refluxed in 70% aqueous acetone, it afforded 22_hydroxy form of furostanol glycoside, which was further indicated by the characteristic ketal carbon signal at δ 110. 9 in ^{13}C_NMR spectrum[3]. A quasi molecular ion peak of **1** was observed at m/z 1 225. 581 7 ([M- H]$^-$) in the high resolution negative ion FAB mass spectrum, indicating the molecular formula as $C_{57}H_{94}O_{28}$ (calcd for $C_{57}H_{93}O_{28}$, 1 225. 585 3). Acid hydrolysis of **1** gave a steroidal sapogenin (**1a**) and the sugar residues which were identified as glucose and rhamnose by TLC. The steroidal sapogenin (**1a**) was confirmed as diosgenin by ^{1}H_ and ^{13}C_NMR spectra[9]. The presence of five sugar units in **1** was indicated by anomeric proton signals (δ 4. 78 (1H, d, *J* = 7. 6 Hz), 4. 91 (1H, d, *J* = 7. 5 Hz), 5. 09 (1H, d, *J* = 7. 3 Hz), 5. 27 (1H, d, *J* = 7. 5 Hz) and 6. 13 (1H, br s)) and anomeric carbon signals (δ 100. 3, 102. 1, 104. 5, 105. 5 and 105. 8) in ^{1}H_ and ^{13}C_NMR spectra. Broad singlet peak at δ 6. 13 indicated the α_orientation at the anomeric center of rhamnose. The *J* values of the other four anomers of the sugar moieties indicated the β_orientation at the anomeric center of the *D*_pyranoses. The above evidence suggested that **1** was (25*R*)_furost_5_en_3β, 22ζ, 26_triol pentaglycoside.

The sugar linkages were determined by the assignment of ^{1}H- and ^{13}C_NMR signals due to the sugar moieties that were established by analyses of 2D NMR experiments. ^{13}C_NMR chemical shifts due to sugar moieties were assigned easily by HMQC_TOCSY spectrum (Table 1). Three_bond ^{1}H_^{13}C long_range correlations were observed in HMBC spectrum. The cross signals were shown at δ 4. 91 (H_1 of inner glucopyranosyl residue) and 78. 3 (C_3 of aglycone), 6. 13 (H_1 of terminal rhamnopyranosyl residue) and 77. 7 (C_2 of inner glucopyranosyl residue), 5. 09 (H_1 of middle glucopyranosyl residue) and 81. 6 (C_4 of inner glucopyranosyl residue), 5. 27 (H_1 of terminal glucopyranosyl residue) and 88. 2 (C_3 of middle glucopyranosyl residue), as well as 4. 78 (H_1 of glucopyranosyl residue) and 75. 2 (C_26 of aglycone) (Fig. 1). Thus, the structure of **1** was determined to be 25(*R*)_26_O_β_glucopyranosyl_furost_5_en_3β, 22ξ, 26_triol 3_O_β_*D*_glucopyranosyl (1→3)_β_*D*_glucopyranosyl (1→4)_[α_*L*_rhamnopyranosyl (1→2)]_β_*D*_glucopyranoside, and it was named parvifloside.

Enzymatic hydrolysis of **1** with cellulase afforded six

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Table 1 ^{13}C-NMR data of compounds **1**, **5** and **6**

Position	**1**[a]	**5**	**6**	Position	**1**[a]	**5**	**6**
1	37.7t	37.5t	37.6t	Glc-1	100.3d	102.7d	100.1d
2	30.3t	30.1t	30.1t	2	77.7d	75.4d	77.5d
3	78.3d	78.2d	78.2d	3	76.4d	78.7d	76.3d
4	39.1t	39.4t	39.0t	4	81.6d	71.8d	82.1d
5	141.0s	141.0s	140.9s	5	77.7d	78.6d	77.8d
6	122.0d	121.8d	121.9d	6	61.7t	62.9t	62.0t
7	32.6t	32.4t	32.4t	Rha-1	102.1d		101.9d
8	31.9d	31.7d	31.8d	2	72.5d		72.5d
9	50.6d	50.4d	50.4d	3	72.9d		72.8d
10	37.3s	37.3s	37.2s	4	74.2d		74.2d
11	21.3t	21.2t	21.2t	5	69.7d		69.5d
12	40.2t	40.0t	40.0t	6	18.8q		18.7q
13	41.0s	40.9s	40.9s	Glc′-1	104.5d		105.0d
14	56.6d	56.7d	56.7d	2	74.2d		75.0d
15	32.6t	32.5t	32.5t	3	88.2d		78.3d
16	81.3d	81.2d	81.2d	4	69.7d		71.3d
17	63.9d	63.9d	63.9d	5	78.1d		78.5d
18	16.6q	16.6q	16.5q	6	62.1t		62.2t
19	19.6q	19.5q	19.5q	Glc″-1	105.8d		
20	40.8d	40.8d	40.8d	2	75.7d		
21	16.5q	16.6q	16.5q	3	78.8d		
22	110.9s	110.8s	110.8s	4	71.8d		
23	37.3t	37.1t	37.2t	5	78.8d		
24	28.5t	28.4t	28.4t	6	62.7t		
25	34.4d	34.4d	34.4d	Glc$_{26}$-1	105.0d	105.0d	105.0d
26	75.2t	75.3t	75.3t	2	75.3d	75.4d	75.2d
27	17.6q	17.5q	17.5q	3	78.7d	78.7d	78.6d
				4	71.9d	71.8d	71.8d
				5	78.5d	78.6d	78.5d
				6	63.0t	62.9t	62.8t

a, spectra obtained at 125 MHz in pyridine-d_5 unless otherwise specified with offset set to δ 149.89 for pyridine. Assignments based on ^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC and HMQC-TOCSY NMR experiments.

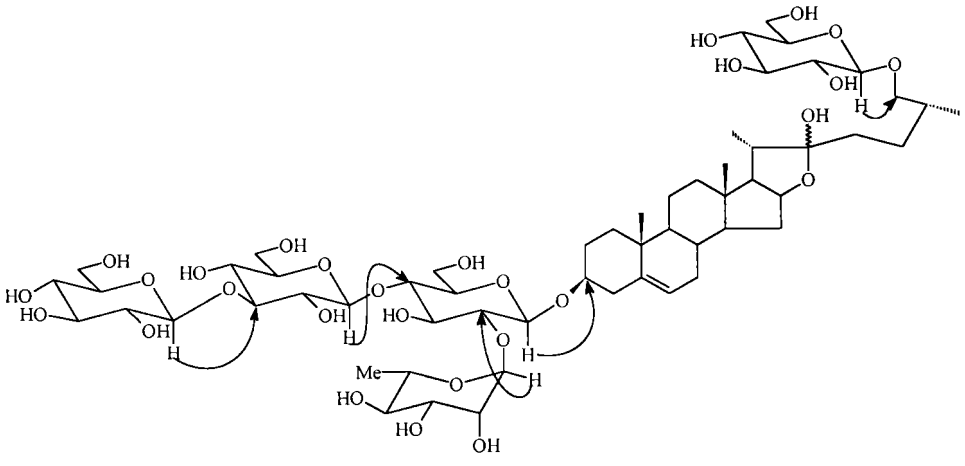

Fig. 1. The key ^{1}H-^{13}C long-range correlations of compound **1** in the HMBC spectrum.

prosapogenins (**2**–**7**), while **1** yielded two prosapogenins, **3** and **4** in the same condition with β-glucosidase. Based on comparison of physical and spectroscopic data with previously reported, six prosapogenins were identified to be prosapogenin A of dioscin (**2**)[10], deltonin (**3**)[11], diosgenin 3-O-β-*D*-glucopyranosyl (1→3)-β-*D*-glucopyranosyl (1→4)-[α-*L*-rhamnopyranosyl (1→2)]-β-*D*-glucopyranoside (**4**)[12,13], 25(*R*)-26-O-β-glucopyranosyl-furost-5-en-3β, 22ξ, 26-triol 3-O-β-*D*-glucopyranoside (lilioglycoside K) (**5**)[14], 25(*R*)-26-

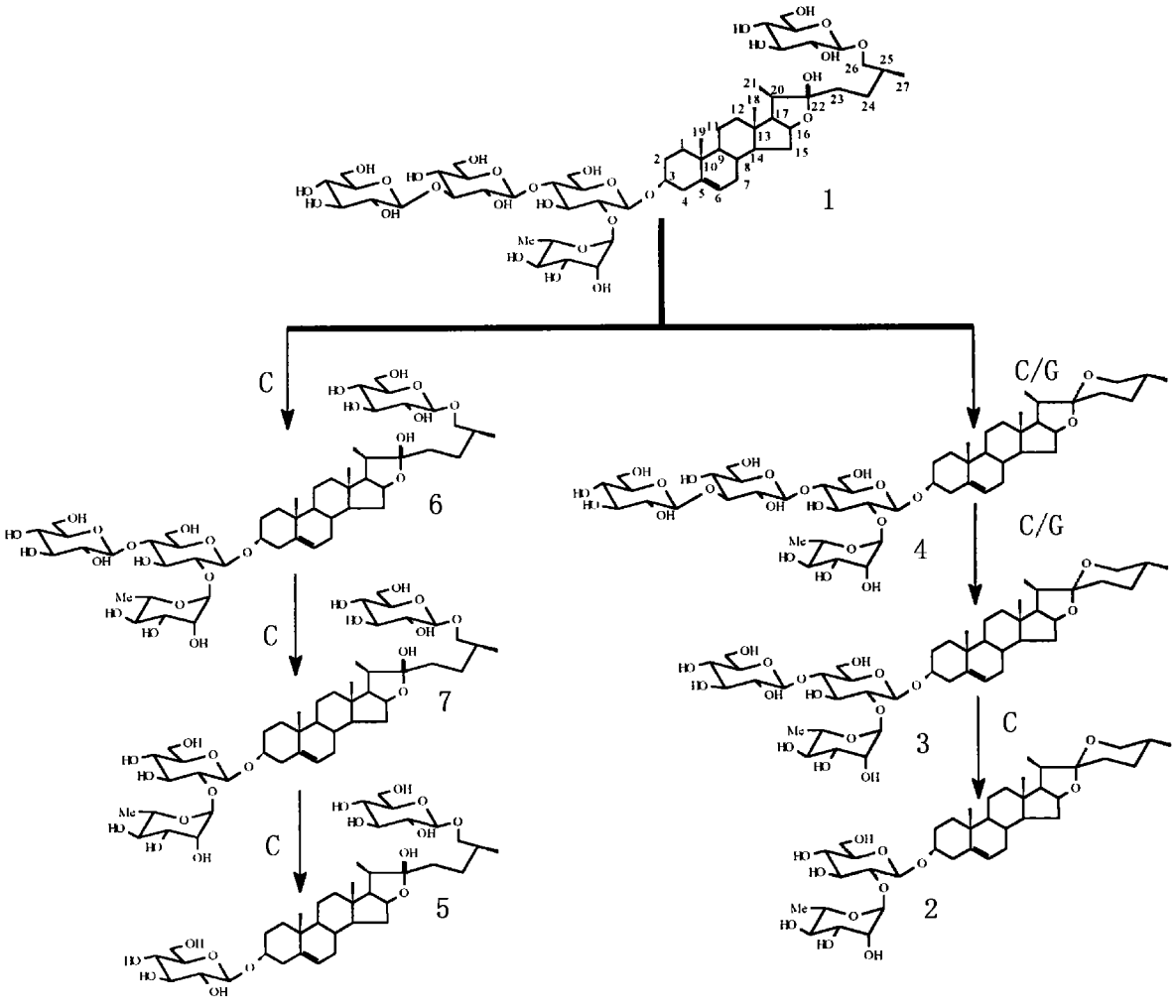

Fig. 2. The enzymatic degradation of saponin **1**.
1, parvifloside; **2**, prosapogenin A of dioscin; **3**, deltonin; **4**, diosgenin 3_O_β_*D*_glucopyranosyl (1→3) _β_*D*_glucopyranosyl (1→4)_[α_*L*_rhamnopyranosyl (1→2)]_β_*D*_glucopyranoside; **5**, lilioglycoside K; **6**, deltoside; **7**, protobioside; C, crude cellulase; G, β_glucosidase.

O_β_glucopyranosyl_furost_5_en_3β, 22ξ, 26_triol 3_O_β_*D*_glucopyranosyl (1→4)_[α_*L*_rhamnopyranosyl (1→2)]_β_*D*_glucopyranoside (deltoside) (**6**)[11] and 25(*R*)_26_O_β_glucopyranosyl_furost_5_en_3β, 22ξ, 26_triol 3_O_α_*L*_rhamnopyranosyl (1→2)_β_*D*_glucopyranoside (protobioside) (**7**).

It is well known that furostanol glycosides were easily transformed to the corresponding spirostanol saponin by β_glucosidase or cellulase[3, 9]. They only lost a glucosyl unit at C_26 position of aglycones. In our research, **1** not only transferred to its spirostanol counterpart **4**, but also gave prosapogenin **3** by β_glucosidase. After hydrolyzed by cellulase, **1** not only yielded spirostanol prosapogenins **2**– **4**, but also gave furostanol glycosides **5**– **7** (Fig. 2).

The glucosyl unit at C_26 position of furostanol glycosides may be the optimal substrate of β_glucosidase, so furostanol glycosides were easily transformed to the corresponding spirostanol saponins by β_glucosidase. Spirostanol saponins can also be degraded by β_glucosidase, if spirostanol saponins have the glucosyl unit which is the optimal substrate of β_glucosidase and are soluble in water. After **4** was incubated with β_glucosidase in a NaAc_HOAc (pH 5.0) buffer at 25 ℃ for 16 h, **3** was detected on TLC. Why was not **3** degraded by β_glucosidase? The glucosyl unit at C_3 position of **3** may not be the optimal substrate of β_glucosidase and **3** is insoluble in water, so **3** could not be degraded by β_glucosidase.

The cellulase used in the experiment was proven to be very impure by electrophoresis. The activity of its β_glucosidase might be very low. We suggested the cellulase used in the experiment was crude cellulase. It is reported for the first time that the furostanol glycoside was degraded partially and formed a series of prosapogenins with both of spirostanol and furostanol skeleton after hydrolyzed by crude cellulase. The enzymatic degradation or transformation of crude cellulase may be a good way to establish molecular bank of steroidal saponins for bioassay test. Moreover, it may be an important route to the formation of molecular diversity of steroidal saponins in plants.

Compounds **2**, **3** and **5** exhibited significant activity of morphological deformation of mycelia germinated from *Pyricularia oaryzae* P_2b conidia and showed minimum

Table 2 ^{13}C_NMR data of compounds **1a, 2– 4**

Position	1a	2	3	4	Position	2	3	4
1	37. 2t	37. 6t	37. 6t	37. 5t	Glc_1	100. 5d	100. 2d	100. 0d
2	31. 4t	30. 3t	30. 2t	30. 2t	2	78. 0d	78. 5d	77. 3d
3	71. 6d	78. 2d	78. 3d	78. 3d	3	79. 7d	76. 2d	76. 2d
4	42. 2t	39. 1t	39. 0t	39. 0t	4	72. 0d	82. 1d	81. 5d
5	140. 8s	141. 0s	140. 9s	140. 8s	5	78. 2d	77. 7d	77. 7d
6	121. 3d	121. 8d	121. 8d	121. 8d	6	62. 8t	62. 1t	61. 6t
7	32. 0t	32. 4t	32. 3t	32. 3t	Rha_1	102. 0d	101. 8d	101. 8d
8	31. 5d	31. 8d	31. 8d	31. 7d	2	72. 6d	72. 4d	72. 5d
9	50. 0d	50. 5d	50. 4d	50. 3d	3	72. 9d	72. 8d	72. 8d
10	36. 6s	37. 2s	37. 2s	37. 2s	4	74. 3d	74. 2d	74. 2d
11	20. 8t	21. 2t	21. 2t	21. 1t	5	69. 5d	69. 4d	69. 4d
12	39. 7t	40. 0t	40. 0t	39. 9t	6	18. 7q	18. 6q	18. 7q
13	40. 2s	40. 6s	40. 6s	40. 5s	Glc′_1		105. 2d	104. 6d
14	56. 4d	56. 8d	56. 8d	56. 7d	2		75. 0d	73. 8d
15	31. 8t	32. 3t	32. 4t	32. 3t	3		78. 3d	88. 3d
16	80. 8d	81. 2d	81. 2d	81. 2d	4		71. 4d	69. 5d
17	62. 0d	63. 0d	63. 0d	62. 9d	5		77. 5d	78. 0d
18	16. 2q	16. 4q	16. 4q	16. 4q	6		62. 2t	61. 8t
19	14. 5q	19. 5q	19. 4q	19. 4q	Glc″_1			105. 9d
20	41. 5d	42. 1d	42. 2d	42. 0d	2			75. 6d
21	14. 5q	15. 0q	15. 0q	15. 1q	3			78. 2d
22	109. 2s	109. 3s	109. 3s	109. 3s	4			71. 7d
23	31. 3t	31. 9t	31. 9t	31. 9t	5			78. 7d
24	28. 7t	29. 3t	29. 3t	29. 3t	6			62. 6t
25	30. 2d	30. 7d	30. 7d	30. 6d				
26	66. 8t	67. 0t	67. 0t	66. 9t				
27	17. 1q	17. 3q	17. 3q	17. 4q				

morphological deformation concentration as 1. 17, 1. 17 and 1. 95 μg/mL, respectively (Table 3). This result suggested that the sugar linkage might play an important role in the biological activity of steroidal saponins.

Table 3 Effect of compounds **1– 6** on deformation of mycelia germinated from *Pyricularia oaryzae* P_2b conidia

Compounds	Sample quantity (mg)	Mmdc (μg/mL)
1	1. 5	187. 50
2	0. 6	1. 17
3	0. 6	1. 17
4	1. 3	75. 00
5	1. 0	1. 95
6	1. 7	13. 30

Mmdc, minimum morphological deformation concentration.

2 Experimental

2. 1 General experimental procedures

The melting point was determined on XRC_1 instrument and was uncorrected. Optical rotations were measured in pyridine with HORIBA SEPA_300 high_sensitive polarimeter. IR (KBr) spectra were measured on Bio_Rad FTS_135 spectrophotometer. NMR spectra were recorded on a Bruker DRX_500 or Brucker AM_400 instrument at 25 ℃, using TMS as an internal standard. The negative ion FAB mass spectra were recorded on a VG AutoSpec mass spectrometer using glycerol as matrix. MPLC was carried out on B chi 681 chromatography system and B chi 684 fraction collector. Diaion HP_20 (Mitsubishi Chemical Industries), silica gel (Qingdao Haiyang Chemical Co.), RP_8 (Merck) were used for column chromatography. Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC and HPTLC. Detection was done by spraying the plates with 10% H_2SO_4, followed by heating.

2. 2 Plant material

The fresh rhizomes of *Dioscorea parviflora* C. T. Ting were collected in Qiubei County, Yunnan Province, China, in May 1999. The plant was identified by Prof. LU Chun_Chao. A voucher specimen was preserved at the herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

2. 3 Extraction and isolation

The fresh rhizomes of *D. parviflora* (70 kg) were crushed and extracted with 70% aqueous ethanol (70 L) for 4 h three times. The concentrated extract was partitioned between *n*_butanol and water. The aqueous layer was subjected to Diaion HP_20 column chromatography and gave a MeOH eluate (81 g), which was fractionated on a silica gel column eluting with $CHCl_3$_MeOH_H_2O (7 ∶ 3∶ 0. 5) and was divided into 18 fractions. Fractions 16 and 17 were combined (15 g), and chromatographed to

yield a pair of 22_hydroxy and 22_methoxy forms of furostanol saponins by silica gel ($CHCl_3$_MeOH_H_2O (7: 2.5: 0.4)) and RP_8 (50% aqueous MeOH). After refluxed with 70% aqueous acetone for 10 h[15], compound **1** was afforded (11.3 g).

2.4 Acid hydrolysis of 1

Compound **1** (80 mg) was refluxed with 2 mol/L HCl_dioxane (1:1, V/V, 4 mL) on water bath for 6 h. After cooling, the reaction mixture was evaporated to dryness. The dry reaction mixture was extracted by $CHCl_3$ four times. The $CHCl_3$ extract was chromatographed on an silica gel column and gave a steroidal sapogenin (**1a**) (19 mg). Sugar residues were checked on an HPTLC silica gel 50000 F_{254} plate using $CHCl_3$_MeOH_Me_2CO_H_2O (3: 3: 3: 1) as developing solvent (R_f 0.51 (glucose), 0.68 (rhamnose)).

2.5 Enzymatic hydrolysis of 1

Compound **1** (350 mg) and cellulase (Dongfeng Biochem. Co., 350 mg) in a NaOAc_HOAc buffer (pH 5.0, 50 mL) were incubated at 25 ℃ for 16 h. The reaction liquid was extracted with *n*_butanol four times. The *n*_butanol layer (320 mg) was chromatographed by silica gel [$CHCl_3$_MeOH, 10: 1] and RP_8 MPLC, and afforded **2** (37 mg), **3** (55 mg), **4** (8 mg), **5** (24 mg), **6** (25 mg) and **7**, respectively. (**7** was only determined by negative ion FAB mass spectrum and comparison of authentic sample on TLC because its quantity was very low).

Compound **1** (90 mg) and β_glucosidase (90 mg) (SIGMA) in a NaOAc_HOAc buffer (pH 5.0, 90 mL) were incubated at 25 ℃ for 16 h. The resulting mixture was extracted by *n*_butanol four times. The *n*_butanol extract (75 mg) was chromatographed to give **3** (15 mg) and **4** (46 mg) by silica gel MPLC ($CHCl_3$_MeOH, 10: 1).

2.6 Bioassay

A 90_well flat_bottomed assay plate was used for the bioassay. The first, middle and last columns were preserved for negative and positive controls. Rhizoxin was used for positive control with the final concentrations of 1 μmol/L, 0.5 μmol/L, 0.25 μmol/L, 0.125 μmol/L, 60 nmol/L, 30 nmol/L, 16 nmol/L, and 8 nmol/L. One column (eight wells) was usually used for one test material with eight different concentrations. Each 50 μL of conidia suspension of *Pyricularia oaryzae* P_2b (4×10^4 conidia/mL) was first poured into each well, and 50 μL of each test solution was then added to the first well. The suspension was mixed and taken 50 μL to the second well. The procedure was repeated to the last well of the column. For negative control, 50 μL of water was added to the first well followed by the procedure as above. The assay plates were incubated at 27 ℃ for 16 h, and the shape of mycelia germinated from conidia was observed and compared with controls under an inverted microscope[16].

2.7 Identification

Parvifloside (1) A white amorphous powder (70% aqueous acetone), mp 194 – 196 ℃. $[\alpha]_{28.5}^{D}$ – 46.07° (*c* 0.036, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3 401, 2 930, 1 073, 1 035. HRFAB_MS m/z 1 225.581 7 [M – H]$^-$ (calcd for $C_{57}H_{93}O_{28}$, 1 225.585 3). Negative ion FAB_MS m/z 1 225 [M – H]$^-$, 1 079 [M – H – 146]$^-$, 1 063 [M – H – 162]$^-$, 901 [M – H – 162 – 162]$^-$, 755 [M – H – 162 – 162 – 146]$^-$. ^{1}H_NMR (C_5D_5N): δ 0.89 (3H, s, H_18), 1.04 (3H, s, H_19), 0.98 (3H, d, *J* = 6.3 Hz, H_27), 1.33 (3H, d, *J* = 6.1 Hz, H_21), 1.73 (3H, d, *J* = 4.8 Hz, Rha H_6), 3.61, 4.05 (1H each, H_26), 3.92 (1H, H_3), 4.78 (1H, d, *J* = 7.6 Hz, Glc$_{26}$ H_1), 4.91 (1H, d, *J* = 6.0 Hz, Glc H_1), 4.95 (1H, H_16), 5.09 (1H, d, *J* = 7.3 Hz, Glc′ H_1), 5.27 (1H, d, *J* = 7.5 Hz, Glc″ H_1), 5.31 (1H, s, H_6), 6.16 (1H, br s, Rha H_1). ^{13}C_NMR data see Table 1.

Diosgenin (1a) A white amorphous powder ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3 392, 2 946, 1 456, 1 375, 1 174, 1 052, 980, 918, 899, 864 (intensity: 899> 918). EI_MS m/z: 414, 355, 342, 326, 300, 282, 271, 253, 139 (base peak), 115. ^{1}H_NMR ($CDCl_3$): δ 0.74 (3H, s, H_18), 0.97 (3H, s, H_19), 0.73 (3H, d, *J* = 6.1 Hz, H_27), 0.92 (3H, d, *J* = 6.96 Hz, H_21). ^{13}C_NMR data see Table 2.

Prosapogenin A of dioscin (2) A white amorphous powder (MeOH), mp 239 – 241 ℃. $[\alpha]_{19.2}^{D}$ – 50.15° (*c* 0.1, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3 414, 2 943, 1 633, 1 455, 1 051, 981, 919, 899 (intensity: 899> 919). Negative ion FAB_MS m/z 721 [M – H]$^-$. ^{1}H_NMR (C_5D_5N): δ 0.67 (3H, d, *J* = 5.4 Hz, H_27), 0.81 (3H, s, H_18), 1.03 (3H, s, H_19), 1.12 (3H, d, *J* = 7.0 Hz, H_21), 1.77 (3H, d, *J* = 5.6 Hz, Rha H_6), 3.95 (1H, m, H_3), 5.03 (1H, d, *J* = 7.2 Hz, Glc H_1), 5.28 (1H, br d, *J* = 4.8 Hz, H_6), 6.37 (1H, br s, Rha H_1). ^{13}C_NMR data see Table 2.

Deltonin (3) A white amorphous powder (MeOH), mp 300 – 304 ℃ (decomposed). $[\alpha]_{19.2}^{D}$ – 83.85° (*c* 0.032 5, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3 440, 2 938, 1 633, 1 455, 1 062, 984, 917, 900 (intensity: 900> 917). Negative ion FAB_MS m/z 883 [M – H]$^-$, 721 [M – H – 162]$^-$. ^{1}H_NMR (C_5D_5N): δ 0.68 (3H, d, *J* = 5.0 Hz, H_27), 0.81 (3H, s, H_18), 1.03 (3H, s, H_19), 1.12 (3H, d, *J* = 6.8 Hz, H_21), 1.78 (3H, d, *J* = 7.0 Hz, Rha H_6), 3.83 (1H, m, H_3), 4.92 (1H, d, *J* = 6.5 Hz, Glc H_1), 5.10 (1H, d, *J* = 7.8 Hz, Glc′ H_1), 5.28 (1H, br s, *J* = 4.2 Hz, H_6), 6.21 (1H, br s, Rha H_1). ^{13}C_NMR data see Table 2.

Compound 4 A white amorphous powder (MeOH), mp 282 – 286 ℃ (decomposed). $[\alpha]_{20.4}^{D}$ – 85.00° (*c* 0.015, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3 410, 2 937, 1 633, 1 455, 1 050, 983, 919, 899 (intensity: 899> 919). Negative ion FAB_MS m/z 1 045 [M – H]$^-$, 883 [M – H – 162]$^-$, 721 [M – H – 162 –

162]$^-$. ^{1}H_NMR (C_5D_5N): 0.68 (3H, d, J = 5.6 Hz, H_27), 0.82 (3H, s, H_18), 1.04 (3H, s, H_19), 1.12 (3H, d, J = 7.0 Hz, H_21), 1.75 (3H, d, J = 6.2 Hz, Rha H_6), 3.88 (1H, m, H_3), 4.93 (1H, d, J = 7.7 Hz, Glc H_1), 4.95 (1H, 16_H), 5.08 (1H, d, J = 7.9 Hz, Glc′ H_1), 5.27 (1H, d, J = 7.9 Hz, Glc″ H_1), 5.31 (1H, s, 6_H), 6.22 (1H, br s, Rha H_1). ^{13}C_NMR data see Table 2.

Lilioglycoside K (5) A white amorphous powder (70% aqueous acetone), mp 204–208 ℃. $[\alpha]_{19.7}^{D}$ –25.00° (c 0.04, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3 401, 2 923, 1 078, 1 028. Negative ion FAB_MS m/z: 755 [M–H]$^-$, 593 [M–H–162]$^-$. ^{1}H_NMR (C_5D_5N): δ 0.88 (3H, s, H_18), 1.04 (3H, s, H_19), 0.95 (3H, d, J = 6.6 Hz, H_27), 1.33 (3H, d, J = 6.3 Hz, H_21), 4.79 (1H, d, J = 7.7 Hz, Glc$_{26}$ H_1), 4.89 (1H, d, J = 7.7 Hz, Glc$_3$ H_1). ^{13}C_NMR data see Table 1.

Deltoside (6) A white amorphous powder (70% aqueous acetone), mp 245–247 ℃. $[\alpha]_{20.7}^{D}$ –48.62° (c 0.145, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3 401, 2 930, 1 073, 1 035. Negative ion FAB_MS m/z: 1 063 [M–H]$^-$, 901 [M–H–162]$^-$, 755 [M–H–162–146]$^-$. ^{1}H_NMR (C_5D_5N): δ 0.88 (3H, s, H_18), 1.04 (3H, s, H_19), 0.97 (3H, d, J = 5.8 Hz, H_27), 1.33 (3H, br d, H_21), 1.74 (3H, d, J = 4.5 Hz, Rha H_6), 3.61, 4.04 (1H each, both dd_like, H_26), 3.92 (1H, dd_like, H_3), 4.79 (1H, d, J = 7.2 Hz, Glc$_{26}$ H_1), 4.93 (1H, d_like, Glc H_1), 5.12 (1H, d, J = 6.6 Hz, Glc′ H_1), 5.27 (1H, s, H_6), 6.22 (1H, br s, Rha H_1). ^{13}C_NMR data see Table 1.

Protobioside (7) Negative ion FAB_MS m/z 901 [M–H]$^-$, 755 [M–H–146]$^-$.

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小花盾叶薯蓣甙的酶降解

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摘要: 从小花盾叶薯蓣(*Dioscorea parviflora* C. T. Ting)的新鲜根状茎中分离到一个新的呋甾烷型配糖体, 命名为小花盾叶薯蓣甙(parvifloside) (**1**), 其结构通过波谱和化学方法鉴定为: (25*R*)_26_O_β_glucopyranosyl_furost_5_en_3β, 22ξ, 26_triol 3_O_β_*D*_glucopyranosyl (1→3)_β_*D*_glucopyranosyl (1→4)_[α_*L*_rhamnopyranosyl (1→2)]_β_*D*_glucopyranoside。化合物 **1** 在纤维素酶粗酶和 β_葡萄糖苷酶中进行水解, 得到降解产物 **2**– **7**。对 **1** 的酶解现象进行了讨论。同时, 对所分离的甾体皂甙的抗稻瘟霉菌活性进行了初步筛选。

关键词: 小花盾叶薯蓣; 小花盾叶薯蓣甙; 甾体皂甙; 酶降解

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