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Oxygenated Geosmins and Plant-Like Eudesmanes from a Bacterial Mangrove Endophyte

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ABSTRACT

Geosmin (**1**) is a microbial terpene metabolite that is responsible for the typical smell of soil and causes an off-odor of food and water. Eudesmane sesquiterpenes are commonly found in plant essential oils. Here we describe the discovery of four geosmin-type metabolites, 7R-hydroxygeosmin (**2**), 3-oxogeosmin (**3**), 2R-hydroxy-7-oxogeosmin (**4**), 5-deoxy-7 β ,9 β -dihydroxygeosmin (**5**), and the plant-like eudesmanes, 4 β ,10 α -eudesmane-5 β ,11-diol (**6**) and (1S,5S,6S,7S,10S)-10 α -eudesm-4(15)-ene-1 α ,6 α -diol (**7**), and the known 1(10)E,5E-germacradiene-2,11-diol (**8**), from a bacterial endophyte (*Streptomyces* sp. JMRC:ST027706) of the mangrove plant *Bruguiera gymnorrhiza*. By means of NMR, MS and ECD spectroscopy, all chemical structures as well as the absolute configurations for the new compounds were elucidated. Compounds **2–5** represent the first geosmin-related metabolites directly as bacterial natural products. The plant-derived eudesmane-5 β ,11-diol (**6**) and (1S,5S,6S,7S,10S)-10 α -eudesm-4(15)-ene-1 α ,6 α -diol (**7**) were reported as bacterial products. The broad antimicrobial activities of **6** against a suite of fungal and bacterial pathogens including methicillin-resistant *Staphylococcus aureus* suggests that this terpene could be an important active principle of the medicinal plant *Cymbopogon distans*. The discovery of geosmin metabolites from one actinomycete indicated that these bacteria could possess enzymes for modifying geosmin and offer a possibility for bioremediation.

Geosmin (**1**) is a terpene produced by a variety of microbes, mainly streptomycetes, cyanobacteria, myxobacteria, and fungi, that is responsible for the strong earthy-musty odor typical for freshly ploughed soil. Owing to its compact, 4,10-substituted decalin scaffold that lacks the typical isopropyl side chain of related eudesmane sesquiterpenoids, geosmin is highly volatile.¹ Because animals and humans are capable of sensing geosmin at extremely low thresholds, various ecological roles have been implicated.² For example, earthworms and springtails are attracted by geosmin as an indicator of humidity – and thus food source – and may in return play a role in the dispersal of streptomycetes spores.³ In contrast, geosmin detection by *Drosophila* flies helps them identifying unsuitable feeding and breeding sites.⁴ Generally, the ability of animals and humans to sense geosmin may raise the awareness of potentially dangerous microbes and thus helps to avoid rotten foods or contaminated water.⁵⁻⁶ Although geosmin does not exhibit any cytotoxicity in mammalian cell assays,⁷ it has received considerable attention due to its off-flavor in food and drinking water.⁸ Therefore, various avenues for geosmin degradation have been explored.⁹⁻¹⁰ A promising approach for bioremediation is the use of microbes that are able to modify geosmin.¹¹⁻¹² For example, two terpene-degrading bacteria, *Pseudomonas* sp. and *Rhodococcus wratislaviensis*, were reported to transform geosmin into its oxidation products, ketogeosmins and hydroxygeosmins.¹³ However, there is no report on geosmin degradation or transformation in typical geosmin producers such as actinomycetes, and naturally occurring geosmin derivatives are extremely scarce. Here we report the isolation, structure elucidation, and characterization of four oxygenated geosmin-type terpenes, along with two plant-like eudesmanes from a mangrove endophyte.

Results and Discussion

From the stem of the mangrove plant *Bruguiera gymnorrhiza*, we obtained the endophyte *Streptomyces* sp. JMRC:ST027706.¹⁴ Thin-layer chromatography (TLC) analysis of its extract showed typical blue to violet color reaction with anisaldehyde–sulfuric acid indicated the presence of bacterial terpenes.¹⁴ To elucidate the structures of these volatiles, the organic extract of a large scale fermentation broth (200 L) was subjected

to open-column chromatography on Amberchrom 161c resin, silica gel, and Sephadex LH-20, yielding compounds **2** (10.0 mg), **3** (2.2 mg), **4** (7.3 mg), **5** (2.1 mg), **6** (1.0 mg), **7** (2.1 mg), and **8** (0.6 mg), which were studied by NMR (Tables 1–2), MS and ECD spectroscopy. Due to the difficulties in ionization of compounds **2–5** using the electron spray ionization method, an electron ionization method was instead to be applied in providing informative data.

The ^1H NMR spectrum of compound **2** revealed the presence of one methyl singlet (δ 0.98, Me-12), one methyl doublet (δ 0.80, Me-11), three oxygen-bonding protons (δ 4.08, H-7; δ 3.55, 7-OH; δ 3.03, 5-OH) as well as other aliphatic protons. ^{13}C NMR data indicated the presence of carbon signals constituting a decahydronaphthalene backbone, which was further corroborated by 2D NMR, especially by HMBC correlations (**Figure 1**). The relative configuration of **2** was deduced from the NOESY spectrum showing correlations between 5-OH (7-OH) and H-6 β , Me-12 and H-6 α , and Me-11 and 5-OH (**Figure 1**). Thus, the structure of **2** was established as 7 β -hydroxygeosmin. Notably, a compound with the same structure has been reported as a synthetic product.¹⁵ Yet, the NMR spectra of the natural product and the synthetic compound differ in the coupling constant of H-7 with neighboring protons (synthetic, $J_{\text{HH}} = 10$ Hz; natural, broad singlet). 3D modeling (**Figure S6**) confirmed the small coupling constant for H-7 in the proposed structure of **2**. The synthetic compound may be a diastereoisomer, with the hydroxy group in an equatorial position (**Figure S6**).

Compound **3** was identified as another geosmin congener. The ^{13}C NMR spectrum showed 12 carbon signals, including a signal for one carbonyl group (δ 212.0, C-3) and one for an oxygen-bonding carbon (δ 78.7, C-5). We concluded that **3** is a geosmin derivative with a carbonyl group located at C-3 position. Its structure was proposed to be 3-oxo-geosmin. Correlations between H-4 and Me-12 in the NOESY spectrum established its relative configuration. The same compound has been reported as a geosmin biotransformation product by a *Pseudomonas* sp. that was investigated as a potential bioremediator strain.¹³ The ^{13}C NMR data of **3** are in agreement with the synthetic compound and confirms the relative configuration.¹⁶

The ^1H NMR spectrum of **4** indicated the presence of an oxygen-bonding methylene (δ 4.18, H-2), two methyls (δ 0.82, Me-11, δ 1.27, Me-12) as well as other aliphatic residues. In addition, the ^{13}C NMR spectrum showed a carbonyl signal (δ 212.1, C-7), which showed correlations with H-6, H-8 and H-9 in the HMBC spectrum (**Figure 1**). Together with COSY and HSQC correlations, compound **4** was established as 2 β -hydroxy-7-oxogeosmin. Its relative configuration was established by correlations between H-5 and Me-11, H-4 and Me-12, and H-2 and Me-12 in the NOESY spectrum (**Figure 1**). 2 β -Hydroxy-7-oxogeosmin, represents a new natural product with a decalin scaffold.

In the ^1H NMR spectrum of **5**, signals for two protons (δ 4.13, H-7, δ 3.31, H-9) indicating it being an alcohol. Two methyl groups (δ 0.83, Me-11, δ 0.79, Me-12) as well other aliphatic protons were observed. The location of two hydroxy groups at C-7 and C-9, was established by both COSY and HMBC correlations (**Figure 1**). The correlations between H-5 and 7-OH, 7-OH and 9-OH, H-5 and Me-11, and Me-12 and H-9 in the NOESY spectrum established its relative configuration (**Figure 1**). The new structure was established as 5-deoxy-7 β ,9 β -dihydroxygeosmin.

Natural geosmin is typically found as the (–)-enantiomer, irrespective of the producing organism (prokaryotes or eukaryotes).⁸ To elucidate the absolute configuration of **3** and **4**, we measured their ECD spectra and observed opposite Cotton effects between them (**Figure S6–S7**). According to the octant rule, we deduced the absolute configurations of all isolated geosmins and confirmed that they are related to the natural (–)-geosmin.

In addition to C₁₂-decalins **2–5** that are, like geosmin, derived from the truncation of a sesquiterpene precursor, we obtained congeners **6** and **7** as intact sesquiterpenes. The molecular formula of C₁₅H₂₈O₂ was deduced for compound **6** by HRMS. The ¹H NMR spectrum showed signals of three methyl groups appearing as singlets (δ 1.24 Me-12, δ 1.24 Me-13, δ 1.03 Me-14) and a signal of one methyl group appearing as a doublet (δ 0.83, Me-15). The ¹³C NMR spectrum exhibited 15 signals, including two of oxygenated carbons resonating at δ 74.3 (C-5) and δ 73.1 (C-11). 2D NMR spectra, in particular COSY and HMBC, revealed a eudesmane skeleton for compound **6**. The locations of the two oxygenated carbons were deduced from HMBC correlations, where correlations between Me-12 (Me-13) and C-7 (δ 40.8) and C-11 (δ 73.1), between Me-15 and C-5 (δ 74.3) and C-10 (δ 36.8), and between Me-15 and C-3 (δ 30.6) and C-5 (δ 74.3) were observed. Compound **6** represents 4 β ,10 α -eudesmane-5 β ,11-diol, which was reported as an essential oil component from the aromatic grass *Cymbopogon distans*.¹⁷ Its absolute configuration was confirmed by X-crystallography.¹⁷ Owing to the same specific rotation, both bacterial and plant eudesmane-5 β ,11-diol share the same absolute configuration. Eudesmanes are relatively rare as bacterial products. Only recently, (4S,5S,7R,10S)-4 β ,10 α -eudesmane-5 β ,11-diol (**6**) was reported as a bacterial sesquiterpene from *Streptomyces anulatus* isolated from *Giraffa camelopardalis*.¹⁸

The ¹H NMR spectrum of **7** exhibits signals of an exo-methylene (δ 4.96, δ 4.82, H-15), two oxygen-bonding methylenes (δ 3.92, H-1, δ 3.49, H-6), three methyl groups (δ 0.82, Me-12, δ 0.92, Me-13, δ 0.85 Me-14), and other aliphatic residues. The ¹³C NMR spectrum shows 15 carbons signals, including those for two olefinic (δ 145.4, δ 114.2) and two oxygen-bonding carbons (δ 68.1, δ 67.1). 2D NMR data (especially HMBC correlations) (**Figure 1**) corroborated the 4(15)-eudesmene-1,6-diol structure. The 2D NOESY

spectrum established the relative configurations from correlations between H-1 and H-6, H-5 and H-7/Me-14, respectively. Compound **7** was elucidated as 4(15)-eudesmene-1 α ,6 α -diol. Interestingly, both enantiomers were reported from plants, such as *Croton caudatus* var. *tomentosus*,¹⁹ *Guarea guidonia* (Meliaceae),²⁰ *Caragana intermedi*,²¹ *Eupatorium fortune*,²² and *Litsea verticillata*.²³ Recently, (+)-10 α -eudesm-4(15)-ene-1 α ,6 α -diol was prepared in total synthesis.²⁴⁻²⁵ Based on the negative specific rotation, compound **7** was confirmed to be (1S,5S,6S,7S,10S)-10 α -eudesm-4(15)-ene-1 α ,6 α -diol, which exhibited the opposite configuration with the synthetic one. This is the first report of (1S,5S,6S,7S,10S)-10 α -eudesm-4(15)-ene-1 α ,6 α -diol as a bacterial metabolite.

Finally, we found that compound **8** is identical with 1(10)*E*,5*E*-germacradiene-2,11-diol, which was previously reported as a metabolite of an endophyte (*Streptomyces griseus* subsp.) isolated from the mangrove plant *Kandelia candel*.²⁶

To test the antimicrobial activities of geosmins and other isolated terpenes, we performed agar diffusion assays against a suite of bacteria and fungi. Compounds **2** and **3** exhibited only weak antibacterial activities (Table 3). Thus, it is unlikely that those geosmin compounds act as antibiotics in nature. In stark contrast, we found that **6** exerts broad antimicrobial activities against all tested bacteria (*Bacillus subtilis*, *Mycobacterium vaccae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis* and *Escherichia coli*) and fungi (*Sporobolomyces salmonicolor*, *Candida albicans*, and *Penicillium notatum*). This finding is particularly remarkable since 4 β ,10 α -eudesmane-5 β ,11-diol (**6**) is the principal component (34%) of *Cymbopogon distans* essential oil^{16, 27} also known as lemongrass. The oil of *Cymbopogon* is used in traditional medicine to treat inflammation, colds and microbial infections.²⁸⁻²⁹ Moreover, the leaves of *Cymbopogon distans*

were employed for protecting books from insects and fungi in ancient China.³⁰ Our findings could rationalize these observations and applications.

Although many microorganisms have been known as producers of geosmin, reports on naturally occurring geosmin derivatives are extremely scarce. Only dehydrogeosmin was detected by mass spectrometry from the flower scent of Cactaceae.¹⁵ In this study we isolated several oxygenated geosmin congeners from an endophyte belonging to the genus *Streptomyces*, which is well-known as geosmin producers.³¹⁻³² The endophytic actinomycete has a remarkable potential to oxygenate the decalin core of geosmin and closely related terpenoids. These geosmin-modifying oxygenases could find application in water bioremediation. Moreover, we provide a rationale for the antimicrobial potency of the medicinal plant *Cymbopogon distans*. Overall, the discovery of the geosmin and eudesmane congeners underlines the immense biosynthetic potential of this mangrove endophyte.¹⁴ Beyond such implications for ecology, our findings may set the basis for biotechnological geosmin remediation.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a Propol digital automatic polarimeter (Dr. Wolfgang Kernchen GmbH). UV spectra were recorded on a Cary 1 Bio UV-visible spectrophotometer (Variant). ECD spectra were obtained on a Jasco J-810-150s spectropolarimeter. IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. NMR spectra were recorded on Bruker Avance III 500 MHz or Avance III 600 MHz spectrometers. Chemical shifts are given on the δ scale and referenced to CDCl₃ at 7.24 ppm for proton and 77.38 ppm for carbon. ESIMS data were obtained on a triple quadrupole mass spectrometer (Quattro; VG Biotech). HRESIMS were recorded on a Finnigan TSQ Quantum Ultra AM Thermo Electron. EI spectra were measured on Thermo Q-Exactive GC. Open column chromatography was performed on silica gel 60 (Merck, 0.04–0.063 mm, 230–400 mesh ASTM) and Sephadex LH-20 (Pharmacia). TLC analysis was performed on silica gel plates (Sil G/UV254, 0.20 mm, Macherey-Nagel). Preparative HPLC was performed on a Waters HPLC system using a Nucleosil 100-5 C18 column (5 μ m, 250 $\times$ 16 mm) with a UV detector.

Strain Isolation and Taxonomic Classification. JMRC:ST027706 was isolated from the stems of *Bruguiera gymnorrhiza*. Isolation and taxonomy can be found in the previous report.¹⁴

Fermentation. Liquid organic medium 79 (dextrose 10 g, bacto peptone 10 g, casamino acids 1 g, yeast extract 2 g, NaCl 6 g, H₂O 1 L) (2 × 100 mL/flask) was inoculated with a suspension of mycelium and spores (about 1 × 1 cm) of *Streptomyces* sp. JMRC:ST027706 grown on agar slants or agar plates. After incubation for 48 h on a rotary shaker at 28 °C, the culture was transferred to 3,200 mL of medium 27 (glucose 20 g, soybean powder 20 g, NaCl 5 g, CaCO₃ 3 g, 1 L H₂O, eight 1,000 mL scale Erlenmeyer flasks with 400 mL of medium 27 each) and incubated at 28 °C under shaking conditions for 48 h to yield pre-fermentation culture, which was poured into a 300 L scale fermenter filled with 200 L of medium 27 and fermented for 5 days.¹⁴

Extraction and Isolation. The fermentation broth of *Streptomyces* sp. JMRC:ST027706 was separated into culture filtrate and mycelia by centrifugation. The fermentation broth was filtered and loaded onto an Amberchrom 161c resin LC column (200 × 20 cm, 6 L). Elution with a linear gradient of H₂O/MeOH (from 30% to 100% v/v, flow rate 0.5 L min⁻¹, in 58 min) afforded seven fractions (F1–F7). F1–F5 were combined and extracted by EtOAc and evaporated to afford the extract (8 g). It was first fractionated by silica gel column chromatography with a CH₂Cl₂/MeOH gradient to yield four fractions, A–D. Fraction A was first fractionated by a Sephadex LH-20 (CH₂Cl₂, 50% MeOH) column, and six fractions (A1–A6) were obtained.¹ Fraction A1 was further separated by a RP-C₁₈ column (MeOH /H₂O gradient) to afford 17 fractions, FA1-1 to FA1-23. FA1-10 was separated by HPLC RP-C₁₈ (MeOH/H₂O as gradient) to afford **3** (2.2 mg). FA1-14 was separated by silica gel column chromatography (DCM/MeOH as gradient) to afford **5** (2.1 mg) and **7** (2.1 mg). FA1-17 was separated by silica gel column chromatography (CH₂Cl₂/MeOH as

gradient) to afford **8** (0.6 mg). FA1-18 was separated by silica gel column chromatography (CH₂Cl₂/MeOH as gradient) to afford **2** (10.0 mg), **4** (7.3 mg), and **6** (1.0 mg).

7R-Hydroxygeosmin (2): Colorless solid; $[\alpha]_D^{23} -6.2$ (*c* 0.1, MeOH); IR (film) $\nu_{\max}$ 3314, 1456, 1410, 1378, 1339, 1309, 1273, 1225, 1210, 1177, 1133, 1067, 1009, 988, 955, 938, 905, 874, 856, 833, 811, 715, 670, 638 cm⁻¹; ¹H NMR data, Table 1, ¹³C NMR data, Table 2; EIMS (14 eV): 198, 180, 165, 162, 151, 137, 126, 110, 86, 32; HREIMS (15 eV): *m/z* 198.1613 (calcd for C₁₂H₂₂O₂, 198.1614, Δ -0.50 ppm).

3-Oxogeosmin (3): colorless solid; $[\alpha]_D^{24} -30.5$ (*c* 0.07, MeOH); ECD (0.0036 mM, MeOH), $\lambda_{\max}$ ($\Delta\epsilon$) 284 (-83.2) nm; IR (film) $\nu_{\max}$ 3448, 2941, 1686, 1437, 1413, 1372, 1318, 1246, 1171, 1097, 1076, 1060, 1010, 958, 945, 888, 848, 652, 611 cm⁻¹; ¹H NMR data, Table 1, ¹³C NMR data, Table 2; EIMS (14 eV): 196, 178, 167, 154, 149, 140, 125, 123, 112, 106, 97, 82, 32. HREIMS (70 eV): *m/z* 196.14595 (calcd for C₁₂H₂₀O₂, 196.14578, Δ 0.87 ppm).

2R-Hydroxy-7-oxogeosmin (4): colorless solid; $[\alpha]_D^{24} +8.4$ (*c* 0.22, MeOH); ECD (0.011 mM, MeOH), $\lambda_{\max}$ ($\Delta\epsilon$) 292 (4.2) nm; ¹H NMR data, Table 1, ¹³C NMR data, Table 2; EIMS (14 eV): 196, 178, 167, 163, 149, 139, 122, 110, 101, 97, 82, 32; HREIMS (70 eV): *m/z* 196.14582 (calcd for C₁₂H₂₀O₂, 196.14578, Δ 0.18 ppm).

7S,9S-Dihydroxy 5-deoxygeosmin (5): colorless oil; $[\alpha]_D^{24} +2.9$ (*c* 0.06, MeOH); ¹H NMR data, Table 1, ¹³C NMR data, Table 2; EIMS (14 eV): 198, 180, 162, 147, 136, 124, 110, 95, 84, 73, 59, 49, 31; HREIMS (70 eV): *m/z* 198.1613 (calcd for C₁₂H₂₂O₂, 198.1614, Δ -0.42 ppm).

(4S,5S,7R,10S)-4 β ,10 α -Eudesmane-5 β ,11-diol (6): colorless oil; $[\alpha]_D^{24} -6.7$ (*c* 0.40, CHCl₃, reported -9.0, *c* 0.8, CHCl₃¹⁷); IR (film) $\nu_{\max}$ 3277, 2934, 2861, 1653, 1559, 1447, 1419, 1378, 1361, 1314, 1281, 1235, 1163, 1127, 1103, 1057, 1027, 1005, 956, 924, 899, 873, 831, 777, 667, 612 cm⁻¹; HRESIMS *m/z* [M + Na]⁺ 263.1981 (calcd for C₁₅H₂₈O₂Na, 263.1982, Δ 0.04 ppm).

(1S,5S,6S,7S,10S)-10 α -Eudesm-4(15)-ene-1 α ,6 α -diol (7): colorless oil; $[\alpha]_D^{23} -16.7$ (*c* 0.21, CHCl₃, reported -88, *c* 0.6, CHCl₃²¹); IR (film) $\nu_{\max}$ 3365, 2933, 2870, 1699, 1652, 1559, 1541, 1507, 1457, 1369, 1261, 1032, 986, 878, 799, 756, 679, 668, 630 cm⁻¹.

1(10)E,5E-Germacradiene-2,11-diol (8): colorless solid; $[\alpha]_D^{23} -30.4$ (*c* 0.10, MeOH, reported -42, *c* 0.52, MeOH²⁶).

Antimicrobial Assays. Sterile filter paper disks were impregnated with 50 μg of the samples using MeOH as the carrier solvent. The impregnated disks were then placed on agar plates previously inoculated with *Bacillus subtilis* ATCC 6633, *Mycobacterium vaccae* IMET 10670, *Pseudomonas aeruginosa* K799/61, *Pseudomonas aeruginosa* BG137 B7, *Staphylococcus aureus* SG511, *Staphylococcus aureus* 134/94 R9 (methicillin-resistant MRSA), *Enterococcus faecalis* 1528 R10 (vancomycin-resistant VRE), *Escherichia coli* SG458, *Sporobolomyces salmonicolor* SBUG 549, *Candida albicans* and *Penicillium notatum* JP36. Chloramphenicol (5 μg) and amphotericin (10 μg) were used as positive controls against bacteria and fungi, respectively. MeOH was used as a negative control. After the plates were incubated at 37 °C for 24 h, antimicrobial activity was recorded as clear zones (in mm) of inhibition surrounding the disk. The test sample was considered active when the zone of inhibition was greater than 7 mm.¹⁴

ASSOCIATED CONTENT

Supporting Information

NMR spectra of compounds **2–7**. The Supporting Information is available free of charge on the ACS Publications website at DOI:

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Notes

The authors declare no competing financial interest.

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Figure 1. Selected HMBC (single arrows) and key NOESY (double arrows) correlations of **2–5**.

Table 1. ¹H NMR Data for **2–5** (CDCl₃, 600 MHz for **2** and **5**; 500 MHz for **3** and **4**)

Position	¹ H NMR δ (J in Hz)			
	2	3	4	5
1	1.62, m, 1.43, m	2.03, td (13.2, 5.4), 1.75, m, 1.30, m 1.38, m		1.77, m, 1.10, m
2	0.98, m	2.47, ddd (15.1, 4.18, m 13.2, 7.2), 2.33, ddd (15.1, 5.4, 1.8)		1.57, m
3	1.35, m	–	1.85, m, 1.25, m	1.66, m, 0.97, m
4	1.62, m	2.57, q (6.7)	1.82, m	1.35, m
5	–	–	1.15, m	1.52, m
5-OH	3.03, s	–	–	–
6	1.87, m 1.48, dd (14.8, 3.5)	1.57, m	2.48, m, 2.27, m	1.91, m, 1.30, m
7	4.08, brs	1.57, m	–	4.13, m
7-OH	3.55, d (5.6)	–	–	2.91, d (6.7)
8	1.83, m, 1.67, m	1.51, m	2.45, m, 2.08, td (14.4, 0.8)	1.93, dd (5.0, 2.7), 1.91 t (3.2)
9	1.93, td (13.6, 3.9), 0.91, 1.62, m, 1.21, m ddd (13.6, 4.4, 2.6)		1.69, ddd (13.3, 6.8, 3.31, m 1.9), 1.39, td (13.3, 5.4)	
9-OH	–	–	–	2.62, d (5.3)
10	–	–	–	–
11	0.80, d (6.8)	0.95, d (6.8)	0.82, d (6.5)	0.83, d (6.4)
12	0.98, s	1.24, s	1.27, s	0.79, s

Table 2. ^{13}C NMR Data for **2–5** (CDCl_3 , 150 MHz for **2** and **5**; 125 MHz for **3** and **4**)

Position	^{13}C NMR, type			
	2	3	4	5
1	20.6, CH_2	35.5, CH_2	46.3, CH_2	34.7, CH_2
2	34.6, CH_2	37.9, CH_2	67.4, CH	21.4, CH_2
3	29.7, CH_2	212.0, C	42.8, CH_2	36.4, CH_2
4	34.0, CH	49.5, CH	27.5, CH	31.2, CH
5	76.4, C	78.7, C	50.8, CH	37.3, CH
6	34.8, CH_2	30.7, CH_2	41.2, CH_2	32.3, CH_2
7	68.0, CH	20.5, CH_2	212.1, C	68.3, CH
8	28.7, CH_2	20.3, CH_2	37.5, CH_2	33.3, CH_2
9	30.3, CH_2	35.1, CH_2	41.0, CH_2	76.9, CH
10	37.5, C	37.3, C	33.0, C	38.5, C
11	14.9, CH_3	6.6, CH_3	19.1, CH_3	20.1, CH_3
12	20.0, CH_3	20.7, CH_3	18.2, CH_3	16.4, CH_3

Table 3. Antimicrobial Activities of Compounds **2**, **3** and **6** (Agar Diffusion Assay, 50 μ g/paper disk).

<i>Cpd</i>	Test strains								
	<i>B.</i> <i>subtilis</i>	<i>S.</i> <i>aureus</i>	<i>E.</i> <i>coli</i>	<i>P.</i> <i>aeruginosa</i>	MRSA	VRE	<i>M.</i> <i>vaccae</i>	<i>S.</i> <i>salmonicolor</i>	<i>P.</i> <i>notatum</i>
2	10	0	10	10	0	0	0	0	0
3	10	0	0	10	0	10	0	0	0
6	12	12	10	11	11	11	14	12	12
chloramphenicol	29	17	23	24	0	17	20	-	-
amphotericin b	-	-	-	-	-	-	13	13	17

TOC Graphic