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Short communication

Highly regioselective glucosylation of alcoholic hydroxyls of protostane triterpenoids mediated by fungal biotransformation

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ABSTRACT

An efficient glucosylation of alcoholic hydroxyls in the structures of protostane triterpenoids catalyzed by fungus has been developed. Four protostanes **1–4** as the substrates have been transformed by *Syncephalastrum racemosum* AS 3.264 and generated corresponding 11-OH glucosylation derivatives. The regioselective characteristics were also identified by various highly oxygenated protostane substrates. The time-courses displayed the highest yields of **1a**, **3a**, and **4a** at >50%, and **1a** had the highest yield of 72%. All of the protostane analogues displayed hCE-2 inhibitory effects in an *in vitro* bioassay. This report describes a rare biocatalytic alcoholic hydroxyl glucosylation with considerable yield and regioselective characteristics.

1. Introduction

Protostane triterpenoids belong to a group of tetracyclic triterpenes that exhibit unique structural characteristics [1]. To date, a number of protostanes have been obtained from natural sources, especially the genus *Alisma* of the Alismataceae family [1]. In our previous chemical investigations, we isolated several vital protostanes from *Alisma orientale*, which is a traditional medicine widely distributed throughout China, Japan, North America, and Europe [2]. Bioassays have also demonstrated that protostane triterpenoids have potential inhibitory effects on vascular contraction [3], immunosuppression [4], and HBV [5]. However, it is noteworthy that no natural or artificial protostane glycosides have been obtained. Therefore, to the best of our knowledge, chemical and biological investigations of protostane glycosides have not been reported in the literature.

To prepare the synthetic glycosides, chemical glycosylation has been conducted and displayed weaknesses, such as relatively low yield of the final glycosides, glycosyl activation, and multiple steps of protection/deprotection to control regio- and stereo-selectivity [6–8]. Compared with chemical glycosylation, biotransformed glycosylation has been thought to be an efficient and environmentally friendly method. Ye et al. conducted the glucosylation of cinobufagin, cardioactive C-24 steroids using suspensions of cells of *Catharanthus roseus* [9]. Compared with the glucosylation of alcoholic hydroxyls, more investigations have been conducted on the phenolic hydroxyls. Glucosides of the phenolic hydroxyls of taxifolin by cultured cells of *Nicotiana tabacum* and *Catharanthus roseus* have been prepared by Shimoda et al. [10] *Cunninghamella echinulata* AS 3.3400 and *Rhizopus japonicus* ZW-4 were also used to glucosylate the phenolic compounds magnolol and honokiol [11]. Xie et al. explored a new glycosyltransferase (UGT73AE1) from *Carthamus tinctorius*, which could catalyze glycosylation of phenolic hydroxyls [12].

In brief, biotransformation has been shown to catalyze the glycosylation of natural compounds efficiently. However, most of these investigations were related to the glycosylation of phenolic hydroxyls. It is still worthwhile to explore a highly efficient transformational method to catalyze the glycosylation of alcoholic hydroxyls of natural compounds selectively. Based on biotransformation studies of various natural compounds [13–16], we conducted the biotransformed glucosylation of alcoholic hydroxyls in the structures of protostanes with regioselective characteristics.

2. Experimental

2.1. Materials

Chemicals, microorganisms and instruments are described in the supplementary information.

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2.2. Experimental procedures

The preparative experiment was carried out in 1000 ml Erlenmeyer flasks containing 400 ml of potato medium. Substrate in acetone (50 mg/ml) was added to the cultures, which was pre-cultured with *Syncephalastrum racemosum* AS 3.264 for 24 h. Also, the incubation was carried out for another 4 days.

The cultures were pooled and filtered, and the filtrate was subjected to a macroporous adsorptive resin column eluted with ethanol-water. The transformed products were obtained in the fraction eluted with ethanol-water (70:30). Pure product could be prepared after recrystallization with ethanol. Detailed procedures are described in the supplementary information.

3. Results and discussion

3.1. Screening experiment of the glucosylation of protostanes

A total of 15 different fungal strains, including *Absidia coerulea* AS 3.3538, *Actinomucor elegans* AS 3.2778, *Aspergillus niger* AS 3.739, *Aspergillus niger* AS 3.795, *Aspergillus niger* AS 3.1858, *Aspergillus niger* AS 3.4627, *Chaetomium globosum* AS 3.4254, *Cunninghamella elegans* AS 3.2028, *Cunninghamella echinulata* AS 3.3400, *Fusarium avenaceum AS* 3.4594, *Mucor rouxianus* AS 3.3447, *Penicillium melinii* AS 3.4474, *Penicillium janthinellum* AS 3.510, *Rhizopus oryzae* AS 3.2380 and *Syncephalastrum racemosum* AS 3.264, were used in the screening experiment to conduct the glucosylation of protostanes (**1**–**4**). The transformed cultures were analyzed using a HPLC-DAD instrument. As a result, most fungi did not transform the protostanes. A number of products were detected for the transformation of several fungi and were suggested to be hydroxylated derivatives by ESI-MS. These experimental results could be explained by the highly oxygenated structures of protostanes, as well as that micro biotransformation medicated most oxidation reactions. Furthermore, only one product was detected by HPLC-DAD in the culture of *Syncephalastrum racemosum* AS 3.264 incubated with protostanes **1**–**4**. On the basis of LC-ESI-MS data, the transformed products of protostanes catalyzed by *S. racemosum* AS 3.264 were deduced to be glucosylation derivatives of protostanes. Therefore, *S. racemosum* AS 3.264 displayed selective capability to perform glucosylation of protostane substrates (Fig. 1).

Table 1
HRESIMS data of products **1a**, **2a**, **3a** and **4a**.

	1a	2a	3a	4a
Ion peak	$[M-H]^-$	$[M + Na]^+$	$[M + Na]^+$	$[M + Na]^+$
Experimental *m/z*	633.3953	677.4241	673.3927	675.4062
Calcd. *m/z*	633.4003	677.4241	673.3922	675.4084
Molecular formula	$C_{36}H_{58}O_9$	$C_{36}H_{62}O_{10}$	$C_{36}H_{58}O_{10}$	$C_{36}H_{60}O_{10}$

3.2. Preparation and identification of glucosylation products

To prepare the transformed products, 150 mg protostane substrates **1**–**4** were transformed by *S. racemosum* AS 3.264. The incubation cultures were separated by chromatographic technology and gave glucosylation derivatives of protostanes **1a** (yield 58%), **2a** (yield 25%), **3a** (yield 55%), and **4a** (yield 53%), respectively. On the basis of spectroscopic data analysis, the structures of transformed products were determined.

Product **1a** was obtained as an amorphous powder, gave the molecular formula $C_{36}H_{58}O_9$ by HRESIMS at the ion peak 633.3953 (calcd for 633.4003, Table 1). The molecular weight of **1a** was 162 amu higher

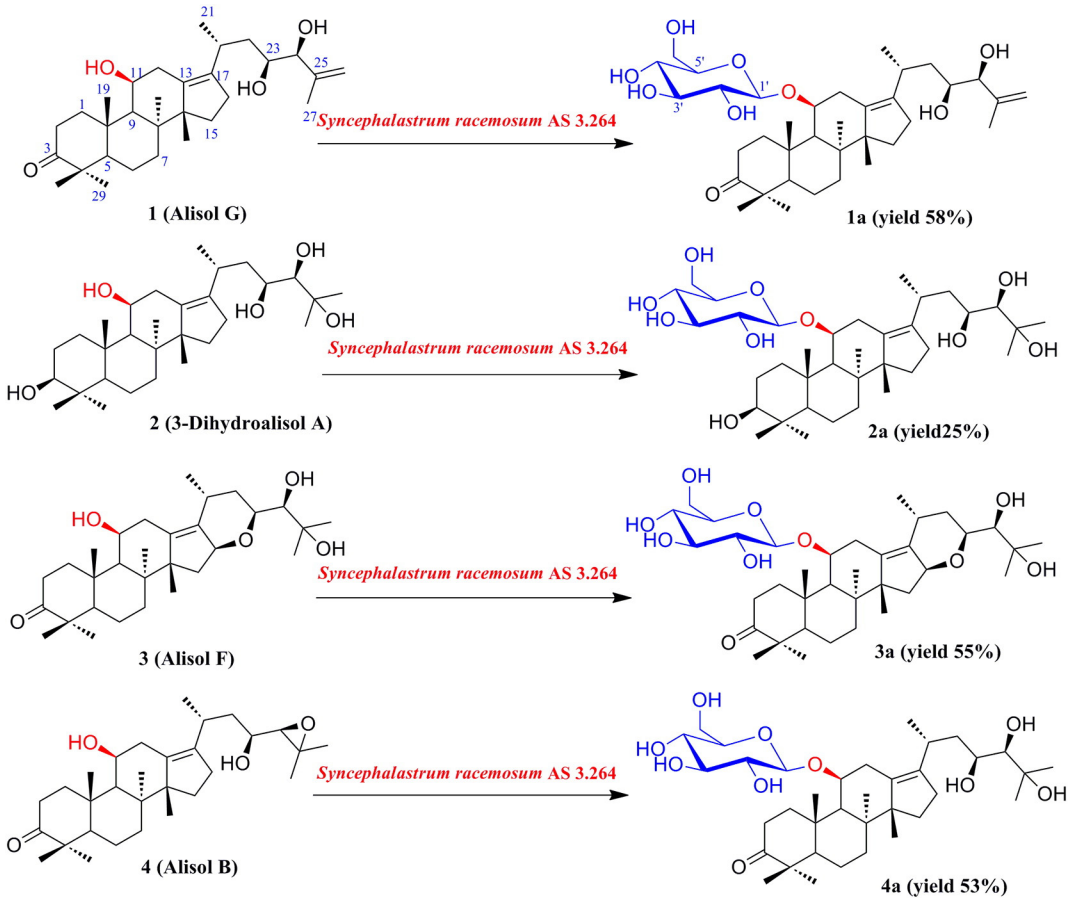

Fig. 1. Glucosylation of protostane triterpenoids **1**–**4** catalyzed by *Syncephalastrum racemosum* AS 3.264.

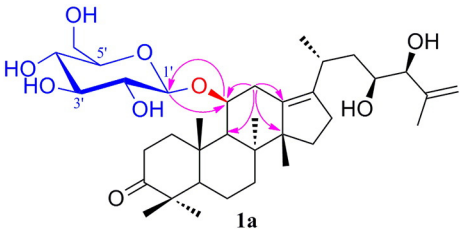

Fig. 2. Key HMBC correlations (H → C) regarding the attachment of saccharide moiety in **1a**.

than that of alisol G (**1**), suggesting an introduction of the hexosyl moiety *via* glucosylation. The ^{1}H NMR spectrum of **1a** was similar to that of protostane **1**, except for the additional proton signals at 3.0–5.0 ppm. The ^{13}C NMR spectrum of **1a** was also similar to that of **1**, except for the six extra carbon signals at 60–110 ppm. The MS and NMR data suggested the presence of a six carbon saccharide moiety (δ_C 105.7, 75.7, 79.9, 72.7, 78.6, 63.8, Table S1). The saccharide moiety was deduced to be D-glucopyranose on the basis of culture preparation and an acid hydrolysis experiment. The anomeric proton singlet at δ_H 4.96 (d, J = 6.5 Hz) (Table S1) indicated the β orientation of glycosidation bond. The long-range correlation between anomeric proton δ_H 4.97 and aglycon carbon δ_C 81.3 observed in the HMBC spectrum suggested that the glucosyl moiety was located at an oxygenated carbon (δ_C 81.3). This aglycon carbon (δ_C 81.3) was determined to be C-11 by HMBC correlations of H-12 (δ_H 3.93)/δ_C 81.3, H-12 (δ_H 3.93)/C-13 (δ_C 139.0) (Fig. 2). Therefore, the glucosyl moiety was positioned at C-11. Also, product **1a** was established to be alisol G-11-O-β-D-glucopyranoside.

The spectroscopic data of product **2a** established the molecular formula $C_{36}H_{58}O_9$ (Table 1). The NMR data δ_C 105.6, 75.6, 78.9, 72.0, 77.8, 63.1 and anomeric proton signal δ_H 4.40 (d, J = 8.0 Hz) indicated the presence of the β-D-glucosyl moiety. The observed HMBC correlations between anomeric proton δ_H 4.40 and δ_C 83.0 suggested the glucosidation of C-11. Thus, the structure of **2a** was established to be a triterpene glucoside, as shown in Fig. 1.

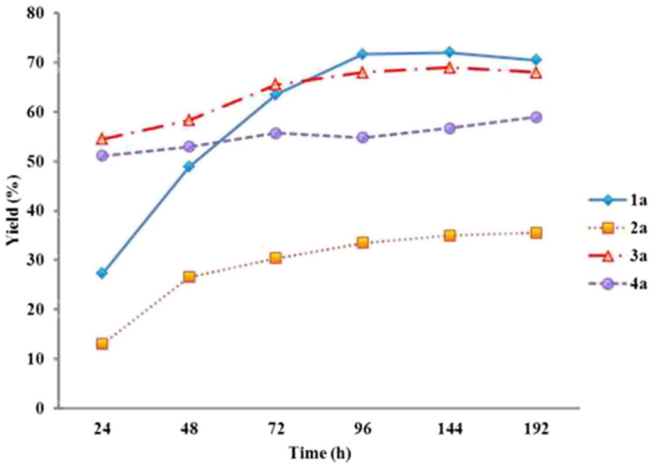

Fig. 4. Time-courses of the transformation of protostanes.

The structures of transformed products **3a** and **4a** were also determined by HRESIMS, 1D NMR, and 2D NMR data. Both **3a** and **4a** were glucosylation derivatives of protostane substrates. Also, the glucosylation position was C-11, which was the same as **1a** and **2a**.

3.3. Regioselective glucosylation characteristics of protostanes

As shown in Fig. 1, protostane substrates **1**–**4** were highly oxygenated polyhydroxy triterpenoids. For the structures of **1**–**4**, hydroxyls existed at C-11 and in the C-17 side chain. In addition, a 3-OH moiety was present in the structure of **2**. However, the biotransformation results suggested the regioseletive glucosylation of 11-OH. The other hydroxyls could not be glucosylated by *S. racemosum* AS 3.264. So, this fungus displayed regiospecific glucosylation capability.

In order to identify the specific glucosylation capability of *S. racemosum* AS 3.264, six protostanes without 11-OH were used

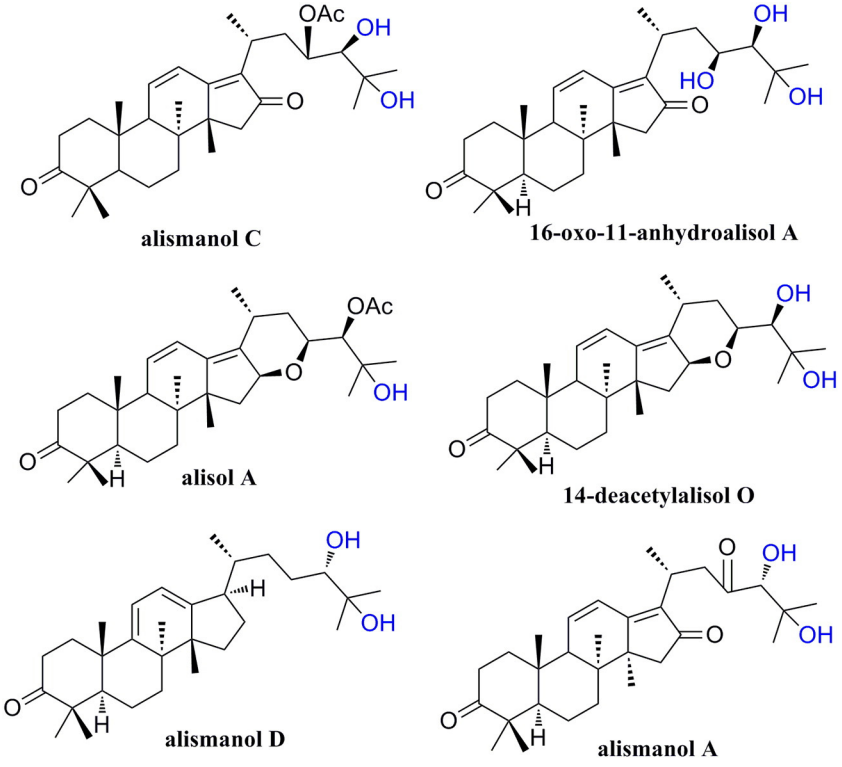

Fig. 3. Six highly oxygenated protostane triterpenoids without 11-OH.

Table 2
Human carboxylesterase 2 (hCE-2) inhibitory effects of protostane analogues.

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
1	3.8 ± 0.14	**1a**	13.3 ± 0.29
2	20.5 ± 0.60	**2a**	23.8 ± 0.46
3	26.6 ± 0.44	**3a**	31.2 ± 0.67
4	18.3 ± 0.35	**4a**	24.5 ± 0.52

[a] The experiments were performed at least in triplicate.

as the substrates in the biotransformation experiment (Fig. 3). These protostanes have a 3-ketone, C11–C12 olefinic bonds and a highly oxygenated C-17 side chain. There are hydroxyls at C-23, or C-24, or C-25. However, no glycosylation product occurred in the incubated cultures of six different nor-11-OH protostanes. Therefore, the regioselective capability was further confirmed.

From the glucosylated products, it was deduced that glycosyltransferases (GTs) existed in the fungus *S. racemosum* AS 3.264. These findings led the researchers to hypothesize that protostanes interacted with GTs in fixed structural conformation. The preferential conformation of protostanes could easily dock into the catalytic site of GTs. On the side of microstructure, the C-11 alcoholic hydroxyl group interacted with the active amino acid residues in GTs. Thus, *S. racemosum* AS 3.264 displayed high biocatalytic capability to mediate the glucosylation of alcoholic hydroxyls in protostane triterpenoids with significant regioselectivity. It is necessary to prepare the enzyme glusocyltransferases from this fungus, which may be a potentially bioengineered enzyme in the carbohydrate chemistry field. Therefore, the enzymatic glucosylation will be an ongoing investigation of this work.

3.4. Biotransformation time course

The biotransformation time-courses of protostanes **1**–**4** by *S. racemosum* AS 3.264 were investigated with LC-MS as the detection method (Fig. 4). Products **1a**, **3a**, **4a** displayed higher yields than **2a**, which suggested that the 3-ketone group was more suitable than 3-OH. The substructure of the C-17 side chain showed minor effects on the glucosylation yield. Although **2a** and **4a** had the highest yields at the incubation time of 192 h, the time-courses of four products suggested that incubation time of 96 h may be most economical for the yield. Finally, **1a**, **3a**, **4a** had yields of >50%, and **1a** had the highest yield of 72%. Once again, *S. racemosum* AS 3.264 was confirmed to be an efficient organism to prepare glucosides of protostanes.

3.5. hCE-2 inhibitory effects of protostanes

Human carboxylesterase-2 (hCE-2) is highly expressed in the intestine and catalyzes the hydrolysis of medical compounds, such as esters, amides, and carbamates. We reported that protostanes displayed significant inhibitory effects on hCE-2 [2]. In this study, the inhibitory effects of glucosylation protostanes were also evaluated in a bioassay [17]. As shown in Table 2, all of the protostane analogues displayed inhibitory effects toward hCE-2, and the IC_{50} value of a reported positive hCEs inhibitor BNNP (bis-para-nitrophenylphosphate) against hCE-2 was 4.03 μM [18,19]. The bioassay results indicated that glucosylation derivatives of protostanes have weaker inhibitory effects compared with the corresponding substrates. However, glucosylation derivatives still displayed moderate inhibitory effects with the IC_{50} values <10–35 μM. In consideration of the glucosyl moiety of structure, the inhibitory effects of glucosylation derivatives may be more significant *in vivo*.

4. Conclusions

We have report a novel glucosylation method of alcoholic hydroxyls in protostane triterpenoids, which are important bioactive natural products. The glucosylation was catalyzed by *S. racemosum* AS 3.264 with significant highly efficient and regioseletive characteristics. For the transformation of four major protostanes, there were only 11-OH glucosylated derivatives presenting the highest yield of 72%. The authors believe that this fungus and even the GTs of this fungus could be developed to be an important method for the glucosylation of alcoholic hydroxyls.

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Experimental sections and copies of spectra of compounds **1a**–**4a** are available free of charge *via* the Internet. Supplementary data associated with this article can be found in the online version, at 10.1016/j.catcom.2016.10.025.

References

[1] M. Zhao, T. Gödecke, J. Gunn, J.A. Duan, C.T. Che, Molecules 18 (2013) 4054–4080.
[2] Z.P. Mai, K. Zhou, G.B. Ge, C. Wang, X.K. Huo, P.P. Dong, S. Deng, B.J. Zhang, H.L. Zhang, S.S. Huang, X.C. Ma, J. Nat. Prod. 78 (2015) 2372–2380.
[3] H. Matsuda, G. Kobayashi, J. Yamahara, H. Fujimura, K. Kurahashi, M. Fujiwara, Life Sci. 41 (1987) 1845–1852.
[4] C.F. Zhang, A.C. Zhou, M. Zhang, Chin. J. Chin. Mater. Med. 34 (2009) 994–998.
[5] Q. Zhang, Z.Y. Jiang, J. Luo, P. Cheng, Y.B. Ma, X.M. Zhang, F.X. Zhang, J. Zhou, J.J. Chen, Bioorg. Med. Chem. Lett. 18 (2008) 4647–4650.
[6] K. Oyama, T. Kondo, Tetrahedron 60 (2004) 2025–2034.
[7] Y. Du, G.H. Wei, R.J. Linhardt, J. Org. Chem. 69 (2004) 2206–2209.
[8] P.W. Needs, G. Williamson, Carbohydr. Res. 330 (2001) 511–515.
[9] M. Ye, J.G. Dai, H.Z. Guo, Y.J. Cui, D.A. Guo, Tetrahedron Lett. 43 (2002) 8535–8538.
[10] K. Shimoda, N. Kubota, M. Hamada, M. Sugamoto, K. Ishihara, H. Hamada, Nat. Prod. Commun. 8 (2013) 903–904.
[11] L. Yang, Z.H. Wang, H. Lei, R.D. Chen, X.L. Wang, Y. Peng, J.G. Dai, Tetrahedron 70 (2014) 8244–8251.
[12] K.B. Xie, R.D. Chen, J.H. Li, R.S. Wang, D.W. Chen, X.X. Dou, J.G. Dai, Org. Lett. 16 (2014) 4874–4877.
[13] M. Xu, X.K. Huo, X.G. Tian, P.P. Dong, C. Wang, S.S. Huang, B.J. Zhang, H.L. Zhang, S. Deng, X.C. Ma, RSC Adv. 5 (2015) 78081–78089.
[14] X.X. Gao, S.S. Huang, P.P. Dong, C. Wang, J. Hou, X.K. Huo, B.J. Zhang, T.H. Ma, X.C. Ma, Catal. Sci. Technol. 6 (2016) 3585–3593.
[15] C. Wang, P.P. Dong, L.Y. Zhang, X.K. Huo, B.J. Zhang, C.Y. Wang, S.S. Huang, X.B. Wang, J.H. Yao, K.X. Liu, X.C. Ma, RSC Adv. 5 (2015) 12717–12725.
[16] Z.P. Mai, X.L. Xin, Z. Sun, N. Zhang, S.S. Huang, C. Wang, L. Chen, Y. Li, X.K. Huo, G.J. Fan, Phytochem. Lett. 13 (2015) 228–233.
[17] Z.M. Liu, L. Feng, J. Hou, X. Lv, J. Ning, G.B. Ge, K.W. Wang, J.N. Cui, L. Yang, Sensors Actuators B Chem. 205 (2014) 151–157.
[18] Y.G. Li, J. Hou, S.Y. Li, X. Lv, J. Ning, P. Wang, Z.M. Liu, G.B. Ge, J.Y. Ren, L. Yang, Fitoterapia 101 (2015) 99–106.
[19] H. Eng, M. Niosi, T.S. McDonald, A. Wolford, Y. Chen, S.T. Simila, J.N. Bauman, J. Warmus, A.S. Kalgutkar, Xenobiotica 40 (2010) 369–380.