Microbial Transformation of 20(*S*)-Protopanaxatriol-Type Saponins by *Absidia coerulea*

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Three 20(*S*)-protopanaxatriol-type saponins, ginsenoside-Rg_1 (**1**), notoginsenoside-R_1 (**2**), and ginsenoside-Re (**3**), were transformed by the fungus *Absidia coerulea* (AS 3.3389). Compound **1** was converted into five metabolites, ginsenoside-Rh_4 (**4**), 3β,2β,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-β-D-glucopyranoside (**5**), 20(*S*)-ginsenoside-Rh_1 (**6**), 20(*R*)-ginsenoside-Rh_1 (**7**), and a mixture of 25-hydroxy-20(*S*)-ginsenoside-Rh_1 and its C-20(*R*) epimer (**8**). Compound **2** was converted into 10 metabolites, 20(*S*)-notoginsenoside-R_2 (**9**), 20(*R*)-notoginsenoside-R_2 (**10**), 3β,12β,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**11**), 3β,12β-dihydroxydammar-(*E*)-20(22),24-diene-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**12**), 3β,12β,20,25-tetrahydroxydammaran-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**13**), and compounds **4**−**8**. Compound **3** was metabolized to 20(*S*)-ginsenoside-Rg_2 (**14**), 20(*R*)-ginsenoside-Rg_2 (**15**), 3β,12β,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**16**), 3β,12β-dihydroxydammar-(*E*)-20(22),24-diene-6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**17**), 3β,12β,20,25-tetrahydroxydammaran-6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**18**), and compounds **4**−**8**. The structures of five new metabolites, **10**−**13** and **16**, were established by spectroscopic methods.

Panax notoginseng (Burk.) F. H. Chen is one of the most prominent herbal medicines used in traditional Chinese medicine (TCM) for the treatment of cardiovascular disease, inflammation, pain, trauma, and internal and external bleeding due to injury.[1−3] Phytochemical and pharmaceutical studies have shown that dammarane-type saponins are the major bioactive principles, which are the derivatives of protopanaxadiol and protopanaxatriol.[4] Microbial transformation is an important tool in the structural modification and metabolism study of organic compounds, especially for natural products, due to its significant regio- and stereoselectivities.[5,6] In the course of our research intended to expand the chemical diversity of ginsenoside and notoginsenoside derivatives for further pharmaceutical research, and to attain a better understanding of microbial models of ginsenoside and notoginsenoside metabolism, we have studied the microbial transformation of three dammarane-type saponins. Ginsenosides-Rg1 (**1**) and -Re (**2**) and notoginsenoside-R_1 (**3**) are the three major 20(*S*)-protopanaxatriol-type saponins in *P. notoginseng* and are the subject of the present work.

Preliminary screening of 42 fungal strains for the microbial biotransformation of **1**−**3** was carried out in 250 mL Erlenmeyer flasks containing 100 mL of liquid medium (Table S1, Supporting Information). After 48 h of preculture, the substrates were added into each flask. The incubation was continued for an additional 4 days, after which metabolites in the *n*-BuOH extract of the broth were detected by HPLC. *Absidia coerulea* (AS 3.3389) showed an ability to convert these substrates with high yield. Spectroscopic data interpretation was used to elucidate the structures of five new metabolites, **10**−**13** and **16**. Ten known metabolites (**4**−**9**, **14**, **15**, **17**, and **18**) were identified by comparison of their spectroscopic data with those published in the literature.[7−9]

The molecular formula of **10** was determined to be $C_{41}H_{70}O_{13}$ by HRESIMS ($[M + Na]^+$, *m*/*z* 793.4658). Comparison of the ^1H and ^{13}C NMR data of **10** (Table 1) with those of substrate **2** suggested that signals belonging to a glucose moiety at C-20 were no longer present. The ^1H NMR spectrum showed an olefinic proton signal at δ_H 5.33 (t, J = 7.2 Hz), together with the two olefinic carbon signals at δ_C 130.8 and 126.1 in its ^{13}C NMR spectrum, indicating the presence of a double bond at C-24(25). The ^1H and ^{13}C NMR spectra of **10** were similar to those of 20(*S*)-notoginsenoside (**9**).[9] The key differences were that the upfield shift of C-17 and C-21 from δ_C 54.7 and 25.8 in **9** to δ_C 50.6 and 22.8 in **10** and the downfield shift of C-22 from δ_C 35.8 in **9** to δ_C 43.3 in **10**. These chemical shifts were also found among 20(*R*)- and 20(*S*)-protopanaxatriol isomers.[9] Thus, the structure of metabolite **10** was elucidated as 20(*R*)-notoginsenoside-R_2. The assignments made from the ^1H and ^{13}C NMR data were confirmed by the HSQC and HMBC spectra.

The molecular formula of **11** was determined to be $C_{41}H_{70}O_{13}$ by HRESIMS ($[M + Na]^+$, *m*/*z* 793.4764), which was consistent with the disappearance of a glucose moiety signal at C-20 in the ^1H and ^{13}C NMR spectra compared with those of substrate **2**. The ^1H NMR spectrum showed an olefinic proton signal at δ_H 5.54 (t, J = 6.8 Hz), as well as two olefinic carbon signals at δ_C 139.2 and 125.2 in its ^{13}C NMR spectrum, indicating that a double bond was not present at C-24(25). The ^1H and ^{13}C NMR signals of Me-21 (δ_H 1.79, s; δ_C 12.7), Me-26 (δ_H 1.33, s; δ_C 29.4), and Me-27 (δ_H 1.37, s; δ_C 29.6) were determined, suggesting that a double bond occurred at C-20(22) and a hydroxyl group was located at C-25. This was confirmed by a HMBC experiment, showing correlations of H-21 with C-20 (δ_C 139.2), C-22 (δ_C 125.2), and C-17 (δ_C 50.1), of H-26 with C-25 (δ_C 69.1), C-24 (δ_C 43.9), and C-27 (δ_C 29.6), and of H-27 with C-25 (δ_C 69.1), C-24 (δ_C 43.9), and C-26 (δ_C 29.4). The stereochemistry of the double bond at C-20(22) was determined to be *E* since the signal of C-21 was observed at δ_C 12.7 in the ^{13}C NMR spectrum, while in the case of a *Z*-type structure, it would be expected at δ_C 25−30.[10] The ^1H and ^{13}C NMR signals for the ring system and the glycosyl units of **11** were very similar to those of **9**. On the basis of these observations, compound **11** was assigned as 3β,12β,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside.

The molecular formula of compound **12** was determined as $C_{41}H_{68}O_{12}$ by HRESIMS ($[M + Na]^+$, *m*/*z* 775.4606). Comparison of its ^1H and ^{13}C NMR data with those of **2** suggested that the glucose moiety at C-20 was missing and a new double bond was

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Table 1. ^{13}C NMR Spectroscopic Data (100 MHz, C_5D_5N) of Compounds **10**−**12**, **13**, and **16**

position	**10**	**11**	**12**	**13**	**16**	position	**10**	**11**	**12**	**13**	**16**
1	39.5	39.3	39.5	39.5	39.5	23	22.6	23.3	27.4	19.1 (18.7)[b]	23.7
2	27.8	28.4	28.8	27.8	28.7	24	126.1	43.9	124.9	45.6 (45.8)[b]	44.3
3	78.8[a]	78.4[a]	78.8[a]	78.7[a]	78.3	25	130.8	69.1	130.8	69.7	69.5
4	40.2	39.9	40.2	40.2	40.0	26	25.8	29.4	25.7	29.9	29.7
5	61.4	61.0	61.3	61.3	60.8	27	17.7[a]	29.6	17.6	30.2	30.0
6	79.4	79.1	79.5	79.4	74.4	28	31.7	31.4	31.7	31.7	32.2
7	45.0	44.8	45.1	45.0	46.2	29	16.7	16.3	16.7	16.7	17.6
8	41.2	41.0	41.3	41.1	41.4	30	17.1	16.5	16.8	16.9 (17.1)[b]	16.9
9	50.2	50.3	50.7	50.1	50.1	6-O-Glc					
10	39.7	39.4	39.7	39.7	39.7	1′	103.5	103.2	103.1	103.5	101.8
11	32.3	32.2	32.3	32.1	32.3	2′	79.9	80.0	80.2	80.3	78.5
12	70.9	72.2	72.5	71.0 (70.9)[b]	72.6	3′	78.1	77.6	78.0	78.0	79.4
13	49.0	50.1	50.9	48.3 (48.9)[b]	50.6	4′	71.8	71.4	71.7	71.8	72.4
14	51.8	50.5	51.2	51.7	50.9	5′	79.4	79.5	79.9	79.8	78.4
15	31.4	31.9	32.5	31.3	32.6	6′	63.0	62.6	62.9	63.0	63.1
16	26.7	27.5	27.8	26.9 (26.7)[b]	27.8	Xyl(or Rha)					
17	50.6	50.1	50.5	54.7 (50.8)[b]	50.4	1″	105.0	104.6	104.5	104.9	101.9
18	17.4	17.0	17.3	17.4	17.2	2″	75.9	75.5	75.9	75.8	72.3
19	17.7[a]	17.4	17.7	17.6	17.7	3″	78.8[a]	78.4[a]	78.8[a]	78.7[a]	72.6
20	73.0	139.2	139.7	73.3	139.6	4″	71.0	70.9	71.3	71.2	74.2
21	22.8	12.7	13.1	27.2 (22.8)[b]	13.0	5″	67.3	66.9	67.3	67.3	69.5
22	43.3	125.2	122.6	36.5 (44.0)[b]	125.5	6″					18.8

[a] Values in vertical column may be interchanged. [b] Chemical shifts in parentheses due to the 20(*R*) epimer of **13**.

apparent. The ^1H NMR spectrum showed two olefinic proton signals at δ_H 5.48 (t, $J = 6.8$ Hz) and 5.21 (t, $J = 7.2$ Hz), which was consistent with the four olefinic carbon signals observed at δ_C 139.7, 130.8, 124.9, and 122.6 in the ^{13}C NMR spectrum. The location of the double bond was supported by a HMBC experiment, which exhibited correlations of H-21 (with C-20, C-22, and C-17), H-26 (with C-24, C-25, and C-27), and H-27 (with C-24, C-25, and C-26). The geometrical isomerism of the double bond at C-20(22) was determined to be *E*.[10] The ^1H and ^{13}C NMR signals for the ring system and the glycosyl units of **12** were very similar to those of **9**. Therefore, **12** was concluded to be 3*β*,12*β*-dihydroxydammarane-(*E*)-20(22),24-diene-6-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside.

Metabolite **13** was assigned a molecular formula of $C_{41}H_{72}O_{14}$, as determined by HRESIMS ($[M + Na]^+$, *m*/*z* 811.4877). When compared with **2**, it was found that the glucose moiety at C-20 was missing and a hydroxyl group was introduced into the substrate molecule. In its NMR spectrum, the olefinic signals were absent, which suggested that **13** possesses a C_8-saturated side chain with two hydroxyl groups. The ^1H NMR signals of the C-21 (δ_H 1.43, overlap), C-26 (δ_H 1.43, overlap), and C-27 (δ_H 1.43, overlap) methyl groups were overlapping, indicating that the hydroxyl groups are located at C-20 and C-25. The HMBC correlations of the ^1H NMR signal at δ_H 1.43 with C-17 (δ_C 54.7), C-20 (δ_C 73.3), C-22 (δ_C 36.5), C-24 (δ_C 45.6), and C-25 (δ_C 69.7) confirmed the hydroxylation positions to be at C-20 and C-25. Consequently, the structure of metabolite **13** was determined as 3*β*,12*β*,20,25-tetrahydroxydammarane-6-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside. Metabolite **13** was isolated as a mixture of C-20 stereoisomers, and the additional signals in the ^1H and ^{13}C NMR spectra were completely assigned.

The molecular formula of metabolite **16** was established as $C_{42}H_{72}O_{13}$ by HRESIMS ($[M + Na]^+$, *m*/*z* 807.4812). Comparison of the ^1H and ^{13}C NMR data of **16** with those of substrate **3** indicated that the glucose moiety at C-20 was absent. The ^1H NMR spectrum showed an olefinic proton signal at δ_H 5.52 (t, $J = 6.8$ Hz), together with the two olefinic carbon signals at δ_C 139.6 and 125.5 in the ^{13}C NMR spectrum, indicating that a double bond was no longer at C-24(25). In the ^{13}C NMR spectrum, the C-21 signal was observed at δ 12.7, which suggested that the double bond is at C-20(22), and the stereochemistry was determined to be *E*.[10] The HMBC correlations of H-21 with C-20 (δ_C 139.6), C-22 (δ_C 125.5), and C-17 (δ_C 50.4), of H-26 with C-25 (δ_C 69.5), C-24 (δ_C 44.3), and C-27 (δ_C 30.0), and of H-27 with C-25 (δ_C 69.5), C-24 (δ_C 44.3), and C-26 (δ_C 29.7) confirmed the location of the double bond at C-20(22) and the hydroxylation at C-25. Thus, the structure of metabolite **16** was concluded to be 3*β*,12*β*,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside.

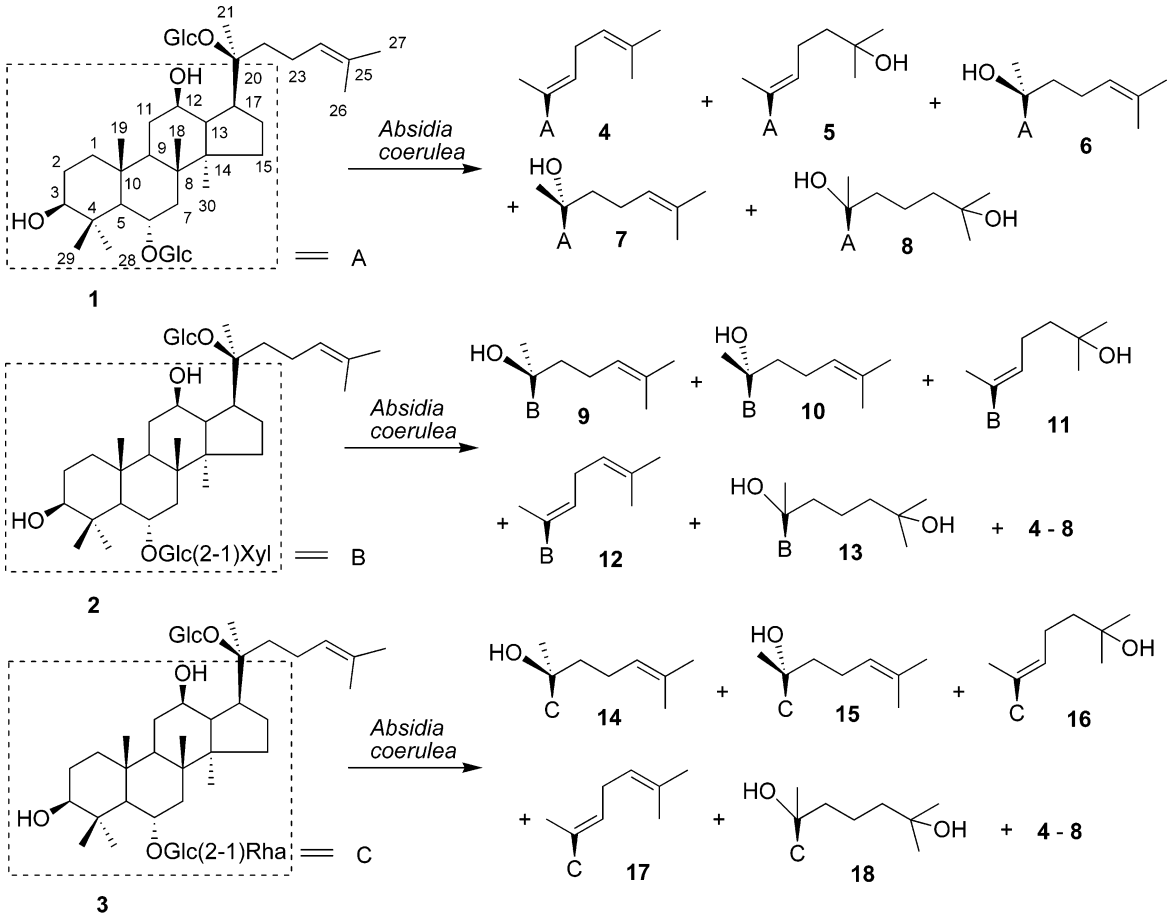
The pH of the broth was determinted to be around 3.4 after growing a culture of *A. coerulea* for 6 days without substrate, which indicated that this organism could decrease the pH of the medium. Therefore, autoclaved culture controls of **2** and **6** were performed in parallel in order to determine whether some of the metabolites obtained were artifacts of acid-catalyzed hydrolysis rather than being produced by enzymatic conversion. The HPLC results obtained showed that no metabolites were present. This suggested that this organism could decrease the pH value of the medium but did not cause any acid-catalyzed transformations.

Thus, the present results show that microbial transformation of three 20(*S*)-protopanaxatriol-type saponins (**1**−**3**) by *A. coerulea* yielded metabolites with C-20 deglycosylation, side-chain oxidation−reduction, and C-20 hydroxyl group isomerization. The deglycosylation of the terminal glycosyl at C-6 was also found in the metabolites of **2** and **3** (**4**−**8**).

The three substrates **1**−**3**, with various glycosyl substituents at C-6, were converted into the same metabolites (**4**−**8**) and the same side-chain-modified metabolites (**9**−**18**) by *A. coerulea*. This suggests that enzymes secreted by *A. coerulea* are capable of transforming the 20(*S*)-protopanaxatriol-type saponins with high yield and regioselectivity but with low selectivity for the glycoside type and linkage at C-6. In our preliminary screening, we also investigated two 20(*S*)-protopanaxadiol-type saponins, ginsenosides-Rb_1 and -Rd, but found that *A. coerulea* was not capable of transforming these two substrates.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were measured on a Perkin-Elmer 577 spectrometer. The ^1H and ^{13}C NMR spectra were carried out on a Bruker AM-400 spectrometer at 400 and 100 MHz in pyridine-d_5, and chemical shifts are expressed in δ (ppm) relative to tetramethylsilane (TMS). ESIMS and HRESIMS were recorded on a Finnigan LCQ^{DECA} instrument. Silica gel (200−300 mesh; Qingdao Haiyang Chemical Co. Ltd., Qingdao, People's Republic of China) was used for open column chromatography. Reversed-phase preparative HPLC was performed on an Agilent 1100 Series HPLC system (Agilent Technologies, Deutschland, Waldbronn, Germany)



Glc: β-D-glucopyranosyl Xyl: β-D-xylopyranosyl Rha: α-L-rhamnopyranosyl

Figure 1. Structures of ginsenoside-Rg_1 (**1**), notoginsenoside-R_1 (**2**), ginsenoside-Re (**3**), and metabolites **4**−**18**.

connected to a UV detector, using a C_{18} column (250 mm × 10.0 mm, 5 μm; Deerfield, IL) eluted with MeOH−H_2O at a flow rate of 1.5 mL/min.

Chemicals and Materials. Ginsenoside-Rg_1 (**1**), notoginsenoside-R_1 (**2**), and ginsenoside-Re (**3**) were purchased from Kunming Zhongzheng Bio-technology Co. Ltd (Kunming, People's Republic of China), and the purity was above 98% by HPLC analysis. All solvents were AR grade (Sinopharm Chemical Reagent Co. Ltd., People's Republic of China).

Microorganisms and Culture Conditions. All microorganisms screened in our experiments were obtained from the China General Microbiology Culture Center, People's Republic of China. Stock cultures of the fungi were stored on potato dextrose agar slants at 4 °C. Seed cultures were obtained by transferring fungi from stock cultures to potato dextrose broth. Screening scale biotransformation was carried out in 250 mL Erlenmeyer flasks containing 100 mL of medium. The flasks were placed on a rotary shaker operating at 160 rpm at 26 °C. After 2 days of incubation, the substrates (0.2 mL, 10 mg/mL, 95% ethanol) were added into each flask, and these flasks were maintained under fermentation conditions for 4 days. Then the cultures were pooled and filtered. The filtrates were extracted three times with equal volumes of *n*-BuOH, and the extractions were evaporated in vacuo and analyzed by HPLC. An autoclaved culture control was carried out in 250 mL Erlenmeyer flasks containing 100 mL of liquid medium. On growing the culture of *A. coerulea* for 6 days without substrate, the pH of the broth was checked. Then, the incubations were autoclaved and the substrates (0.2 mL, 10 mg/mL, 95% ethanol) were added. Incubation was carried out for another 4 days, and the *n*-BuOH extract of the broth was analyzed by HPLC. Culture controls consisted of fermentation blanks in which fungi were grown without substrate but fed with the same amount of 95% ethanol. Substrate controls were composed of sterile medium to which the substrate was added and incubated without fungi.

Biotransformation of 1 and Isolation of Metabolites. The preparative scale biotransformation of **1** was carried out in twenty 1000 mL flasks each containing 400 mL of potato broth medium. The fungus was incubated for 2 days before 25 mg of substrate **1** (1 mL, 95% ethanol) was fed to each flask. Incubation conditions and the extraction process were the same as described above and afforded a crude extract (2.52 g). The extract was subjected to column chromatography on MCI gel (100 g). The column was eluted with H_2O (600 mL), methanol (800 mL), and acetone (500 mL). The fraction that eluted with methanol (827 mg) was chromatographed on a silica gel column (60 g), with $CHCl_3$−MeOH (10:1−7:1) as solvent, which yielded fractions A−D. Fraction B was purified by HPLC (MeOH−H_2O, 85:15) to yield metabolite **4** (14.9 mg). Fraction C was purified using HPLC (MeOH−H_2O, 80:20) to yield metabolites **5** (8.4 mg), **6** (10.6 mg), and **7** (6.3 mg). A compound obtained from fraction D was identified as **8** (11.5 mg), by ^1H and ^{13}C NMR spectroscopic analysis.

Biotransformation of Notoginsenoside-R_1 (2) and Isolation of Metabolites. Biotransformation of **2** by the procedure described above yielded a methanol fraction (868 mg), which was chromatographed on silica gel (65 g). Elution of the column with $CHCl_3$−MeOH (20:1−7:1) afforded fractions A′−G′. Fractions B′ and E′ were subjected to HPLC eluted with MeOH−H_2O (80:20) to yield metabolites **4** (12.7 mg) and **12** (21.1 mg), respectively. HPLC (MeOH−H_2O, 70:30) of fraction F′ afforded **9** (20.8 mg), **10** (15.6 mg), and **11** (7.3 mg). Fraction C′, upon HPLC (MeOH−H_2O, 80:20), gave **5** (4.0 mg), **6** (8.6 mg), and **7** (5.5 mg). Further chromatography on silica gel of fractions D′ and G′ yielded the metabolites **8** (4.0 mg) and **13** (11.0 mg), respectively.

20(*R*)-Notoginsenoside-R_2 (10): fine needles (MeOH); mp 239−240 °C; $[\alpha]_D^{20}$ +17 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3405, 1637, 1047 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 0.87, 1.01, 1.24, 1.40, 1.48, 1.65, 1.70, 2.10 (3H each, all s, H_3-30, 19, 18, 21, 29, 27, 26, 28), 4.97 (1H, d, J = 7.2 Hz, H-1′), 5.80 (1H, d, J = 7.2 Hz, H-1″); ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 1; HRESIMS *m*/*z* 793.4658 $[M + Na]^+$ (calcd for $C_{41}H_{70}O_{13}Na$, 793.4714).

3β,12β,25-Trihydroxydammar-(*E*)-20(22)-ene-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (11): white, amorphous powder;

$[\alpha]_D^{20}$ +12 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3405, 1639, 1049 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 0.79, 0.96, 1.19, 1.33, 1.37, 1.48, 1.79, 2.07 (3H each, all s, H_3-30, 19, 18, 26, 27, 29, 21, 28), 4.93 (1H, d, J = 6.8 Hz, H-1′), 5.75 (1H, d, J = 7.2 Hz, H-1″); ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 1; HRESIMS *m*/*z* 793.4764 $[M + Na]^+$ (calcd for $C_{41}H_{70}O_{13}Na$, 793.4714).

3β,12β-Dihydroxydammarane-(*E*)-20(22),24-diene-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (12): white, amorphous powder; $[\alpha]_D^{20}$ +14 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3415, 1637, 1047 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 0.83, 1.22, 1.23, 1.49, 1.58, 1.65, 1.82, 2.10 (3H each, all s, H_3-30, 18, 19, 29, 27, 26, 21, 28), 4.96 (1H, d, J = 7.2 Hz, H-1′), 5.78 (1H, d, J = 7.2 Hz, H-1″); ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 1; HRESIMS *m*/*z* 775.4606 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{12}Na$, 775.4658).

3β,12β,20,25-Tetrahydroxydammarane-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (13): white, amorphous powder; $[\alpha]_D^{20}$ +14 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3415, 1641, 1047 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 0.82 (3H, s, H_3-30), 0.98 (3H, s, H_3-19), 1.23 (3H, s, H_3-18), 1.43 (9H, overlap, H_3-21, 26, 27), 1.49 (3H, s, H_3-29), 2.11 (3H, s, H_3-28), 4.97 (1H, d, J = 7.2 Hz, H-1′), 5.79 (1H, d, J = 7.2 Hz, H-1″); ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 1; HRESIMS *m*/*z* 811.4877 $[M + Na]^+$ (calcd for $C_{41}H_{72}O_{14}Na$, 811.4820).

Biotransformation of Ginsenoside-Re (3) and Isolation of Metabolites. Biotransformation of **3** by the procedure described above yielded a methanol fraction (892 mg), which was further chromatographed on silica gel (65 g). Elution of the column with $CHCl_3$−MeOH (20:1−7:1) gave fractions A″−E″. Fraction A″ was purified by HPLC (MeCN−H_2O, 60:40) and yielded metabolite **4** (10.8 mg). HPLC (MeOH−H_2O, 80:20) of fraction B″ afforded **5** (1.7 mg), **6** (7.8 mg), and **7** (5.0 mg). Fraction D″ was subjected to HPLC (MeCN−H_2O, 50:50) to yield **17** (21.5 mg). HPLC (MeOH−H_2O, 75:25) of fraction E″ afforded **14** (30.0 mg), **15** (13.8 mg), and **16** (3.8 mg). Fractions C″ and F″ were subjected to further chromatography on silica gel and yielded metabolites **8** (7.0 mg) and **18** (11.3 mg).

3β,12β,25-Trihydroxydammar-(*E*)-20(22)-ene-6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (16): white, amorphous powder; $[\alpha]_D^{20}$ −14 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3417, 1637, 1049 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 0.97, 0.99, 1.26, 1.37, 1.80, 2.01 (3H each, all s, H_3-30, 19, 18, 27, 29, 21, 28), 1.36 (6H, s, H_3-26, 29), 1.80 (3H, d, J = 4.4 Hz, H_3-6″), 5.28 (1H, d, J = 6.8 Hz, H-1′), 6.51 (1H, brs, H-1″); ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 1; HRESIMS *m*/*z* 807.4812 $[M + Na]^+$ (calcd for $C_{42}H_{72}O_{13}Na$, 807.4871).

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Supporting Information Available: Table listing microorganisms used for preliminary screening. This information is available free of charge via the Internet at http://pubs.acs.org.

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