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Oxygenated Pimarane Diterpenes from *Kaempferia marginata*

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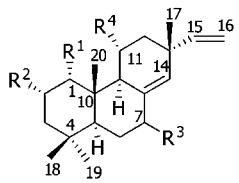
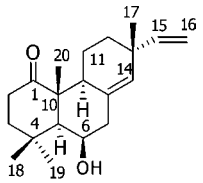
Six new diterpenes, (1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2,7-trihydroxypimara-8(14),15-diene (**1**), (1*R*,2*S*,5*S*,9*S*,-10*S*,11*R*,13*R*)-1,2,11-trihydroxypimara-8(14),15-diene (**2**), (1*S*,5*S*,7*R*,9*R*,10*S*,11*R*,13*R*)-1,7,11-trihydroxypimara-8(14),15-diene (**3**), (1*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,11-dihydroxypimara-8(14),15-diene (**4**), (5*S*, 6*R*,9*S*,-10*S*,13*R*)-6-hydroxypimara-8(14),15-diene-1-one (**5**), and (1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2-dihydroxypimara-8(14),15-diene-7-one (**6**), along with four known diterpenes, have been isolated from the dichloromethane extract of whole plants of *Kaempferia marginata*. The structures were assigned by spectroscopic methods. The absolute configuration of **1** was established by the Mosher ester method. Substances obtained were evaluated against a panel of bioassays including antimalarial, antituberculous, and antifungal activity.

Kaempferia marginata Carey (Zingerberaceae, local name: tup mup) is a Thai medicinal plant, and its roots have been used in the treatment of allergy, fever, and swollen leg.[1] Chemical constiuents that have been reported for the genus *Kaempferia* include cyclohexane oxide derivatives,[2] chalcone derivatives,[3] cinnamates,[4] diterpenes,[5,6] monoterpenes,[7] and flavonoids.[8] A preliminary biological assay on *K. marginata* indicated that the dichloromethane extract of whole plants exhibited activity against the malarial parasite *Plasmodium falciparum* with an IC_{50} value of 26.4 μg/mL. In the present study on this plant we have isolated six new pimarane-type diterpenes, namely, (1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2,7-trihydroxypimara-8(14),-15-diene (**1**), (1*R*,2*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,2,11-trihydroxypimara-8(14),15-diene (**2**), (1*S*,5*S*,7*R*,9*R*,10*S*,11*R*,13*R*)-1,7,-11-trihydroxypimara-8(14),15-diene (**3**), (1*S*,5*S*,9*S*,10*S*,11*R*,-13*R*)-1,11-dihydroxypimara-8(14),15-diene (**4**), (5*S*,6*R*,9*S*,-10*S*,13*R*)-6-hydroxypimara-8(14),15-diene-1-one (**5**), and (1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2-dihydroxypimara-8(14),15-diene-7-one (**6**), along with four known compounds, sandaracopimaradiene,[9] sandaracopimaradien-1α-ol,[10] 2α-acetoxysandaracopimaradien-1α-ol,[5] and sandaracopimaradien-1α,2α-diol.[5] We herein report the isolation, structure elucidation, and biological activity of these compounds.





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1 $R^1 = R^2 = OH$, $R^3 = \beta$-OH, $R^4 = H$
2 $R^1 = R^2 = R^4 = OH$, $R^3 = H$
3 $R^1 = R^4 = OH$, $R^3 = \alpha$-OH, $R^2 = H$
4 $R^1 = R^4 = OH$, $R^2 = R^3 = H$
6 $R^1 = R^2 = OH$, $R^3 = O$, $R^4 = H$

Results and Discussion

Compound **1** was isolated as a white solid, mp 131−133 °C. A molecular formula of $C_{20}H_{32}O_3$ was determined from HRFABMS. The ^{13}C NMR spectrum indicated 20 carbon signals, including four methyls, five methylenes, seven methines, and four quaternary carbons. The ^1H NMR spectrum displayed signals characteristic for vinylic protons at δ_H 5.82 (1H, dd, J = 17.4, 11.2 Hz, H-15), 4.95 (1H, dd, J = 17.5, 1.3 Hz, H-16a), and 4.93 (1H, dd, J = 11.2, 1.3 Hz, H-16b), as well as an olefinic proton at δ_H 5.67. Three oxygenated methine groups were observed at δ_H 4.00 (δ_C 72.1, d), 3.91 (δ_C 66.8, d), and 3.71 (δ_C 74.8, d) in addition to four tertiary methyl group singlet signals at δ_H 1.09, 0.99, 0.93, and 0.83. On the basis of the molecular formula and the presence of two unsaturated double bonds inferred from the ^1H and ^{13}C NMR data, it was apparent that three rings were present in the molecule. The long-range $^1H-^{13}C$ NMR correlations between a methyl proton signal at δ_H 1.09 and the ^{13}C signal at δ_C 148.1(d) as well as ^3J correlations between a vinylic proton at δ_H 4.95 and 4.93 and a quaternary ^{13}C signal at δ_C 37.0 indicated a pimarane diterpene skeleton with a vinyl group attached to C-13.[11] The $^1H-^{13}C$ HMBC NMR correlations between H-1/C-2, C-3, C-5, C-10, and C-20; H-3/C-2, C-18, and C-19; H-6/C-5, C-7, and C-10; and H-7/C-6, C-8, and C-14 placed the three hydroxyl groups at C-1, C-2, and C-7, respectively. The ^3J correlations between H-14/C-7, C-9, C-12, C-13, C-15, and C-17 indicated the double bond at C-8(14). The NOESY spectrum of **1** showed correlations between H-1, H-2, H_3-18, and H_3-20, indicating that both OH-1 and OH-2 adopt an α-orientation. NOE correlations between H-7/H-5 and H-9 as well as the large vicinal coupling constant (J = 11.4 Hz) between H-6a and H-7α detected from the H-7 signal at δ_H 4.00 (ddd, J = 11.4, 5.5, and 1.0 Hz) further indicated the β-oriented hydroxyl group at C-7. The NOE correlation between H_3-17 and H-11β could also be observed.

The absolute stereochemistry of **1** was determined using the Mosher ester method. The presence of vicinal hydroxyl groups on C-1 and C-2 was not favorable for the application of this method. The 1,2-diol was converted to the 1,2-acetonide prior to the esterification step, which was further treated with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (Experimental Section) at room temperature. MTPA (methoxytrifluoromethyl phenylacetic acid) esters of the 1,2-acetonide of **1**, which showed the observed chemical shift differences ($\Delta\delta_{S-R}$) indicated in Figure 1, unambiguously determined the absolute con-

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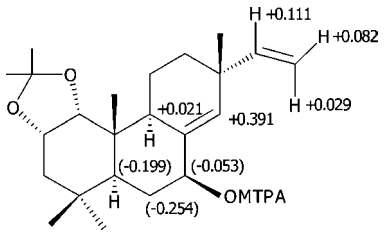

Figure 1. Distribution of $\Delta\delta_{S-R}$ Values of the *S*- and *R*-MTPA esters of the 1,2-acetonide of **1**.

figuration of C-7 of **1** to be *S*. Compound **1** was thus assigned as (1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2,7-trihydroxypimara-8(14),15-diene.

Compound **2** was obtained as a colorless solid, mp 93−95 °C. A positive ion HRFABMS showed a $[M + H]^+$ ion at *m/z* 321.2433, corresponding to the molecular formula $C_{20}H_{32}O_3$. Absorption bands at ν_{max} 3354, 1640, 960, and 759 cm^{-1} in the IR spectrum of **2** suggested the presence of hydroxyl and unsaturation groups, respectively. The ^1H NMR spectrum of **2** was similar to that of **1** except for the presence of an oxymethine proton at δ_H 4.10 (ddd, $J = 12.0$, 7.1, and 4.3 Hz) instead of a multiplet signal at δ_H 4.00 as found for H-7 in **1**. HMBC correlations observed between the oxymethine proton at δ_H 4.10 and the ^{13}C signals at δ_C 44.0 (t, C-12), 52.7 (d, C-9), and 43.5 (s, C-10) indicated the third hydroxy group in **2** to be at C-11. Detailed assignments of ^1H and ^{13}C chemical shifts were based on the COSY, HMQC, and HMBC NMR experiments. Relative stereochemistry of **2** was based on NOESY data. The locations of the three hydroxyl groups at C-1, C-2, and C-11 were found to be all α-oriented due to the NOEs between H-1/H-2, H-2/H_3-20, and H-11/H_3-17 and H_3-20, respectively. Compound **2** was therefore elucidated as (1*R*,2*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,2,11-trihydroxypimara-8(14),15-diene.

Compound **3** had the same molecular formula as **1** and **2** ($C_{20}H_{32}O_3$) on the basis of HRFABMS. The IR spectrum of **3** showed absorptions of hydroxyl (ν_{max} 3430 cm^{-1}) and olefinic (ν_{max} 1722, 1644 cm^{-1}) groups. Analysis of the ^1H and ^{13}C NMR data of **3** revealed the presence of three oxymethine protons at δ_H 3.68 (br s), 3.97 (m), and 4.10 (t). Long-range correlations observed in the HMBC spectrum particularly between H-1/C-20, H-7/C-5 and C-14, and H-11/C-10 suggested the hydroxyl groups to be at C-1, C-7, and C-11, respectively. NOE correlations between H-1/H_3-20 and H-11/H_3-17 and H_3-20 revealed the hydroxyl groups at C-1 and C-11 to be α-oriented. The configuration of OH-7, which was first presumed to be as found in **1**, was in fact different. The H-7 signal resonating at δ_H 4.10 observed as a triplet ($J = 2.7$ Hz), indicating a rather small vicinal coupling constant, together with the NOE interactions between H-7/H-14, H-6α, and H-6β indicated OH-7 to be α-oriented. Compound **3** was thus proposed to be (1*S*,5*S*,7*R*,9*R*,10*S*,11*R*,13*R*)-1,7,11-trihydroxypimara-8(14),15-diene.

Compound **4** was obtained as colorless crystals, mp 158 °C, with elemental formula $C_{20}H_{32}O_2$. The IR spectrum showed the presence of hydroxyl (ν_{max} 3246 cm^{-1}) and double-bond (ν_{max} 1637 cm^{-1}) groups. Although most of the ^1H and ^{13}C NMR data were similar to those of sandaracopimaradien-1β,11α-diol,[12] the melting points and specific rotations of the two compounds were rather different. The relative stereochemistry of **4** was obtained using coupling constant data, as well as the NOEs in the NOESY spectrum. NOE interactions between H-11/H_3-17 and H_3-20 indicated the β-axial orientation of H-11, revealing the OH-11 to be α-equatorial. The NOE effects between H-1/H_3-20, H-2β, and H-2α as well as the small $J_{H-1,H-2}$ detected from the H-1 signal (δ_H 3.79) as a broad singlet indicated the OH-1 as α-axial. Compound **4** was thus proposed as a 1-epimer of sandaracopimaradien-1β,11α-diol and was assigned as (1*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,11-dihydroxypimara-8(14),15-diene.

The positive ion HRFABMS spectrum of **5** indicated that the $[M + H]^+$ ion at *m/z* 303.2322 corresponded to $C_{20}H_{33}O_2$. The IR spectrum showed the presence of hydroxyl (ν_{max} 3600 cm^{-1}), carbonyl (ν_{max} 1703 cm^{-1}), and olefinic (ν_{max} 1630 cm^{-1}) groups. The ^1H NMR spectrum of **5** was similar to that of **4**, but the oxymethine proton was less shielded (δ_H 4.22) and there were additional signals for methylene protons bonded to a carbonyl group (δ_H 2.84 and 2.09). The hydroxyl group at C-6 and a keto group at C-1 were implied from HMBC correlations, particularly between H-6/C-4, C-5, C-8, and C-10 and between H_3-20/C-1 and C-5, respectively. NOE effects between H-6/H-5 and H-9 in addition to the appearance of the oxymethine proton signal at δ_H 4.22, assigned for H-6, as a broad multiplet with width at half-peak height of 7.7 Hz revealing small $J_{H-5/H-6}$ and $J_{H-6/H-7}$, indicated that the hydroxyl group at C-6 was β-oriented. Compound **5** was thus elucidated as (5*S*,6*R*,9*S*,10*S*,13*R*)-6-hydroxypimara-8(14),15-dien-1-one.

Compound **6** was obtained as a colorless amorphous solid having a molecular formula of $C_{20}H_{32}O_3$. The presence of ^{13}C chemical shifts at δ_C 200.0 (s), 145.1 (d), and 135.0 (s), the IR absorption (ν_{max} 1678 cm^{-1}), and the UV maximum at 253 nm indicated an α,β-unsaturated carbonyl group. The key $^1H-^{13}C$ long-range correlations between the signal at δ_H 6.77 (H-14) and carbon signals at δ_C 43.5 (d, C-9), 146.2 (d, C-15), and 200 (s) indicated a carbonyl group at C-7. The two oxygenated secondary carbons at δ_C 74. 2 (δ_H 3.77) and 66.6 (δ_H 4.06) were assigned to C-1 and C-2, respectively, from the HMBC correlations, especially between H-1/C-2, C-3, C-5, and C-20. The relative stereochemistry of **6** was assigned from the NOESY experiment. The key NOE correlations between H-1/H-2 and H_3-20 and between H-2/H_3-18 and H_3-20 indicated that both OH-1 and OH-2 adopt α-orientations. Compound **6** was thus assigned as (1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2-dihydroxypimara-8(14),-15-dien-7-one.

All isolates except for compounds **5** and sandaracopimaradiene were evaluated for their antimalarial, antituberculous, and antifungal activity. Compounds **2** and **4** exhibited in vitro antimalarial activity against *Plasmodium falciparum* K-1 strain, with IC_{50} values of 8.8 and 3.2 μg/mL, respectively. Compounds sandaracopimaradien-1α-ol and 2α-acetoxysandaracopimaradien-1α-ol showed antituberculous activity against *Mycobacterium tuberculosis* H37Ra with MIC values of 25 and 50 μg/mL, respectively, whereas compounds **2**, **3**, **6**, and sandaracopimaradien-1α,2α-diol were less active, with MIC values greater than 100 μg/mL. In an antifungal activity assay against *Candida albicans*, only compounds **2** and sandaracopimaradien-1α,2α-diol showed weak inhibition activity, with IC_{50} values of 17.5 and 49.9 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi 535 apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1020 digital polarimeter. UV spectra were obtained with a Shimadzu UV−vis 2001S spectrophotometer. IR spectra were measured on a Perkin-Elmer FT-IR 1760X spectrophotometer. NMR data were recorded at room temperature on Bruker AM400 spectrometers with tetramethylsilane (TMS) as internal standard.

EIMS data were obtained on a Finnigan Polaris mass spectrometer, and HRFABMS were obtained on a MAT 90 mass spectrometer. Column chromatography was carried out with silica gel 60 (Merck, 230−400 mesh), silica gel 60 (Merck, 70−230 mesh), flash silica gel, silica gel 60 RP C_{18} (Merck, 40−60 μm), and Sephadex LH 20 (Pharmacia). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thickness Merck silica gel 60 F_{254} and silica gel 60 RP-18 F_{254S} aluminum sheets, visualized with a UV lamp, and also by spraying with an anisaldehyde/concentrated H_2SO_4 solution and heating.

Plant Material. The whole plant of *K. marginata* (Zingiberaceae) was collected from Ubonratchathani Province in August 2001. The plant material was kindly identified by Assoc. Prof. Dr. Wongsatit Chuakul of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. A voucher specimen (CRINP 2244) was deposited at the Laboratory of Natural Products, Chulabhorn Research Institute, Bangkok, Thailand.

Extraction and Isolation. The dried and milled whole plant (619 g) was extracted three times (3 × 2 L) by maceration with MeOH at room temperature. After filtration and evaporation of the solvent under reduced pressure, the combined crude MeOH extract was partitioned between CH_2Cl_2 and H_2O to afford CH_2Cl_2 (41 g) and H_2O−MeOH (22.8 g) extracts.

The CH_2Cl_2-soluble extract was subjected to silica gel column chromatography by elution with *n*-hexane−CH_2Cl_2 (0−100%) and CH_2Cl_2−MeOH (0−20%) and finally pure MeOH to afford 13 fractions. Fraction 1 gave sandaracopimaradiene[9] (0.274 g, 0.044% w/w). Fraction 5 gave sandaracopimaradien-1α-ol[10] (0.863 g, 0.139% w/w). Fraction 7 (1.19 g) was rechromatographed with a gradient of *n*-hexane−EtOAc (0 to 100%) to afford 10 subfractions (7/1−7/10). 2α-Acetoxysandaracopimaradien-1α-ol[5] (0.016 g, 0.003% w/w) was obtained from the subfraction 7/3. Subfraction 7/2 was further purified over a silica gel column with a gradient of *n*-hexane−EtOAc (0 to 10%) and further purified on a silica gel column with 2% EtOAc−*n*-hexane to give compound **5** (0.015 g, 0.002% w/w). Only 7.65 g of fraction 9 was rechromatographed with 8% EtOAc−*n*-hexane to afford 16 subfractions (9/1−9/16). Compound **4** (0.367 g, 0.059% w/w) was obtained from the subfraction 9/9. Subfraction 9/11 was separated over a silica gel column with a gradient of *n*-hexane−EtOAc (2% to 20%) to give sandaracopimaradien-1α,2α-diol[5] (0.756 g, 0.122% w/w). Fraction 10 (1.36 g) was rechromatographed using 8% EtOAc−*n*-hexane to afford eight subfractions (10/1−10/8). Subfraction 10/8 was further purified over RP C_{18} silica gel with 60% MeOH−H_2O to give compounds **1** (0.031 g, 0.005% w/w) and **2** (0.088 g, 0.014% w/w) and seven additional subfractions (1−7). Subfraction 4 from the subfraction 10/8 was further purified over PTLC C_{18} with 70% MeOH−H_2O to give compound **6** (0.008 g, 0.001% w/w). Fraction 11 (0.778 g) was separated over a Sephadex-LH 20 column with 80% CH_2Cl_2−*n*-hexane and was further chromatographed over silica gel with 40% EtOAc−*n*-hexane and then over C_{18} silica gel with 80% MeOH−H_2O to give compound **3** (0.008 g, 0.001% w/w).

(1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2,7-Trihydroxypimara-8(14),-15-diene (1): white solid, mp 131−133 °C; $[\alpha]_D$ −7.7° (*c* 1.23, $CHCl_3$); IR (KBr) ν_{max} 3397 (OH), 2948, 2877, 1636, 1460, 1162, 1041, 996, 913, 660 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.82 (1H, dd, *J* = 17.4, 11.2 Hz, H-15), 5.67 (1H, br s, H-14), 4.95 (1H, dd, *J* = 17.4, 1.3 Hz, H-16a), 4.93 (1H, dd, *J* = 11.2, 1.3 Hz, H-16b), 4.00 (1H, ddd, *J* = 11.4, 5.6, 1.0 Hz, H-7), 3.91 (1H, ddd, *J* = 12.2, 4.0, 1.9 Hz, H-2), 3.71 (1H, d, *J* = 1.9 Hz, H-1), 2.35 (1H, t, *J* = 7.4 Hz, H-9), 2.00 (1H, ddd, *J* = 11.7, 5.6, 2.2 Hz, H-6a), 1.76 (1H, m, H-11a), 1.70 (1H, t, *J* = 12.4 Hz, H-3), 1.59 (1H, m, H-12a), 1.49 (1H, m, H-11b), 1.47 (1H, m, H-5), 1.45 (1H, m, H-12b), 1.43 (1H, td, *J* = 12.4, 4.0 Hz, H-3b), 1.31 (1H, t, *J* = 11.7 Hz, H-6b), 1.09 (3H, s, H-17), 0.99 (3H, s, H-19), 0.93 (3H, s, H-18), 0.83 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 148.1 (d, C-15), 138.7 (s, C-8), 126.3 (d, C-14), 110.7 (t, C-16), 74.8 (d, C-1), 72.1 (d, C-7), 66.8 (d, C-2), 43.9 (d, C-5), 42.7 (s, C-10), 42.4 (t, C-3), 42.1 (d, C-9), 37.0 (s, C-13), 34.2 (s, C-4), 33.9 (t, C-6), 33.4 (q, C-19), 31.8 (t, C-12), 26.1 (q, C-17), 23.3 (q, C-18),18.0 (t, C-11), 15.1 (q, C-20); EIMS *m/z* 320 [M]$^+$ (45), 302 [M − H_2O]$^+$ (7), 287 [M − Me − H_2O]$^+$ (10), 269 [M − Me − 2H_2O]$^+$ (100), 251 [M − Me − 3H_2O]$^+$ (23); HRFABMS (positive ion) [M + H]$^+$ *m/z* 321.2429 (calcd for C_{20} H_{33} O_3, 321.2430).

1,2-Acetonide of 1. To a CH_2Cl_2 solution (1 mL) of **1** (10 mg) were added 2,2-dimethoxypropane (1.0 mL) and pyridinium *p*-toluenesulfonate (1 crystal), and the mixture was stirred at room temperature for 4 h. The reaction was then worked up with buffer pH 7.00 in CH_2Cl_2 and NaCl, respectively. After evaporating the solvent, the residue was passed through a silica gel column [1 × 4 cm, 5% EtOAc in *n*- hexane] to obtain the 1,2-acetonide of **1**: ^{1}H NMR data of the 1,2-acetonide of **1** ($CDCl_3$, 400 MHz; data were assigned from the ^{1}H−^{1}H COSY spectrum) δ_H 5.73 (1H, ddd, *J* = 16.7, 10.6, 1.4 Hz, H-15), 5.56 (1H, t, *J* = 1.4 Hz, H-14), 4.88 (1H, dd, *J* = 16.7, 1.4, Hz, H-16a), 4.86 (1H, br d, *J* = 10.6 Hz, H-16b), 4.23 (1H, q, *J* = 5.9 Hz, H-2), 3.95 (1H, dd, *J* = 11.8, 4.8 Hz, H-7), 3.87 (1H, d, *J* = 5.9 Hz, H-1), 2.24 (1H, t, *J* = 6.7 Hz, H-9), 1.97 (1H, ddd, *J* = 11.8, 5.0, 2.9 Hz, H-6), 1.71 (1H, m, H-11), 1.56 (1H, m, H-11), 1.53 (2H, m, H-3), 1.48 (1H, d, *J* = 2.9 Hz, H-5), 1.42 (3H, s, 1,2-acetonide), 1.39 (2H, m, H-12), 1.26 (1H, m, H-6), 1.19 (3H, s, 1,2-acetonide), 1.02 (3H, s, H_3-17), 0.95 (3H, s, H_3-19), 0.78 (6H, s, H_3-18 and H_3-20); HRFABMS (positive ion) [M + H]$^+$ *m/z* 361.2739 (calcd for $C_{23}H_{37}O_3$, 361.2743).

Preparation of the MTPA Ester of the 1,2-Acetonide of 1.[13,14] The 1,2-acetonide of **1** (5.0 mg) was dissolved in 1 mL of dry CH_2Cl_2, and either *R*-(−)- or *S*-(+)-MTPACl (4 μL) in *n*-hexane was added to this solution. The mixture was stirred at room temperature for 2 h. After evaporating the solvent, the residue was passed through a silica gel column (1 × 4 cm, 10% EtOAc in *n*-hexane) to furnish the MTPA esters.

***S*-(−)-MTPA ester of the 1,2-acetonide of 1:** colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz; data were assigned from the ^{1}H−^{1}H COSY spectrum) δ_H 5.63 (1H, ddd, *J* = 17.4, 10.6 Hz, H-15), 5.35 (1H, br s, H-14), 5.32 (1H, d, *J* = 6.2 Hz, H-7), 4.84 (1H, dd, *J* = 10.6, 1.4 Hz, H-16a), 4.78 (1H, dd, *J* = 17.4, 1.4, Hz, H-16b), 4.26 (1H, q, *J* = 6.0 Hz, H-2), 3.87 (1H, d, *J* = 6.0 Hz, H-1), 2.31 (1H, t, *J* = 7.4 Hz, H-9), 1.93 (1H, m, H-6), 1.58 (1H, m, H-3), 1.43 (3H, s, 1,2-acetonide), 1.38 (1H, m, H-5), 1.26 (1H, s, H-6), 1.19 (3H, s, 1,2-acetonide), 0.98 (3H, s, H_3-17), 0.94 (3H, s, H_3-19), 0.79 (6H, s, H_3-18 and H_3-20).

***R*-(+)-MTPA ester of the 1,2-acetonide of 1:** colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.52 (1H, dd, *J* = 10.6, 17.4 Hz, H-15), 5.37 (1H, dd, *J* = 10.8, 5.6 Hz, H-7), 4.96 (1H, t, *J* = 1.9 Hz, H-14), 4.81 (1H, dd, *J* = 10.6, 1.4 Hz, H-16a), 4.69 (1H, dd, *J* = 17.4, 1.4, Hz, H-16b), 4.27 (1H, q, *J* = 6.0 Hz, H-2), 3.86 (1H, d, *J* = 6.0 Hz, H-1), 2.29 (1H, t, *J* = 7.1 Hz, H-9), 1.93 (1H, ddd, *J* = 10.8, 5.6, 2.5 Hz, H-6), 1.64 (1H, m, H-11), 1.58 (H-3 and H-5), 1.51 (H-6 and H-11), 1.43 (3H, s, 1,2-acetonide), 1.18 (3H, s, 1,2-acetonide), 0.98 (3H, s, H_3-19), 0.81 (3H, s, H_3-17), 0.77 (6H, s, H_3-18 and H_3-20).

(1*R*,2*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,2,11-Trihydroxypimara-8(14),15-diene (2): white solid, mp 93−95 °C; $[\alpha]_D$ +21.2° (*c* 4.38, $CHCl_3$); IR (KBr) ν_{max} 3354 (OH), 1462, 1013, 913 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.80 (1H, dd, *J* = 17.5, 10.6 Hz, H-15), 5.33 (1H, br s, H-14), 4.98 (1H, dd, *J* = 17.5, 0.9 Hz, H-16a), 4.93 (1H, dd, *J* = 10.6, 0.9 Hz, H-16b), 4.10 (1H, ddd, *J* = 12.0, 7.3, 4.8 Hz, H-11), 3.94 (1H, br d, *J* = 11.6 Hz, H-2), 3.82 (1H, br s, H-1), 2.32 (1H, d, *J* = 7.5 Hz, H-9), 2.28 (1H, dd, *J* = 14.3, 2.7 Hz, H-7a), 2.01 (1H, br dt, *J* = 13.8, 5.3 Hz, H-7b), 1.73 (1H, dd, *J* = 12.0, 4.6 Hz, H-12a), 1.71 (1H, t, *J* = 11.6 Hz, H-3), 1.62 (1H, br d, *J* = 12.5 Hz, H-6a), 1.51 (1H, t, *J* = 12.0 Hz, H-12b), 1.47 (1H, m, H-3), 1.47 (1H, m, H-5), 1.32 (1H, br td, *J* = 12.9, 4.6 Hz, H-6b), 1.05 (3H, s, H-17), 0.96 (3H, s, H-19), 0.90 (3H, s, H-18), 0.83 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 148.2 (d, C-15), 135.7 (s, C-8), 128.6 (d, C-14), 110.3 (t, C-16), 76.1 (d, C-1), 66.2 (d, C-2), 65.3 (d, C-11), 52.7 (d, C-9), 46.7 (d, C-5), 44.0 (t, C-12), 43.5 (s, C-10), 42.5 (t, C-3), 38.2 (s, C-13), 35.1 (t, C-7), 34.6 (s, C-4), 33.3 (q, C-19), 25.3 (q, C-17), 23.1 (t, C-6), 22.1 (q, C-18), 15.2 (q, C-20); EIMS *m/z* 302 [M − H_2O]$^+$ (76), 287 [M − Me − H_2O]$^+$ (40), 269 [M − Me − 2H_2O]$^+$ (52), 251 [M − Me − 3H_2O]$^+$ (100);

HRFABMS (positive ion) $[M + H]^+$ *m/z* 321.2433 (calcd for $C_{20}H_{33}O_3$, 321.2430).

(1*S*,5*S*,7*R*,9*R*,10*S*,11*R*,13*R*)-1,7,11-Trihydroxypimara-8(14),15-diene (3): colorless amorphous solid; $[\alpha]_D$ +1.6° (*c* 0.55, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3430 (OH), 2956, 1722, 1644, 1468, 1370, 1234, 1197, 1028, 958, 919, 874 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.69 (1H, dd, *J* = 17.5, 10.6 Hz, H-15), 5.47 (1H, br s, H-14), 4.88 (1H, dd, *J* = 17.5, 1.4 Hz, H-16a), 4.83 (1H, dd, *J* = 10.6, 1.4 Hz, H-16b), 4.10 (1H, t, *J* = 2.7 Hz, H-7), 3.97 (1H, m, H-11), 3.68 (1H, b s, H-1), 2.55 (1H, d, *J* = 6.8 Hz, H-9), 1.88 (1H, dd, *J* = 13.5, 2.7 Hz, H-5a), 1.71(1H, m, H-2a), 1.68 (1H, m, H-6a), 1.66 (1H, m, H-3a), 1.63 (1H, β-H, m, H-12a), 1.54 (1H, ddd, *J* = 13.4, 6.4, 3.2 Hz, H-2b), 1.46 (1H, β-H, m, td, *J* = 13.9, 3.5 Hz, H-6b), 1.41 (1H, t, *J* = 11.9 Hz, H-12b), 1.07 (1H, td, *J* = 13.0, 3.2 Hz, H-3b), 0.93 (3H, s, H-17), 0.81 (3H, s, H-19), 0.74 (3H, s, H-18), 0.68 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 147.5 (d, C-15), 138. 5 (s, C-8), 133.3 (s, C-14), 110.9 (d, C-16), 72.7 (d, C-1), 72.6 (d, C-7), 65.4 (d, C-11), 48.2 (d, C-9), 43.9 (t, C-12), 43.0 (S, C-10), 40.3 (d, C-5), 38.2 (s, C-13), 34.2 (t, C-6), 33.1 (q, C-19), 32.8 (s, C-4), 29.4 (t, C-3), 25.3 (t, C-2), 25.1 (q, C-17), 22.0 (q, C-18), 14.8 (q, C-20); EIMS *m/z* 302 $[M - H_2O]^+$ (53), 284 $[M - 2H_2O]^+$ (76), 269 $[M - Me - 2H_2O]^+$ (96), 268 (22), 266 $[M - 3H_2O]^+$ (34), 240 (22), 225 (67); HRFABMS (positive ion) $[M + H]^+$ *m/z* 321.2428 (calcd for $C_{20}H_{33}O_3$, 321.2429).

(1*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,11-Dihydroxypimara-8(14),-15-diene (4): colorless crystals, mp 158 °C; $[\alpha]_D$ +28° (*c* 1.46, $CHCl_3$); IR (KBr) ν_{max} 3246, 1637, 1370, 918, 856 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.80 (1H, dd, *J* = 17.5, 10.6 Hz, H-15), 5.31 (1H, d, *J* = 1.4 Hz, H-14), 4.97 (1H, dd, *J* = 17.5, 1.1 Hz, H-16a), 4.93 (1H, dd, *J* = 10.6, 1.1 Hz, H-16b), 4.10 (1H, ddd, *J* = 11.9, 7.2, 4.8 Hz, H-11), 3.79 (1H, br s, H-1), 2.29 (1H, β-H, dd, *J* = 4.5, 1.7 Hz, H-7a), 2.26 (1H, br d, *J* = 7.2 Hz, H-9), 2.01 (1H, dt, *J* = 13.0, 5.6 Hz, H-7a), 1.89 (1H, β-H, tt, *J* = 13.4, 2.5 Hz, H-2a), 1.78 (1H, dd, *J* = 13.0, 2.9 Hz, H-3a), 1.70 (1H, β-H, m, H-12a), 1.65 (1H, m, H-2b), 1.65 (1H, m, H-6a), 1.56 (1H, dd, *J* = 13.0, 3.1 Hz, H-5), 1.50 (1H, t, *J* = 11.8 Hz, H-12), 1.34 (1H, dd, *J* = 12.9, 4.6 Hz, H-6a), 1.17 (1H, β-H, dt, *J* = 13.0, 3.1 Hz, H-3b), 1.05 (3H, s, H-17), 0.93 (3H, s, H-19), 0.87 (3H, s, H-18), 0.83 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 148.3 (d, C-15), 136.6 (s, C-8), 128.3 (d, C-14), 110.2 (t, C-16), 72.6 (d, C-1), 65.6 (d, C-11), 52.7 (d, C-9), 47.5 (d, C-5), 44.3 (t, C-12), 42.6 (s, C-10), 38.3 (s, C-13), 35.4 (d, C-7), 34.2 (t, C-3), 33.4 (s, C-4), 33.3 (q, C-19), 25.3 (q, C-17), 25.2 (t, C-2), 22.5 (t, C-6), 22.2 (q, C-18), 15.6 (q, C-20); EIMS obsd *m/z* (%): 286, $[M - H_2O]^+$ (100), 271 (30), 268 (15), 253 (50), 227 (73); HRFABMS (positive ion) $[M + H]^+$ *m/z* 305.2485 (calcd for $C_{20}H_{33}O_2$, 305.2481).

(5*S*,6*R*,9*S*,10*S*,13*R*)-6-Hydroxypimara-8(14),15-dien-1-one (5): colorless amorphous solid; $[\alpha]_D$ −6.7° (*c* 0.76, $CHCl_3$); IR (KBr) ν_{max} 3010, 2928, 2850, 1703, 1454, 1372, 1253, 1197, 1078 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.69 (1H, dd, *J* = 17.3, 11.0 Hz, H-15), 5.39 (1H, br s, H-14), 4.86 (1H, dd, *J* = 11.0, 1.5 Hz, H-16a), 4.83 (1H, dd, *J* = 17.3, 1.5 Hz, H-16b), 4.22 (1H, m, $w_{1/2}$ = 7.7 Hz, H-6), 2.84 (1H, β-H, ddd, *J* = 13.6, 12.5, 5.2 Hz, H-2a), 2.33 (1H, br t, *J* = 5.6 Hz, H-9), 2.21 (1H, dt, *J* = 14.3, 3.0 Hz, H-7), 2.09 (1H, dt, *J* = 12.5, 4.2 Hz, H-2b), 1.96 (1H, m, H-11a), 1.71 (1H, ddd, *J* = 13.0, 5.1, 4.2 Hz, H-3a), 1.54 (1H, m, H-3b), 1.54 (1H, β-H, m, H-11b), 1.39 (3H, s, H-18), 1.36 (1H, m, H-12), 1.36 (3H, s, H-20), 1.27 (1H, d, *J* = 1.1 Hz, H-5), 1.02 (3H, s, H-17), 0.97 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 215.6 (s, C-1), 147.5 (d, C-15), 134.6 (d, C-14), 111.2 (t, C-16), 69.1 (d, C-6), 57.8 (d, C-5), 53.7 (s, C-10), 45.7 (t, C-7), 43.9 (t, C-3), 42.2 (d, C-9), 37.7 (s, C-13), 36.3 (t, C-2), 34.3 (s, C-4), 34.3 (t, C-12), 32.6 (q, C-19), 27.4 (q, C-17), 24.2 (q, C-18), 20.5 (t, C-11), 17.4 (q, C-20); EIMS obsd *m/z* (%): 302 $[M^+, 100]$, 287 (8), 285 (33), 284 (8); HRFABMS (positive ion) $[M + H]^+$ *m/z* 303.2323 (calcd for $C_{20}H_{31}O_2$, 303.2324).

(1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,13*R*)-1,2-Dihydroxypimara-8(14),-15-dien-7-one (6): colorless amorphous solid; $[\alpha]_D$ −8.9° (*c* 0.42, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ϵ) 253 (3.68); IR (KBr) ν_{max} 3610, 2962, 1678, 1607, 1468, 1400, 1257, 1152, 1028, 1005, 932, 926, 889, 631 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 6.77 (1H, dd, *J* = 2.8, 1.4 Hz, H-14), 5.85 (1H, dd, *J* = 17.5, 10.6 Hz, H-15), 5.04 (1H, dd, *J* = 17.5, 0.8 Hz, H-16a), 5.01 (1H, dd, *J* = 10.6, 0.8 Hz, H-16b), 4.06 (1H, ddd, *J* = 12.2, 4.4, 2.6 Hz, H-2), 3.77 (1H, br s, H-1), 2.78 (1H, ddd, *J* = 10.5, 5.0, 2.9 Hz, H-9), 2.59 (1H, dd, *J* = 18.5, 5.0 Hz, H-6a), 2.29 (1H, dd, *J* = 18.5, 14.0 Hz, H-6b), 1.90 (1H, dd, *J* = 14.0, 5.0 Hz, H-5), 1.85 (1H, β-H, dd, *J* = 14.0, 5.0 Hz, H-11a), 1.64 (1H, m, H-12), 1.48 (1H, β-H, m, H-3b), 1.48 (1H, m, H-11b), 1.14 (3H, s, H-17), 0.98 (3H, s, H-18), 0.96 (3H, s, H-19), 0.89 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 200.2 (s, C-7), 146.2 (d, C-15), 145.1 (d, C-14), 135.0 (s, C-8), 111.9 (t, C-16), 74.2 (d, C-1), 66.6 (d, C-2), 43.5 (d, C-9), 42.5 (d, C-5), 42.3 (t, C-3), 40.5 (s, C-10), 38.6 (s, C-13), 36.8 (t, C-6), 34.3 (s, C-4), 33.9 (t, C-12), 32.4 (q, C-19), 25.7 (q, C-17), 22.4 (q, C-18), 18.4 (t, C-11), 13.8 (q, C-20); EIMS obsd *m/z* (%): 318 $[M]^+$, (65), 300 (26); HRFABMS (positive ion) $[M + H]^+$ *m/z* 319.2272 (calcd for $C_{20}H_{33}O_3$, 319.2273).

Bioassays. Antimalarial activity was evaluated against *Plasmodium falciparum* (K1 multidrug-resistant strain) cultured continuously according to Trager and Jensen.[15] Quantitative determination of antimalarial activity in vitro was achieved using the microculture radioisotope technique based on the method of Desjardins et al.[15] The standard drugs, rifempicin, kanamycin, and isoniazide, used as positive controls for the antimycobacterial activity showed minimum inhibitory concentrations (MIC) of 0.0023, 2.5, and 0.1 μg/mL, respectively. The antimycobacterial activity (anti-TB) assay was performed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay.[16] Antifungal tests were undertaken against *Candida albicans* (ATCC 90028) using the tetrazolium/formazan assay method.[17] Amphotericin B and DMSO were used as a positive (IC_{50} value of 0.068−0.092 μg/mL) and a negative control, respectively.

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Supporting Information Available: $^1H-^1H$ COSY, HMBC, and NOESY correlations of **1**−**6** (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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