Microbial Transformation of Protopanaxadiol and Protopanaxatriol Derivatives with *Mycobacterium* sp. (NRRL B-3805)

K. C. Wang, Perng-Haur Wang, and Shoei-Sheng Lee*

School of Pharmacy, College of Medicine, National Taiwan University, 1 Jen-Ai Road, Section 1, Taipei 100, Taiwan, Republic of China

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Transformation of the dammaranes (20*R*)-dihydroprotopanaxadiol (**3**) and a mixture of (20*S*)- and (20*R*)-dihydroprotopanaxatriol (**4a**, **4b**) by *Mycobacterium* sp. (NRRL B-3805) yielded the corresponding 3-oxo- (**5** and **7**) and 3-oxo-25-hydroxylated (**6** and **8**) derivatives. Incubation of (20*R*)-hydroxydammarane-3,12-dione (**9**), a pyridinium chlorochromate oxidation product of **3**, with the same microorganism yielded 3β,20*R*-dihydroxydammaran-12-one (**10**); 20*R*,24-dihydroxypropakisnordammarane-3,12-dione (**11**); and 3β,20*R*,24-trihydroxypropakisnordammaran-12-one (**12**). These results indicated that the uptake of the compound into the bacterial cells might be a critical factor in the side-chain degradation process because it occurred only in the less polar compound **9**. It has also been demonstrated that hydroxylation at C-25 by this microorganism using **3** and **4** represents a diverse reaction mechanism.

Recent work in our laboratory has revealed that the 4,4,14α-trimethyl groups in lanosterol, cycloartenol, and 24-methylenecycloartanol, together with their C-17 side chains, are oxidatively cleaved by *Mycobacterium* sp. (NRRL B-3805) to give C_{19} sterols.[1,2] This finding attracted our attention to the microbial transformation of structurally related triterpenes. Protopanaxatriol and protopanaxadiol are the corresponding aglycons of ginsenosides Rg_1 and Rb_1, the major saponins in the plant "Sanchi", *Panax notoginseng* (Burk.) F. H. Chen (Araliaceae).[3] They possess the dammarane skeleton, which is similar to lanostane except for the replacement of Me-13 by Me-8. It was considered of interest to investigate whether transformation of the derivatives of protopanaxatriol and protopanaxadiol with this same microorganism would follow a similar oxidative degradation pathway and afford C_{19} steroids, which might be biologically active. The following reports the outcome of this study.

Results and Discussion

Both the genuine *P. notoginseng* aglycons protopanaxadiol and protopanaxatriol can be obtained via enzymatic hydrolysis of the corresponding saponins Rb_1 and Rg_1, but only on a small scale.[4] The large-scale preparation of these aglycons can be achieved generally through acid hydrolysis of saponins. However, both aglycons contain Δ^{24} and OH-20 functions and yield pseudo aglycons with a pyran moiety under acidic conditions.[5] To avoid these problems and to obtain a larger quantity of substrate for the microbial transformation, (20*R*)-dihydroprotopanaxadiol (**3**) and (20*S*)- and (20*R*)-dihydroprotopanaxatriol (**4a** and **4b**) were chosen as starting materials. They were prepared from the catalytic hydrogenation of a selected portion of crude *P. notoginseng* saponins, followed by acid hydrolysis.[5] These saponins were obtained on the fractionation, inclusive of centrifugal partition chromatography, of the BuOH-soluble fraction of the EtOH extract of the roots. The ratio of ginsenoside Rb_1 and Rg_1 is about 1.42:1, as determined from a reversed-phase HPLC analysis.[6] These three compounds were separated by a Si gel column. Among them, only small amounts of the pure C-20 epimers **4a** and **4b** were separated. Most of these two epimers were collected as a mixture, which was used for the microbial transformation study. Their structures were identified by direct comparison of their ^{13}C-NMR data to those reported.[7] The C-20 epimers **4a** (20*S*) and **4b** (20*R*) were distinguished by the more downfield shifted C-21 signals (δ 26.6 vs δ 22.3, respectively) and upfield shifted C-22 signals (δ 36.5 vs δ 43.6, respectively), which possibly result from intramolecular H-bonding between OH-12β and OH-20.[7]

The transformation of **3** with *Mycobacterium* sp. (NRRL B-3805) produced compounds **5** and **6** in the isolated yields of 24.7% and 1.4%, respectively (Scheme 1).

Compound **5** had a molecular formula $C_{30}H_{52}O_3$, as deduced from HRFABMS, and was found to contain a carbonyl function (IR 1710 cm^{-1} and δ_C 217.8). Its ^{1}H-NMR spectrum revealed only one carbinol proton signal at δ 3.57 (ddd, J = 5.2, 10.4, 10.4 Hz), assignable to H-12. Except for the lack of an H-3 signal, its ^{1}H-NMR data were similar to those of **3**. These spectral data established **5** as the 3-dehydro analogue of **3**, and **5** was named 12β,20*R*-dihydroxydammaran-3-one.

Compound **6** had a molecular formula $C_{30}H_{52}O_4$ as deduced from HRFABMS, having one more oxygen atom than that of **5**. It was also found to contain a carbonyl function (IR 1700 cm^{-1} and δ_C 217.8). Its ^{1}H-NMR data were similar to those of **5** except for the appearance of one additional two-methyl singlet at δ 1.21, and the absence of a two-methyl doublet at δ 0.85 (J = 6.6 Hz, Me-26 and Me-27). These data suggested **6** to be the C-25 hydroxylated analogue of **5**. The replacement of the C-25 doublet (δ 27.9) in **5** by a singlet (δ 71.1) in **6** in their respective ^{13}C-NMR spectra also supported this suggestion. Based on this analysis, **6** was established as 12β,20*R*,25-trihydroxydammaran-3-one.

Transformation of the mixture of **4a** and **4b** by *Mycobacterium* sp. (NRRL B-3805) produced compounds **7** and **8** in the isolated yields of 23.3% and 20.9%, respectively (Scheme 1). Compound **7**, mp 130−132 °C

* To whom correspondence should be addressed. Phone: (886) 2-391-6127. FAX: (886) 2-391-9098. E-mail: shoeilee@ha.mc.ntu.edu.tw.
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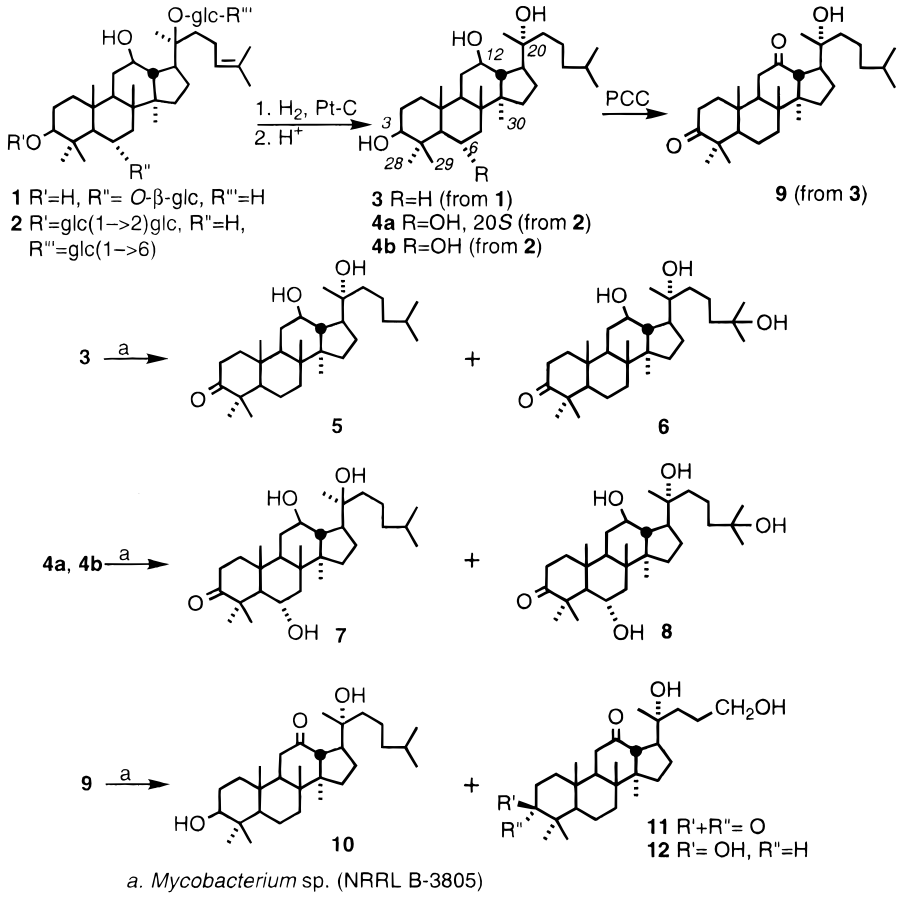
Scheme 1. Preparation and Microbial Transformation of Protopanaxadiol and Protopanaxatriol Derivatives



a. Mycobacterium sp. (NRRL B-3805)

from MeOH, $[\alpha]^{20}_D$ + 12.4° (*c* 0.5, MeOH), had the molecular formula $C_{30}H_{52}O_4$ as deduced from HREIMS. In comparison with **4a**, **7** showed two fewer protons, which accounted for one more double bond equivalent. The IR spectrum of **7** showed one carbonyl absorption at 1710 cm^{-1}. As discussed for those of **3** and **5**, the ^{1}H-NMR spectrum of **7** also lacked an H-3 signal, and the ^{13}C-NMR data of **7** were similar to those of **4a** except for the replacement of a hydroxylated carbon (δ 79.5, d, C-3) in **4a** by a carbonyl carbon (δ 219.3) in **7**. Because the carbon signals of C-17 side chain in **7** were almost superimposable with those in **4a**, the structure of **7** was established as 6α,12*β*,20*S*-trihydroxydammaran-3-one.

Compound **8**, mp 218−220 °C from $CHCl_3$, had a molecular formula $C_{30}H_{52}O_5$ as deduced from HR-FABMS, having one more oxygen atom than **7**. The IR absorptions at 3350 and 1700 cm^{-1} indicated the presence of hydroxyl and carbonyl functions. As discussed for those of **3** and **6**, the ^{1}H-NMR spectra of **8** and **7** were very similar, and the two-methyl doublet (δ 0.83, J = 6.6 Hz) for Me-26 and Me-27 in **7** was replaced by a two-methyl singlet at δ 1.18. Consequently, **8** was assigned as the C-25 hydroxylated analogue of **7**. Comparison of the ^{13}C-NMR data, however, indicated the similarity of the C-21 (δ 22.4 vs δ 22.3) and C-22 (δ 44.0 vs δ 43.6) signals in **8** and **4b**, and large differences (δ_{C-21} 22.4 vs δ 26.6, δ_{C-22} 44.0 vs δ 36.5) in **8** and **4a**, indicating the (20*R*)-configuration for **8**. The inverse heteronuclear multiple bond correlation (HMBC) spectrum of **8** displayed the coupling of a two-methyl singlet at δ 1.18 (Me-26 and Me-27) with the hydroxylated quaternary carbon at δ 71.5, proving C-25 to be hydroxylated. Analysis of this 2D NMR spectrum also assisted in the unambiguous assignment of ^{13}C-NMR data. These observations established the structure of **8** as 6α,12*β*,20*R*,25-tetrahydroxydammaran-3-one.

The results of this biotransformation study using dihydro analogues as substrate indicated simple oxidation at C-3, and subsequent hydroxylation at C-25. Further oxidative degradation on the C-17 side chain and ring-bound methyls did not occur even using **6** or **8** as substrate. We thought this might be due to the relatively high polarity of the highly hydroxylated substrate as compared with lanosterol. To prove this, we incubated the less polar substrate, (20*R*)-hydroxydammarane-3,12-dione (**9**), the pyridinium chlorochromate (PCC) oxidation product of **3**, with the same bacterium under similar conditions. Three products, compounds **10**−**12**, were isolated from this study in the yield of 9.5%, 7.4%, and 10.5%, respectively (Scheme 1).

Compound **9** had the molecular formula $C_{30}H_{50}O_3$ as deduced from HRFABMS, having four hydrogen atoms fewer than that of **3**. This mass spectral data together with the IR absorption at 1710 cm^{-1} and ^{13}C-NMR signals at δ 216.6 (s, C-3) and 214.0 (s, C-12) suggested that the structure of **9** was (20*R*)-hydroxydammarane-3,12-dione.

Compound **10**, mp 145−146 °C, had a molecular formula $C_{30}H_{52}O_3$ as deduced from HRFABMS. Its IR spectrum showed absorptions for hydroxyl (3402 cm^{-1}) and carbonyl (1708, 1690 cm^{-1}) functions. Its ^{1}H-NMR spectrum was similar to that of **3** except for the absence of the H-12 signals, indicating **10** as the 12-dehydro analogue of **3**. The H-13 signal appeared as a doublet at δ 2.81 (J = 10.3 Hz) in **10**, and the C-12 signal at δ

70.8 (d) in **3** has been replaced by a carbonyl carbon at δ 214.9 (s) in **10**. Consequently, compound **10** was structurally assigned as 3β,20*R*-dihydroxydammaran-12-one. The ^{1}H- and ^{13}C-NMR data of **10** were also assigned with the aid of its HMQC and HMBC spectra, with the results being listed in the Experimental Section.

Compound **11** had the molecular formula $C_{27}H_{44}O_4$, as deduced from HREIMS. The ^{1}H-NMR spectrum showed six methyl singlets and an oxygenated methylene signal at δ 3.62 (2H, br s, $W_{1/2}$ = 16.7 Hz, H$_2$-24). These methyl signals resonated at almost identical frequency to the corresponding signals observed for **9**, suggesting an intact ring moiety. These data, hence, established **11** to be the side-chain degradative product of **9**, and the structure of **11** was assigned as 20*R*,24-dihydroxy-propakisnordammarane-3,12-dione.

The FABMS of **12** showed a $[M - H]^-$ ion at *m*/*z* 433, consistent with a molecular formula of $C_{27}H_{46}O_4$, representing two more hydrogen atoms than in **11**. The ^{1}H-NMR spectrum ($CDCl_3$−CD_3OD, 3:1) showed an additional signal for a carbinol proton (δ 3.07, dd, J = 5.0, 8.5 Hz, H-3β) besides six methyl singlets and one oxygenated methylene signal (δ 3.49, dd, J = 4.0, 6.0 Hz). These data suggested that **12** was a C-3 hydroxyl analogue of **11**. The resemblance of the ^{1}H- and ^{13}C-NMR data (see Experimental Section) in the steroid nucleus between **12** and **10** further supported the structure of **12** being 3β,20*R*,24-trihydroxypropakisnordammaran-12-one.

The later work in the present investigation indicated that *Mycobacterium* sp. (NRRL B-3805) can degrade the terminal C_3 unit in the side chain of the less polar dammarane substrate **9**. However, no degradation occurred for the ring-bound methyl residues as in lanosterol. It is interesting to note that the reduction of the C-3 carbonyl group yielded equatorially oriented C-3 hydroxyl groups instead of the more commonly found axial carbinols. This could be due to the steric effect caused by the C-4 and C-10 substitutions. During the study of cholesterol side-chain degradation with *Mycobacterium* sp. (NRRL B-2805), Sih and co-workers[8] showed that the hydroxylation of one of the terminal methyl groups was the initial step of the degradation process and that the terminal C_3 unit was then cleaved as propionic acid. In our study, isolation of the 25-hydroxylated products, **6** and **8**, respectively, from the incubation of **3** and **4b** showed an inconsistency with the expected degradation pathway. Furthermore, when **8** was incubated with this same microorganism for 192 h, we recovered mostly unchanged substrate. Based on these results, it might be concluded that the C-25 hydroxylation of **3** and **4b**, respectively, must be a distinct reaction from the usual cholesterol side-chain degradation pathway.

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. IR spectra were recorded on a JASCO A-100 infrared spectrophotometer. Mass spectra were recorded on Finnigan TSQ-700 and JEOL JMS−HX 110 mass spectrometers. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ or CD_3-OD using residual solvent peak as reference standard. 2D NMR spectra were recorded using Bruker's standard pulse programs with the HMQC and HMBC experiments, Δ = 1 s and J = 140 and 8 Hz, respectively. The correlation maps consisted of 512 × 1 K data points per spectrum, each composed of 32 to 64 transients.

Plant Material. The dry roots of *Panax notoginseng* were purchased in August 1994, from Chua-yuan herbal store, Taipei, Taiwan. A voucher herbarium specimen is deposited at the School of Pharmacy, National Taiwan University.

Preparation of (20*R*)-Dihydroprotopanaxadiol (3), (20*S*)-Dihydroprotopanaxatriol (4a), and (20*R*)-Dihydroprotopanaxatriol (4b). The EtOH extract (2.5 kg) of plant material (10 kg) was partitioned between H_2O and organic solvents to give fractions soluble in $CHCl_3$ (77 g), *n*-BuOH (1365 g), and H_2O. Part of the BuOH-soluble fraction (100 g) was fractionated on a Sanki centrifugal partition chromatograph (LLI-7 type; $CHCl_3$−MeOH−H_2O, 10:10:5) with the organic layer as mobile phase and the aqueous layer as stationary phase; flow rate of 3.5 mL·min^{-1}; rotation speed 1000−1100 rpm; and pressure 30 to 45 kg·cm^{-2}) to give a fraction (25 g) containing ginsenosides Rg_1 and Rb_1 as major components. A reversed-phase HPLC analysis (10 μm; CH_3CN−H_2O 34:66; flow rate, 1 mL; detection, 203 nm)[6] indicated the ratio of Rg_1 (retention time 3.6 min) and Rb_1 (retention time 7.5 min) to be 1:1.42. Part of this saponin mixture (20.0 g) dissolved in MeOH (250 mL) was catalytically hydrogenated (10% Pt/C,1.5 g; H_2, 15.2 psi) overnight at room temperature. The suspension was filtered over a Celite pad and the residue washed with $CHCl_3$. The combined filtrate was evaporated in vacuo to give an amorphous residue (18.3 g) that, without further purification, was heated under reflux with a mixture of concentrated HCl (100 mL), EtOH (200 mL), and H_2O (200 mL) for 2 h. After cooling, the resultant white precipitate (10.8 g) was taken up in $CHCl_3$ via partitioning. Chromatography of this residue on a Si gel column (400 g) eluted with 0−5% MeOH in $CHCl_3$ gave (20*R*)-dihydroprotopanaxadiol (**3**, 453 mg), 20*S*-dihydroprotopanaxatriol (**4a**) (144 mg), and (20*R*)-dihydroprotopanaxatriol (**4b**) (1.1 g), and mixture of **4a** and **4b** (1.4 g) after working up in the usual method and recrystallization of the corresponding fractions from MeOH and $CHCl_3$.

(20*R*)-Dihydroprotopanaxadiol (3): mp 258−260 °C (MeOH) [lit.[9] 246−248 °C (Me_2CO)]; $[\alpha]^{26}_D$ +16.0° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.18 (1H, dd, J = 4.8 and 11.2 Hz, H-3α), 0.71 (1H, d, J = 10.8 Hz, H-5α), 3.57 (1H, ddd, J = 5.2, 10.3, 10.3 Hz, H-12α), 2.02 (1H, ddd, J = 7.2, 10.4, 10.4 Hz, H-17α), 0.96 (3H, s, Me-18), 0.86 (3H, s, Me-19), 1.11 (3H, s, Me-21), 0.86 (6H, d, J = 6.6 Hz, Me-26, 27), 0.95 (3H, s, Me-28), 0.76 (3H, s, Me-29), 0.87 (3H, s, Me-30); NOE data, Me-28 to H-3α 9%, H-5α 8%, and Me-29 7%; Me-29 to Me-28 4%, and Me-19 7%; Me-19 to Me-29 1%, and Me-18 0.6%; Me-18 to Me-19 7%; H-17α to Me-30 4%, and H-12α 4%.

(20*S*)-Dihydroprotopanaxatriol (4a): mp 223−224 °C ($CHCl_3$); $[\alpha]^{20}_D$ +3.8° (*c* 1.7, MeOH); IR (KBr) ν_{max} 3400, 2950, 2870, 1460, 1390, 1370, 1080, 1040 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.11 (1H, dd, J = 5.3, 11.4 Hz, H-3α), 4.02 (1H, ddd, J = 3.7, 10.6, 10.6 Hz, H-6β), 3.54 (1H, ddd, J = 5.2, 10.4, 10.4 Hz, H-12α), 2.02 (1H, ddd,

J = 7.1, 10.6, 10.6 Hz, H-17α), 1.08 (3H, s, Me-18), 0.95 (3H, s, Me-19), 1.13 (3H, s, Me-21), 0.89 (6H, d, J = 6.6 Hz, Me-26, 27), 1.28 (3H, s, Me-28), 0.95 (3H, s, Me-29), 0.94 (3H, s, Me-30); ^{13}C NMR (CD_3OD) δ 40.1 (t, C-1), 27.8 (t, C-2), 79.5 (d, C-3), 40.2 (s, C-4), 62.1 (d, C-5), 68.9 (d, C-6), 47.3 (t, C-7), 42.0 (s, C-8), 50.8 (d, C-9), 40.5 (s, C-10), 32.0 (t, C-11), 72.0 (d, C-12), 48.6 (d, C-13), 52.4 (s, C-14), 31.9 (t, C-15), 27.3 (t, C-16), 55.1 (d, C-17), 17.6 (q, C-18), 17.7 (q, C-19), 74.5 (s, C-20), 26.6 (q, C-21), 36.5 (t, C-22), 22.4 (t, C-23), 41.1 (t, C-24), 29.3 (d, C-25), 23.1 (q, C-26), 23.0 (q, C-27), 31.4 (q, C-28), 16.1 (q, C-29), 17.1 (q, C-30); EIMS m/z 461 (1), 443 (2), 393 (8), 358 (6), 207 (23), 109 (100), 85 (21); HREIMS m/z $[M]^+$ 478.4002 (calcd for $C_{30}H_{54}O_4$, 478.4022).

(20*R*)-Dihydroprotopanaxatriol (4b): mp 277−278 °C ($CHCl_3$) (lit.[10] 273−274 °C); $[\alpha]^{20}_D$ +3.7° (*c* 1.1, MeOH); IR (KBr) ν_{max} 3500, 3300, 2950, 2870, 1470, 1390, 1370, 1030 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.11(1H, dd, J = 5.2 and 11.4 Hz, H-3α), 4.02 (1H, ddd, J = 3.8, 10.6, and 10.6 Hz, H-6β), 3.55 (1H, ddd, J = 5.3, 10.4, 10.4 Hz, H-12α), 2.04 (1H, m, H-17α), 1.08 (3H, s, Me-18), 0.96 (3H, s, Me-19), 1.09 (3H, s, Me-21), 0.89 (6H, d, J = 6.6 Hz, Me-26, 27), 1.28 (3H, s, Me-28), 0.96 (3H, s, Me-29), 0.95 (3H, s, Me-30); NOE data, Me-28 to H-3α 8%, and Me-29 8%; Me-29 to Me-28 3%, and H-6β 8%; Me-19 to Me-18 4%; Me-18 to Me-19 8%, and H-6β 8%; Me-30 to H-17α 2%; ^{13}C NMR (CD_3OD) δ 40.1 (t, C-1), 27.8 (t, C-2), 79.5 (d, C-3), 40.1 (s, C-4), 62.1 (d, C-5), 68.9 (d, C-6), 47.3 (t, C-7), 42.0 (s, C-8), 50.7 (d, C-9), 40.5 (s, C-10), 32.0 (t, C-11), 71.8 (d, C-12), 49.2 (d, C-13), 52.5 (s, C-14), 31.9 (t, C-15), 27.1 (t, C-16), 51.0 (d, C-17), 17.6 (q, C-18), 17.7 (q, C-19), 74.7 (s, C-20), 22.3 (q, C-21), 43.6 (t, C-22), 21.8 (t, C-23), 40.9 (t, C-24), 29.2 (d, C-25), 23.2 (q, C-26), 23.0 (q, C-27), 31.5 (q, C-28), 16.1 (q, C-29), 17.4 (q, C-30); EIMS m/z 461 (0.4), 443 (1.3), 428 (0.8), 410 (1.3), 393 (3.9), 358 (4.6), 207 (32), 109 (100), 85 (18); HREIMS m/z $[M]^+$ 478.4034 (calcd for $C_{30}H_{54}O_4$, 478.4022).

Biotransformation of (20*R*)-Dihydroprotopanaxadiol (3) with *Mycobacterium* sp. (NRRL B-3805). The microorganism was grown in Nutrient broth (1.6%)−dextrose (4%) medium (1.4 L) contained in four 2-L Erlenmeyer flasks at 26−27 °C on a rotary shaker (250 rpm, 1-in stroke). Compound **3** (353 mg) was dissolved in DMF (18 mL) and distributed evenly among the flasks. After 4 days of incubation, the culture broth was acidified with HOAc to a pH of 3.0 and was extracted with $CHCl_3$ (400 mL × 3). The combined $CHCl_3$ layer was dried over Na_2SO_4 and evaporated in vacuo to give a brownish semisolid (2.06 g) that was chromatographed over a Si gel column (60 g) eluted with 0−10% MeOH in $CHCl_3$. Compound **5** (87 mg, 24.74% yield) and the recovered **3** (96 mg) were obtained, respectively, by recrystallization with MeOH of the residues obtained from the 1% MeOH elution (150 mg) and 1.5% MeOH elution (159 mg). A fraction (23 mg) from 5% MeOH elution was further purified on a Sephadex LH-20 column (100 g, MeOH) to give **6** (5 mg, 1.44% yield).

12β,20*R*-Dihydroxydammaran-3-one (5): mp 225−227 °C (MeOH); $[\alpha]^{27}_D$ +40.0° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3300, 2950, 1710, 1460, 1380, 1360 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (2H, m, H-2), 3.57 (1H, ddd, J = 5.2, 10.4, 10.4 Hz, H-12α), 1.72 (1H, dd, J = 10.4, 10.4 Hz, H-13), 2.02 (1H, m, H-17α), 0.95 (3H, s, Me-18), 1.02 (3H, s, Me-19), 1.11 (3H, s, Me-21), 0.85 (6H, d, J = 6.6 Hz, Me-26, 27), 1.05 (3H, s, Me-28), 1.00 (3H, s, Me-29), 0.87 (3H, s, Me-30); ^{13}C NMR ($CDCl_3$) δ 39.5 (t, C-1), 34.0 (t, C-2), 217.8 (s, C-3), 47.3 (s, C-4), 55.3 (d, C-5), 19.6 (t, C-6), 34.1 (t, C-7), 39.6 (s, C-8), 49.3 (d, C-9), 36.8 (s, C-10), 31.5 (t, C-11), 70.5 (d, C-12), 48.7 (d, C-13), 51.6 (s, C-14), 30.9 (t, C-15), 26.3 (t, C-16), 49.8 (d, C-17), 15.8 (q, C-18), 15.3 (q, C-19), 74.5 (s, C-20), 21.8 (q, C-21), 43.0 (t, C-22), 20.7 (t, C-23), 39.7 (t, C-24), 27.9 (d, C-25), 22.7 (q, C-26), 22.6 (q, C-27), 26.7 (q, C-28), 21.0 (q, C-29), 17.0 (q, C-30); EIMS m/z 460 (2), 424 (10), 409 (11), 375 (11), 357 (77), 339 (14), 314 (34), 299 (53), 205 (27), 108 (100); HRFABMS m/z $[M + H]^+$ 461.3994 (calcd for $C_{30}H_{53}O_3$, 461.3995).

12β,20*R*,25-Trihydroxydammaran-3-one (6): mp 200−202 °C (MeOH−H_2O, 1:1); $[\alpha]^{27}_D$ +2.5° (*c* 0.4, MeOH); IR (KBr) ν_{max} 3300, 2950, 1700, 1460, 1380, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (2H, m, H-2), 3.57 (1H, ddd, J = 5.2, 10.4, 10.4 Hz, H-12α), 1.72 (1H, dd, J = 10.4, 10.4 Hz, H-13), 2.02 (1H, m, H-17α), 0.96 (3H, s, Me-18), 1.02 (3H, s, Me-19), 1.12 (3H, s, Me-21), 1.21 (6H, s, Me-26, 27), 1.06 (3H, s, Me-28), 1.01 (3H, s, Me-29), 0.88 (3H, s, Me-30); ^{13}C NMR ($CDCl_3$) δ 39.7 (t, C-1), 34.0 (t, C-2), 217.8 (s, C-3), 47.3 (s, C-4), 55.3 (d, C-5), 19.6 (t, C-6), 34.1 (t, C-7), 39.6 (s, C-8), 49.3 (d, C-9), 36.8 (s, C-10), 31.6 (t, C-11), 70.6 (d, C-12), 48.6 (d, C-13), 51.6 (s, C-14), 31.0 (t, C-15), 26.3 (t, C-16), 49.9 (d, C-17), 15.9 (q, C-18), 15.3 (q, C-19), 74.3 (s, C-20), 21.9 (q, C-21), 43.0 (t, C-22), 17.6 (t, C-23), 44.1 (t, C-24), 71.1 (s, C-25), 29.4 (q, C-26), 29.4 (q, C-27), 26.7 (q, C-28), 21.0 (q, C-29), 17.0 (q, C-30); EIMS m/z 458 (2), 443 (10), 425 (11), 375 (11), 357 (37), 339 (8), 299 (17), 205 (16), 145 (26), 127 (58), 109 (64), 59 (100); HRFABMS m/z $[M + H]^+$ 477.3932 (calcd for $C_{30}H_{53}O_4$, 477.3944).

Biotransformation of the Mixture of (20*S*)- and (20*R*)-Dihydroprotopanaxatriol (4a and 4b) with *Mycobacterium* sp. (NRRL B-3805). A mixture of **4a** and **4b** (600 mg) divided evenly in eight 2-L Erlenmeyer flasks was incubated with *Mycobacterium* sp. (NRRL B-3805) in a manner similar to that described for **3**. Similar workup as mentioned above yielded 7.10 g residue from the $CHCl_3$ fraction that was chromatographed over a Si gel column (200 g) eluted with 0−10% MeOH in $CHCl_3$. The fraction (377 mg) obtained from elution with 3% MeOH was repeatedly recrystallized with MeOH to give **7** (139 mg, 23.3% yield). The fraction (363 mg) obtained from the 5% MeOH elution was further purified on a Sephadex LH-20 column (100 g, MeOH) to give compound **8** (129 mg, 20.9% yield).

6α,12β,20*S*-Trihydroxydammaran-3-one (7): mp 130−132 °C (MeOH); $[\alpha]^{20}_D$ +12.4° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3350, 2950, 1710, 1460, 1380, 1360, 1180 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.22 (1H, ddd, J = 2.8, 10.0, 15.0 Hz, H-2α), 2.68 (1H, ddd, J = 6.4, 12.0, 15.0 Hz, H-2β), 1.63 (1H, d, J = 10.0 Hz, H-5), 3.94 (1H, ddd, J = 5.2, 9.7, 10.0 Hz, H-6β), 3.54 (1H, ddd, J = 4.7, 10.0, 10.1 Hz, H-12α), 1.69 (1H, dd, J = 10.6, 10.6 Hz, H-13), 2.01 (1H, m, H-17α), 0.99 (3H, s, Me-18), 0.74 (3H, s, Me-19), 1.14 (3H, s, Me-21), 0.83 (6H, d, J = 6.6 Hz, Me-26, 27), 1.29 (3H, s, Me-28), 1.27 (3H, s, Me-29), 0.88 (3H, s, Me-30); ^{13}C NMR ($CDCl_3$) δ 39.6 (t, C-1), 32.9 (t, C-2), 219.3 (s, C-3), 47.1 (s, C-4), 58.8 (d, C-5), 67.5 (d, C-6), 44.8 (t, C-7), 40.2 (s, C-8), 48.3 (d, C-9), 37.9 (s, C-10), 31.7 (t, C-11), 70.3 (d, C-12), 47.3 (d, C-13), 51.4 (s, C-14), 30.9 (t, C-15), 26.3 (t, C-16), 53.2 (d, C-17),

16.6 (q, C-18), 17.4 (q, C-19), 74.2 (s, C-20), 26.9 (q, C-21), 34.9 (t, C-22), 21.2 (t, C-23), 39.7 (t, C-24), 28.1 (d, C-25), 22.6 (q, C-26), 22.5 (q, C-27), 31.8 (q, C-28), 19.4 (q, C-29), 15.8 (q, C-30); EIMS *m/z* 476 (2), 391 (6), 315 (20), 108 (100), 95 (50); HREIMS *m/z* $[M]^+$ 476.3851 (calcd for $C_{30}H_{52}O_4$, 476.3866).

6α,12*β*,20*R*,25-Tetrahydroxydammaran-3-one (8): mp 218−220 °C ($CHCl_3$); $[\alpha]^{27}_D$ +117.0° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3300, 2950, 1710, 1460, 1380, 1360 cm^{-1}; ^1H NMR (CD_3OD) δ 2.20 (1H, ddd, J = 3.3, 9.7, 15.3 Hz, H-2α), 2.83 (1H, ddd, J = 6.7, 11.8, 15.3 Hz, H-2*β*), 1.74 (1H, d, J = 10.4 Hz, H-5), 3.92 (1H, ddd, J = 5.0, 10.4, 10.4 Hz, H-6*β*), 3.57 (1H, ddd, J = 5.1, 10.4, 10.4 Hz, H-12α), 2.10 (1H, m, H-17α), 1.06 (3H, s, Me-18), 0.79 (3H, s, Me-19), 1.11 (3H, s, Me-21), 1.18 (6H, s, Me-26, 27), 1.32 (3H, s, Me-28), 1.29 (3H, s, Me-29), 0.98 (3H, s, Me-30); ^{13}C NMR (CD_3OD) δ 40.7 (t, C-1), 34.0 (t, C-2), 222.6 (s, C-3), 49.2 (s, C-4), 59.6 (d, C-5), 68.0 (d, C-6), 45.4 (t, C-7), 41.4 (s, C-8), 49.5 (d, C-9), 39.2 (s, C-10), 32.7 (t, C-11), 71.6 (d, C-12), 49.3 (d, C-13), 52.6 (s, C-14), 32.1 (t, C-15), 27.1 (t, C-16), 50.8 (d, C-17), 16.2 (q, C-18), 18.2 (q, C-19), 74.7 (s, C-20), 22.4 (q, C-21), 44.0 (t, C-22), 18.9 (t, C-23), 45.4 (t, C-24), 71.5 (s, C-25), 29.4 (q, C-26), 29.1 (q, C-27), 32.4 (q, C-28), 19.9 (q, C-29), 17.3 (q, C-30); FABMS (neg) *m/z* $[M - H]^-$ 491(100); HRFABMS (pos) *m/z* $[M + H]^+$ 493.3894 (calcd for $C_{30}H_{53}O_5$, 493.3893).

Pyridinium Chlorochromate Oxidation of 3. To **3** (149 mg, 0.32 mmol) dissolved in CH_2Cl_2 (10 mL) in a 25-mL round-bottom flask was added PCC (139 mg, 0.64 mmol). The resultant solution was stirred at room temperature for 2 h, then Et_2O (10 mL) was added and the grayish brown precipitate was removed by filtering through a Celite pad. The filtrate was evaporated, and the residue was recrystallized repeatedly from MeOH to give colorless needles of **9** (100 mg, 0.22 mmol, 68% yield).

(20*R*)-Hydroxydammarane-3,12-dione (9): mp 125−126 °C ($CHCl_3$); $[\alpha]^{27}_D$ +30.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3450, 2950, 1710, 1460, 1380 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.46 (2H, m, H-2), 2.25 (2H, m, H-11), 2.82 (1H, d, J = 10.3 Hz, H-13*β*), 2.38 (1H, m, H-17α), 1.18 (3H, s, Me-18), 1.03 (3H, s, Me-19), 0.95 (3H, s, Me-21), 0.84 (6H, d, J = 6.6 Hz, Me-26, 27), 1.06 (3H, s, Me-28), 1.00 (3H, s, Me-29), 0.77 (3H, s, Me-30); ^{13}C NMR ($CDCl_3$) δ 39.0 (t, C-1), 33.2 (t, C-2), 216.6 (s, C-3), 47.3 (s, C-4), 55.1 (d, C-5), 19.6 (t, C-6), 33.7 (t, C-7), 40.0 (s, C-8), 52.5 (d, C-9), 37.0 (s, C-10), 39.0 (t, C-11), 214.0 (s, C-12), 56.5 (d, C-13), 54.4 (s, C-14), 30.8 (t, C-15), 25.0 (t, C-16), 43.9 (d, C-17), 15.4 (q, C-18), 15.4 (q, C-19), 73.4 (s, C-20), 21.7 (q, C-21), 42.9 (t, C-22), 20.9 (t, C-23), 39.6 (t, C-24), 28.0 (d, C-25), 22.7 (q, C-26), 22.6 (q, C-27), 26.5 (q, C-28), 21.0 (q, C-29), 17.3 (q, C-30); EIMS *m/z* $[M - H_2O]^+$ 440 (6), 373 (46), 234 (12), 205 (8), 129 (1), 124 (100), 109 (6); HREIMS *m/z* $[M]^+$ 458.3767 (calcd for $C_{30}H_{50}O_3$, 458.3760).

Biotransformation of (20*R*)-hydroxydammarane-3,12-dione (9) with *Mycobacterium* sp. (NRRL B-3805). Compound **9** (95 mg) was dissolved in DMF (5 mL) and distributed evenly in the flask containing the microorganism and 400 mL of medium in a manner similar to that of **3**. After 4 days of incubation, similar workup as mentioned above yielded 0.87 g of residue from the $CHCl_3$ fraction (400 mL × 3). The residue was chromatographed over a Si gel column (40 g) eluted with 0−10% MeOH in $CHCl_3$. Compounds **10** (9 mg, 9.5%) and **11** (7 mg, 7.4%) were purified, in turn, from fractions from 0.5% MeOH (40 mg) and 1% MeOH elution (11 mg) via a Sephadex LH-20 column (100 g, MeOH). Compound **12** (10 mg, 10.5%) was purified by direct crystallization of the fraction (82 mg) of 5% MeOH elution from $CHCl_3$.

3*β*,20*R*-Dihydroxydammaran-12-one (10): mp 145−146 °C (MeOH); $[\alpha]^{27}_D$ +25.0° (*c* 0.8, MeOH); IR (KBr) ν_{max} 3402, 3312, 2931, 2854, 1708, 1690, 1466, 1381 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.17 (1H, dd, J = 5.0, 11.2 Hz, H-3α), 0.75 (1H, d, J = 11.0 Hz, H-5α), 2.28 (1H, dd, J = 4.5, 14.0 Hz, H-11α), 2.19 (1H, dd, J = 14.0, 14.0 Hz, H-11*β*), 2.81 (1H, d, J = 10.3 Hz, H-13*β*), 2.40 (1H, ddd, J = 6.6, 10.4, 10.4 Hz, H-17α), 1.15 (3H, s, Me-18), 0.91 (3H, s, Me-19), 0.96 (3H, s, Me-21), 0.85 (6H, d, J = 6.6 Hz, Me-26, 27), 0.97 (3H, s, Me-28), 0.78 (6H, s, Me-29, 30); ^{13}C NMR ($CDCl_3$) δ 38.4 (t, C-1), 27.1 (t, C-2), 78.5 (d, C-3), 38.9 (s, C-4), 55.7 (d, C-5), 18.3 (t, C-6), 33.9 (t, C-7), 40.1 (s, C-8), 53.2 (d, C-9), 37.4 (s, C-10), 39.0 (t, C-11), 214.9 (s, C-12), 56.5 (d, C-13), 54.4 (s, C-14), 30.8 (t, C-15), 25.1 (t, C-16), 43.9 (d, C-17), 15.8 (q, C-18), 15.8 (q, C-19), 73.5 (s, C-20), 21.7 (q, C-21), 42.9 (t, C-22), 20.9 (t, C-23), 39.6 (t, C-24), 28.0 (d, C-25), 22.8 (q, C-26), 22.6 (q, C-27), 28.0 (q, C-28), 15.3 (q, C-29), 17.4 (q, C-30); FABMS (neg) *m/z* $[M - H]^-$ 459; HRFABMS (pos) *m/z* $[M + H]^+$ 461.3995 (calcd for $C_{30}H_{53}O_3$, 461.3995).

20*R*,24-Dihydroxypropakisnordammarane-3,12-dione (11): amorphous solid; $[\alpha]^{27}_D$ +40.0° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3386, 2924, 2854, 1703, 1459, 1384 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.46 (2H, m, H-2), 2.25 (2H, m, H-11), 2.82 (1H, d, J = 10.2 Hz, H-13*β*), 2.38 (1H, m, H-17α), 1.01 (3H, s, Me-18), 1.08 (3H, s, Me-19), 3.62 (2H, br s, $W_{1/2}$ = 16.7 Hz, C*H*$_2$OH), 1.05 (3H, s, Me-25), 1.00 (3H, s, Me-26), 0.80 (3H, s, Me-27); ^{13}C NMR ($CDCl_3$) δ 38.9 (t, C-1), 33.1 (t, C-2), 216 (s, C-3), 47.3 (s, C-4), 55.1 (d, C-5), 19.6 (t, C-6), 33.7 (t, C-7), 40.0 (s, C-8), 52.3 (d, C-9), 37.0 (s, C-10), 39.6 (t, C-11), 214.5 (s, C-12), 56.7 (d, C-13), 54.3 (s, C-14), 30.6 (t, C-15), 25.1 (t, C-16), 44.6 (d, C-17), 15.5 (q, C-18), 15.4 (q, C-19), 73.2 (s, C-20), 21.5 (q, C-21), 38.9 (t, C-22), 26.9 (t, C-23), 63.4 (t, C-24), 26.5 (q, C-25), 21.0 (q, C-26), 17.5 (q, C-27); EIMS *m/z* $[M]^+$ 432 (0.5), 414 (5), 399 (15), 384 (6), 373 (100); HREIMS *m/z* $[M]^+$ 432.3193 (calcd for $C_{27}H_{44}O_4$, 432.3239), $[M - H_2O]^+$ 414.3114 (calcd for $C_{27}H_{42}O_3$, 414.3134), $[M - H_2O - Me]^+$ 399.2921 (calcd for $C_{26}H_{39}O_3$, 399.2900).

3*β*,20*R*,24-Trihydroxypropakisnordammaran-12-one (12): mp 280−282 °C ($CHCl_3$); $[\alpha]^{26}_D$ +10.0° (*c* 0.1, pyridine); IR (KBr) ν_{max} 3397, 2917, 2850, 1733, 1468, 1378, 1180, 1057 cm^{-1}; ^1H NMR ($CDCl_3$−CD_3OD, 3:1) δ 3.07 (1H, dd, J = 5.0, 8.5 Hz, H-3α), 2.18 (2H, m, H-11), 2.77 (1H, d, J = 10.3 Hz, H-13*β*), 2.30 (1H, ddd, J = 7.1, 10.3, 10.3 Hz, H-17α), 1.10 (3H, s, Me-18), 0.85 (3H, s, Me-19), 0.93 (3H, s, Me-21), 3.49 (2H, dd, J = 4.0, 6.0 Hz, C*H*$_2$OH), 0.89 (3H, s, Me-25), 0.72 (3H, s, Me-26), 0.71 (3H, s, Me-27); ^{13}C NMR ($CDCl_3$−CD_3OD, 3:1) δ 38.3 (t, C-1), 26.3 (t, C-2), 77.9 (d, C-3), 38.6 (s, C-4), 55.5 (d, C-5), 18.0 (t, C-6), 33.6 (t, C-7), 39.9 (s, C-8), 53.1 (d, C-9), 37.1 (s, C-10), 38.7 (t, C-11), 215.8 (s, C-12), 56.4 (d, C-13), 54.4 (s, C-14), 30.3 (t, C-15), 24.7 (t, C-16), 44.0 (d, C-17), 15.4 (q, C-18), 15.4 (q, C-19), 73.2 (s, C-20), 21.5 (q, C-21), 38.2 (t, C-22), 26.1 (t, C-23), 62.5 (t, C-24), 27.5 (q, C-25), 15.0 (q, C-26),

17.2 (q, C-27); FABMS (neg) *m*/*z* $[M - H]^-$ 433; HR-FABMS (pos) *m*/*z* $[M - H_2O + H]^+$ 417.3355 (calcd for $C_{27}H_{45}O_3$, 417.3369).

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