A Novel 5-HT Receptor Ligand and Related Cytotoxic Compounds from an Acid Mine Waste Extremophile

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Berkeley Pit Lake in Butte, Montana, is an acid mine waste reservoir rich in toxic metals. A *Pithomyces* sp. isolated from the Pit Lake yielded three tyrosine derivatives (**1**−**3**), one of which acts as a $5\text{-HT}_{(2a)}$ receptor ligand. This type of activity has been associated with migraine preventative and antihypertensive drugs. The isolation and characterization of compounds **1**−**3** and three sesquiterpenes (**5**−**7**) that have been isolated previously from higher plants are reported here.

The Berkeley Pit Lake mine waste system in Butte, Montana, is the second largest EPA Superfund site in North America. It includes Berkeley Pit Lake, an abandoned open-pit copper mine, 1500 feet deep and one mile across. Infiltrating groundwater seeps into the Pit, dissolving rich veins of pyrite and other minerals, generating acid in the process. There are currently 30 billion gallons of water in the Pit, with an inflow rate of 4 million gallons/day. The water is acidic (pH 2.7) and contaminated with high concentrations of dissolved metal sulfates (including 1200 ppm iron, 240 ppm copper, 290 ppm aluminum, and 650 ppm zinc).[1] Unfortunately, the Pit Lake system sits at the headwaters of the Columbia River. If the water rises another 200 feet, it will reach the critical overflow level. At the current rate of rise, the critical level will be reached in approximately 10 years.

The chemistry of the water and possible remediation methods of the Pit Lake have been studied for 20 years. Yet the microbial ecology has been neglected. With its low pH and high dissolved metal ion content, it was considered too toxic to support life. In 1995, our first water samples yielded many different fungi and bacteria. Subsequent collections (our own, and those of phycologist Grant Mitman) have yielded over 42 fungi and bacteria as well as euglenoids, algae, and protozoans.[2] Although conditions within the Pit Lake system are toxic for "normal" aquatic biota, these same conditions represent an ideal environment for extremophiles that can benefit from lack of competition in this unique habitat. This hostile environment may also select for species never before observed. Such species may produce novel chemistry that can be exploited in many ways. The organisms themselves may also be effective bioremediators of the mine wastewater in which they grow. Their metabolic byproducts could impact the overall ecology of the Pit Lake system by raising the pH of the Pit water, by providing nutrients for other heterotrophs, or by adsorbing metal contaminants. The research potential of this site is tremendous and may represent a renaissance for a geographic area characterized by years of mining, milling, and smelting waste.

We are in the process of systematically studying the Pit Lake microbes for biologically active compounds. In the early stages of this investigation we isolated a filamentous fungus from the surface waters that was identified as a *Pithomyces* Ellis = *Sporodesmia* species.[3] The $CHCl_3$ extract from this fungus was active in the brine shrimp lethality assay. Although several compounds have been reported from this genus in the past, they were either epipolythiopiperazine-3,6-diones (sporodesmins), cyclic depsipeptides, or polyketide derivatives[4,5] We report the isolation of three new cytotoxic tyrosine derivatives and three guaiane sesquiterpenes from this fungus.

The *Pithomyces* sp. was grown in potato dextrose broth that was acidified to pH 2.7 and shaken for 11 days. The culture was killed with MeOH, the mycelia were removed by filtration, and the filtrate was extracted with $CHCl_3$. This organic extract was active in the brine shrimp lethality assay, which was used to guide compound isolation. Sephadex LH-20 chromatography followed by RPHPLC gave the three cytotoxic compounds **1**−**3** and the three inactive sesquiterpenes **5**−**7**.

Compound **1** had a molecular formula of $C_{13}H_{18}O_2$ by HRCIMS. The ^1H NMR spectrum showed three isolated spin systems. The first was a *para*-disubstituted benzene ring with proton absorbances at δ 7.09 (2H, d, $J = 8.6$ Hz) and 6.80 (2H, d, $J = 8.6$ Hz). A chemical shift at δ 6.80 for an aromatic proton indicated either an *ortho* or *para* oxygen substituent. The second spin system indicated a prenylated moiety, with a methylene absorbance at δ 4.45 (d, $J = 6.7$ Hz) coupled to an olefinic proton at δ 5.46 (br t, $J = 6.7$ Hz), which was long-range coupled to two broad methyl singlets at δ 1.72 and 1.67. The third spin system included two mutually coupled methylene triplets at δ 3.77 and 2.76. Acetylation of **1** gave a monoacetate with a ^1H NMR spectrum identical to that of **2** and differing from that of **1** by a downfield shift of the methylene triplet from δ 3.77 to 4.21 and an additional methyl singlet at δ 2.02. These data indicated that the structures of compounds **1** and **2** are as shown.

The HRMS of compound **3** indicated a molecular formula of $C_{16}H_{21}NO_4$. The compound readily formed a methyl ester (**4**) with diazomethane, indicating the presence of a carboxylic acid. The IR spectrum showed an acid carbonyl stretch at 1734 cm^{-1} and an additional carbonyl stretch at 1647 cm^{-1}, attributed to an amide. The ^1H NMR spectrum

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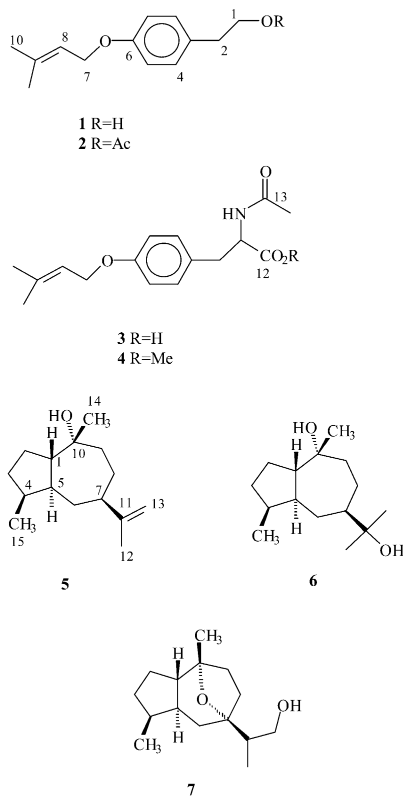

of **3** exhibited the *para*-disubstituted aromatic ring and prenylated side chain of **1** and **2**. The ^{1}H NMR spectrum of **3**, however, showed that the coupled methylenes in **1** and **2** were replaced with a broad multiplet at δ 4.57 (1 H) and two absorbances at δ 3.10 (1H, J = 13.7, 3.9 Hz) and 2.85 (1H, J = 13.7, 8.7 Hz). There was an additional singlet at δ 1.88 (3H). ^{13}C NMR, HSQC, and HMBC spectra supported assignment of the structure to that of the prenylated, *N*-acetylated tyrosine derivative **3**.

Simple aromatic amino acid derivatives often exhibit neurotransmitter activity. Therefore, compounds **1**–**4** were evaluated using the 5-HT$_{(2a)}$ receptor. Studies suggest that 5-HT$_{(2a)}$ (serotonin) receptor antagonists might act as migraine preventatives[6] or as antihypertensive agents.[7] This assay determines the ability of a compound to displace radiolabeled ketanserin from the binding site of the rat 5-HT$_{(2a)}$ receptor. Since 100% of the control is bound in the absence of the test substances, positive results are indicated by lower percent binding of ketanserin. Smaller numbers indicated positive results as less ketanserin is bound when the test compound is a good displacer. Compounds were tested at 100 μM concentrations.[8,9] Compounds **1**, **3**, and **4** resulted in ketanserin percent binding of approximately 85%, while compound **2** lowered the ketanserin binding to 11%. These data suggest that **2** acts as a 5-HT$_{(2a)}$ receptor ligand, while the other compounds are only marginally active. Drugs that act as 5-HT$_{(2a)}$ ligands include ketanserin, methysergide, the tricyclic antidepressant amitryptiline, and certain calcium channel and beta blockers.[6]

In addition to these prenylated tyrosine derivatives, the $CHCl_3$ extract yielded three sesquiterpenes, **5**–**7**. These compounds were not isolated because of their activity, but rather because the ^{1}H NMR spectra of crude fractions suggested the presence of sesquiterpenes, which are more commonly found in higher plants. Compound **5** gave a $(M + 1)^+$ peak in CIMS at m/z 223, which corresponded to a molecular formula of $C_{15}H_{26}O$ and three sites of unsaturation. EIMS of compound **5** gave an $(M - 18)^+$ at m/z 204 that showed a loss of H_2O. The ^{13}C NMR spectrum showed two olefinic carbons typical of an exocyclic methylene double bond, one oxygen-bearing quaternary carbon, four methines, five methylenes, and three methyl groups. One of the methyl groups was a doublet in the ^{1}H NMR spectrum, and the other two were attached to quaternary centers. These data required a bicyclic structure for **5**. Detailed analysis of the ^{1}H–^{1}H COSY and HMBC spectra led to assignment of the guaiane ring system. The ^{1}H NMR and IR spectral data for this compound were identical to those of pogostol, reported from the oil of the patchouli plant, *Pogostemon cablin*.[10] The ^{13}C NMR spectral data for pogostol were not previously reported and are reported here. NOESY and 1D difference NOE experiments show that the relative stereochemistry for pogostol and compound **5** are the same, while optical rotation data show that the absolute stereochemistry is also the same.

The molecular formula for sesquiterpene **6** was also established by HRCIMS as $C_{15}H_{28}O_2$. The ^{13}C NMR spectral data showed no sp^2-hybridized carbons, two oxygen-bearing quaternary carbons, and an additional singlet methyl signal when compared to spectral data for compound **5**. The molecular formula indicated a bicyclic skeleton. These data were accommodated by structure **6**. Detailed analysis of the ^{1}H–^{1}H COSY and HMBC spectra were consistent with the proposed structure. Analysis of the NOESY and 1D NOE data suggested that the relative stereochemistries of compounds **5** and **6** were the same. Although 11-hydroxypogostol has been reported from the arial parts of *Leuceria floribunda*, compound **6** has a *trans* ring juncture consistent with compound **5** and pogostol, while 11-hydroxypogostol has a *cis* ring juncture.[11]

HRCIMS established the molecular formula of compound **7** as $C_{15}H_{26}O_2$. Sesquiterpene **7** lacked carbonyl or olefinic carbons but had two quaternary and one primary oxygen-bearing carbons. The molecular formula required three rings, accounted for by the bicyclic guaiane skeleton and the cyclic ether. Acetylation of **7** yielded a monoacetate, and the ^{1}H NMR spectrum showed a downfield shift of the methylene protons at C-13. The relative stereochemistry was established from the NOESY and 1D NOE difference spectra and was found to be identical to that of compounds **5** and **6**, indicating the structure of **7** as shown.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were run on Bruker DPX-300 or DRX-500 spectrometers. ^{1}H NMR spectra were recorded at 500 MHz and the ^{13}C NMR spectra were recorded at 125 MHz unless otherwise noted. All of the chemical shifts were recorded with respect to the deuterated solvent shift ($CDCl_3$, δ 7.24 for the proton resonance and δ 77.0 for the carbon; CD_3OD, δ 3.30 for the proton resonance and δ 47.9 for the carbon). IR spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter using a 1 mL cell. Mass spectral data were provided by the Montana State Mass Spectrometer Facility at Montana State University. All solvents used were spectral grade.

Collection, Extraction, and Isolation Procedures. Water samples from different depths of the Berkeley Pit Lake were collected in sterile plastic containers.[12] Drops of these water samples were placed on sterile agar plates, and the plates were incubated at room temperature for 3–10 days, during which time proliferating hyphae and bacterial colonies were transferred to fresh DIFCO mycological agar or acidified (pH 2.7) potato dextrose agar plates. Microbes were established as pure cultures using standard procedures. Each microbe was grown in a variety of microbiological media either shaken for

6 days or still for 21 days. At time of harvest, organisms were killed by the addition of 20 mL of MeOH, then homogenized with an Omnimixer. The cultures were extracted with 100 mL of $CHCl_3$ (3×), and the organic layer was reduced in vacuo to an oil. The aqueous layer was lyophilized, then thoroughly extracted with $CHCl_3$/MeOH (1:1, v/v). The freeze-dried extract (FDX) and the $CHCl_3$ extract were tested against the following bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Vibrio harveyii*, and *Escherichia coli*; and fungi: *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium oxysporum*. The extracts were also tested for brine shrimp lethality. Microbes that produced active extracts were marked for further study.

A fungus identified as a *Pithomyces* sp. by Microbial Identification, Inc. was chosen for study. The $CHCl_3$ extract of the *Pithomyces* pilot culture showed no antimicrobial activity, but good brine shrimp lethality. The fungus was regrown in 32 × 200 mL of DIFCO potato dextrose broth (acidified to pH 2.7 with H_2SO_4) in 500 mL Erlenmeyer flasks for 11 days, shaken at 200 rpm. At harvest time, the fungus was killed with the addition of 20 mL of MeOH/flask. The culture was filtered through cheesecloth to remove the mycelial mat. The filtrate was extracted three times with $CHCl_3$ (1 L), and the extract was reduced in vacuo to an oil (287 mg). This $CHCl_3$ extract demonstrated good brine shrimp lethality. All subsequent isolation procedures were guided by this bioassay.

The $CHCl_3$ extract was fractionated using a Sephadex LH-20 column (3 cm × 115 cm, $CHCl_3$/MeOH, 1:1). Fractions E and F were further fractionated by preparative RPHPLC on a Rainin C-4 column with a MeOH/H_2O gradient to give pure brine shrimp lethal compounds **1** (2.2 mg), **2** (1.3 mg), and **3** (15.3 mg), and RPHPLC with a Rainin C-8 column to afford the sesquiterpenes **5** (11.2 mg), **6** (2.0 mg), and **7** (1.1 mg).

Neurotransmitter Assay. Details published elsewhere.[8,9]

Cell Culture. NIH 3T3 cells expressing the rat 5-$HT_{(2a)}$ receptor (gift of Dr. David Julius; UCSF) were cultured in DMEM fortified with 10% calf serum and 200 ug/mL geneticin. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Cells were subcultured or assayed upon confluency (5−8 days).

Receptor Preparation. Cells were harvested by trypsinization and centrifuged at low speed in ice-cold medium. The pellet was resuspended in ice-cold Earle's Balanced Salt Solution followed by centrifugation. Pelleted cells were resuspended in 10 mL of ice-cold bindng buffer (50 mM Tris, 4 mM $CaCl_2$, 10 μM pargyline, pH 7.4), homogenized with Teflon-glass, and centrifuged for 450 000 *g* min at 4 °C. The pellet was resuspended in 30 mL of ice-cold binding buffer and homogenized, first with Teflon-glass and then with a Polytron (setting 4) for 5 s. The crude membranes were assayed immediately.

Drug-Receptor Binding. Binding of the agonist followed well-characterized in vitro protocols.[8,9] Reaction mixtures, 1 mL in triplicate, were incubated for 30 min in a 30 °C shaker bath. Composition of the 1 mL reaction mixture was 700 μL of receptor preparation, 100 μL of either binding buffer (for total binding) or 10 μM 5-HT (final concentration for nonspecific binding), 100 μL of the tritiated agonist (final concentration of 0.5 nM), and 100 μL of either displacer (various concentrations) or binding buffer in the case of controls. Reactions were stopped by addition of 4 mL of ice-cold 50 mM Tris buffer, pH 7.4, and subsequent vacuum filtration on glass fiber filters (Whatman GF/B). Filters were rinsed twice in 5 mL of ice-cold Tris buffer and counted in 5 mL of Ecoscint (National Diagnostics) liquid scintillation fluid in a Beckman LS 6500 instrument. The procedures for the 5-$HT_{(2a)}$ receptor were parallel with the following exceptions: 10 μM mianserin was added for the nonspecific determinations; radioligand was the antagonist, ^{3}H-ketanserin (0.2 nM); filters were S & S #32.

Compound 1: clear oil, UV (MeOH) λ_{max} (log ϵ) 204 (3.88), 224 (3.78), 277 (3.07); IR ($CHCl_3$) ν_{max} 3350, 2928, 2870, 1617, 1511, 1384, 1236, 1000, 822 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.09 (2H, d, J = 8.6 Hz, H-4), 6.80 (2H, d, J = 8.6 Hz, H-5), 5.46 (1H, t, J = 6.7 Hz, H-8), 4.45 (2H, d, J = 6.7 Hz, H-7), 3.77 (2H, d, J = 8.0 Hz, H-1), 2.76 (2H, t, J = 8.0 Hz, H-2), 1.72 (3H, br s, H-10), 1.67 (3H, br s, H-11); ^{13}C NMR ($CDCl_3$) δ 157.7 (s, C-6), 131.8 (s, C-9), 130.1 (s, C-3), 129.9 (d, 2C, C-4), 119.6 (d, C-8), 114.7 (d, 2C, C-5), 64.7 (t, C-7), 63.8 (t, C-1), 38.2 (t, C-2), 25.8 (q, C-10), 18.2 (q, C-11); EIMS *m/z* 206 (2), 138 (38), 107 (100), 69 (27); HREIMS *m/z* 206.1312 (M^+ calcd for $C_{13}H_{18}O_2$, 206.1307).

Acetylation of 1. Compound **1** (0.5 mg) was dissolved in pyridine (50 μL) and Ac_2O (50 μL) and stirred for 24 h. The solvents were removed in vacuo to give **2** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.10 (2H, d, J = 8.6 Hz, H-4), 6.84 (2H, d, J = 8.6 Hz, H-5), 5.47 (m, H-8), 4.46 (2H, d, J = 6.7 Hz, H-7), 4.21 (2H, t, J = 7.0 Hz, H-1), 2.85 (2H, t, J = 7.0 Hz, H-2), 2.20 (3H, s, Ac), 1.77 (3H, br s, H-10), 1.72 (3H, br s, H-11); EIMS *m/z* 248 (1), 188 (25), 120 (100).

Compound 3: white solid, $[\alpha]^{25}_D$ +42.5° (*c* 1.46, MeOH); UV (MeOH) λ_{max} (log ϵ) 277 (3.17), 229 (3.68), 212 (3.79); IR ($CHCl_3$) ν_{max} 3314, 2923, 1734, 1647, 1610, 1509, 1235, 1003 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.09 (2H, d, J = 8.6 Hz, H-4), 6.80 (2H, d, J = 8.6 Hz, H-5), 5.42 (br t, J = 6.6 Hz, H-8), 4.57 (br m, H-1), 4.46 (2H, d, J = 6.6 Hz, H-7), 3.10 (dd, J = 13.7, 3.9, H-2), 2.85 (dd, J = 13.7, 8.7, H-2), 1.88 (3H, s, H-14), 1.75 (3H, s, H-10), 1.71 (3H, s, H-11): ^{13}C NMR (CD_3OD) δ 172.8 (s, C-13), 171.1 (s, C-12), 157.8 (s, C-6), 136.4 (s, C-9), 129.2 (2C, d, C-4), 128.3 (s, C-3), 119.3 (d, C-8), 113.6 (2C, d, C-5), 63.8 (t, C-2), 35.6 (d, C-1), 23.9 (q, C-10), 20.3 (q, C-14), 16.2 (q, C-11); EIMS *m/z* 291 $[M]^+$ (0.7), 223 (10), 107 (100), 69 (45); CIMS *m/z* 292 $[M + 1]^+$ (100); HREIMS *m/z* 223.0841 ($M^+ - C_5H_9$ calcd for $C_{11}H_{13}NO_4$, 223.0844).

Methylation of 3. Compound **3** (1 mg) was dissolved in 100 μL of MeOH, and a solution of diazomethane in diethyl ether added until the solution stayed yellow. The excess diazomethane and solvents were removed with a stream of nitrogen to give 1 mg of a clear oil (**4**): ^{1}H NMR ($CDCl_3$) δ 6.96 (2H, d, J = 8.7 Hz, H-4), 6.82 (2H, d, J = 8.7 Hz, H-5), 5.85 (br d, NH), 5.46 (br t, J = 6.8 Hz, H-8), 4.83 (m, H-1), 4.46 (2H, d, J = 6.8 Hz, H-7), 3.71 (3H, s, Me ester), 3.04 (2H, m, H-2), 1.97 (3H, s, H-14), 1.78 (3H, s, H-10), 1.72 (3H, s, H-11); EIMS *m/z* 305 (2), 254 (2), 178 (100), 107 (94), 67 (50); HREIMS 305.1643 (M^+ calcd for $C_{17}H_{23}NO_4$, 305.1627).

Compound 5: clear oil, $[\alpha]^{25}_D$ −19.9° (*c* 0.93, MeOH); IR ($CHCl_3$) ν_{max} 3440, 2950, 2870, 1643, 1455, 1377, 1098, 891 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.64 (br s, H-13), 4.56 (br s, H-13), 2.23 (m, H-7), 2.10 (m, H-1), 2.20 (2H, m, H-4, H-5), 1.89 (m, H-9), 1.80 (m, H-8), 1.68 (5H, m, H-2, H-3, H-12), 1.52 (2H, m, H-2, H-9), 1.40 (2H, m, H-6, H-8), 1.25 (2H, m, H-3, H-6), 1.17 (3H, s, H-14), 0.86, d, J = 6.8 Hz, H-15); ^{13}C NMR ($CDCl_3$) δ 152.4 (s, C-11), 107.7 (t, C-13), 74.9 (s, C-10), 55.3 (d, C-1), 46.1 (d, C-7), 45.8 (d, C-5), 38.9 (d, C-4), 36.0 (t, C-9), 31.1 (t, C-3), 29.7 (q, C-14), 28.5 (2C, t, C-8, C-6), 26.1 (t, C-2), 19.9 (q, C-12), 16.1 (q, C-15); EIMS *m/z* 204 $[M - H_2O]^+$ 204 (12), 189 (23), 161 (25), 121 (43), 107 (100), 55 (84); HRCIMS *m/z* 222.2216 ($[M + NH_4 - H_2O]^+$ calcd for $C_{15}H_{28}N$; 222.2222), 205.1966 ($[M + 1 - H_2O]^+$ calcd for $C_{15}H_{25}$, 205.1956).

Compound 6: clear oil, $[\alpha]^{25}_D$ −25.0° (*c* 0.040, MeOH); IR ($CHCl_3$) ν_{max} 3445, 2955, 2870, 1459, 1375, 1169, 1097, 907 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (m, H-1), 2.00 (m, H-4), 1.90 (3H, m, H-5, H-8, H-9), 1.69 (3H, m, H-2, H-3, H-6), 1.60 (2H, m, H6, H-7), 1.47 (2H, m, H-2, H-9), 1.24 (2H, m, H-3, H-8), 1.16 (3H, s, H-12), 1.14 (3H, s, H-13), 1.12 (3H, s, H-13), 0.92 (3H, d, J = 6.8 Hz, H-15), 0.87 (m, H-6); ^{13}C NMR ($CDCl_3$) δ 74.8 (s, C-10), 74.2 (s, C-11), 55.4 (d, C-1), 48.9 (d, C-7), 47.0 (d, C-5), 39.1 (d, C-4), 33.3 (t, C-9), 31.2 (q, C-14), 30.7 (t, C-3), 27.6 (q, C-12), 25.5 (q, C-13), 24.3 (t, C-8), 22.6 (t, C-6), 16.2 (q, C-15); EIMS *m/z* 222 $[M - H_2O]^+$ (2), 204 (8), 189 (12), 149 (37), 81 (56), 59 (100); HRCIMS *m/z* 240.2334 ($[M + NH_4 - H_2O]^+$ calcd for $C_{15}H_{30}NO$, 240.2327); 222.2181 ($[M + NH_4 - 2H_2O]^+$ calcd for $C_{15}H_{28}N$, 222.2222).

Compound 7: clear oil, $[\alpha]^{25}_D$ −13.6° (*c* 0.11, MeOH); IR ($CHCl_3$) ν_{max} 3456, 2956, 2871, 1459, 1377, 1102, 1026, 897 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.67 (br t, J = 10.5 Hz, H-12), 3.45 (dd, J = 10.5, 3.5 Hz, H-12), 2.43 (m, H-1), 2.09 (m, H-11), 2.01 (m, H-5), 1.98 (m, H-4), 1.82 (m, H-6), 1.71 (m, H-9), 1.63 (2H, m, H-2, H-3), 1.52 (m, H-8), 1.37 (m, H-9), 1.25 (7H, m,

H-2, H-3, H-8, H-9, H-16), 0.90 (3H, d, $J = 6.8$ Hz, H-15), 0.80 (3H, d, $J = 6.6$ Hz, H-13), 0.79 (m, H-6); ^{13}C NMR ($CDCl_3$) δ 84.9 (s, C-7), 82.4 (s, C-10), 66.0 (t, C-12), 50.6 (d, C-1), 43.0 (d, C-11), 40.6 (t, C-8), 38.7 (d, C-5), 37.2 (d, C-4), 33.1 (t, C-3), 29.2 (t, C-9), 29.1 (q, C-14), 26.8 (t, C-6), 26.2 (t, C-2), 14.6 (q, C-15), 12.2 (q, C-13); EIMS *m/z* 220 ($M^+ - H_2O$) (4), 202 (8), 161 (32), 143 (35), 121 (55), 95 (90), 81 (94), 55 (100); HRCIMS *m/z* 339.2050 ($[M + 1]^+$ calcd for $C_{15}H_{27}O_2$, 239.2011); 221.1928 ($[M + 1 - H_2O]^+$ calcd for $C_{15}H_{25}O$, 221.1905).

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