

Production of rare ginsenosides by biotransformation of *Panax notoginseng* saponins using *Aspergillus fumigatus*

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Abstract

Panax notoginseng saponins (PNS) are the main active components of *Panax notoginseng*. But after oral administration, they need to be converted into rare ginsenosides by human gut microbiota and gastric juice before they can be readily absorbed into the bloodstream and exert their effects. The sources of rare ginsenosides are extremely limited in *P. notoginseng* and other medical plants, which hinders their application in functional foods and drugs. Therefore, the production of rare ginsenosides by the transformation of PNS using *Aspergillus fumigatus* was studied in this research. During 50 days at 25 °C and 150 rpm, *A. fumigatus* transformed PNS to 14 products (**1–14**). They were isolated by varied chromatographic methods, such as silica gel column chromatography, Rp-C₁₈ reversed phase column chromatography, semi-preparative HPLC, Sephadex LH-20 gel column chromatography, and elucidated on the basis of their NMR and MS spectroscopic data. Then, the transformed products (**1–14**) were isolated and identified as ginsenosides C-K, 20 (*R/S*)-Rg₃, Rg₅, 20 (*R*)-Rh₁, Rk₁, Rh₄, Rk₃, 20(*S*)-protopanaxatriol, 20(*S*)-I, 20 (*R/S*)-Rg₂, and notoginsenosides 20 (*R/S*)-R₂, respectively. In addition, all transformed products (**1–14**) were tested for their antibacterial activity. Among them, compounds **5** and **7** showed moderate antibacterial activities against *Staphylococcus aureus* and *Candida albicans* with MIC values of 6.25/25.00, and 1.25/25.00 µg/mL, respectively. This study lays the foundation for production of rare ginsenosides.

Introduction

Panax notoginseng (Burk.) F.H. Chen is a well-known traditional Chinese medicine, which has various biological activities (Cicero et al. 2003; Ye et al. 2010; Li et al. 2006). *P. notoginseng* saponins (PNS) are the main active components of *P. notoginseng*. Among them, ginsenosides Rg₁, Rb₁, Rd, Re and notoginsenoside R₁ are the major saponins, accounting for over 80% of the PNS. Saponins have pharmacological effects, such as anti-inflammatory, antioxidant, inhibition of platelet aggregation, regulation of blood glucose and blood pressure, inhibition of neuronal apoptosis, protection of neurons, etc. (Duan et al. 2018; Lin et al. 2015; Luan et al. 2006; Xiong et al. 2019). However, the major ginsenosides in PNS (including Rg₁, Rb₁, Rd, Re, R₁) are difficult to be absorbed by the human body due to their high molecular weight, low membrane permeability, and low bioavailability (Cui et al. 2016; Upadhyaya et al. 2016). After oral administration, they need to be converted into minor ginsenosides by human gut microbiota and gastric juice before they can be readily absorbed into the bloodstream and exert their effects. In addition, pharmacological studies showed that rare saponins have better biological activity, but their content in *P. notoginseng* is very low (Park et al. 2010; Wei et al. 2011; Wu et al. 2012). Thus, a lot of studies have been focused on the conversion of major ginsenosides to rare ginsenosides. Biotransformation is the most promising method to produce rare ginsenosides, which has the advantages of strong specificity, high yield, low cost and environmental friendliness (Zhao et al. 2009; Chang et al. 2014). Studies showed that the genus *Aspergillus* has the ability of transformation saponins to rare ginsenosides. Such as ginsenoside Re can be transformed to ginsenoside Rh₁ via ginsenoside Rg₁ by *Aspergillus niger*, ginsenoside Re also can be transformed to Rg₂ by *Aspergillus usamii* (Chi et al.

2005). However, there have no reports on the separation, purification, and structural identification of PNS transformation products by the genus of *Aspergillus*. This study aimed to transform major ginsenosides into rare ginsenosides through fermentation of PNS by the fungus *Aspergillus fumigatus*. During 50 days at 25 °C and 150 rpm, *A. fumigatus* transformed PNS to 14 products (**1–14**). They were isolated by varied chromatographic methods and elucidated on the basis of their NMR and MS spectroscopic data. Then, the transformed products (**1–14**) were isolated and identified as ginsenosides C-K, 20 (*R/S*)-Rg₃, Rg₅, 20 (*R*)-Rh₁, Rk₁, Rh₄, Rk₃, 20(*S*)-protopanaxatriol, 20(*S*)-I, 20 (*R/S*)-Rg₂, and notoginsenosides 20 (*R/S*)-R₂, respectively. In addition, all transformed products (**1–14**) were tested for their antibacterial activity. Quantitative analysis by HPLC showed that the conversion rate of ginsenoside Rb₁, Rg₁, Re and notoginsenoside R₁ (four major ginsenosides of PNF), were 100%, 32.52%, 19.35% and 24.74%, respectively. Then, the transformation pathways of five major ginsenosides of PNF were deduced.

Materials and methods

Strains

The strain of *A. fumigatus* was isolated from fresh *P. notoginseng* root soil in our previous research, and was conserved in Potato Dextrose Agar(PDA) Medium. A voucher specimen (No. Yang20210907) was deposited at the Faculty of Life Science and Technology, Kunming University of Science and Technology.

Sample, chemical, and reagents

Reference standards, including ginsenosides Rg₁, Re, Rb₁, Rd, 20 (*S/R*)-Rg₂, 20 (*R*)-Rh₁, Rg₆, Rk₃, Rh₄, 20 (*S/R*)-Rg₃, Rk₁, Rg₅, CK, 20 (*S/R*)-Rh₂, and notoginsenosides R₁, 20 (*S/R*)-R₂ were purchased from the Sichuan Victory Biological Technology Co., Ltd. (Sichuan, China). PNS was supported by professor of Xiuming Cui, Kunming University of Science and Technology. The solvents methanol and acetonitrile for HPLC were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The Welchrom C₁₈ Column (4.6×250 mm, 5 μm) was purchased from Yuexu Technology Co, Ltd. (Sichuan China). The Agilent 1260 High Performance liquid chromatograph was purchased from Agilent (Grand Island, NY, USA). The GF₂₅₄ Silica gel plate was purchased Qingdao Marine Chemical Plant Co., Ltd. (Shandong, China). The standard strains, *Staphylococcus aureus* (CMCC(B)26003) and *Candida albicans* (BNCC109047), were purchased from Engineering Research Center of Industrial Microbiology (Henan, China). Ciprofloxacin was purchased from Beijing Solarbio Science & Technology Co., LTD (Beijing, China).

Morphological observation of *A. fumigatus*

A. fumigatus was inoculated on PDA medium and cultured in 25 °C incubator for 3 days. Colony characteristics and morphological characteristics (under optical microscope) were observed.

Medium

PDA medium: potato extract powder 5 g/L, glucose 15 g/L, and agar 25 g/L. PDB medium: potato extract powder 5 g/L and glucose 15 g/L.

Microbial transformation of PNS by *A. fumigatus*

Transfer well-developed fungal hyphae from the surface of the agar slant to three 500 mL Erlenmeyer flasks containing 300 mL PDB medium. The cultures were grown for 3 d on a rotating shaker at 25 °C with shaking at 150 rpm to produce seed liquid. Then, the seed liquids were transferred to 500 mL reagent bottles that contained 300 mL of medium for expand fermentation. The cultures were then incubated using the same conditions as before. After 4 d, PNS (transformation substrate) were added to the cultures at the concentration of 5 mg/mL. The cultures were incubated for additional 46 days at 25°C with shaking at 150 rpm. Finally, A total of 172.5 g PNS was transformed and a total of 34.5 L fermentation broth were prepared. The mycelia were separated by filtration and the filtrate was extracted five times with n-butanol. The organic layer was concentrated under reduced pressure to afford a residue (161.5 g).

Chromatographic conditions

A total of 161.5 g residue was obtained from the broth. The residue was purified by D101 macroporous resin column chromatography, silica gel column chromatography, Rp-C₁₈ reversed phase column chromatography, semi-preparative HPLC, and Sephadex LH-20 gel column chromatography. The structures of the compounds were elucidated on the basis of their NMR and MS spectroscopic data.

Analysis the conversion rate and products yield by HPLC

HPLC was performed using an Agilent 1260 system (Grand Island, NY, USA). A reverse phase column (4.6×250 mm, 5 µm; Yuexu Technology Co., Ltd. Sichuan China) at 30 °C was used. To determine the conversion rate of five main ginsenosides of PNF, using the conversion products by *A. fumigatus* (10 mg), PNS (10 mg) and 14 rare ginsenosides (0.4 mg) were dissolved in 1 mL methanol and analyzed by HPLC. H₂O and CH₃CN were used as the mobile phases A and B, respectively. The gradient elution was programmed as follows: 0–30 min, 20% (B); 30–60 min, 20–37% (B); 60–65 min, 37–38% (B); 65–70 min, 38–45% (B); 70–75 min, 45–50% (B); 75–90 min, 50–56% (B); 90–93 min, 56–62% (B); 93–103 min, 62–75% (B). The flow rate and detection wavelength were set as 1 mL/min and 203 nm respectively. The injection volume was 30 µL.

Antimicrobial activity

Transformation products (**1–14**) were evaluated for antibacterial activity against 2 human pathogenic bacteria. The tested bacteria strains were: *Staphylococcus aureus* and *Candida albicans*. The activity of compounds **1–14** against human pathogenic bacteria was tested by double dilution method.

Compounds **1–14** and positive control ciprofloxacin were dissolved with DMSO and the concentration was 1 mg/mL. Pathogenic bacteria were incubated in LB medium at 37 °C and 160 rpm for 8–12 h, bacterial solution and compounds **1–14** were added to 96-well plates, incubated at 37 °C, and observed

after 12 h. The experiment was repeated three times. LB broth medium component: 20 g LB Broth dissolved in 1000 mL distilled water.

Results and Discussion

Morphological observation of *A. fumigatus*

A. fumigatum was inoculated on PDA and cultured in 25 °C incubator for 3 days. The following colony characteristics were observed: *A. fumigatus* is fluffy or flocculent, dark green (Fig. 1a). The conidial head is columnar, green when young, dark green when mature; The surface of the conidial stem is smooth, and many spores are scattered around it (Fig. 1b).

Separation of transformation products

The 161.5 g residue was eluted by D101 macroporous resin column chromatography with a gradient elution of an ethanol-water solvent system to obtain four fractions (Frs. A ~ D). Frs D was further subjected over silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 20:1 \rightarrow 15:1 \rightarrow 10:1 \rightarrow 5:1 \rightarrow 1:1$), Rp- C_{18} reversed phase column chromatography ($\text{MeOH}/\text{H}_2\text{O} = 3:7 \rightarrow 1:1 \rightarrow 7:3 \rightarrow 1:0$) to give compounds **1** (7 mg), **3** (19.3 mg), **5** (9 mg), **7** (50.5 mg), and **11** (8 mg); semi-preparative HPLC (50% $\rightarrow$ 70% $\rightarrow$ 90% $\rightarrow$ 100% MeOH) to give compounds **12** (3 mg) and **14** (6 mg); Sephadex LH-20 gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 1:1$) to give compounds **2** (9.5 mg), **6** (10 mg), and **13** (2 mg); semi-preparative HPLC conditions are as follows: H_2O and MeOH were used as the mobile phases A and B, respectively. The gradient elution was programmed as follows: 0–35 min, 70% (B); 35–50 min, 100% (B) to give compound **4** (6 mg), **8** (6 mg), and t_R is 32.1, 37.3 min, respectively. 0–10 min, 70% (B); 10–30 min, 90% (B); 30–40 min, 100%(B) to give compounds **9** (2 mg), **10** (13 mg), and t_R is 35.5, 31.4 min, respectively.

Structural characterization of products

The structures of products were identified on the basis of their spectroscopic data. Data of ^1H and ^{13}C NMR spectra of products **1–14** were in agreement with the reported literatures' data. Compounds **1–14** were identified as ginsenoside Rk_3 , Rh_4 , 20 (*R*)- Rh_1 , 20 (*S*)-Protopanaxatriol, C-K, 20 (*R*)- Rg_3 , 20 (*S*)- Rg_3 , 20 (*S*)- Rg_2 , notoginsenoside 20 (*R*)- R_2 , Gnsenoside Rk_1 , Rg_5 , notoginsenoside 20 (*S*)- R_2 , Gnsenoside 20 (*R*)- Rg_2 , and 20 (*S*)-I (Fig. 2).

Ginsenoside Rk_3 (**1**) white powder; ^1H NMR (600 MHz, $\text{C}_5\text{D}_5\text{N}$): δ 5.29 (1H, t, $J = 6.8$ Hz, H-24), 5.13 (1H, br s, H-21b), 5.06 (1H, d, $J = 7.8$ Hz, H-1'), 4.91 (1H, br s, H-21a), 3.91 (1H, m, H-12), 3.56 (1H, dd, $J = 11.7, 4.7$ Hz, H-3), 2.11 (3H, s, Me-28), 1.67 (3H, s, Me-26), 1.64 (3H, s, Me-27), 1.60 (3H, s, Me-29), 1.24 (3H, s, Me-18), 1.05 (3H, s, Me-19), 0.83 (3H, s, Me-30); ^{13}C NMR (150 MHz, $\text{C}_5\text{D}_5\text{N}$) δ 155.3 (s, C-20), 131.1 (s, C-25), 125.3 (d, C-24), 108.0 (t, C-21), 106.0 (d, C-1'), 80.0 (d, C-6), 79.7 (d, C-3'), 78.5 (d, C-3), 78.2 (d, C-5'), 75.4 (d, C-2'), 72.3 (d, C-12), 71.7 (d, C-4'), 63.0 (t, C-6'), 61.4 (d, C-5), 52.0 (d, C-13), 51.1 (s, C-14), 50.6 (d, C-9),

48.3 (d, C-17), 45.2 (t, C-7), 41.2 (s, C-8), 40.4 (s, C-4), 39.6 (s, C-10), 39.4 (t, C-1), 33.6 (t, C-22), 32.7 (t, C-11), 32.4 (t, C-15), 31.7 (q, C-28), 30.7 (t, C-16), 27.9 (t, C-2), 26.9 (t, C-23), 25.7 (q, C-26), 17.7 (q, C-18), 17.7 (q, C-19), 17.3 (q, C-27), 16.6 (q, C-30), 16.3 (q, C-29) (Park et al. 2002).

Ginsenoside Rh₄ (**2**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.45 (1H, overlapped, H-22), 5.19 (1H, overlapped, H-24), 5.05 (1H, d, *J* = 7.8 Hz, H-1'), 4.44 (1H, d, *J* = 11.5 Hz, H-6), 3.97 (1H, m, H-12), 3.55 (1H, d, *J* = 11.5, 4.2 Hz, H-3), 2.10 (3H, s, Me-28), 1.84 (3H, s, Me-21), 1.64 (3H, s, Me-26 and M-29), 1.63 (3H, s, Me-27), 1.58 (3H, s, Me-27), 1.25 (3H, s, Me-18), 1.04 (3H, s, Me-19), 0.84 (3H, s, Me-30); ¹³C NMR (150 MHz, C₅D₅N) δ 139.8 (s, C-20), 131.0 (s, C-25), 125.4 (d, C-24, overlapped), 123.0 (d, C-22), 105.7 (d, C-1'), 79.8 (d, C-6), 79.3 (d, C-3'), 78.3 (d, C-3), 77.9 (d, C-5'), 75.2 (d, C-2'), 72.3 (d, C-12), 71.4 (d, C-4'), 62.7 (t, C-6'), 61.1 (d, C-5), 50.6 (d, C-17), 50.3 (d, C-9 and C-13), 50.1 (s, C-14), 45.0 (t, C-7), 41.0 (s, C-8), 40.1 (s, C-4), 39.4 (s, C-10), 39.2 (t, C-1), 32.2 (t, C-11), 32.0 (t, C-15), 31.4 (q, C-28), 28.6 (t, C-2), 27.6 (t, C-23), 27.2 (t, C-16), 25.4 (q, C-26), 17.4 (q, C-27), 17.4 (q, C-18), 17.1 (q, C-19), 16.5 (q, C-30), 16.1 (q, C-29), 12.9 (q, C-21) (Teng et al. 2002).

Ginsenoside 20(*R*)-Rh₁ (**3**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.30 (1H, t, *J* = 6.8 Hz, H-24), 5.06 (1H, d, *J* = 7.9 Hz, H-1'), 4.56 (1H, m, H-6), 2.10 (3H, s, H-28), 1.69 (3H, s, Me-26), 1.64 (3H, s, Me-27), 1.63 (3H, s, Me-29), 1.40 (3H, s, Me-21), 1.24 (3H, s, Me-18), 1.06 (3H, s, Me-19), 0.86 (3H, s, Me-30); ¹³C NMR (150 MHz, C₅D₅N) δ 130.7 (s, C-25), 125.9 (d, C-24), 105.9 (d, C-1'), 80.0 (d, C-6), 79.6 (d, C-3'), 78.4 (d, C-3), 78.1 (d, C-5'), 75.4 (d, C-2'), 72.9 (s, C-20), 71.7 (d, C-4'), 70.8 (d, C-12), 62.9 (t, C-6'), 61.3 (d, C-5), 51.6 (s, C-14), 50.4 (d, C-17), 50.1 (d, C-9), 48.8 (d, C-13), 45.1 (t, C-7), 43.1 (t, C-22), 41.0 (s, C-8), 40.3 (s, C-4), 39.5 (s, C-10), 39.3 (t, C-1), 32.1 (t, C-11), 31.6 (q, C-28), 31.2 (t, C-15), 27.8 (t, C-2), 26.5 (t, C-16), 25.8 (q, C-26), 22.7 (q, C-21), 22.5 (t, C-23), 17.6 (q, C-27), 17.6 (q, C-19), 17.3 (q, C-18), 16.9 (q, C-30), 16.3 (q, C-29) (Teng et al. 2002).

20(*S*)-Protopanaxatriol (**4**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.33 (1H, t, *J* = 7.2 Hz, H-24), 4.42 (1H, m, H-6), 3.56 (1H, dd, *J* = 11.6, 4.6 Hz, H-3), 3.97 (1H, m, H-12), 2.03 (3H, s, Me-28), 1.66 (3H, s, Me-26), 1.63 (3H, s, Me-27), 1.47 (3H, s, Me-29), 1.43 (3H, s, Me-21), 1.12 (3H, s, Me-19), 1.01 (3H, s, Me-18), 0.97 (3H, s, Me-30); ¹³C NMR (150 MHz, C₅D₅N) δ 130.8 (s, C-25), 126.2 (d, C-24), 78.3 (d, C-3), 72.9 (s, C-20), 71.0 (d, C-12), 67.6 (d, C-6), 61.7 (d, C-5), 54.7 (d, C-17), 51.6 (s, C-14), 50.0 (d, C-9), 48.2 (d, C-13), 47.5 (t, C-7), 41.1 (s, C-8), 41.1 (s, C-4), 40.3 (s, C-4), 39.3 (t, C-1), 39.3 (s, C-10), 35.8 (t, C-22), 32.0 (t, C-11), 31.9 (q, C-28), 31.3 (t, C-15), 28.1 (t, C-2), 27.0 (q, C-21), 26.8 (t, C-16), 25.8 (q, C-26), 22.9 (t, C-23), 17.6 (q, C-27), 17.5 (q, C-18), 17.4 (q, C-19), 17.0 (q, C-30), 16.5 (q, C-29) (Usami et al. 2008).

Ginsenoside C-K (**5**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.23 (1H, t, *J* = 7.3 Hz, H-24), 5.22 (1H, d, *J* = 7.3 Hz, H-1'), 3.43 (1H, d, *J* = 10.9 Hz, H-3), 1.64 (3H, s, Me-21), 1.59 (3H, s, Me-26 and Me-27), 1.24 (3H, s, Me-28), 1.05 (3H, s, Me-29), 0.98 (3H, s, Me-30), 0.95 (3H, s, Me-18), 0.89 (3H, s, Me-19); ¹³C NMR (150 MHz, C₅D₅N) δ 130.8 (s, C-25), 125.9 (d, C-24), 98.2 (d, C-1'), 83.2 (s, C-20), 79.3 (d, C-3'), 78.3 (d, C-5'), 77.9 (d, C-3), 75.1 (d, C-2'), 71.5 (d, C-4'), 70.1 (d, C-12), 62.8 (t, C-6'), 56.3 (d, C-5), 51.5 (d, C-17), 51.3 (s, C-14), 50.2 (d, C-9), 49.4 (d, C-13), 40.0 (s, C-8), 39.5 (s, C-4), 39.3 (t, C-1), 37.3 (s, C-10), 36.1 (t, C-22), 35.1 (t,

C-7), 30.9 (t, C-15), 30.7 (t, C-11), 28.6 (q, C-28), 28.2 (t, C-2), 26.6 (t, C-16), 25.7 (q, C-26), 23.1 (t, C-23), 22.3 (q, C-21), 18.7 (t, C-6), 17.7 (q, C-27), 17.3 (q, C-30), 16.3 (q, C-29 and C-18), 15.9 (q, C-19) (Zhou et al. 2009).

Ginsenoside 20(*R*)-Rg₃ (**6**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.42 (1H, d, J = 7.7 Hz, H-1"), 5.33 (1H, t, J = 7.5 Hz, H-24), 4.97 (1H, d, J = 7.6 Hz, H-1'), 3.30 (1H, dd, J = 11.8, 4.5 Hz, H-3), 1.71 (3H, s, Me-26), 1.67 (3H, s, Me-27), 1.41 (3H, s, Me-21), 1.32 (3H, s, Me-28), 1.13 (3H, s, Me-29), 1.02 (3H, s, Me-18), 1.00 (3H, s, Me-30), 0.83 (3H, s, Me-19); ¹³C NMR (150 MHz, C₅D₅N) δ 130.8 (s, C-25), 125.9 (d, C-24), 106.1 (d, C-1"), 105.1 (d, C-1'), 88.8 (d, C-3), 83.4 (d, C-2'), 78.3 (d, C-5" and C-3"), 78.2 (d, C-5'), 77.9 (d, C-3'), 77.2 (d, C-2"), 72.9 (s, C-20), 71.5 (d, C-4' and C-4"), 70.8 (d, C-12), 62.8 (t, C-6'), 62.6 (t, C-6"), 56.3 (d, C-5), 51.7 (s, C-14), 50.5 (d, C-17), 50.3 (d, C-9), 49.1 (d, C-13), 43.2 (t, C-22), 39.9 (s, C-8), 39.7 (s, C-4), 39.0 (t, C-1), 36.8 (s, C-10), 35.1 (t, C-7), 32.1 (t, C-11), 31.3 (t, C-15), 28.2 (q, C-28), 26.7 (t, C-16), 26.6 (t, C-2), 25.8 (q, C-26), 22.8 (q, C-21), 22.6 (t, C-23), 18.4 (t, C-6), 17.7 (q, C-27), 17.2 (q, C-30), 16.6 (q, C-29), 16.3 (q, C-19), 15.7 (q, C-18) (Teng et al. 2004).

Ginsenoside 20(*S*)-Rg₃ (**7**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.41 (1H, d, J = 7.6 Hz, H-1"), 5.32 (1H, t, J = 7.4 Hz, H-24), 4.95 (1H, d, J = 7.9 Hz, H-1'), 3.95 (1H, m, H-12), 3.29 (1H, dd, J = 11.8, 4.5 Hz, H-3), 1.65 (3H, s, Me-26), 1.62 (3H, s, Me-27), 1.43 (3H, s, Me-21), 1.30 (3H, s, Me-28), 1.11 (3H, s, Me-29), 0.96 (3H, s, Me-18), 0.95 (3H, s, Me-30), 0.79 (3H, s, Me-19); ¹³C NMR (150 MHz, C₅D₅N) δ 130.8 (s, C-25), 126.0 (d, C-24), 106.1 (d, C-1"), 105.1 (d, C-1'), 88.8 (d, C-3), 83.4 (d, C-2'), 78.3 (d, C-3" and C-5"), 78.2 (d, C-5'), 77.9 (d, C-3'), 77.2 (d, C-2"), 72.9 (s, C-20), 71.5 (d, C-4' and C-4"), 70.8 (d, C-12), 62.8 (t, C-6'), 62.6 (t, C-6"), 56.3 (d, C-5), 54.8 (d, C-17), 51.7 (s, C-14), 50.3 (d, C-9), 48.5 (d, C-13), 39.9 (s, C-8), 39.7 (s, C-4), 39.0 (t, C-1), 36.8 (s, C-10), 35.8 (t, C-22), 35.1 (t, C-7), 32.1 (t, C-11), 31.3 (t, C-15), 28.1 (q, C-21), 26.7 (t, C-16), 26.6 (t, C-2), 25.8 (q, C-26), 22.9 (t, C-23), 18.4 (t, C-6), 17.7 (q, C-30), 17.2 (q, C-27), 16.6 (q, C-29), 16.3 (q, C-19), 15.7 (q, C-18) (Teng et al. 2004).

Ginsenoside 20(*S*)-Rg₂ (**8**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 6.54 (1H, s, H-1"), 5.34 (1H, t, J = 7.0 Hz, H-24), 5.29 (1H, d, J = 7.3 Hz, H-1'), 4.72 (1H, m, H-6), 3.49 (1H, dd, J = 11.9, 4.8 Hz, H-3), 2.15 (3H, s, Me-28), 1.82 (3H, d, J = 7.0 Hz, Me-rha), 1.68 (3H, s, Me-26), 1.63 (3H, s, Me-27), 1.39 (3H, s, Me-21), 1.36 (3H, s, Me-29), 1.19 (3H, s, Me-18), 0.98 (3H, s, Me-19), 0.95 (3H, s, Me-30); ¹³C NMR (150 MHz, C₅D₅N) δ 130.7 (s, C-25), 126.2 (d, C-24), 101.9 (d, C-1'), 101.7 (d, C-1"), 79.4 (d, C-3'), 78.4 (d, C-5'), 78.3 (d, C-3), 78.2 (d, C-2'), 74.1 (d, C-6), 74.1 (d, C-4"), 72.9 (s, C-20), 72.8 (d, C-4'), 72.4 (d, C-2"), 72.2 (d, C-3"), 70.9 (d, C-12), 69.4 (d, C-5"), 62.9 (t, C-6'), 60.7 (d, C-5), 54.6 (d, C-17), 51.6 (s, C-14), 49.6 (d, C-9), 48.0 (d, C-13), 46.0 (t, C-7), 41.0 (s, C-8), 40.1 (s, C-4), 39.9 (t, C-1), 39.5 (s, C-10), 35.7 (t, C-22), 31.6 (t, C-11), 31.1 (t, C-15), 27.7 (q, C-28), 27.6 (t, C-2), 26.9 (t, C-16), 25.8 (q, C-26), 22.9 (t, C-23), 22.7 (q, C-21), 18.7 (q, C-6"), 17.6 (q, C-27), 17.6 (q, C-30), 17.5 (q, C-18), 17.0 (q, C-19), 16.8 (q, C-29) (Wang et al. 2007).

Ginsenoside 20(*R*)-R₂ (**9**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.82 (1H, d, J = 7.1 Hz, H-1"), 4.98 (1H, d, J = 7.3 Hz, H-1'), 2.11 (3H, s, Me-28), 1.69 (3H, s, Me-26), 1.64 (3H, s, Me-27), 1.49 (3H, s, Me-29), 1.41 (3H, s, Me-21), 1.22 (3H, s, Me-18), 0.99 (3H, s, Me-19), 0.85 (3H, s, Me-30); ¹³C NMR (150 MHz,

C₅D₅N) δ 130.7 (s, C-25), 125.9 (d, C-24), 104.8 (d, C-1"), 103.5 (d, C-1'), 80.0 (d, C-2'), 79.9 (d, C-5'), 79.4 (d, C-6), 78.7 (d, C-3"), 78.7 (d, C-3), 78.1 (d, C-3'), 75.8 (d, C-2"), 72.9 (s, C-20), 71.6 (d, C-4'), 71.2 (d, C-4"), 70.8 (d, C-12), 67.2 (t, C-5"), 62.8 (t, C-6'), 61.2 (d, C-5), 51.7 (s, C-14), 50.4 (d, C-17), 50.0 (d, C-9), 48.8 (d, C-13), 44.9 (t, C-7), 43.1 (t, C-22), 41.0 (s, C-8), 40.2 (s, C-4), 39.9 (s, C-10), 39.5 (t, C-1), 32.1 (t, C-11), 31.6 (q, C-28), 31.2 (t, C-15), 27.7 (t, C-2), 26.5 (t, C-16), 25.8 (q, C-26), 22.8 (q, C-21), 22.5 (t, C-23), 17.6 (q, C-19), 17.6 (q, C-27), 17.3 (q, C-18), 17.0 (q, C-30), 16.7 (q, C-29) (Chen et al. 2007).

Ginsenoside Rk₁ (**10**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.29 (1H, d, J = 7.3 Hz, H-1"), 5.23 (1H, br.t, J = 6.7 Hz, H-24), 5.16 (1H, br. s, H-21), 4.95 (1H, t, J = 7.4 Hz, H-1), 4.92 (1H, br. s, H-21), 3.95 (1H, m, H-12), 3.29 (1H, dd, J = 11.6, 4.9 Hz, H-3), 1.65 (3H, s, Me-18), 1.60 (3H, s, Me-26), 1.30 (3H, s, Me-27), 1.12 (3H, s, Me-28), 1.02 (3H, s, Me-29), 0.97 (3H, s, Me-30), 0.81 (3H, s, Me-19); ¹³C NMR (150 MHz, C₅D₅N) δ 155.4 (s, C-20), 131.1 (s, C-25), 125.3 (d, C-24), 108.0 (t, C-21), 106.1 (d, C-1"), 105.1 (d, C-1'), 88.8 (d, C-3), 83.4 (d, C-2'), 78.3 (d, C-3"), 78.1 (d, C-3'), 78.1 (d, C-5"), 77.9 (d, C-5'), 77.2 (d, C-2"), 72.3 (d, C-12), 71.5 (d, C-4"), 71.5 (d, C-4'), 62.7 (t, C-6"), 62.5 (t, C-6'), 56.3 (d, C-5), 52.4 (d, C-13), 51.1 (s, C-14), 50.8 (d, C-17), 48.2 (d, C-9), 40.1 (s, C-8), 39.6 (s, C-4), 39.2 (t, C-1), 36.9 (s, C-10), 35.2 (t, C-7), 33.7 (t, C-22), 32.7 (t, C-15), 32.5 (t, C-11), 30.7 (t, C-16), 28.0 (q, C-28), 26.9 (t, C-23), 26.7 (t, C-2), 25.7 (q, C-26), 18.4 (t, C-6), 17.7 (q, C-27), 16.9 (q, C-30), 16.5 (q, C-29), 16.4 (q, C-18), 15.7 (q, C-19) (Park et al. 2002).

Ginsenoside Rg₅ (**11**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.51 (1H, t, J = 7.3 Hz, H-22), 5.41 (1H, d, J = 7.8 Hz, H-1"), 5.23 (1H, t, J = 6.4 Hz, H-24), 4.95 (1H, d, J = 7.4 Hz, H-1'), 3.96 (1H, m, H-12), 3.30 (1H, dd, J = 11.6, 4.0 Hz, H-3), 1.83 (3H, s, Me-21), 1.62 (3H, s, Me-26), 1.58 (3H, s, Me-27), 1.30 (3H, s, Me-28), 1.12 (3H, s, Me-18), 1.02 (3H, s, Me-19), 0.97 (3H, s, Me-30), 0.81 (3H, s, Me-29); ¹³C NMR (150 MHz, C₅D₅N) δ 140.1 (s, C-20), 131.2 (s, C-25), 123.5 (d, C-24), 123.2 (d, C-22), 106.1 (d, C-1"), 105.1 (d, C-1'), 88.8 (d, C-3), 83.4 (d, C-2'), 78.3 (d, C-3"), 78.3 (d, C-3'), 78.1 (d, C-5"), 77.9 (d, C-5'), 77.2 (d, C-2"), 72.5 (d, C-12), 71.5 (d, C-4"), 71.5 (d, C-4'), 62.8 (t, C-6"), 62.5 (t, C-6'), 56.3 (d, C-5), 50.9 (s, C-14), 50.8 (d, C-17), 50.7 (d, C-9), 50.4 (d, C-13), 40.2 (s, C-4), 39.6 (s, C-8), 39.2 (t, C-1), 36.9 (s, C-10), 35.3 (t, C-7), 32.6 (t, C-15), 32.2 (t, C-11), 28.8 (q, C-28), 28.0 (t, C-2), 27.4 (t, C-23), 26.7 (t, C-16), 25.7 (q, C-26), 18.4 (t, C-6), 17.7 (q, C-27), 16.9 (q, C-30), 16.5 (q, C-19), 16.4 (q, C-18), 15.7 (q, C-29), 13.1 (q, C-21) (Kim et al. 1996).

Notoginsenoside 20(S)-R₂ (**12**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.82 (1H, d, J = 7.0 Hz, H-1"), 4.96 (1H, d, J = 7.5 Hz, H-1'), 3.51 (1H, dd, J = 11.5, 4.8 Hz, H-3), 2.11 (3H, s, Me-28), 1.66 (3H, s, Me-26), 1.63 (3H, s, Me-27), 1.47 (3H, s, Me-29), 1.40 (3H, s, Me-21), 1.16 (3H, s, Me-18), 0.96 (3H, s, Me-19), 0.79 (3H, s, Me-30); ¹³C NMR (150 MHz, C₅D₅N) δ 130.7 (s, C-25), 126.2 (d, C-24), 104.8 (d, C-1"), 103.5 (d, C-1'), 79.9 (d, C-6), 79.9 (d, C-2'), 79.4 (d, C-5'), 78.7 (d, C-3"), 78.7 (d, C-3), 78.0 (d, C-3'), 75.8 (d, C-2"), 72.9 (s, C-20), 71.6 (d, C-4'), 71.2 (d, C-4"), 70.9 (d, C-12), 67.2 (t, C-5"), 62.8 (t, C-6'), 61.2 (d, C-5), 54.7 (d, C-17), 51.6 (s, C-14), 50.0 (d, C-9), 48.1 (d, C-13), 44.9 (t, C-7), 41.0 (s, C-8), 40.1 (s, C-4), 39.5 (s, C-10), 39.3 (t, C-1), 35.7 (t, C-22), 32.0 (t, C-11), 31.7 (q, C-28), 31.1 (t, C-15), 27.7 (t, C-16), 26.9 (t, C-2), 26.7 (q, C-26), 25.8 (q, C-21), 22.9 (t, C-23), 17.6 (q, C-19), 17.6 (q, C-18), 17.3 (q, C-27), 16.7 (q, C-30), 16.7 (q, C-29) (Teng et al. 2002).

Ginsenoside 20(*R*)-Rg₂ (**13**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 6.49 (1H, s, H-1"), 5.31 (1H, d, J = 6.8 Hz, H-1'), 4.73 (1H, m, H-6), 3.95 (1H, m, H-12), 3.51 (1H, m, H-3), 2.17 (3H, s, Me-28), 1.83 (3H, d, J = 6.3 Hz, Me-rha), 1.39 (3H, s, Me-29), 1.70 (3H, s, Me-26), 1.65 (3H, s, Me-27), 1.38 (3H, s, Me-21), 1.26 (3H, s, Me-19), 0.99 (3H, s, Me-30); ¹³C NMR (150 MHz, C₅D₅N) δ 130.7 (s, C-25), 126.0 (d, C-24), 102.0 (d, C-1'), 101.7 (d, C-1''), 79.4 (d, C-2'), 78.5 (d, C-3), 78.4 (d, C-3'), 78.2 (d, C-5'), 74.2 (d, C-4''), 74.1 (d, C-6), 72.9 (s, C-20), 72.5 (d, C-2''), 72.4 (d, C-3''), 72.2 (d, C-4'), 70.8 (d, C-12), 69.4 (d, C-5''), 63.0 (t, C-6'), 60.7 (d, C-5), 51.7 (s, C-14), 50.4 (d, C-9), 49.6 (d, C-17), 48.8 (d, C-13), 46.0 (t, C-7), 43.2 (t, C-22), 41.1 (s, C-10), 40.0 (s, C-4), 39.5 (t, C-1), 39.2 (s, C-8), 32.1 (t, C-11), 31.3 (q, C-28), 30.0 (t, C-15), 27.7 (t, C-2), 26.5 (t, C-16), 25.8 (q, C-26), 22.7 (q, C-21), 22.5 (t, C-23), 18.7 (q, C-6''), 17.6 (q, C-18), 17.6 (q, C-19), 17.6 (q, C-27), 17.1 (q, C-29), 17.1 (q, C-30) (Yang et al. 2000).

3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*S*-*O*-β-D-glucopyranosyl-3β-12β,20(*S*)-trihydroxy-24ε-hydroperoxydammar-25-ene (**14**) white powder; ¹H NMR (600 MHz, C₅D₅N) δ 5.40 (1H, d, J = 7.0 Hz, H-1"), 5.22 (1H, d, J = 7.0 Hz, H-1'''), 5.20 (1H, br s, H-26), 5.04 (1H, br s, H-26), 4.95 (1H, d, J = 7.3 Hz, H-1'), 4.72 (1H, dd, J = 8.1, 4.3 Hz, H-24), 3.27 (1H, dd, J = 4.6 Hz, 11.3 Hz, H-3), 1.89 (3H, s, Me-27), 1.63 (3H, s, Me-21), 1.28 (3H, s, Me-28), 1.11 (3H, s, Me-29), 0.94 (3H, s, Me-18), 0.93 (3H, s, Me-30), 0.80 (3H, s, Me-19); ¹³C NMR (150 MHz, C₅D₅N) δ 146.0 (s, C-25), 113.3 (t, C-26), 106.1 (d, C-1''), 105.1 (d, C-1'), 98.2 (d, C-1'''), 89.9 (d, C-24), 88.8 (d, C-3), 83.4 (s, C-20), 83.2 (d, C-2'), 79.2 (d, C-3'''), 78.3 (d, C-3''), 78.3 (d, C-3'), 78.1 (d, C-5'), 77.9 (d, C-5'''), 77.9 (d, C-5''), 77.2 (d, C-2''), 75.0 (d, C-2'''), 71.6 (d, C-4''), 71.5 (d, C-4'), 71.5 (d, C-4'''), 70.1 (d, C-12), 64.8 (t, C-6'''), 62.8 (t, C-6'), 62.5 (t, C-6''), 56.2 (d, C-5), 51.4 (d, C-17), 51.3 (s, C-14), 50.1 (d, C-9), 49.4 (d, C-13), 39.9 (s, C-8), 39.9 (s, C-4), 39.6 (t, C-1), 36.8 (s, C-10), 35.8 (t, C-7), 32.8 (t, C-22), 30.9 (t, C-11), 30.6 (t, C-15), 28.0 (q, C-28), 26.7 (t, C-2), 26.6 (t, C-16), 25.3 (t, C-23), 22.5 (q, C-21), 18.3 (t, C-6), 17.6 (q, C-27), 17.2 (q, C-30), 16.5 (q, C-29), 16.2 (q, C-18), 15.9 (q, C-19) (Masayuki et al. 1997).

Conversion rate of four products of PNS

The conversion rate of substrates and the yield of products was defined as follows:

$$\text{Conversion Rate (\%)} = \frac{m - m_1}{m} \times 100\%$$

$$\text{Productivity (\%)} = \frac{m_2}{m} \times 100\%$$

m: the mass of substrate; m₁: the mass of remaining substrate; m₂: the mass of products

The major ginsenosides of PNF was ginsenoside R₁, ginsenosides Rg₁, Re, and Rb₁, the conversion rate of them were 32.52, 19.35, 24.74, and 100%, respectively. The conversion products of PNF were shown in Table S1.

Propose possible biosynthetic pathways of major ginsenosides Rg₁, Re, Rb₁ and notoginsenoside R₁ of PNF

The transformation pathway of ginsenoside Rb₁ is proposed in Fig. S1A. The ginsenoside Rb₁ contains four glucopyranosyl moieties at the C-3 and C-20 position of aglycone. According to the isolated ginsenosides 20 (*R/S*)-Rg₃, Rg₅ and Rk₁, C-K, 20 (*S*)-I, the conversion pathway of Rb₁ can be predicted. The first pathway is the *A. fumigatus* attacked the outer β(1→6)-glucosidic bond linkages to C-20 position of aglycone to produce Rd from Rb₁, and was then followed by the hydrolysis of the outer β(1→6)-glucosidic bond to C-3 position to produce F₂, later followed by the hydrolysis of the inner β(1→6)-glucosidic bond to C-3 position to produce CK or by heating and oxidizing the air to get 20 (*S*)-I. Another pathway was followed by the hydrolysis of the inner β(1→6)-glucosidic bond to the C-20 position to produce 20 (*R/S*)-Rg₃ from Rd, then through dehydration reaction at the C-20 position to form a double bond with C-21 to get Rk₁, or to form a double bond with C-22 to get Rg₅.

The transformation pathway of ginsenoside Rg₁ is proposed in Fig. S1B. Using the same method as above, we proposed the pathway of ginsenoside Rg₁ as follows: Rg₁→20 (*R*)-Rh₁→Rh₄; Rg₁→20 (*R*)-Rh₁→20 (*S*)-protopanaxatriol; Rg₁→20 (*R*)-Rh₁→Rk₃, respectively (Fig. S1B). We also proposed the pathway of ginsenoside Re as follow: Re→20 (*R/S*)-Rg₂→20 (*R*)-Rh₁→20 (*S*)-protopanaxatriol (Fig. S1C). Lastly, we proposed the pathway of notoginsenoside R₁ as follow: R₁→20 (*R/S*)-R₂→20 (*R*)-Rh₁→20 (*S*)-protopanaxatriol (Fig. S1D).

Antibacterial activity

The antibacterial activity of compounds **1–14** against 2 pathogenic bacteria were tested. As shown in Table S2, Compounds **5** and **7** showed moderate antibacterial activities against *Staphylococcus aureus* and *Candida albicans* with MIC values of 6.25/25.00, and 1.25/25.00 µg/mL, respectively. Additionally, compounds **10** and **11** also had certain antibacterial activity against *Staphylococcus aureus* and *Candida albicans*.

Conclusion

Rare ginsenosides is a group of dammarane triterpenoids that exist in low natural abundance, which can be produced by physicochemical processing or metabolic transformation of major ginsenosides. Due to their small polarity and molecular weight, they exhibited potent biological activity comparing to the primary ginsenosides. The fungus *A. fumigatus* has the ability of transform PNF to rare ginsenosides. We isolated 14 rare ginsenosides from the transformation products. The structure analysis of 14 rare ginsenosides showed that they were the metabolites of ginsenosides Rb₁, Rg₁, Rd, Re and notoginsenoside R₁ (they are the major ginsenosides of PNF), respectively. Based on the structure of the transformation products, we speculate on the possible biological transformation pathways of saponins (Fig. S1). The conversion rates of four ginsenosides were calculated by HPLC analysis, and it was found that the conversion rates of ginsenosides Rb₁, R₁, Rg₁, Re were 100, 32.52, 19.35, 24.74%, respectively. The yield of ginsenoside 20 (*R*)-Rg₃, which has good anti-tumor effect, can reach 22.29%. The yield of other ginsenosides 20 (*S*)-Rg₃, Rh₄, 20 (*S*)-I, Rg₅, Rk₃, Rk₁ and C-K could reach 17.90, 6.42, 5.60, 6.30, 3.61,

3.25 and 0.19%, respectively. We found that the products of PNS converted by *A.fumigatus* were rich and varied, and the conversion rate of ginsenoside Rb₁ could reach 100%, which completely transformed components in PNS.

Rare saponins have significant pharmacological activity, but their low natural content has formed a serious contradiction between supply and demand. The content of rare saponins in *Panax notoginseng* is far from meeting its medicinal needs, so how to quickly obtain a large number of rare saponins with high pharmacological activity has become a research hotspot. Through the transformation of PNS by *A.fumigatus*, this study can provide a method for obtaining the rare saponins, lay a foundation for the efficient utilization *P. notoginseng*, improve the pharmacological activity and economic value of saponins in *P. notoginseng*, and provide a basis and theoretical support for large-scale industrial preparation of rare saponins.

Abbreviations

PNS: *Panax notoginseng* saponins, mainly including notoginsenoside R₁, ginsenosides Rg₁, Re, Rb₁, Rd

TLC: Thin Layer chromatography

HPLC: High Performance Liquid Chromatography

PDA: Potato Dextrose Agar

PDB: Potato Dextrose Broth

MIC: minimum inhibitory concentration

Declarations

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Author contributions

LY designed and conducted experiments, performed the data analysis and drafted the manuscript. DL and FL coordinated the study. XC reviewed the manuscript. DL and XY conceived the idea for this study. All authors read and approved the final manuscript.

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Availability of data and materials

The data and the materials are all available in this article and its supporting information document, which will be given access on the journal's website.

Ethics approval and consent for participate

Not applicable.

Consent for publication

All authors read and approved the final manuscript and potential publication. **Competing interests**

The authors declare that they have no competing interests.

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Figures

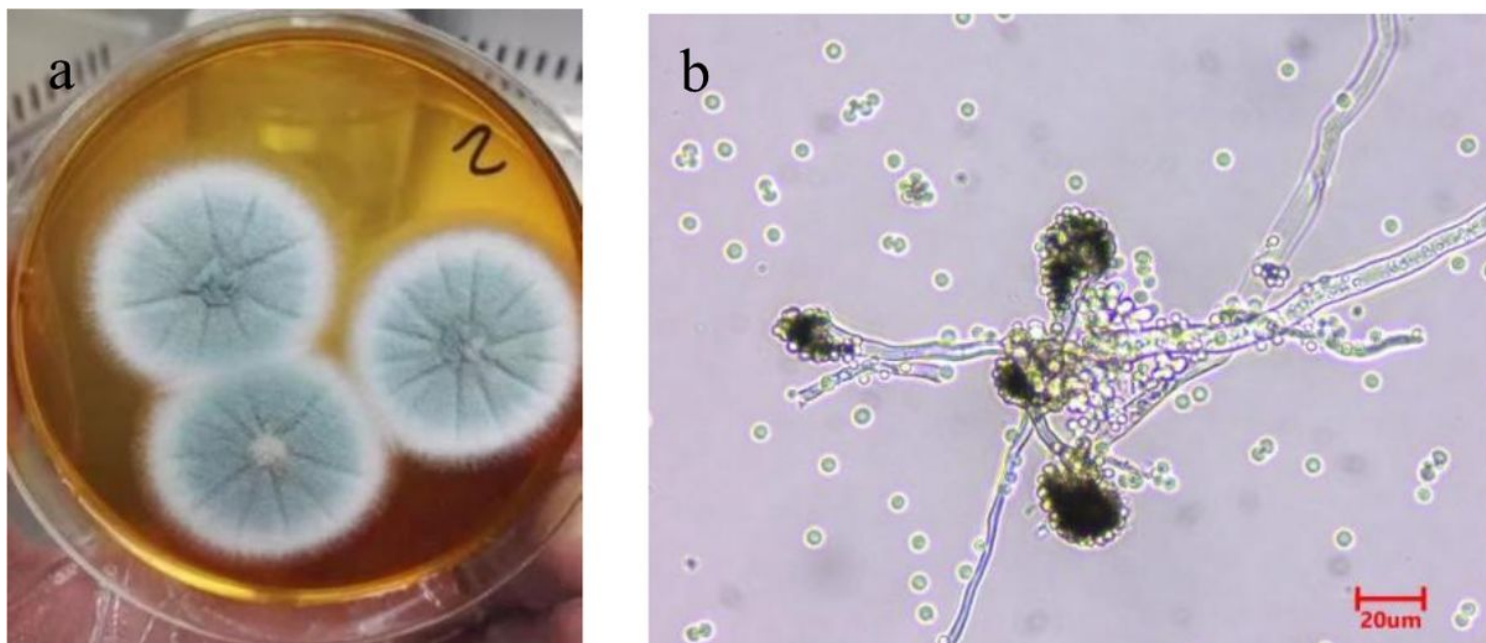


Figure 1

Morphology of *A. fumigatus*. a, Colony morphology diagram; b, Spore map of *A. fumigatus*.

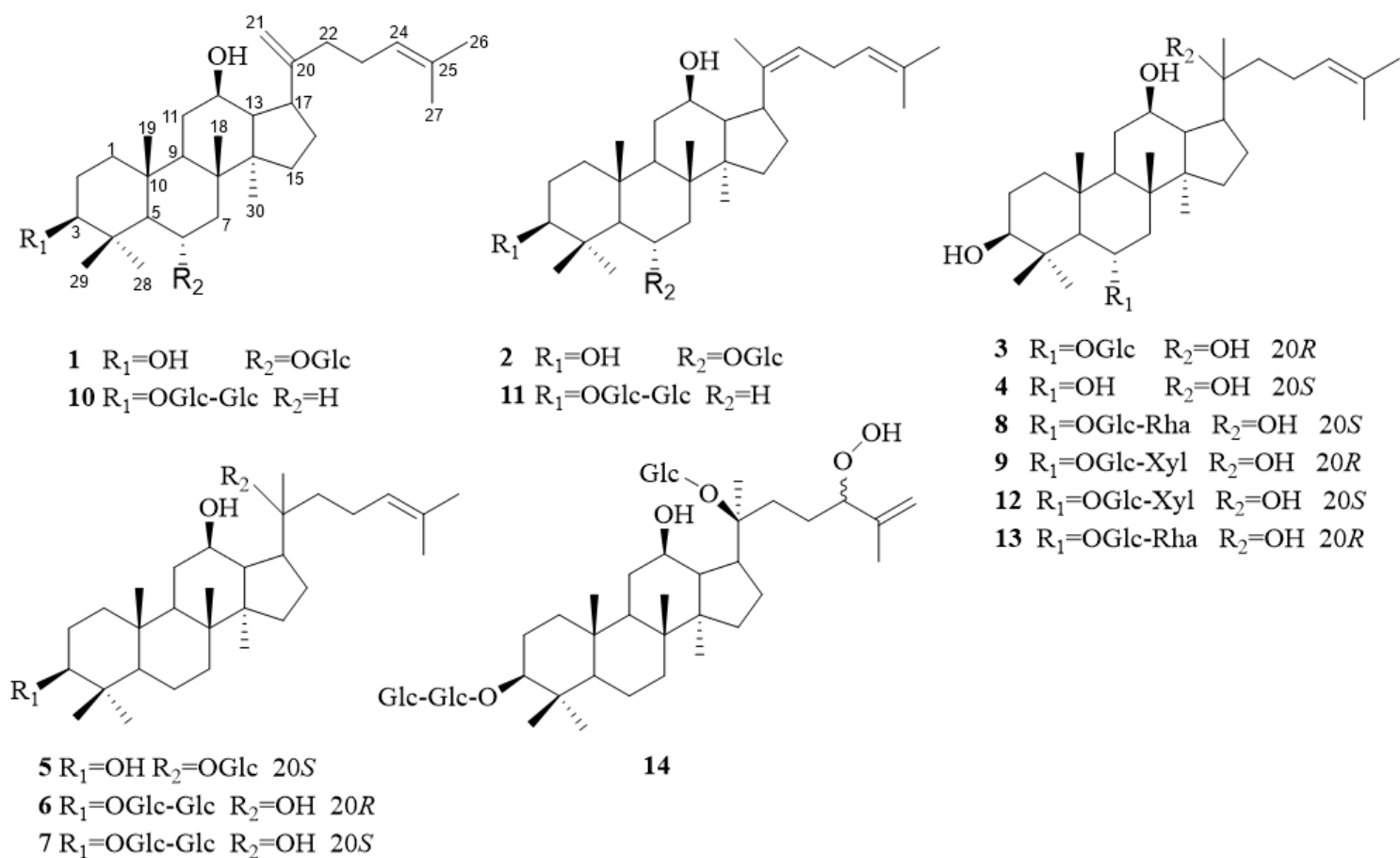


Figure 2

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