Highly Efficient and Regio-selective Glucosylation of 25(*S*) Ruscogenin by *Gliocladium deliquescens* NRRL1086

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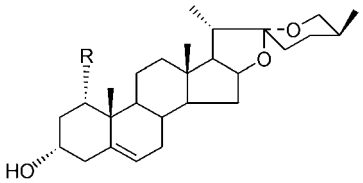
A new steroidal glycoside, 25(*S*) ruscogenin 1-*O*-*β*-*D*-glucopyranoside (**2**) was obtained through the microbial transformation of 25(*S*) ruscogenin (**1**) by *G. deliquescens* NRRL1086 in 54% isolated yield. The structure of the product was elucidated by IR, MS and NMR spectra. This is the first report on the preparation of steroidal saponins by microbial transformation.

Keywords ruscogenin, biotransformation, microbial glucosylation, steroid, *Gliocladium deliquescens* NRRL1086

Introduction

Ruscogenin (**1**), an important aglycon of natural steroidal saponins, was first isolated from *Ruscus aculeatus* L. It has strong anti-inflammatory activities and acts as an anti-elastase, decreases capillary permeability.[1-3] Our previous studies showed that ruscogenin and its glycosides markedly suppressed the adherence of HL-60 cells to human endothelial ECV304 cells and the overexpression of ICAM-1 induced by TNF-*α* in endothelial cell.[4-6] However, the low water-solubility affected its pharmacodynamics and bioavailability *in vivo*, a problem occurs on many natural products in the drug discovery pipeline. Microbial transformation is a versatile tool for the structural modification of bioactive natural compounds under mild conditions.[7-9] With the aim to obtain more active and/or better water-soluble derivatives of ruscogenin, the microbial transformation of ruscogenin was carried out. The screening tests of dozens of microbe strains revealed that **1** could be glycosylated by *G. deliquescens* NRRL1086 to give a good-water-solubility steroidal saponin (**2**) with excellent yield. The product was further identified as 25(*S*) ruscogenin 1-*O*-*β*-*D*-glucopyranoside (**2**, Figure 1) based on its IR, HR-ESIMS, ^{1}H NMR, ^{13}C NMR, HSQC and HMBC spectra.

In the research of microbial glucosylations, many microbes were found to be able to glycosylate exogenous substrates with their own enzymic systems which were presumed to be inducible UDP-glucose-dependent glycosyltransferase and speculated as a reasonable interpretation for microbial detoxification.[10-12] The mainly used substrates for microbial glycosylations were focused on antibiotics, flavonoids, anthraquinones, diterpenes,[13-15] *etc*. Up to now, there were no reports about microbial glycosylation of steroidal saponins. In this study, we demonstrated a microbial glucosylation of 25(*S*) ruscogenin (a spirostane steroidal sapogenin) to ruscoside.



Compound **1** R = OH
Compound **2** R = *O*-*β*-*D*-glucose

Figure 1 Chemical structure of compound **1** and **2**.

Experimental

G. deliquescens was obtained from a courtesy of Prof. J. P. N. Rosazza of University of Iowa, USA. 25(*S*) ruscogenin (compound **1**) was prepared from *Liriope spicata* (Thunb.) Lour. var. *prolifera* Y. T. Ma and the purity was determined to be higher than 98% by

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normalization of the peak areas detected by HPLC-ELSD.

The screening scale biotransformation was performed by the standard two-stage fermentation protocol with 30 mL of potato medium (PDA) held in 150 mL flasks. Cultures were incubated on rotary shakers at 180 r/min at 28 ℃. 1 mL inoculum derived from 24-h-old stage I cultures was used to initiate stage II cultures, which were incubated for 24 h before receiving 5 mg of 25(*S*) ruscogenin as substrate. After a further fermentation for 5 d, the cultures were filtered and the broth was extracted with equal volume of ethyl acetate 3 times. The organic extracts were dried over anhydrous sodium sulfate and then chromatographed by TLC with $CHCl_3$-MeOH (V/V=8∶1). Detection was carried out by spraying 10% H_2SO_4-EtOH ($V_{conc.\ sulfuric\ acid}$∶$V_{ethanol}$=10∶90) on the plate and heating at 120 ℃ for 1—2 min.

The procedures of preparative scale biotransformation[16,17] of **1** by *G. deliquescens* were carried out in 60 flasks each of which contained 30 mL of liquid PDA medium. Other procedures were the same as screening scale biotransformation. The cultures were filtered and the broth was extracted with 6 L of ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate and concentrated by rotary evaporation, which was subjected to silica gel chromatographic separation by elution with chloroform-methanol in gradient manner. The product was determined based on IR, HR-ESIMS, ^{1}H-NMR, ^{13}C-NMR, HSQC and HMBC spectra.

Results and discussion

The screening test showed that *G. deliquescens* could transform ruscogenin to one single more polar product (compound **2**). 200 mg of the substrate was added to the biotransformation culture and 148.9 mg of **2** (54% yield, *m*/*m*) was obtained as amorphous powder.

Compound **2** showed a positive Liebermann-Burchard reaction. The molecular formula was established as $C_{33}H_{52}O_9$ by the HR-ESIMS showing $[M+H]^+$ at *m/z* 593.3655 (calcd for 593.3684), indicating a glucosyl group might be introduced to the substrate. The ^{1}H NMR, ^{13}C NMR and DEPT spectra of **2** showed new proton and carbon signals with chemical shifts that are characteristic of *β-D*-glucose (Table 1), which further confirmed the presence of a glucose moiety in compound **2**. Comparisons of the ^{13}C NMR spectra of **1** and **2** indicated that **2** is a glycoside of **1** on C-1 position based on the downfield shift of C-1 from δ 78.2 to 83.2 and the chemical shift of C-2 and C-10 were shifted up-field δ 6.1 and 0.9, respectively, but the other carbon signals remained unaffected (Table 1). In addition, the HMBC data of **2** (Figure 2) showed a correlation between H-1 (δ_H 4.96) and C-1' (δ 101.7), which further confirmed that the glucosyl group was introduced at C-1. The anomeric proton H-1' of the glucose moiety in **2** resonated as a doublet at δ 4.96. A coupling constant of 7.68 Hz for H-1' indicated that the stereochemistry of the glucosidic linkage at C-1' of *D*-glucose is *β*. Accordingly, the structure of compound **2** was determined as 25(*S*) ruscogenin 1-*O*-*β*-*D*-glucopyranoside.

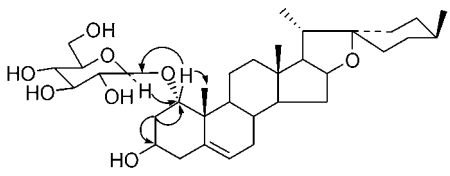

Figure 2 Key H-C HMBC spectra of compound **2**.

There are two hydroxyl groups on the skeleton of 25(*S*) ruscogenin, but only one glucosylation product on C-1 hydroxyl group was obtained. To test whether the enzyme(s) can catalyze the reaction on the C-3 position, compound **2** was directly added to culture as substrate and incubated for another 5 d, no additional product but compound **1** was observed.

Glucosylations using biotransformation methods have been subjected of increasing attention because they facilitate the conversion of water-insoluble compounds to those that are more water-soluble, and as one-step enzymatic glucosylation, it is useful for preparation of glycosides compared with chemical glycosylation that requires tedious steps including the protection and the deprotection of hydroxyl groups of sugar moieties.[18-22] In the biosynthetic pathway of steroidal saponin, the sugar chain can be linked to either C-1 or C-3, and the plant-origin monosides of ruscogenin were very rare.[23] Therefore, the enzemy catalyzed this reaction in *G. deliquescens* may differ from the enzymatic systems in herbs which contain the ruscogenin glycoside.

To our knowledge, microbial glycosylation of ectogenic substrates was rather difficult to implement and mostly proved to be of low yield.[24-26] Plant cells suspension cultures and hairy root cultures as the most commonly utilized to glycosylation cultures[27-32] require relatively more procedures and much longer culture periodicity. Hereby, the glucosylation by *G. deliquescens* could provide us a high efficient and practical method to obtain ruscogenin glycoside. The unique catalytic capability of *G. deliquescens* to regio-selective glucosylation of ruscogenin deserves further exploration. Such studies could provide new platforms for combinatorial synthesis[33] and the development of new, active steroidal saponin. The bioactivity of the new ruscogenin glycoside and the characters of the enzyme are now in progress.

Table 1 NMR spectral data of compounds **1** and **2** (pyridine-d_6, 500 MHz)

Carbon	**1**	**2**			
	δ_C	δ_C	δ_H	HMBC(H→C)	DEPT
1	78.2	83.2	3.93 (dd, J=6.3, 19.5 Hz)	C-2,19,6'	CH
2	44.0	37.9	2.76 (d, J=12.2 Hz), 2.11 (t, J=11.85 Hz)	C-4	CH_2
3	68.3	68.0	3.74—3.92 (m)	C-2,4	CH
4	43.7	43.8	2.56 (dd, J=4.1, 11.7 Hz)		CH_2
5	140.5	139.5			C
6	124.3	124.7	5.57 (d, J=5.5 Hz)	C-4	CH
7	33.2	32.4		C-6	CH_2
8	32.5	33.0		C-7,9	CH
9	51.6	50.3		C-1,8,12,19	CH
10	43.7	42.8		C-1,2,4,6,19	C
11	24.4	23.9	2.84 (d, J=11.6 Hz), 1.43—1.47 (m)		CH_2
12	40.8	40.4		C-14,18	CH_2
13	40.4	40.2		C-8,12,14,16,17	C
14	57.2	56.9		C-12,18	CH
15	32.6	32. 0			CH_2
16	81.2	81.1	4.45—4.51 (m)	C-15	CH
17	63.4	63.1	1.9 (t, J=6.8 Hz)	C-14,15,16,18	CH
18	16.7	16. 8	0.87 (s)		CH_3
19	14.0	14. 8	1.25 (s)	C-1,9	CH_3
20	42.2	42. 0		C-17,18,21	CH
21	15.1	15.0	1.10 (d, J=6.5 Hz)	C-17,18,20	CH_3
22	109.3	109.2			C
23	26.4	27.5		C-24	CH_2
24	26.3	26.4		C-23,25	CH_2
25	27.6	28.2		C-24,27	CH
26	65.2	65.0	4.05 (d, J=2.6 Hz), 3.35 (d, J=11.3 Hz)	C-27	CH_2
27	16.3	16.3	1.06 (d, J=7.2 Hz)		CH_3
1'		101.7	4.96 (d, J=7.7 Hz)	C-1,2',3'	CH
2'		75.4	4.03 (t, J=13.9 Hz)	C-2,1',5'	CH
3'		78.1		C-2',4'	CH
4’		72.4	4.14 (t, J=15.2 Hz)	C-5'	CH
5'		78.6	4.23 (t, J=14.5 Hz)	C-4',6'	CH
6'		63.7	4.55 (dt, J=2.8, 11.3 Hz), 4.35 (dd, J=9.5, 18.8 Hz)	C-5'	CH_2

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