New Neoverrucosane Diterpenoids Produced by the Marine Gliding Bacterium *Saprospira grandis*

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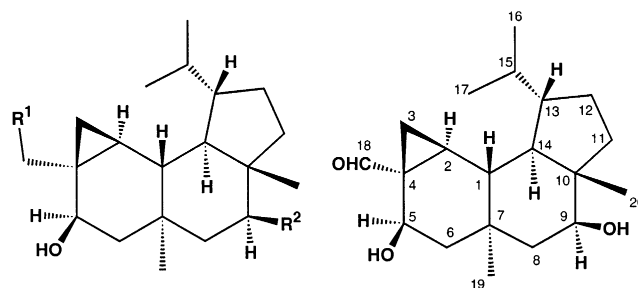
Chemical examination of the culture broth extracts of the marine gliding bacterium *Saprospira grandis* (ATCC 23116) has resulted in the isolation of four new diterpenoids of the neoverrucosane class. The structures of the new diterpenoids, compounds **1**−**4**, were assigned by combined spectroscopic methods emphasizing 2D NMR experiments. The relative stereochemistry of **1** was determined by 2D ROESY NMR methods, while the absolute stereochemistry was assigned by application of the modified Mosher method. This study adds to the rare observation of terpene production by prokaryotic microorganisms and suggests that marine gliding bacteria may be a significant source for new terpenoid secondary metabolites.

As part of an effort to access the chemical potential of biologically diverse marine microorganisms, we turned our attention to a group of Gram-negative gliding bacteria that are not readily isolated using normal agar plate cultivation methods. Bacteria with gliding motility are moderately common in the marine environment, but they are relatively difficult to isolate, requiring selective media and isolation procedures guided by microscopic examination of colonies isolated from sand grains and other marine inanimate surfaces. Gliding bacteria are not a uniform taxonomic group, but rather have representatives within the Cyanobacteria, Bacteriodes-Flavobacteria, Green Sulfur Bacteria, Green Non-sulfur Bacteria, and Proteobacteria divisions. Most bacteria with gliding motility, however, fall within the Bacteriodes-Flavobacterium-Cytophaga group. These bacteria have the ability to glide on solid surfaces, which is achieved without the use of flagella, using a mechanism that remains unknown. The most chemically well-known of the gliding bacteria are the myxobacteria, a group of mainly terrestrial microorganisms[1] with the demonstrated capacity to produce a wide diversity of bioactive secondary metabolites.[2] The most recent of the myxobacterial metabolites to receive considerable attention are the epothilones, anticancer macrolides from *Sorangium cellulosum* with potent stabilizing effects against intracellular microtubules.[3]

As part of this exploratory program, we examined culture extracts of *Saprospira grandis*, a Gram-negative marine gliding bacterium that forms helical, multicellular filaments.[4] This highly pigmented organism belongs to the Bacteriodes-Flavobacterium-Cytophaga group and is common in sandy marine littoral and other coastal habitats.[5] Although the secondary metabolite chemistry of *S. grandis* is unknown, the major pigment from this species has been identified as the red xanthophyll saproxanthin (1′,2′-dihydro-3′,4′-dehydro-3′,1′-dihydroxy-γ-carotene).[6] *Saprospira grandis* is also of biological interest because of its predatory behavior in capturing and digesting other bacteria.[7]

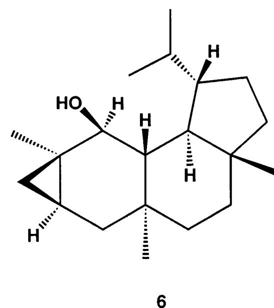
Results and Discussion

Saprospira grandis, ATCC strain # 23116, was cultured at 37 °C by rotary shaking in replicate 2.8 L Fernbach flasks containing 1 L of culture medium. The whole cultures were extracted by adding Amberlite XAD-16 resin; the resin was stirred, filtered, and finally eluted with 1:1 isooctane/ethyl acetate to obtain a crude extract after solvent removal under vacuum. The diterpenoids **1**−**4** were subsequently isolated from the extract by silica column chromatography and reversed-phase, C_8, HPLC methods.



1, R^1= H, R^2= OH
2, R^1= OH, R^2= H
3, R^1= R^2= OH
5, R^1= H, R^2= H

4



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Neoverrucosan-5β,9β-diol (**1**) was isolated as a viscous oil that analyzed for the molecular formula $C_{20}H_{34}O_2$ by HR-MALDI mass spectrometry ($[M + H - 2H_2O]^+$ *m/z* 271.2419, Δ 0.1 mmu and $[M + H - H_2O]^+$ *m/z* 289.2529, Δ 0.3 mmu), combined with analysis of ^{1}H and ^{13}C NMR spectral data (Table 1). This formula suggested 4° of unsaturation in the molecule. The IR spectrum of **1** showed broad absorption for multiple hydroxyl groups (ν_{max} 3354 cm^{-1}) and lacked any evidence of carbonyl absorptions.

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Table 1. ^{1}H and ^{13}C NMR Assignments for Compounds **1**−**4** in $CDCl_3$

	compound **1**		compound **2**		compound **3**		compound **4**	
#	δ_C	δ_H, mult, (J^b)	δ_C	δ_H, mult, (J^b)	δ_C	δ_H, mult, (J^b)	δ_C	δ_H, mult, (J^b)
1	47.2 CH	1.03 nr	46.8 CH	1.10 nr	46.4 CH	1.08 nr	46.3 CH	1.18 nr
2	25.0 CH	0.85 nr	21.5 CH	0.90 nr	21.9 CH	0.95 nr	25.5 CH	0.90 nr
3	20.1 CH_2	0.31 t (4.8)	17.8 CH_2	0.49 t (4.5)	18.0 CH_2	0.50 t (4.8)	20.1 CH_2	0.75 nr
		0.61 dd (8.7, 4.8)		0.80 nr		0.80 nr		0.87 nr
4	22.0 C		28.0 C		27.9 C		22.0 C	
5	71.2 CH	4.02 dd (10.5, 7.5)	69.2 CH	4.43 dd (10.7, 7.2)	68.8 CH	4.30 dd (10.4, 7.2)	75.0 CH	5.00 dd (9.6, 8.4)
6	47.2 CH_2	1.75 nr	45.2 CH_2	1.72 nr	46.1 CH_2	1.80 nr	43.3 CH_2	1.90 nr
		0.75 nr		0.75 nr		0.80 nr		0.85 nr
7	38.3 C		37.1 C		38.0 C		37.6 C	
8	43.7 CH_2	1.20 nr	35.4 CH_2	1.10 nr	43.5 CH_2	1.42 nr	43.4 CH_2	1.45 nr
		1.40 nr		1.45 nr		1.28 nr		1.20 nr
9	75.7 CH	3.57 dd (11.5, 4.5)	34.6 CH_2	1.45 nr	75.3 CH	3.64 dd (11.2, 4.8)	61.3 CH	3.65 dd (11.4, 4.0)
10	49.6 C		44.1 C		49.4 C		49.3 C	
11	37.1 CH_2	1.13 nr	39.2 CH_2	1.10 nr	37.0 CH_2	1.15 nr	36.8 CH_2	1.15 nr
		1.64 nr		1.45 nr		1.65 nr		1.65 nr
12	22.0 CH_2	1.47 nr	21.7 CH_2	1.45 nr	20.6 CH_2	1.50 nr	21.8 CH_2	1.50 nr
		1.65 nr		1.60 nr		1.65 nr		1.70 nr
13	45.1 CH	2.08 m	46.2 CH	1.95 m	44.9 CH	2.05 m	44.9 CH	2.12 m
14	46.8 CH	1.27 nr	46.7 CH	1.23 nr	46.0 CH	1.28 nr	45.6 CH	1.25 nr
15	28.7 CH	2.19 m	28.8 CH	2.15 m	28.7 CH	2.15 m	28.6 CH	2.12 m
16	15.1 CH_3	0.84 d (6.9)	15.1 CH_3	0.79 d (6.9)	15.0 CH_3	0.81 d (6.8)	15.0 CH_3	0.82 d (6.8)
17	23.0 CH_3	0.94 d (6.9)	22.9 CH_3	0.92 d (6.9)	22.9 CH_3	0.92 d (6.8)	22.8 CH_3	0.95 d (6.8)
18a	26.2 CH_3	1.19 s	73.6 CH_2	3.16 d (10.5)	73.3 CH_2	3.85 d (10.8)	201.0 CH	8.74 s
18b				3.91 d (10.5)		3.16 d (10.8)		
19	18.8 CH_3	0.90 s	18.7 CH_3	0.75 s	18.5 CH_3	0.89 s	18.7 CH_3	0.79 s
20	12.4 CH_3	0.76 s	17.1 CH_3	0.82 s	12.3 CH_3	0.77 s	12.1 CH_3	0.78 s

^{a} Assignments made on the basis of DEPT, COSY, HMQC, and HMBC experiments. ^{b} Coupling constants are in Hz. nr = not resolved.

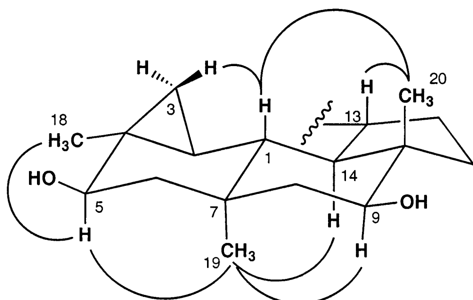

Figure 1. Three-dimensional structure of compound **1** based on ROESY correlation shown.

Analysis of ^{1}H and ^{13}C NMR spectral data (Table 1) illustrated signals for two secondary alcohols [δ ^{1}H: 4.02 (dd, 10.5, 7.5); 3.75 (dd, 11.5, 4.5)] and one cyclopropane ring [δ ^{1}H: C-2, (0.85, m); C-3 (0.31, t, 4.8)] and showed that olefinic bonds were not present in the molecule. These data, and the presence of five methyl groups, led to the preliminary conclusion that **1** was a tetracarbocyclic diterpenoid diol. Analysis of 2D ^{1}H NMR COSY correlations showed the presence of four spin systems: one involving correlations of the C-3 and C-2 cyclopropane protons and protons at C-1, C-14, C-13, C-12, and C-11; one defining the protons at C-5 and C-6; one involving the protons at C-8 and C-9; and one involving the isopropyl protons at C-15, C-16, and C-17. Further analysis of 2D NMR HMQC and HMBC data allowed all the protons and carbons to be assigned. Key HMBC correlations from C-16 and C-17 to H-13, between C-20 and H-11, H-14, and H-9, between C-19 and H-8, H-6, and H-1, and between C-3 and H-5 and H-18 clearly established connections of the four spin systems and allowed the planar structure of neoverrucosane **1** to be fully assigned.

The relative stereochemistry of compound **1** was assigned by interpretation of 2D ^{1}H NMR NOE data (Figure 1). A typical *trans*-diaxial configuration of the A, B, and C rings was clear from the correlations observed. The C-19 bridgehead methyl group showed NOE correlations to the H-5, H-9, and H-14 protons, placing them in 1,3-diaxial positions on the bottom face of the molecule. Conversely, correlations from H-1 to H-3β and to the C-20 methyl group allowed these protons to be arranged on the top face of the molecule. These data strongly suggested that the secondary alcohols at C-5 and C-9 were in equatorial positions on the six-membered A and B rings. This was confirmed by analysis of the H-5 and H-9 coupling constants, each of which showed the typically large (ca. 11 Hz) axial−axial and smaller (ca. 6−7 Hz) axial−equatorial coupling J values to their neighboring methylene protons. A strong correlation from H-13 to the C-20 methyl group allowed these protons to be positioned on the top face of the molecule and placed the isopropyl substituent down in a pseudoequatorial configuration.

The absolute stereochemistry of the neoverrucosane **1** was determined using the modified Mosher method.[8−10] Acylation of **1**, in separate experiments, with *R*-(+)- and *S*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride yielded all six expected products: from *R*(+)-MTPACl, the C-5 *S* ester **1a** (32%), the C-9 *S* **1c** ester (22%), and the bis *S* ester (34%); from (*S*)-MTPACl, the C-5 *R* ester **1b** (36%), the C-9 *R* ester **1d** (28%), and the bis *R* ester (38%). The products were separated by normal-phase HPLC, and the chemical shifts of the four monoesters were analyzed separately by ^{1}H NMR spectrometry. Acylation was shown to occur at C-5 or C-9 from the downfield shift of the C-5 or C-9 methine protons (Experimental Section). The bis esters were not examined because of the difficulty in separating the effects on the proximate protons near each group. Analysis of the NMR shifts of the C-5 *S* and C-5 *R* esters showed negative values ($\Delta\delta_{S-R}$) for H-18, H-3, H-1, H-15, H-16, and H-17 and positive values for H-6, H-19, H-8, H-9, and H-20, indicating that the absolute configuration of the C-5 chiral center was *R* (Figure 2). Analysis of the NMR shifts for the C-9 *S* and C-9 *R* esters showed negative values for H-8, H-19, H-6, H-5, H-3, H-2, and H-14 and positive values for H-20, H-13, H-15, H-16, and H-17, allowing the absolute stereochemistry of the chiral center

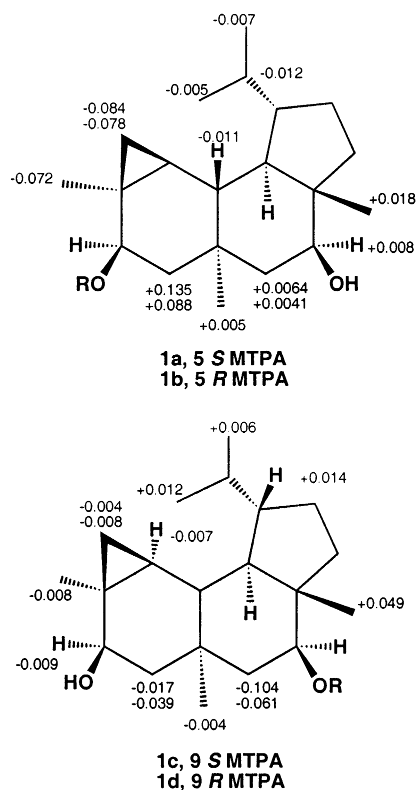

Figure 2. Delta values ($\Delta\delta_{S-R}$) for 5*S* and 5*R* esters (**1a** and **1b**) and 9*S* and 9*R* esters (**1c** and **1d**). $\Delta\delta$ values are expressed in ppm.

at C-9 to be assigned as *S* (Figure 2). The chiral centers at C-1, C-2, C-7, C-10, C-13, and C-14 were then assigned as *S, S, R, S, S,* and *R,* on the basis of their relative configurations determined by NOE measurements.

Neoverrucosan-5β,18-diol (**2**) analyzed for $C_{20}H_{34}O_2$ by HR-MALDI mass spectrometry ([M + Na]$^+$ *m/z* 329.2460, Δ 0.9 mmu), and this formula was confirmed by analysis of ^{1}H and ^{13}C NMR spectral features (Table 1). As in **1**, the IR spectrum of **2** showed broad absorption for multiple hydroxyl groups (ν_{max} 3401 cm^{-1}) and lacked carbonyl absorptions. Analysis of ^{1}H and ^{13}C proton NMR spectral data showed the presence of one primary and one secondary alcohol, one cyclopropane ring, and four methyl groups. The position of the primary alcohol was easily assigned since the signals for the C-18 methyl group in **1** were lost and replaced by a double doublet at δ 3.2 and 3.9 for the diastereotopic protons of the C-18 methylene pair in **2**. As in compound **1**, ^{1}H NMR COSY correlations showed four spin systems: one involving the C-3 and C-2 cyclopropane protons and protons at C-1, C-14, C-13, C-12, and C-11; one involving the protons at C-5 and C-6; another involving the protons at C-8 and C-9; and one involving the isopropyl protons at C-15–C-17. Interpretation of 2D HMQC and HMBC NMR experiments allowed all the protons and carbons to be assigned. Numerous HMBC correlations were observed, but those between C-16 and C-17 and H-13, between C-20 and H-11, H-14, and H-9, between C-19 and H-8, H-6, and H-1, between C-3 and H-5, and between C-18 and H-3 clearly established connections of the four spin systems and allowed the structure of the neoverrucosane **2** to be assigned. The correlation between C-18 and H-3 provided convincing evidence that the primary alcohol was positioned at C-18. Acetylation of **2** with acetic anhydride in dichloromethane and pyridine yielded a diacetate, **2a**, [M + Na]$^+$ *m/z* 413, which confirmed the presence of two alcohol functionalities. In the diacetate, the H-5 ^{1}H NMR signal shifted downfield about 1.0 ppm and the H-18 methylene protons shifted by 0.5 ppm. The two acetate methyl groups were observed at δ 2.05 (Experimental Section).

Neoverrucosan-5β,9β,18β-triol (**3**) analyzed for $C_{20}H_{34}O_3$ by HR-MALDI mass spectrometry ([M + Na]$^+$ *m/z* 345.2411, Δ 1.1 mmu), and this assignment was also supported by the ^{1}H and ^{13}C NMR spectral features of **3**. The IR spectrum for this metabolite showed broad absorption for multiple hydroxyl groups (ν_{max} 3366 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **3** showed signals for two secondary alcohols and one primary alcohol, a cyclopropane ring, and four methyl groups. Proton NMR COSY correlations defined the same four spin systems as in **1** and **2**. Heterocorrelation NMR methods, specifically HMQC and HMBC experiments, allowed all the protons and carbons to be assigned (Table 1). As in the other metabolites, numerous HMBC correlations were observed, but those between C-9 and H-8, H-14, and H-20, between C-5 and H-3, H-2, H-6, and H-18, between C-10 and C-11 and H-20, between C-13 and H-16 and H-17, between C-1, C-7, and C-8 and H-19, and between C-18 and H-5, H-3, and H-2 clearly established connections of the four spin systems and allowed the full structure of compound **3** to be assigned. As in **1**, NMR spectral analyses showed that the C-5 and C-9 alcohols were in equatorial positions. Comparisons of the NMR data obtained for **3** with that from the neoverrucosane **1** allowed the relative stereochemistry to be identically assigned. We assume that the absolute stereochemistry of **3** is identical to that derived for compound **1**, but this has not been rigorously shown.

Neoverrucosan-5β,9β-diol-4-carboxaldehyde (**4**) showed [M + Na]$^+$ *m/z* 345.3 by low-resolution electrospray mass spectrometry, indicating the molecular formula $C_{20}H_{34}O_3$. To firmly establish the molecular formula, HR-FABMS and MALDI-FTMS experiments were attempted several times without success. Hence, the molecular formula was assumed initially by comprehensive analysis of 2D NMR data (Table 1). Analysis of ^{1}H and ^{13}C NMR data showed signals for two secondary alcohols, one aldehyde, a cyclopropane ring, and four methyl groups, but showed the lack of olefinic protons or carbons. The IR spectrum of **4** showed broad absorption for multiple hydroxyl groups (ν_{max} 3351 cm^{-1}) and a sharp absorption for a conjugated aldehyde (ν_{max} 1696 cm^{-1}). The absence of olefinic protons and the presence of the conjugated aldehyde led to the conclusion that the cyclopropane ring must be conjugated with the carbonyl group. Rogers, Cromwell, and others carried out pioneering investigations defining conjugation of the cyclopropane ring.[11–13] They suggested that the conformational arrangement of the cyclopropyl group dictates the degree of p-orbital overlap with the adjacent carbonyl group and that conjugation is most effective when the plane of the ring and the p-orbitals of the carbonyl group are parallel.[14] The carbonyl and cyclopropane ring must be oriented such that the axes of their π-orbitals are parallel with the plane of the three-ring and symmetrically arranged with respect to the bent bonds.[15]

Molecular modeling shows that a favorable configuration affording conjugation exists in **4**. This configuration is stabilized by hydrogen bonding of the 5β hydroxyl group to the aldehyde carbonyl, forming a favorable six-membered ring. The UV spectrum of **4** showed λ_{max} (log ϵ) = 241 nm (2.16), in further support of the cyclopropyl conjugated aldehyde.

Proton NMR COSY correlations for **4** showed the same four spin systems as in **1**–**3**. The neoverrucosane **4** was thus recognized as the C-18 aldehyde derivative of com-

pound **1**. Treatment of the diterpenoid **4** with $LiBH_4$ in THF reduced the aldehyde and converted compound **4** to **3** in excellent yield. Comparison of rotation data, and all NMR spectral data, of the synthetic compound with the natural product **1** showed that they were identical. Hence, the comparable stereocenters in **4** are identical to those in compound **3**.

During the extract fractionation process, we also isolated (−)-neoverrucosan-5β-ol (**5**, $[\alpha]_D$ −16°, lit[17] $[\alpha]_D$ −10°), a neoverrucosane diterpenoid previously isolated from the sponge *Axinyssa aplysinoides*[16] and the liverworts *Mylvia verrucosa*[17] and *Schistochila rigidula*.[18]

Analysis of the literature shows that the production of diterpenoids is rare in bacteria. There is one important exception: the photosynthetic Gram-negative terrestrial bacterium *Chloroflexus aurantiacus* also produces diterpenoids.[19] *C. aurantiacus,* isolated from a hot spring, also uses gliding motility, and it is part of the green non-sulfur branch in bacterial phylogeny.[20] Remarkably, *C. aurantiacus* is reported to produce the related diterpenoid verrucosan-2β-ol (**6**).[19] In *C. aurantiacus*, this metabolite has been proposed to function in modulating membrane fluidity, in a manner analogous to hopanoids and steroids in other microorganisms.[19] *S. grandis* shares gliding motility with *C. aurantiacus*; however, it is an obligate marine bacterium and it is quite distinct both physiologically and phylogenetically. To assess whether the neoverrucosanes **1**−**4** might function as membrane components, we recultured *S. grandis* using the same conditions used for the isolation of **1**−**4**. The cells and broth were separated by centrifugation, each was extracted with 1:1 isooctane/ethyl acetate, and the crude extracts were analyzed by C_8 reversed-phase LCMS. Compounds **1**−**4** were identified in the broth extracts by their MS spectra and retention time comparison with authentic standards. The results of these experiments showed conclusively that the diterpenoids **1**−**4** were not present in the cells, but are excreted into the broth. These results suggest that the saprospirols are not part of the cell membrane in *S. grandis*.

The biosynthesis of the neoverrucosane terpenoids is known to occur via both the mevalonic acid and deoxyxylulose pathways. The neoverrucosane diterpenoids in the liverwort *Fossombronia alaskana* are fully labeled when [1-^{13}C]- and [U-$^{13}C_6$]-glucose is incorporated, suggesting the involvement of the deoxyxylulose pathway.[21] However, incorporation of singly or doubly ^{13}C-labeled acetate into the verrucosan-2β-ol (**6**), isolated from the bacterium *C. aurantiacus*, showed that the isoprenoid precursors were derived from the mevalonate pathway.[22] These results add to the observations that very similar compounds can be produced by different pathways. The biosynthetic origin of the neoverrucosane diterpenes in *S. grandis* remains a subject for further investigation.

Experimental Section

General Experimental Procedures. Optical rotations were measured in chloroform using a Rudolph Research Autopol III polarimeter with a path length of 10 cm at the sodium D line. UV spectra were obtained with a Beckman Coulter DU 640 spectrophotometer. IR spectra were obtained with a Perkin-Elmer 1600 Series FTIR spectrophotometer. Proton and carbon NMR spectra were recorded on Varian Inova Instruments at 400 or 300 MHz for ^{1}H and 75 MHz for ^{13}C. All spectra were recorded in deuteriochloroform, and chemical shifts were referenced to the deuteriochloroform solvent signal (δ 7.24/ 77.0 ppm). 1D and 2D NOE, ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMBC, and HMQC NMR experiments were also performed on these instruments, using the standard pulse sequences supplied with the instruments. Low-resolution mass spectra were obtained on a Hewlett-Packard MSD 1100 instrument, and high-resolution mass spectra were obtained using a IonSpec Ultima FTMS. Normal-phase column chromatography was carried out using Si gel (70−230 mesh). Reversed-phase HPLC separations were carried out using an Acuflow Series II pump, a Rainin Dynamax RI-1 photodiode array detector, and a Zorbax C_8 reversed-phase column (9.4 × 25 mm), at a flow rate of 3.0 mL/min. TLC analyses were performed using Whatman glass-backed plates coated with a 0.25 mm layer of Si gel 60 F_{254}.

Bacterial Strain and Cultivation. The gliding bacterium *Saprospira grandis,* originally isolated by R. A. Lewin and deposited as ATCC # 23116, was acquired from the American Type Culture Collection. The bacterium was inoculated in 40 × 1 L of "RL1" medium each containing 2 g of yeast extract (Difco), 3 g of peptone (Difco), 0.5 g of potassium nitrate, vitamin B_{12} (0.5 μg/L), trace metals (4.3 μg/L NaB_4O_7, 2.5 μg/L $FeSO_4$, 1.8 μg/L $MnCl_2$, 0.4 μg/L $CoCl_2$, 0.3 μg/L Na_2MoO_4, 0.4 μg/L $ZnCl_2$), and 1 L of seawater. After 2 days of growth at 37 °C on a shaker at 180 rpm, 20 g/L of autoclaved Amberlite XAD-16 resin was added to the culture to absorb the lipophilic compounds. The culture was then returned to the shaker for 5 additional days.

Extraction and Isolation. After 7 days the resin was filtered through cheesecloth and washed with 1 L of DI water to remove salts. The resin was allowed to dry at room temperature for 2 h and then eluted with 1 L of 1:1 isooctane/ethyl acetate, 1 L of 100% ethyl acetate, and 1 L of 100% methanol. The 1:1 isooctane/ethyl acetate fraction, which showed terpene signals in the ^{1}H NMR spectrum, was further separated by silica flash column chromatography eluting with an increasing gradient of ethyl acetate in isooctane to give 10 fractions, which were analyzed by TLC and NMR methods. Fractions that showed the presence of secondary metabolites were further fractionated by HPLC. Compounds **1** and **2** were isolated by reversed-phase HPLC chromatography using a C_8 column eluting with 10% H_2O/methanol at 3.0 mL/min flow. Compound **4** was isolated with the same method, but eluting the system with 20% H_2O/methanol. Compound **3** was isolated after reversed-phase HPLC chromatography using a C_{18} column with a gradient of water/methanol and then reversed-phase HPLC using a C_8 column eluting with 40% water/methanol. The yields of **1**−**4** were as follows: **1**, 5 mg/L, **2**, 2 mg/L, **3**, 2 mg/L, **4**, 0.5 mg/L.

Neoverrucosan-5β,9β-diol (1): viscous oil; $[\alpha]_D$ −2.39° (*c* 0.795, $CHCl_3$); IR (KBr) ν_{max} 3354 (broad), 2931, 1008, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MALDI $[M + H - 2H_2O]^+$ *m/z* 271.2419 and $[M + H - H_2O]^+$ *m/z* 289.2529.

Neoverrucosan-5β,18-diol (2): viscous oil; $[\alpha]_D$ −11.5° (*c* 0.175, $CHCl_3$); IR (KBr) ν_{max} 3401 (broad), 2907, 1008 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MALDI $[M + Na]^+$ *m/z* 329.2460.

Neoverrucosan-5β,9β,18β-triol (3): viscous oil; $[\alpha]_D$ −18.4° (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3366 (broad), 2919, 1014 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MALDI $[M + Na]^+$ *m/z* 345.2411.

Neoverrucosan-5β,9β,-diol-4-carboxaldehyde (4): viscous oil; $[\alpha]_D$ −101.3° (*c* 0.15, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (2.16) nm; IR (KBr) ν_{max} 3351 (broad), 2936, 1696, 1055, 1013 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; ESIMS *m/z* 345.3 $[M + Na]^+$.

Preparation of *S*- and *R*-MTPA Ester Derivatives of Neoverrucosan-5β,9β-diol (1). To a solution of compound **1** (5.0 mg in 2 mL of $CHCl_3$) were added sequentially *N,N*-diisopropylethylamine (27 μL), (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (3.81 μL/0.020 μmol), and catalytic amounts of pyridine and 4-(dimethylamino)pyridine (DMAP). In a separate experiment, the neoverrucosane **1** (5.1 mg) was treated with the (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as described above. Each mixture was heated at 50 °C for 3 h under N_2. The solutions were diluted with water and extracted with ethyl acetate. The crude ester

mixtures obtained were purified by silica HPLC eluting with 1:1 ethyl acetate/isooctane. Compound **1** treated with (*R*)-MTPA-Cl furnished the *S*-esters **1a** (32%) and **1c** (22%), while treatment of **1** with (*S*)-MTPA-Cl furnished the *R*-ester derivatives **1b** (36%) and **1d** (28%). Ester **1a** analyzed for the molecular formula $C_{30}H_{41}F_3O_4$ by ES mass spectrometry, $[M + Na]^+$ *m*/*z* 545, and showed the following ^{1}H NMR features: ($CDCl_3$, 300 MHz): δ 5.56 (1H, dd, J = 10.2, 7.8, H-5), 3.69 (1H, dd, J = 11.4, 4.2, H-9), 3.56 (3H, s, OCH_3), 2.11 (1H, m, H-15), 2.01 (1H, m, H-15), 1.12 (3H, s, H-18), 0.99 (3H, *s*, H-19), 0.91 (3H, d, J = 6.6, H-17), 0.84 (3H, d, J = 6.6, H-16), 0.75 (3H, s, H-20), 0.62 (1H, dd, J = 8.1, 4.8, H-3a), and 0.43 (1H, t, J = 5.1 H-3b). Ester **1b** analyzed for the molecular formula $C_{30}H_{41}F_3O_4$ by ESMS, $[M + Na]^+$ *m*/*z* 545, and showed the following ^{1}H NMR features ($CDCl_3$, 300 MHz): δ 5.57 (1H, dd, J = 10.2, 7.8, H-5), 3.68 (1H, dd, J = 11.4, 4.5, H-9), 3.60 (3H, s, OCH_3), 2.12 (1H, m, H-15), 2.01 (1H, m, H-13), 1.18 (3H, s, H-18), 0.99 (3H, s, H-19), 0.92 (3H, d, J = 6.6, H-17), 0.85 (3H, d, J = 6.3, H-16), 0.73 (3H, s, H-20), 0.71 (1H, dd, J = 7.8, 4.8, H-3a), and 0.51 (1H, t, J = 5.1, H-3b). The ester **1c** analyzed for the molecular formula $C_{30}H_{41}F_3O_4$ by ES mass spectrometry, $[M + Na]^+$ *m*/*z* 545, and showed the following ^{1}H NMR features ($CDCl_3$, 300 MHz): δ 4.08 (1H, dd, J = 7.2, 10.5, H-5), 5.11 (1H, dd, J = 11.7, 4.5, H-9), 3.52 (3H, s, OCH_3), 2.12 (1H, m, H-15), 2.04 (1H, m, H-13), 1.19 (3H, s, H-18), 0.98 (3H, s, H-19), 0.94 (3H, d, J = 6.6, H-17), 0.86 (3H, d, J = 6.6, H-16), 0.78 (3H, s, H-20), 0.64 (1H, dd, J = 8.1, 4.8, H-3a), and 0.33 (1H, t, J = 4.8, H-3b). Ester **1d** analyzed for the molecular formula $C_{30}H_{41}F_3O_4$ by ES mass spectrometry, $[M + Na]^+$ *m*/*z* 545, and showed the following ^{1}H NMR features: ($CDCl_3$, 300 MHz) δ 4.09 (1H, dd, J = 10.2, 7.2, H-5), 5.16 (1H, dd, J = 11.7, 4.5, H-9), 3.54 (3H, s, OCH_3), 2.16 (1H, m, H-15), 2.01 (1H, m, H-13), 1.20 (3H, s, H-18), 0.99 (3H, s, H-19), 0.93 (3H, d, J = 6.9, H-17), 0.85 (3H, d, J = 6.9, H-16), 0.73 (3H, s, H-20), 0.64 (1H, dd, J = 8.4, 4.5, H-3a) and 0.34 (1H, t, J = 4.8, H-3b).

Neoverrucosan-5β,18-diol Diacetate (2a). Neoverrucosan-5β,18-diol (**2,** 5 mg) was treated with acetic anhydride (1 mL) in dichloromethane (1 mL), pyridine (1 mL), and 4-(dimethylamino)pyridine (0.1 mL) for 3 h at room temperature. The solution was diluted with water and extracted with ethyl acetate to give the diacetate **2a** (90%), which analyzed for the molecular formula $C_{24}H_{38}O_4$ by ES mass spectrometry, $[M + Na]^+$ *m*/*z* 413. The diacetate showed the following ^{1}H NMR spectral features ($CDCl_3$, 300 MHz): δ 5.56 (1H, dd, J = 10.5, 7.8, H-5), 4.05 (1H, d, J = 11.4, H-18$_a$), 3.70 (1H, d, J = 11.4, H-18$_b$), 2.15 (1H, m, H-15), 2.05 (3H, s,-OAc), 2.04 (3H, s, −OAc), 1.95 (1H, m, H-13), 1.85 (1H, nr, H-6), 1.61 (1H, nr, H-12), 1.47 (1H, nr, H-12), 1.45 (1H, nr, H-9), 1.44 (1H, nr, H-8), 1.42 (1H, nr, H-11), 1.23 (1H, nr, H-14), 1.13 (1H, nr, H-8), 1.13 (1H, nr, H-11), 1.10 (1H, nr, H-1), 0.95 (3H, s, H-19), 0.93 (3H, d, J = 6.6, H-17), 0.86 (1H, nr, H-2), 0.85 (1H, nr, H-6), 0.83 (3H, d, J = 6.9, H-16), 0.82 (3H, s, H-20), 0.81 (1H, nr, H-3a), and 0.63 (1H, t, J = 5.1, H-3b).

$LiBH_4$ Reduction of Neoverrucosan-5β,9β,-diol-4-carboxaldehyde (4). To a solution of compound **4** (2 mg in 0.5 mL of THF) was added 37.5 μL of a 2 M solution of $LiBH_4$ in THF. The mixture was stirred for 2 h under N_2 at room temperature. Water was then added, the solution was extracted with ether (3 × 2 mL), and the ether extracts were combined and dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure, followed by C_8 reversed-phase HPLC separation of the crude product, eluting with 20% H_2O/80% methanol, yielded 1.7 mg (85%) of a triol which analyzed for the molecular formula $C_{20}H_{34}O_3$ by ES mass spectrometry, $[M + Na]^+$ *m*/*z* 345.3. Comparison of the ^{1}H NMR features, and the sodium D line rotation, of the product obtained with those recorded for compound **3** showed they were identical.

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Supporting Information Available: Spectral data for the new neoverrucosane diterpenoids **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP: The Supporting Information paragraph was missing in the version posted on the Web on May 30, 2003. The correct version was posted on June 19, 2003.

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