Genetics and Molecular Research

Synthesis and structure-activity relationship of oleanolic mono- or di-glycosides against *Magnaporthe oryzae*

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Genet. Mol. Res. 15 (3): gmr.15038998
Received July 21, 2016
Accepted August 1, 2016
Published September 23, 2016
DOI http://dx.doi.org/10.4238/gmr.15038998

ABSTRACT. Saponins are naturally-occurring units with broad diversity and are usually recognized as phytoanticipins. In order to develop new saponin chemical entities with high activity against *Magnaporthe oryzae*, we selected oleanolic acid (OA), which has wide natural distribution and rich content in plants. We used the ability of OA to act as an aglycone for glycosylation to obtain information on the structure-activity relationship (SAR) for rational molecular pesticide design. Oleanolic mono- or di-glycosides were synthesized at either the C_3-hydroxy and/or C_{28}-carboxyl position, using trichloroacetimidate or glycosyl bromide donors, respectively. Structures were confirmed by [^{1}H]-,[^{13}C]-NMR. Furthermore, the activity of the synthesized glycosides against *M. oryzae* was assessed *in vitro*, based on the mycelium growth rate. The twenty five oleanolic mono- or di-glycosides

comprised fourteen saponins with 3-monosaccharide residue 1a-1n, six saponins with 28-monosaccharide residue 2a-2f, and five saponins with 3, 28-monosaccharide residue 3a-3e; all showed different activities against *M. oryzae* according to their different structures. We concluded that the optimal oleanolic mono- and di-glycoside structure for activity against *M. oryzae* is a C_3 connection of a hexose such as mannose, galactose, or glucose, in combination with a C_{28} connection to a small group such as allyl or a C_3 connection to a pentose accompanied by a larger group such as another pentose or heptenyl at C_{28}.

Key words: Oleanolic mono-glycosides; Oleanolic di-glycosides; Saponins; *Magnaporthe oryzae*; Structure-activity relationship

INTRODUCTION

Rice blast is caused by the fungus *Magnaporthe oryzae* and is the most prevalent disease in rice (*Oryza sativa*). The disease results in an average loss of about 157 million tons of rice per year worldwide, an amount that would be enough to feed 60 million people in one year (Shi and Wang, 2008). To control rice blast, major efforts have been made to develop disease-resistant varieties of rice. To date, over 85 blast-resistance genes have been identified, 20 of which have been cloned (Sun et al., 2013). However, owing to the pathogen's high adaptive variability, these genes do not provide permanent resistance to rice blast and typically last only 2-3 seasons (Normile, 2009). Furthermore, the resistant varieties often result in a lower quality of rice (Fukuoka et al., 2009). Rice species and cultivar diversity in ecological management are also being explored (Zhu et al., 2000; Han et al., 2013), but chemical control is still an indispensable means for controlling rice blast. Unfortunately, the chemicals used to control rice blast in the field, such as tricyclazole, have resulted in environmental residues (Zhou et al., 2013) and in some cases, isoprothiolane-resistant isolates of *M. oryzae* have emerged (Zhang et al., 2013). Thus, there is high demand for novel natural products with unique structures, modes of action, and high environmental safety, which can counteract the high adaptive variability of rice blast.

Saponins are natural phytoanticipins. Preliminary research has shown that schimasuoside, a new oleanane-type saponin from *Schima superba*, has a strong inhibitory activity against *M. oryzae* (Huo et al., 2014) and also has the potential to control rice blast in the field (Tan et al., 2014). Its aglycone structure 21,22-di-*O*-angeloyl-R_1-barrigenol-3β,15α,16β-trihydroxyl-17-hydroxymethyl-21,22-di-*O*-angeloyl-olea-12-en) is similar to that of oleanolic acid (OA; 3β-hydroxy-olea-12-en-28-oic acid). OA has two glycosylation sites, C(3)-OH and C(28)OOH, which can be glycosylated to form 3-monoglycosides, 28-monoglycosides, and 3,28-diglycosides. Glycosylated saccharides may originate from commercially available D-glucose, D-galactose, D-xylose, D-arabinose, D-mannose, L-rhamnose, and maltose. OA is a favorable raw material that can be obtained from a wide number of sources at low cost. Moreover, glycosylation of OA can improve its weak aqueous solubility and bioavailability (Ghanbari et al., 2012). Therefore, it is of considerable interest to utilize OA as an initial aglycone to synthesize saponins with inhibitory activity against rice blast.

MATERIAL AND METHODS

General experimental procedures

Thin-layer chromatography (TLC) was performed using silica gel GF_{254} plates and column chromatography was performed using silica gel (100-200 mesh). NMR spectra were obtained using a Bruker 400 MHz NMR spectrometer. Chemical shifts (δ) were expressed as ppm and coupling constants (J) were expressed in Hz. Tetramethylsilane (TMS) was used as internal reference.

Procedures for preparing the synthetic compounds

Preparation of bromoglycosides and trichloroacetimidates

Monosaccharides (D-glucose, D-galactose, D-xylose, D-arabinose, D-mannose, L-rhamnose) were successively reacted by benzoylation, bromination to yield benzoylbromoglycoside-6 (Glc-6, Gal-6, Xyl-6, Ara-6, Man-6, Rha-6). Benzoyltrichloroacetimidate-8 (Glc-8, Gal-8, Xyl-8, Ara-8, Man-8, Rha-8) was obtained from benzoylbromoglycoside-6 by hydrolysis and a trichloroacetic amine esterification reaction, according to previous reports (Zheng et al., 2005; Li et al., 2011).

Synthesis of oleanolic 28-allyl ester (10a)

Oleanolic acid (2 g, 4.38 mmol) was dissolved in dry 10 mL dimethyl formamide (DMF) and stirred, and then allyl bromide (0.5 mL, 5.78 mmol) and 1,8-diazabicycloundec-7-ene (DBU; 0.5 mL, 3.35 mmol) were added. Reaction completion was determined by TLC (4:1 petroleum ether-EtOAc) after stirring for 24 h at room temperature. The mixture was extracted using EtOAc and water. The EtOAc phase was dried using anhydrous sodium sulfate. The filtrate was concentrated and purified by silica gel column chromatography (4:1 petroleum ether-EtOAc) to afford oleanolic 28-allyl ester (10a) (1.74 g, 82.9% yield) as a white amorphous solid. R_f = 0.50 (4:1 petroleum ether-EtOAc in TLC). [^{1}H] NMR (400 MHz, $CDCl_3$) δ 5.90 (ddd, J = 16.8, 10.7, 5.5 Hz, 1H,-CH_2-CH=CH_2), δ 5.29 (brs, 1H,H-12), δ 5.36-5.16 (m, 2H,-CH_2-CH=CH_2), δ 4.53 (m, 2H,-CH_2-CH=CH_2), δ 3.21 (m, 1H,H-3), δ 2.88 (d, J = 10.2 Hz,1H, H-18), δ 1.98 (m, 1H,H-16-1), δ 1.87 (m, 2H,H-11), δ 1.14 (s, 3H,H-27), δ 0.99 (s, 3H,H-25), δ 0.93 (s, 3H,H-23), δ 0.90 (s, 6H,H-29,H-30), δ 0.78 (s, 3H,H-24), δ 0.73 (s, 3H,H-26).

Synthesis of oleanolic-28-allylation ester-3-glycosides (1a-1f) and oleanolic-3-glycosides (1i-1n)

Oleanolic-28-allylation ester -3-O-β-D-glucopyranoside (1a): Glc-8 (0.72 g, 0.97 mmol) and 10a (0.37 g, 0.74 mmol) and 0.5 g powdered 4- Å molecular sieves (0.50 g) were stirred at room temperature in dry 5 mL CH_2Cl_2. When the reaction temperature dropped to 0°C, a solution of TMSOTf (10 μL, 0.055 mmol) was added dropwise and stirred for 2 h at room temperature. The reaction was quenched with triethylamine, filtered through Celite, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography to yield oleanolic 3-*O*-β-D-2,3,4,6-benzoyl-glucopyranosyl-28-allyl ester (Glc-11) (0.65 g, 80.6% yield) as a white solid. R_f = 0.52 (4:1 petroleum ether-EtOAc). Glc-11

(0.40 g, 0.37 mmol) was dissolved in 10 mL 1:1 CH_2Cl_2-MeOH solution followed by addition of 20 mg sodium methoxide. The mixture was stirred for 2 h at room temperature. Completion of the reaction was determined by TLC (10:1, CH_2Cl_2-MeOH), then the reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20:1→10:1, CH_2Cl_2-MeOH) to give oleanolic-28-allyl-3-*O*-β-D-glucopyranoside (1a) (0.21 g, 83.8% yield) as a white solid. R_f = 0.26 (10:1 CH_2Cl_2-MeOH).

Oleanolic-3-O-β-D-glucopyranoside (1i)*:* Glc-8 (200 mg, 0.30 mmol) was dissolved in 4 mL anhydrous MeOH, followed by addition of 10 mg palladium dichloride. The reaction mixture was stirred for 24 h at room temperature then leached through diatomite. The filtrate was purified by silica gel column chromatography (20:1→10:1, CH_2Cl_2-MeOH) to yield oleanolic-3-O-β-D -glucopyranoside (1i) (88 mg, 0.14 mmol, 46.7% yield) as a white solid. R_f = 0.21 (10:1 CH_2Cl_2-MeOH). [^{1}H] NMR and [^{13}C] NMR data consistent with the assigned structure were the same as that in previous reports (Sha et al. 2008).

Compounds 1b-1f were prepared according to the procedure described for 1a, and compounds 1j-1n according to that described for 1i. [^{1}H]- and [^{13}C]-NMR data from 1c, 1k, 1b, 1j, 1d, 1l, 1n are consistent with those in previous reports (Zang, 2004; Sha et al., 2008; Zhao et al., 2011).

Oleanolic -28 -allyl ester-3-O-β-D-galactopyranoside (1c): 58.4% yield, R_f = 0.26 (10:1 CH_2Cl_2- MeOH).

Oleanolic 3-O-β-D-galactopyranoside (1k): 32.2% yield, R_f = 0.22 (10:1 CH_2Cl_2- MeOH).

Oleanolic -28 -allyl ester-3-O-β-D-xylopyranoside (1b): 76.4% yield, R_f = 0.36 (10:1 CH_2Cl_2- MeOH).

Oleanolic 3-O-β-D-xylopyranoside (1j): 45.0% yield, R_f = 0.33 (10:1 CH_2Cl_2-MeOH).

Oleanolic -28 -allyl ester-3-O-α-D-arabinopyranoside (1d): 83.5% yield, R_f = 0.38 (10:1 CH_2Cl_2- MeOH).

Oleanolic 3-O-α-D-arabinopyranoside (1l): 48.0%yield, R_f = 0.34 (10:1 CH_2Cl_2-MeOH).

Oleanolic -28 -allyl ester- 3-O-β-D-mannopyranoside (1e): 33.8% yield, R_f = 0.25 (10:1 CH_2Cl_2- MeOH). [^{1}H] NMR (400 MHz, $CDCl_3$) δ 5.90 (ddd, J = 22.6, 10.8, 5.6 Hz, 1H), 5.33 (dd, 1H), 5.29 (s, 1H), 5.20 (dd, J = 10.4 Hz, 1H), 4.98 (s, 1H), 4.53 (m, 2H), 3.95 (m, 1H), 3.85 (m, 2H), 3.76 (d, 1H), 3.65 (m, 1H), 3.16 (d, J = 9.0 Hz, 1H), 2.88 (d, J = 12.7 Hz, 1H), 2.00 (m, 1H), 1.85 (m, 2H), 1.13 (s, 3H), 0.93 (s, 6H), 0.91 (s, 3H), 0.89 (s, 3H), 0.74 (s, 3H), 0.72 (s, 3H). [^{13}C] NMR (101MHz, DMSO-d6) δ 177.20, 144.10, 132.10, 123.00, 118.20, 113.50, 92.50, 81.50, 76.80, 74.10, 71.50, 66.50, 62.20, 55.70, 47.70, 47.10, 46.40, 42.20, 42.10, 39.90, 39.10, 38.80, 37.90, 34.20, 33.10, 32.70, 30.90, 29.00, 26.90, 26.90, 26.00, 25.50, 24.00, 23.80, 23.70, 23.70, 18.60, 17.30, 16.20.

Oleanolic 3-O-β-D-mannopyranoside (1m): 16.2% yield, R_f = 0.22 (10:1 CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 12.00 (s, 1H), 5.16 (s, 1H), 4.77 (s, 1H), 4.71 (d, J = 4.5 Hz, 1H), 4.66 (d, J = 4.0 Hz, 1H), 4.53 (d, J = 5.6 Hz, 1H), 4.37 (s, 1H), 3.61 (m, 1H), 3.51 (br s, 1H), 3.49-3.37 (m, 2H), 3.22-3.15 (m, 2H), 3.06 (m, 1H), 2.74 (d, J = 12.1 Hz, 1H), 1.96-1.86 (m, 1H), 1.81 (m, 2H), 1.10 (s, 3H), 0.95 (s, 3H), 0.88 (s, 9H), 0.73 (s, 3H), 0.72 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 178.54, 143.85, 121.44, 95.86, 80.26, 74.46, 71.16, 71.04, 66.89, 61.34, 54.90, 47.03, 45.68, 45.43, 41.33, 40.79, 38.89, 37.89, 37.49, 36.54, 33.30, 32.80, 32.36, 32.07, 30.37, 28.51, 27.17, 25.58, 23.35, 22.91, 22.60, 21.07, 17.84, 16.84, 16.56, 15.07.

Oleanolic -28 -allyl ester-3-O-α-L-rhamnopyranoside (1f): 87.3.% yield, R_f = 0.44 (10:1 CH_2Cl_2- MeOH). [^{1}H] NMR (400 MHz, $CDCl_3$) δ 5.90 (ddd, J = 22.8, 10.8, 5.6 Hz, 1H),

5.32 (dd, 1H), 5.29 (s, 1H), 5.21 (dd, J = 10.5 Hz, 1H), 4.80 (s, 1H), 4.52 (m, 2H), 3.92 (dd, 1H), 3.76 (m, 2H), 3.41 (m, 1H), 3.09 (dd, J = 11.3, 4.8 Hz, 1H), 2.88 (d, J = 9.6 Hz, 1H), 1.96 (m, 1H), 1.87 (m, 2H), 1.26 (s, 3H), 1.13 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.90 (s, 6H), 0.76 (s, 3H), 0.72 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 177.20, 144.10, 132.10, 123.00, 118.20, 113.70, 92.50, 74.20, 74.10, 73.70, 72.40, 66.50, 55.40, 47.70, 47.10, 46.40, 42.20, 42.10, 39.90, 39.10, 38.80, 37.90, 34.20, 33.10, 32.70, 30.90, 29.00, 26.90, 26.90, 26.00, 25.50, 24.00, 23.80, 23.70, 23.70, 18.60, 17.30, 17.00, 16.20.

Oleanolic -3-O-α-L-rhamnopyranoside (1n): 48.0% yield, R_f = 0.35 (10:1 CH_2Cl_2-MeOH).

Oleanolic -28 -heptenyl ester-3-O-β-D-galactopyranoside (1g): 82.0% yield, R_f = 0.29 (10:1 CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 5.77 (m, 1H), 5.18 (s, 1H), 4.96 (m, 2H), 4.70 (s, 1H), 4.59 (s, 1H), 4.48 (s, 1H), 4.28 (s, 1H), 4.10 (d, J = 6.6 Hz, 1H), 3.93 (m, 2H), 3.62 (s, 1H), 3.52 (m, 1H), 3.41 (m, 1H), 3.27 (m, 3H), 3.02 (d, J = 7.9 Hz, 1H), 2.79 (d, J = 12.5 Hz, 1H), 1.98 (m, 3H), 1.80 (m, 3H), 1.10 (s, 3H), 0.98 (s, 3H), 0.88 (s, 6H), 0.86 (s, 3H), 0.75 (s, 3H), 0.66 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 176.50, 143.44, 138.47, 121.84, 114.70, 106.02, 87.87, 74.82, 73.56, 71.10, 68.00, 63.56, 60.29, 55.01, 46.99, 46.00, 45.40, 41.20, 40.89, 38.89, 38.68, 38.10, 36.24, 33.18, 33.11, 32.70, 32.32, 32.09, 30.33, 27.90, 27.78, 27.62, 27.05, 25.63, 25.53, 25.05, h23.29, 22.90, 22.53, 17.75, 16.67, 16.43, 15.03.

Oleanolic -28 - heptenyl ester-3-O-α-D-arabinopyranoside (1h): 85.0% yield, R_f = 0.41 (10:1 CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 5.78 (m, 1H), 5.18 (s, 1H), 4.96 (m, 2H), 4.79 (d, J = 3.8 Hz, 1H), 4.49 (d, J = 4.9 Hz, 1H), 4.46 (d, J = 3.8 Hz, 1H), 4.11 (d, J = 5.8 Hz, 1H), 3.93 (m, 2H), 3.71-3.54 (m, 2H), 3.38-3.24 (m, 3H), 3.00 (d, J = 10.7 Hz, 1H), 2.79 (d, J = 13.4 Hz, 1H), 1.99 (m, 3H), 1.80 (m, 2H), 1.72 (d, J = 12.0 Hz, 1H), 1.10 (s, 3H), 0.97 (s, 3H), 0.88 (s, 6H), 0.86 (s, 3H), 0.76 (s, 3H), 0.66 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 176.51, 143.43, 138.47, 121.83, 114.71, 105.87, 87.64, 72.69, 70.97, 67.61, 65.09, 63.57, 54.91, 46.96, 46.01, 45.40, 41.21, 40.90, 38.88, 38.74, 38.02, 36.25, 33.16, 33.11, 32.70, 32.30, 32.09, 30.33, 27.90, 27.78, 27.58, 27.04, 25.62, 25.52, 25.05, 23.29, 22.89, 22.53, 17.74, 16.67, 16.39, 15.04.

Synthesis of oleanolic28-glycosides

β-D-glucopyranosyl oleanolate (2a): OA (200 mg,0.44 mmol), benzoylbromo-glucoside (Glc-6) (320 mg, 0.49 mmol), 10 mg tetrabutyl ammonium bromide, and 300 mg potassium carbonate were dissolved in a mixture of CH_2Cl_2/H_2O (10/0.5 mL). The mixture was stirred vigorously for 6 h under reflux. The reaction mixture was diluted with $CHCl_3$ and washed successively with water, diluted in 0.5 mol HCl, and saturated with NaCl. The organic layer was separated and concentrated. The residue was subjected to silica gel column chromatography (4:1, petroleum ether-EtOAc) to afford benzoylglucosyl-28-oleanolate (OA-glc-12) (360 mg, 0.35 mmol, 79.5% yield) as a white solid. R_f = 0.24 (3:1 petroleum ether-EtOAc). OA-glc-12 (300 mg, 0.29 mmol) was dissolved in a solution of CH_2Cl_2-MeOH (1:1) and 20 mg NaOMe was added. The mixture was stirred for 2 h at room temperature. The filtrate was concentrated and purified with a silica gel column chromatography (20:1 →10:1, CH_2Cl_2-MeOH) to give β-D-glucopyranosyl oleanolate(2a) (140 mg, 0.23 mmol, 79.3% yield), R_f = 0.25 (10:1, CH_2Cl_2-MeOH) .^{1}H NMR (400 MHz, DMSO) δ 5.24 (d, *J* = 8.0 Hz, 1H,OH-2'), 5.17 (s, 1H,H-12), 4.96 (brs, 2H,0H-3',OH-4') 4.41 (brs, 1H,OH-6'), 4.28 (d, *J* = 4.0 Hz, 1H,H-1'), 3.62 (d, *J* = 10.6 Hz, 1H,H-6'-1), 3.44 (m,1H,H-6'-2), 3.20 (m,1H,H-3'), 3.12 (m, 3H,H-4',H-5',H-2'), 2.99 (m, 1H,H-3), 2.74 (d, *J* = 10.6 Hz, 1H,H-18), 1.95 (t, *J* = 11.9 Hz,

1H,H-16-1), 1.78 (m,3H,H-2-1,H-11), 1.08 (s, 3H,H-27), 0.89 (s, 3H,H-23), 0.88 (s, 3H,H-29), 0.87 (s, 3H,H-30), 0.85 (s, 3H,H-25), 0.69 (s, 3H,H-24), 0.68 (s, 3H,H-26).

Compounds **2b-2f** were prepared according to the same procedure described for **2a**. [^{1}H] NMR and [^{13}C] NMR data of 2c, 2b, 2f are identical with literature reports (Li et al., 2009; Zhao et al., 2011).

β-D-galactopyranosyl oleanolate (2c): 68.0% yield, R_f = 0.25 (10:1, CH_2Cl_2- MeOH).

β-D-xylopyranosyl oleanolate (2b): 75.0% yield, R_f = 0.35 (10:1, CH_2Cl_2-MeOH).

α-L-arabinopyranosyl oleanolate (2d): 68.0% yield, R_f = 0.35 (10:1, CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO) δ 5.36 (d, J = 4.5 Hz, 1H,OH-2'), 5.20 (brs, 1H,H-12), 5.16 (d, J = 4.9 Hz, 1H,0H-3'), 4.66 (m,1H,OH-4'), 4.28 (d, J = 5.0 Hz, 1H,H-1'), 3.77 - 3.66 (m, 1H,H-5'-1), 3.55 (brs, 1H,H-4'), 3.49 (t, 1H,H-2'), 3.38 (m, 1H,H-3'), 3.17 (d, J = 5.0 Hz, 1H,H-5' -2), 3.04 - 2.95 (m, 1H,H-3), 2.82 (d, J = 10.4 Hz, 1H,H-18), 1.95 (m, 1H,H-16-1), 1.80 (d, J = 5.7 Hz, 2H,H-11), 1.09 (s, 3H,H-27), 0.89 (s,3H,H-23), 0.88 (s, 6H,H-29,H-30), 0.85 (s, 3H,H-25), 0.68 (s, 6H,H-24,H-26). [^{13}C] NMR (101 MHz, DMSO-d6) δ 175.29, 143.47, 121.78, 93.76, 76.81, 71.22, 69.59, 65.52, 63.50, 54.81, 47.08, 46.10, 45.47, 41.23, 40.64, 38.66, 38.36, 38.11, 36.55, 33.22, 32.77, 32.34, 31.74, 30.36, 28.21, 27.15, 26.94, 25.57, 23.36, 22.93, 22.26, 17.96, 16.67, 16.01, 15.11.

β-D-mannopyranosyl oleanolate (2e): 22.0% yield, R_f = 0.24 (10:1, CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO) δ 5.81 (s, 1H), 5.19 (d, J = 17.1 Hz, 1H), 5.08 (t, J = 6.3 Hz, 1H), 4.91 (t, J = 5.1 Hz, 1H), 4.73 (d, J = 4.5 Hz, 1H), 4.49 (t, J = 6.0 Hz, 1H), 4.28 (d, J = 5.1 Hz, 1H), 4.02 (dd, J = 6.8, 3.6 Hz, 1H), 3.55 - 3.47 (m, 1H), 3.00 (d, J = 5.2 Hz, 1H), 2.91 - 2.65 (m, 1H), 1.98 (dd, J = 16.6, 10.0 Hz, 1H), 1.82 (s, 1H), 1.10 (s, 1H), 0.89 (s, 3H), 0.85 (s, 1H), 0.68 (s, 2H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 174.76, 143.22, 122.01, 93.32, 76.79, 76.68, 70.83, 69.30, 66.10, 60.66, 54.71, 47.04, 46.38, 45.28, 41.25, 40.89, 38.73?38.89, 38.34, 38.10, 36.51, 33.05, 32.69, 32.25, 31.82, 30.36, 28.20, 26.93, 26.93, 25.52, 23.28, 22.98, 22.54, 17.99, 16.75, 15.97, 15.06.

α-L-rhamnopyranosyl oleanolate (2f): 80.0% yield, R_f = 0.36 (10:1, CH_2Cl_2-MeOH).

Synthesis of oleanolic -3, 28-diglycosides

3-O-β-D-glucopyranosyl-28-O-β-D-glucopyranosyloleanolate(3a):Trichloroacetimidate donor Glc-8 (0.5 g, 0.68 mmol) and intermediate OA-glc-12 (0.6 g, 0.58 mmol) were dissolved in dry 10 mL CH_2Cl_2. The solution was added to a 1.0 g 4-Å molecular sieve and stirred at room temperature for 20 min. The reaction temperature was reduced to 0 °C, TMSOTf (10 μL, 0.055 mmol) was added dropwise and stirred for 2 h at room temperature. The reaction was stopped by dropwise adding triethylamine, according to the reaction process detected by TLC (4:1, petroleum ether-ethyl acetate). The reaction solution was concentrated and subjected to column chromatography to afford a white solid OA-3,28-glc-13 (0.75 g, 0.46 mmol) with a rate of 79.3%. R_f = 0.22 (3:1, petroleum ether-ethyl acetate).

OA-3,28-glc-13 was dissolved in 20 mL 1:1 MeOH-CH_2Cl_2 solution. NaOMe (20 mg) was added and stirred for 4 h at room temperature. The mixture was neutralized with acidic cation exchange resin to pH 7 and filtered according to the process detected by TLC (5:1, CH_2Cl_2-MeOH). The filtrate was concentrated and purified with a silica gel column chromatography (20:1→10:1, CH_2Cl_2-MeOH) to give a white solid 3a (Sha et al., 2008) (0.25 g, 0.32 mmol) with 69.6% yield (total 55.2% yield) and R_f = 0.29 (5:1, CH_2Cl_2- MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 5.23 (d, J = 8.0 Hz, 1H), 5.16 (s, 1H), 5.13 (d, J = 5.7 Hz, 1H),

4.99 (d, J = 4.5 Hz, 1H), 4.93 (d, J = 4.5 Hz, 1H), 4.83 (m, 3H), 4.40 (t, J = 5.3 Hz, 1H), 4.33 (t, J = 5.3 Hz, 1H), 4.09 (dd, J = 10.4, 5.1 Hz, 1H), 3.62 (m, 2H), 3.43 (m, 2H), 3.22-3.06 (m, 5H), 3.04 (m, 3H), 2.98-2.91 (m, 1H), 2.74 (d, J = 12.7 Hz, 1H), 1.93 (m, 1H), 1.80 (m, 2H), 1.08 (s, 3H), 0.97 (s, 3H), 0.87 (s, 9H), 0.75 (s, 3H), 0.68 (s, 3H). [^{13}C] NMR (100 MHz, DMSO-d6) δ 175.20, 143.47, 121.67, 105.37, 94.09, 87.93, 77.71, 76.91, 76.68, 76.57, 73.98, 72.38, 70.17, 69.58, 61.21, 60.71, 55.08, 47.13, 45.93, 45.56, 41.27, 40.76, 38.99, 38.71, 38.24, 36.29, 33.25, 32.75, 32.29, 31.61, 30.31, 27.66, 27.19, 25.52, 25.52,23.36, 22.96, 22.52, 17.76, 16.68, 16.49, 15.20.

Compounds **3b-3e** were prepared according to the same procedure described for **3a**.

3-O-β-D-xylopyranosyl-28-O-a-L- arabinopyranosyl oleanolate (3b): 62.0% yield, R_f = 0.62 (5:1, CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 5.36 (d, J = 4.5 Hz, 1H), 5.19 (s, 1H), 5.14 (d, J = 4.8 Hz, 1H), 4.87 (br s, 3H), 4.65 (s, 1H), 4.61 (s, 1H), 4.11 (d, J = 7.5 Hz, 1H), 3.71 (m, 2H), 3.64 (dd, J = 11.3, 5.3 Hz, 1H), 3.54 (m, 1H), 3.51-3.46 (m, 1H), 3.36 (d, J = 8.5 Hz, 1H), 3.24 (m, 1H), 3.01 (m, 4H), 2.81 (d, J = 10.3 Hz, 1H), 1.92 (m, 1H), 1.78 (m, 2H), 1.09 (s, 3H), 0.97 (s, 3H), 0.88 (s, 6H), 0.86 (s, 3H), 0.75 (s, 3H), 0.68 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 175.24, 143.42, 121.72, 106.11, 93.74, 87.60, 76.73, 73.73, 71.19, 69.57, 69.57,65.53, 65.48, 63.44, 54.86, 46.98, 46.07, 45.44, 41.21, 40.61, 38.90, 38.72, 38.05, 36.23, 33.20, 32.71, 32.27, 31.70, 30.31, 27.49, 27.12, 25.69, 25.48, 23.33, 22.89, 22.24, 17.69, 16.64, 16.36, 15.08.

3-O-β-D-galactopyranosyl-28-O-a-L-arabinopyranosyl oleanolate (3c): 60.0% yield, R_f = 0.41 (5:1, CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 5.37 (s, 1H), 5.20 (s, 1H), 5.12 (s, 1H), 4.68-4.56 (m, 3H), 4.54 (s, 1H), 4.45 (s, 1H), 4.24 (s, 1H), 4.11 (d, 1H), 3.70 (m, 2H), 3.63 (s, 1H), 3.59-3.46 (m, 3H), 3.45-3.34 (m, 2H), 3.29 (m, 3H), 3.04 (d, 1H), 2.82 (d, J = 11.5 Hz, 1H), 1.93 (m, 1H), 1.80 (s, 2H), 1.09 (s, 3H), 0.98 (s, 3H), 0.88 (s, 9H), 0.76 (s, 3H), 0.68 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 175.30, 143.48, 121.78, 106.00, 93.77, 87.89, 74.85, 73.56, 71.24, 71.12, 69.59, 68.06, 65.55, 63.54, 60.36, 55.04, 47.04, 46.10, 45.47, 41.21, 40.64, 38.74, 38.70, 38.16, 36.25, 33.22, 32.77, 32.30, 31.75, 30.37, 27.66, 27.16, 25.65, 25.54, 23.37, 22.93, 22.26, 17.75, 16.68, 16.48, 15.13.

3-O-a-L-arabinopyranosyl-28-O-β-D-galactopyranosyl oleanolate(3d): 70.0% yield, R_f = 0.50 (5:1, CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 5.21 (d, J = 7.8 Hz, 1H), 5.16 (s, 1H), 4.94 (d, 1H), 4.76 (m, 2H), 4.54 (s, 1H), 4.50-4.42 (m, 3H), 4.11 (m, 1H), 3.67 (br s, 1H), 3.63 (br s, 1H), 3.60 (br s, 1H), 3.50 (m, 1H), 3.45-3.30 (m, 7H), 3.00 (d, J = 10.5 Hz, 1H), 2.75 (d, J = 12.8 Hz, 1H), 1.93 (m, 1H), 1.78 (s, 2H), 1.08 (s, 3H), 0.96 (s, 3H), 0.87 (s, 9H), 0.76 (s, 3H), 0.68 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 175.26, 143.42, 121.63, 105.77, 94.59, 87.68, 75.88, 73.38, 72.68, 70.99, 69.45, 67.72, 67.56, 65.00, 59.90, 54.98, 47.06, 45.90, 45.55, 41.25, 40.72, 38.96, 38.74, 38.10, 36.27, 33.22, 32.72, 32.25, 31.60, 30.27, 27.62, 27.15, 25.63, 25.51, 23.35, 22.92, 22.49, 17.75, 16.66, 16.41, 15.15.

3-O-β-D-galactopyranosyl-28-O-β-D-galactopyranosyl oleanolate(3e): 72.0% yield, R_f = 0.29 (5:1, CH_2Cl_2-MeOH). [^{1}H] NMR (400 MHz, DMSO-d6) δ 5.21 (d, J = 7.8 Hz, 1H), 5.16 (s, 1H), 4.97 (d, J = 5.6 Hz, 1H), 4.78 (d, J = 4.9 Hz, 1H), 4.70 (d, 1H), 4.60 (d, 1H), 4.56 (t, 1H), 4.50 (m, 2H), 4.29 (d, J = 3.8 Hz, 1H), 4.10 (d, J = 5.6 Hz, 1H), 3.68 (br s, 1H), 3.61 (br s, 1H), 3.51 (m, 2H), 3.45-3.20 (m, 8H), 3.03 (d, J = 11.0 Hz, 1H), 2.75 (d, J = 12.4 Hz, 1H), 1.93 (m, 1H), 1.78 (m, 3H), 1.08 (s, 3H), 0.98 (s, 3H), 0.87 (s, 6H), 0.86 (s, 3H), 0.75 (s, 3H), 0.68 (s, 3H). [^{13}C] NMR (101 MHz, DMSO-d6) δ 175.27, 143.44, 121.62, 105.94, 94.58, 87.87, 75.88, 74.84, 73.55, 73.41, 71.13, 69.44, 68.06, 67.70, 60.36, 59.87, 55.04, 47.06, 45.89, 45.52, 41.22, 40.70, 38.96, 38.68, 38.16, 36.24, 33.19, 32.70, 32.21, 31.60, 30.27, 27.63, 27.13, 25.60, 25.49, 23.33, 22.89, 22.46, 17.71, 16.65, 16.45, 15.13.

Antifungal activity bioassay

Antifungal activities of the target compounds against *M. oryzae* were assessed by the mycelium growth rate method (Agricultural Industry Standard of the People's Republic of China, 2006). Each synthetic saponin (100 mg/mL), isoprothiolane (0.11, 0.78, 1.56, 3.13, 6.25, 12.50, 25.00 mg/mL) as a positive control, and a negative control (0 mg/mL) in PDA were mixed thoroughly and sterilized (121°C, 1.2 atm, 30 min), then poured separately into sterilized Petri dishes and allowed to solidify. Three 8-mm-diameter plugs of actively growing *M. oryzae* mycelium colonies were placed in a triangular shape onto each plate (replicated, two plates, six plugs per treatment). The plates were incubated at 27°C. The colony diameter and the radial mycelium growth were measured on the fourth day.

Diameter of mycelia and inhibition rate were calculated using the following formula:

$$I = \frac{\overline{D_0} - \overline{D_1}}{\overline{D_0}} \times 100\% \qquad \text{(Equation 1)}$$

where I is the inhibition rate, $\overline{D_0}$ is the average diameter of mycelia in the blank test, and $\overline{D_1}$ is the average diameter of mycelia in the presence of synthetic saponins or isoprothiolane. The inhibition rates of these compounds at 100 μg/mL are shown in Table 1.

Table 1. The structure, inhibitory rate, R_f and yield of synthetic saponins (100 μg/mL) on *Magnaporthe oryzae*.

No.	Compound	Yield (%)	R_f	C-3(OH)	C-28(COOH)	*I* (%)
1	1a(Glc)	83.8	0.26	-β-D-Glc	-$CH_2CH=CH_2$	60.52 ± 1.52
2	1c(Gal)	58.4	0.26	-β-D-Gal	-$CH_2CH=CH_2$	63.44 ± 1.51
3	1b(Xyl)	76.4	0.36	-β-D-Xyl	-$CH_2CH=CH_2$	6.24 ± 0.92
4	1d(Ara)	83.5	0.38	-α-L-Ara	-$CH_2CH=CH_2$	8.56 ± 1.14
5	1e(Man)	33.8	0.25	-α-D-Man	-CH2CH=CH2	75.46 ± 0.61
6	1f(Rha)	87.3	0.44	-α-L-Rha	-$CH_2CH=CH_2$	27.29 ± 3.64
7	1i(Glc)	46.7	0.21	-β-D-Glc	H	41.29 ± 1.14
8	1k(Gal)	32.2	0.22	-β-D-Gal	H	32.43 ± 4.12
9	1j(Xyl)	45.0	0.33	-β-D-Xyl	H	13.29 ± 2.69
10	1l(Ara)	48.0	0.34	-α-L-Ara	H	16.31 ± 1.32
11	1m(Man)	16.2	0.22	-α-D-Man	H	48.24 ± 4.85
12	1n(Rha)	48.0	0.35	-α-L-Rha	H	15.21 ± 1.82
13	1g(Gal)	82.0	0.29	-β-D-Gal	-$(CH_2)_5CH=CH_2$	40.14 ± 6.31
14	1h(Ara)	85.0	0.41	-α-L-Ara	-$(CH_2)_5CH=CH_2$	35.17 ± 0.40
15	2a(Glc)	79.3	0.25	H	-β-D-Glc	14.40 ± 5.30
16	2c(Gal)	68.0	0.25	H	-β-D-Gal	23.77 ± 7.00
17	2b(Xyl)	75.0	0.35	H	-β-D-Xyl	13.60 ± 1.98
18	2d(Ara)	68.0	0.35	H	-α-L-Ara	16.41 ± 2.87
19	2e(Man)	22.0	0.24	H	-β-D-Man	39.48 ± 5.34
20	2f(Rha)	80.0	0.36	H	-α-L-Rha	25.08 ± 3.15
21	3a(Glc,Glc)	55.2	0.29	-β-D-Glc	-β-D-Glc	16.97 ± 3.60
22	3b(Xyl,Ara)	62.0	0.62	-β-D-Xyl	-α-L-Ara	34.21 ± 0.84
23	3c(Gal,Ara)	60.0	0.41	-β-D-Gal	-α-L-Ara	31.88 ± 4.28
24	3d(Ara,Gal)	70.0	0.50	-α-L-Ara	-β-D-Gal	9.79 ± 1.49
25	3e(Gal,Gal)	72.0	0.29	-β-D-Gal	-β-D-Gal	38.76 ± 2.65
Ck1	OA			H	H	14.00 ± 1.49
Ck2	OA-10a	82.6		H	-CH2CH=CH2	12.99 ± 1.98
Ck3	OA-10b			H	-(CH2)5CH=CH2	20.68 ± 2.52
Ck4	Isoprothiolane (μg/mL)			1.30 ± 1.32 (0.11) to 84.82 ± 2.64 (12.50)		

RESULTS AND DISCUSSION

Synthesis of oleanolic mono- and di-glycosides

Synthesis of oleanolic C_3 monoglycosides

The two-methyl groups at the C-4 position in OA make the hydroxy C-3 glycosylation reaction difficult; a glycosyl donor with high activity is therefore required. Trichloroacetimidate glycosyl as a donor has the advantages of high activity, high stability, and a single spatial structure that can efficiently catalyze glycosylation reactions promoted by trimethylsilyl trifluoromethanesulfinate (TMSOTf). During the glycosylation reaction, other hydroxyl groups in the saccharide-ring must to be operated through protection. The common protection group contains benzyl, alkyl, acetyl, and benzoyl. We chose benzoyl-protected trichloroacetimidate as a glycosyl donor for glycosylation because of its high stability and neighbor-participating effect (Deng et al., 1999), and a glycosyl product with a single beta space configuration (Figure 1).

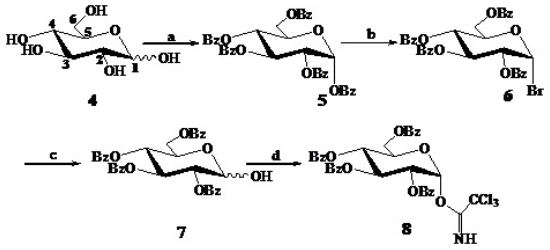

Figure 1. The synthetic routes of glycosyl bromide and trichloroacetimidates donors. Reagen s and conditions: (**a**) BzCl, pyridine, rt, 24 h, 95% yield; (**b**) HBr-HOAc, CH_2Cl_2, rt, 2 h; (**c**) Ag_2CO_3, acetone-H_2O, rt, 2 h, 92.6% yield; (**d**) CCl_3CN, DBU, CH_2Cl_2, rt, 4 h, 84.4% yield.

Activity against *M. oryzae* was higher with oleanolic 3-monoglycosides containing allyl at the C_{28}-carboxyl position than without the protection group (Table 1). A similar report showed that the activity of an inhibiting HIV proteolytic enzyme was enhanced after oleanolic C_{28}-carboxyl was introduced, and an optimal chain length of 6-8 carbon atoms was used (Ma et al., 2000). Therefore, a heptenyl with seven carbon atoms was available to link oleanolic C_{28}-carboxyl before oleanolic C_3-hydroxy glycosylation with galactose and arabinose, consequently affording saponins with an oleanolic 3-monosaccharide 28-heptenyl ester (Figures 2 and 3).

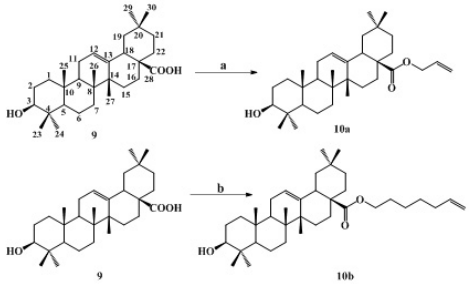

Figure 2. Synthesis of glycosylacceptor. Reagents and conditions: **a.** Allyl Br, DBU, DMF, rt, 24h; **b.** 7-bromo-1-heptene, DBU, DMF, rt, 24h.

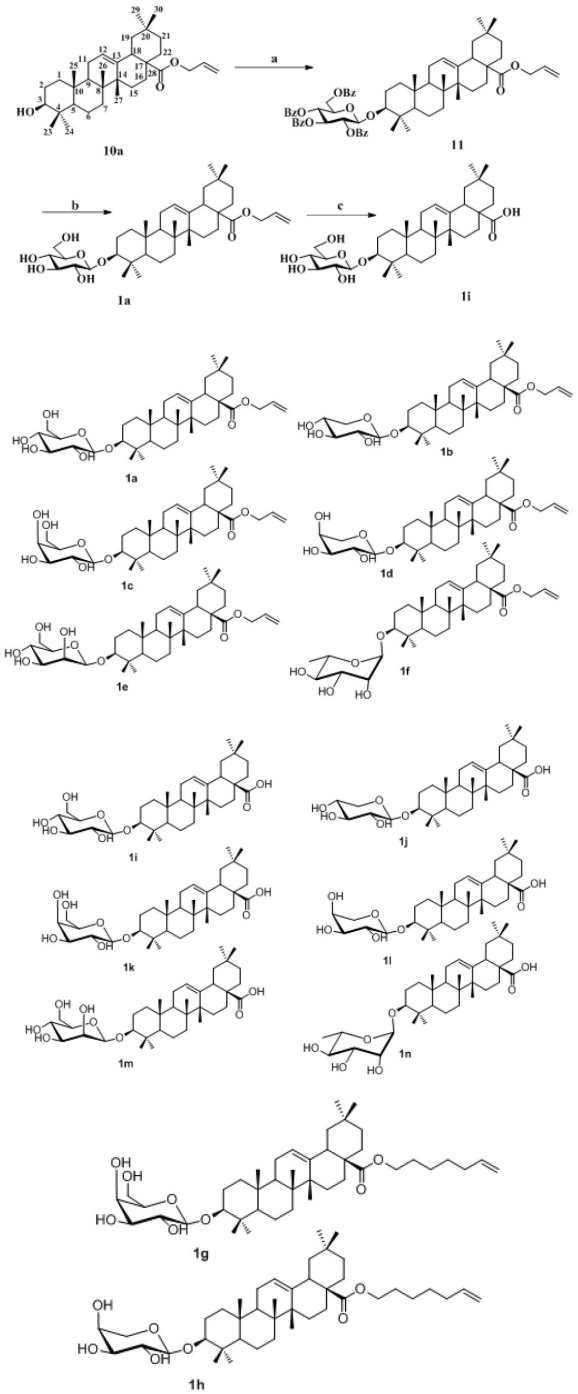

Figure 3. The synthetic routes and their products of oleanolic3-glycosides. Reagents and conditions: **a.** TMSOTf, 0°C, 4Å MS, CH_2Cl_2, 4 h; **b.** MeONa, CH_2Cl_2-MeOH, rt, 2 h; **c.** $PdCl_2$,CH_3OH, rt, 24 h.

Oleanolic 3-monoglycosides and 3-monoglycosides 28-allyl or heptenyl ester were verified by [^{1}H], [^{13}C] NMR data analysis. The [^{1}H] NMR data displayed olefinic C_{12}-H at δ5.29br s in $CDCl_3$ or δ5.16s in DMSO-d_6, C_3-H at δ3.14m in $CDCl_3$ or δ3.02m in DMSO-d6, C_{18}-H at δ2.88d in $CDCl_3$ or δ2.74d in DMSO-d6; seven H from -CH_3 at δ0.72s-1.16s; δ5.32dd, δ4.90ddd, δ4.53m; C_{28}-allyl at δ5.32dd, δ4.90ddd, δ4.53m while C_{28}-heptenyl at δ5.78m, δ4.96m, δ3.93m or C_{28}-OOH at δ12.00s; anomeric proton at δ4.32d (β-Glc), δ4.11d (β-Gal), δ4.11d (β-Xyl), δ4.12d (α-Ara), δ4.37s (α-Man), δ4.66s (α-Rha). The [^{13}C] NMR data showed 36 carbons at saponin Glc-9, Gal-9, Man-9, Rha-9 and 35 carbons at saponin Xyl-9, Ara-9; carboxyl C_{28} at δ178.5 while the carbon signal of C_{28}-heptenyl at δ138.47, δ114.70, δ63.56, d33.11, δ27.90, δ27.78, δ25.05; C_{13}=C_{12} at δ143.8, δ121.5; C_3 at δ80.26-87.95; anomeric carbon at δ105.44 (β-Glc), δ106.12 (β-Gal), δ106.18 (β-Xyl), δ105.85 (α-Ara), δ95.86 (α-Man), δ102.85 (α-Rha). These NMR data are consistent with those in previous reports (Zang, 2004; Sha et al., 2008; Zhao et al., 2011).

Synthesis of oleanolic 28 monoglycosides

The usual strategy for preparing oleanolic C_{28} glycosyl ester involves two methods. In the first method, trichloroacetimidate glycoside as glycosyl donor is coupled with oleanolic C_{28}-carboxyl after C_3-hydroxyl is protected by acetylation. Then an additional deprotection step is performed to provide oleanolic C_{28} monoglycoside. The second method adopts the "phase-transfer-catalyzed method" (Bliard et al., 1994) where bromoglycoside as a glycosyl donor is coupled with OA C_{28}-carboxyl promoted by potassium carbonate-tetrabutylammonium bromide in a two-phase water-chloroform solution. Oleanolic C_3 hydroxyl in such conditions does not participate in the reaction and therefore, there is no need to protect in advance. It has been reported that the second method is simpler, higher-yielding, and better than the first method (Yan, 2008), therefore the second method was adopted in this study (Figure 4).

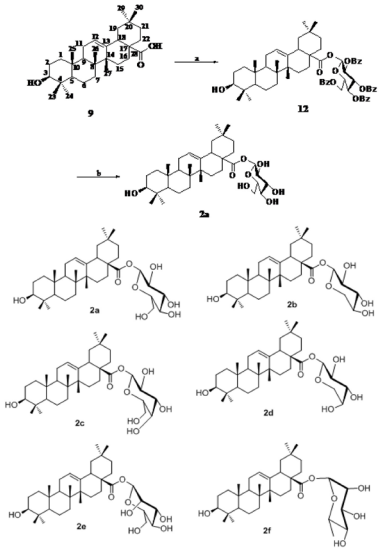

Figure 4. The synthetic routes and their products of oleanolic 28-glycosides. Reagents and conditions: **a.** K_2CO_3, Bu_4NBr, CH_2Cl_2-H_2O, reflux, 6 h. **b.** CH_3ONa, CH_2Cl_2-CH_3OH, rt, 2 h.

Oleanolic 28 monoglycosides were confirmed by [^{1}H]-, [^{13}C]-NMR data analysis. The [^{1}H] NMR data demonstrated C_{12}-H at δ5.16s; C_3-H at δ2.99m; C_3-OH at δ4.27s; C_{18}-H at δ2.74-2.82d; H from seven -CH_3 at δ0.67s-1.11s; anomeric proton at δ5.24d (β-Glc), δ5.21d (β-Gal), δ5.21d (β-Xyl), δ5.36d (α-Ara), δ5.81s (α-Man), δ5.76s (α-Rha), δ6.19d and 5.95s (β-Mal in Pyr-d5). The [^{13}C] NMR indicated 42 carbons in Mal-11, 36 carbons in Glc-11, Gal-11, Man-11, Rha-11, and 35 carbons in Xyl-11, Ara-11; carboxyl C_{28} at δ175.2; C_{13}=C_{12} at δ143.4, δ121.6; C_3 linked hydroxyl at δ76.8 while anomeric carbon at δ94.05 (β-Glc), δ94.58 (β-Gal), δ94.68 (β-Xyl), δ93.76 (α-Ara), δ93.32 (α-Man), δ93.37 (α-Rha), δ102.97 and 95.48 (β-Mal in Pyr-d5). These NMR data are in accordance with previous reports (Li et al., 2009; Zhao et al., 2011).

Synthesis of oleanolic 3, 28 diglycosides

The synthesis of oleanolic 3,28 diglycosides must take into consideration which site is glycosylated first. There are more complex routes in the first glycosylation of oleanolic C_3-hydroxyl than C_{28}-carboxyl because the former bears the operation of protection and deprotection. In some situations, the former has obvious advantages. For example, in preparing a series of saponins with the same C_3 glycosyl and different C_{28} glycosyl chains, this method can reduce workload and improve efficiency. Moreover, it can effectively avoid too much reaction damage to C_{28} containing sensitive groups that are coupled to the C_{28} glycosyl chain in later reactions. In short, the method should be selected according to the needs of the target compounds. The latter method was adopted for preparing five oleanolic diglycosides, i.e., glycosylation modification of the carboxyl C_{28} first, then of the hydroxyl C_3, and finally the deprotecting group in the hydroxyl of the sugar ring giving oleanolic 3,28 diglycosides (Figure 5).

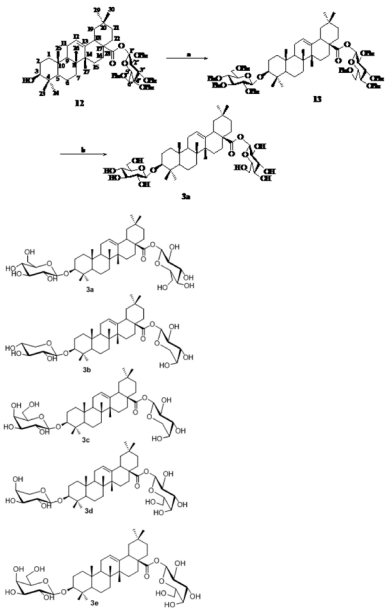

Figure 5. Synthetic routes and their products of oleanolic 3,28-disglycosides. Reagents and conditions: **a.** TMSOTf, 0°C, 4Å MS, CH_2Cl_2, 4 h; **b.** CH_3ONa, CH_2Cl_2-CH_3OH, rt, 2 h.

Oleanolic 3, 28 diglycosides were demonstrated by [^{1}H]-, [^{13}C]-NMR data analysis. The [^{1}H] NMR revealed C_{12}-H at δ5.16s; C_3-H at δ3.00m; C_{18}-H at δ2.74-2.82d; H of seven -CH_3 at δ0.68s-1.09s; proton signal linked anomeric carbon C_3 and C_{28} at δ4.09d (β-Glc) and δ5.23d (β-Glc), δ4.11d (β-Xyl) and δ5.36d (α-Ara),δ4.11d (β-Gal) and δ5.37s (α-Ara),δ4.11m (α-Ara) and δ5.21d (β-Gal),δ4.10d (β-Gal) and δ5.21d (β-Gal) respectively. The [^{13}C] NMR brought out 42 carbons in saponin 3a and 3e, 41 carbons in 3c and 3d, 40 carbon in 3b; C_{28} at δ175.20; C_{13}=C_{12} at δ143.47 and δ121.67; C_3 at δ87.60-87.93 while anomeric carbon C_3 and C_{28} at δ105.37 (β-Glc) and δ94.09 (β-Glc), δ106.11 (β-Xyl) and δ93.74 (α-Ara), δ106.00 (β-Gal) and δ93.77 (α-Ara), δ105.77 (α-Ara) and δ94.59 (β-Gal), δ105.94 (β-Gal) and δ94.58 (β-Gal). These NMR data are consistent with those in previous reports (Zhao et al., 2011).

Activity of oleanolic mono- and di-glycosides against *M. oryzae* and their SAR

Twenty five oleanolic mono- and di-glycosides were synthesized, containing different structural characteristics: seven saccharide residues (pentose 2, hexose 4 and disaccharide 1), two linkage sites (C_3, C_{28}), and aglycone C_{28} with or without a group such as allyl or heptenyl. These 25 synthesized oleanolic mono- or di-glycosides included fourteen oleanolic 3-monoglycosides, six oleanolic 28-monoglycosides, and five oleanolic 3-,28-diglycosides. Their inhibiting rates against *M. oryzae* at a concentrations of 100 μg/mL corresponded to 0.11-12.5 μg/mL for isoprothiolane (Table 1).

The SAR of the synthesized oleanolic monoglycosides showed significant differences in activity between different monosaccharide residues linked to C_3 or C_{28} of OA. In general, activity against *M. oryzae* of oleanolic hexose glycosides was stronger than one of the oleanolic pentose glycosides. The saccharide residue order of enhancing activity in oleanolic C_3 monoglycosides (1i-1n) from strong to weak was mannose, glucose, and galactose, while rhamnose, arabinose, and xylose were ineffective. The saccharide residue order of enhancing activity in oleanolic C_{28} monoglycosides (2a-2f) from strong to weak was mannose, rhamnose, and galactose, while glucose, arabinose, and xylose were ineffective.

The glycosylation site in OA was another important factor affecting the activity of oleanolic monoglycosides. There was stronger activity in oleanolic C_3 mannose, glucose, or galactose glycoside than in their C_{28} ester glycoside, but there was stronger activity in oleanolic C_{28} rhamnose ester glycoside than in its C_3 glycoside. For oleanolic C_3 or C_{28} pentose glycoside, arabinose, and xylose, they showed very little activity difference between the C_3 and C_{28} sites.

Allyl and its length in C_{28} with a symmetrical C_3 monosaccharide residue in OA obviously influenced their activity against *M. oryzae*. When OA C_{28} was linked by allyl, the activity of oleanolic C_3 hexose glycoside (1a,1c,1e,1f) was significantly enhanced. The hexose residue order of enhancing activity from strong to weak was galactose, mannose, glucose, and rhamnose. However, the activity of oleanolic C_3 pentose glycosides with C_{28} allyl (1b,1d) decreased to half that of aglycone OA. When OA C_{28} was linked by heptenyl, the activity of both hexose and pentose glycosides in its C_3 was increased (1g and 1k, 1h and 1l). The activity enhanced in oleanolic hexose glycoside was less in C_{28} heptenyl than in C_{28} allyl (1g and 1c), while the activity enhanced in its pentose glycoside was more in C_{28} heptenyl than in C_{28} allyl (1h and 1d).

Activity of oleanolic diglycosides against *M. oryzae* was influenced by the saccharide residue type, linkage site, and a combination of both. When C_3 and C_{28} of OA were linked to the same saccharide such as galactose or glucose, the activity linked to galactose (3e) was stronger than that linked to glucose (3a). When oleanolic C_3 and C_{28} both were linked to a

pentose such as arabinose or xylose (3b), activity was stronger than when either C_3 or C_{28} alone was linked to a pentose (1j, 1l; 2b, 2d). When oleanolic C_3 and C_{28} were linked to a different saccharide such as hexose galactose or pentose arabinose, the glycoside C_3 linked a hexose than a pentose or the glycoside C_{28} linked a pentose than a hexose played more important role in activity (3c, 3d).

In summary, the optimal oleanolic mono- and di-glycoside structure for activity against *M. oryzae* is a C_3 connection of a hexose such as mannose, galactose, or glucose in combination with a C_{28} connection to a small group such as allyl, or a C_3 connection to a pentose accompanied by a larger group such as another pentose or heptenyl in C_{28}.

ACKNOWLEDGMENTS

The authors would like to thank Professor Harald W. Scherm from the Department of Plant Pathology at the University of Georgia, USA for his helpful advice and proofreading the English style of our manuscript, and Dr. Satish Sreedharamurthy from the Department of Studies in Microbiology, University of Mysore, India for his literal advice. Research supported by the National Natural Sciences Foundation of China (grant #21266010 to G. Huo), the Natural Sciences Fund of Jiangxi Province (grant #20132BAB204028 to G. Huo), and China Scholarship Council Program (grant #201308360006 to G. Huo).

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