Bisnortriterpenes from *Salacia madagascariensis*

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A new bisnortriterpene quinone methide, 20-*epi*-isoiguesterinol (**2**), and a new 6-oxophenolic triterpene, 6-oxoisoiguesterin (**5**), as well as two known compounds, isoiguesterin (**1**) and isoiguesterinol (**4**), were isolated from the petroleum ether extract of the roots of *Salacia madagascariensis*. Isoiguesterin (**1**) and 20-*epi*-isoiguesterinol (**2**) showed potent activity against *Leishmania*.

Salacia madagascariensis (Lam.) DC. (Celastraceae) is a shrub found in East Africa and Madagascar whose roots have been used in traditional medicine to treat malaria, fever, and menorrhagia in Tanzania.[1,2] Previous phytochemical investigation of the roots resulted in the isolation of isoiguesterin (**1**), a bisnortriterpene quinone methide that showed moderate activity against leukemia.[3] Triterpene quinone methides are a unique class of compounds found in the higher order plant families such as Celastraceae. A literature review in 1996 contains a thorough discussion of triterpene quinone methides and related compounds reported through June 1994.[4] To date, isoiguesterin (**1**) has been the only triterpene quinone methide isolated from *S. madagascariensis*. In more recent testing for biological activity, isoiguesterin (**1**) showed very potent antileishmanial activity against *L. donovani* and *L. mexicana* (IC_{50} 0.084 and 0.035 μg/mL, respectively).[5] Given these results, *S. madagascariensis* was reinvestigated for other triterpene quinone methides that may exhibit similar antileishmanial activity.

The petroleum ether extract of the dried ground roots of *S. madagascariensis* was initially fractionated using silica gel column chromatography to give 18 fractions. Isoiguesterin (**1**) was reisolated as the principal triterpene quinone methide, but TLC analysis of other fractions showed orange bands, suggesting the presence of additional triterpene quinone methides. Column chromatography and preparative TLC of appropriate fractions resulted in the isolation of **2**, an orange compound.

High-resolution electrospray mass spectrometry of **2** gave a molecular ion at *m/z* 423.2992, indicating a molecular formula of $C_{28}H_{38}O_3$. The FTIR spectrum contained peaks at 3373 and 1953 cm^{-1}, corresponding to a chelated hydroxy group and a conjugated carbonyl group, respectively.

The ^1H NMR spectrum of **2** was very similar to that of **1**. The characteristic resonances for the protons of the A−B rings of triterpene quinone methides were present as resonances at δ 7.03 (dd), 6.53 (d), and 6.37 (d), corresponding to H-6, H-1, and H-7, respectively. A one-proton singlet at δ 6.98 corresponded to the proton of the hydroxyl group on C-3. Five methyl singlets typical for this class of compounds were present at δ 2.22, 1.48, 1.30, 1.09, and 0.82, corresponding to methyl groups 23, 25, 26, 28, and 27, respectively. However, the two-proton singlet for the H_2-29 alkene protons at δ 4.58 in the spectrum of **1** was replaced in the spectrum of **2** by a two-proton doublet at δ 3.40, indicating a carbon bearing an oxygen atom, most likely a hydroxyl moiety.

The ^{13}C NMR spectrum contained 27 distinct carbon signals ranging from δ 10 to 178, with eight representing sp^2-hybridized carbons and 19 representing sp^3-hybridized carbons. The presence of one resonance at δ 70, shown to couple to the methylene doublet at δ 3.40 in the $^1H-^{13}C$ HSQC spectrum, was consistent with a carbon bearing a hydroxyl moiety. Reduction of the quinone methide moiety of **2**, followed by acetylation, gave a triacetate, **3**. In the ^1H NMR spectrum of **3**, the two-proton doublet at δ 3.40 shifted downfield to 3.81, due to deshielding by the acetate group, confirming the presence of a single additional hydroxyl group in **2**.

The $^1H-^1H$ COSY spectrum confirmed the assignments of H-1, H-6, and H-7 in the ^1H NMR spectrum, and the resonance at δ 3.40 coupled to a resonance at δ 1.8, corresponding to alkyl protons. The $^1H-^{13}C$ HMBC spectrum and data from the literature[4] were used to determine the placement and relative stereochemistry of the five methyl groups, as well as the assignments of most of the carbon resonances. A key correlation was the correlation of the methylene resonance at δ 3.40 to methylene carbons at δ 24.7 and 24.8 and a methine resonance at 35.7, none of which showed correlations to any of the methyl resonances. Thus, these resonances must be due to C-19, C-21, and C-20, respectively, although due to the inability to differentiate protons in the alkyl region, it was not possible to ascertain which resonance corresponded specifically to C-19 or C-21. Consequently, the hydroxymethylene group must be located at C-20. The stereochemistry at C-20 was determined by comparison to the literature. The known triterpene quinone methide isoiguesterinol (**4**) also has a CH_2OH group at C-20. Dhanabalasingham and co-workers first reported the isolation of isoiguesterinol (**4**) from *Salacia reticulata* var. diandra in 1996.[6] On the basis of NOE difference experiments, the CH_2OH group of isoiguesterinol (**4**) was determined to be in the α position. The C-29 protons in isoiguesterinol (**4**) are a doublet of doublets, coupling to each other (J = 10 Hz) and to the proton on C-20 (J = 6 Hz). Since the C-29 protons of **2** were a doublet (J = 6 Hz) and coupled to the proton on carbon 20, the stereochemistry at C-20 must be different, placing C-29 in the β position. Subsequently, isoiguesterinol (**4**) was isolated from other fractions of the extract of *S. madagascariensis*, allowing a direct NMR comparison of the two compounds and confirming the structure of **2** as 20-*epi*-isoiguesterinol.

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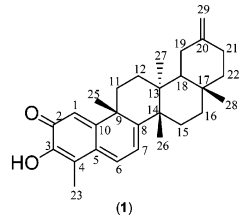
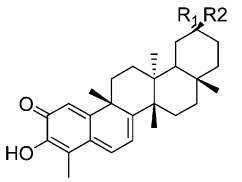
A second extraction of *S. madagascariensis* was carried out using a modified extraction scheme, involving percolation (Soxhlet) with petroleum ether after an initial extraction using petroleum ether at room temperature. The petroleum ether Soxhlet extract contained a precipitate that was removed by filtration and chromatographed to yield additional quantities of 20-*epi*-isoiguesterinol (**2**) as well as isoiguesterinol (**4**).

The supernatant from the Soxhlet extraction was concentrated in vacuo, then the residue was dissolved in toluene and hexanes were added until a precipitate formed. The solids were removed by filtration. The filtrate was concentrated and subjected to column chromatography and preparative TLC to yield **5** as a pale yellow film.

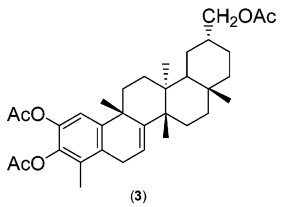


(**1**)

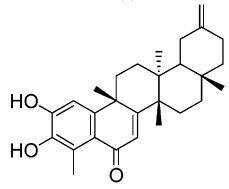


(**2**) R_1= H, R_2 = CH_2OH

(**4**) R_1 = CH_2OH, R_2 = H



(**3**)



(**5**)

High-resolution ESI mass spectrometry of **5** gave a parent ion at *m/z* 421.2557, corresponding to a molecular formula of $C_{28}H_{36}O_3$. The ^{1}H NMR spectrum of **5** did not show the typical resonances for the protons of the A−B ring of quinone methides, but was similar to the spectrum of **3**. There were one-proton singlets at δ 6.91 and 6.23, corresponding to protons attached to conjugated sp^2-hydridized carbons. A broad two-proton singlet at δ 4.56 suggested a C-20 exocyclic double bond as in isoiguesterin (**1**). The ^{1}H NMR spectrum displayed five methyl singlets at δ 2.66, 1.54, 1.31, 1.15, and 0.71, typical resonances for the five methyl groups of these triterpenes, C-23, C-25, C-26, C-28, and C-27, respectively.

The ^{13}C NMR spectrum revealed 28 resonances, with 11 representing sp^2-hybridized carbons and 17 representing sp^3-hybridized carbons. In the ^{1}H−^{13}C HSQC spectrum the proton resonances at δ 6.91 and 6.23 correlated to carbon resonances at δ 108.8 and 125.7, respectively. The proton resonance at δ 4.56 correlated to a carbon resonance at δ 106.5, which is approximately the same chemical shift as C-29 in isoiguesterin (**1**).

In the ^{1}H−^{13}C HMBC spectrum, the proton resonance at δ 6.91 correlated to carbon resonances at δ 147.7, 140.3, and 122.6 and the proton resonance at δ 6.23 correlated to carbon resonances at δ 44.4, 40.1, and 122.6. Combining this information along with the correlations for the protons of methyl groups 23 and 25, the resonance at δ 6.91 corresponded to H-1 and the resonance at δ 6.23 corresponded to H-7. These data, when compared to the data for **3**, confirmed that the A ring of **5** was a pentasubstituted diphenol with two phenolic moieties at C-2 and C-3 and a methyl group at C-4. The resonances for the remainder of the carbons on the triterpene skeleton, with the exception of carbons 6, 19, 20, and 21, were assigned on the basis of HMBC correlations with the five methyl resonances. The proton resonance at δ 4.56 correlated with carbon resonances at δ 148.2 (C-20), 30.5, and 30.3, which corresponded to carbons 20, 19, and 21. As with **2**, the carbon resonances at δ 30.5 and 30.3 only correlated to the proton resonance at δ 4.56 and were not specifically assignable to either C-19 or C-21. No correlations were found to assign the resonance for C-6. However, one remaining carbon resonance at δ 187.7, typical for a conjugated carbonyl carbon, had not been assigned. In addition, the resonance for C-8 was at δ 172.1, on the basis of correlations with the protons of C-25 and C-26, and was further downfield than the chemical shift corresponding to an sp^2-hybridized carbon of an isolated double bond, suggesting deshielding due to a conjugated carbonyl. Furthermore, the chemical shift for H-7 was also shifted downfield to δ 6.23 in comparison to the resonance for H-7 in **3**. On the basis of these data, the carbonyl was placed at C-6, and **5** was determined to be a 6-oxophenolic triterpene and was named 6-oxoisoiguesterin. The stereochemistry of the four methyl groups at positions 9, 13, 14, and 17 was determined to be the same as that of isoiguesterin (**1**) and other triterpene quinone methides based on agreement with the chemical shifts in the literature, and the NMR data were consistent with those of other 6-oxophenolic triterpenes.[4]

Given the potent activity of isoiguesterin (**1**) in the initial antileishmanial screening, further antileishmanial testing on isoiguesterin (**1**) and 20-*epi*-isoiguesterinol (**2**) was carried out at the National Center for Natural Products Research at the University of Mississippi. In addition to the antileishmanial testing, **1** and **2** were screened for antimicrobial activity, antiprotozoal activity, and cytotoxicity.

Isoiguesterin (**1**) and 20-*epi*-isoiguesterinol (**2**) were tested for antimicrobial activity against seven microorganisms, *Candida albicans, Cryptococcus neoformans*, *Aspergillus fumigatus, Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus,* and *Mycobacterium intracellulare*. Ciprofloxacin was the positive control. Isoiguesterin (**1**) and 20-*epi*-isoiguesterinol (**2**) showed moderate activity against *S. aureus* (IC_{50} = 0.85 and 0.65 μg/mL, respectively) and methicillin-resistant *S. aureus* (IC_{50} = 0.85 and 0.7 μg/mL, respectively) and weak activity against *Mycobacterium intracellulare* (IC_{50} = 4.5 and 15 μg/mL, respectively).

In the antiprotozoal screening assay, the compounds were tested against two clones of *Plasmodium falciparum* (Table 1). Isoiguesterin (**1**) was more than an order of magnitude less active than both chloroquine and artemisinin against the D6 clone. 20-*Epi*-isoiguesterinol (**2**) showed slightly better activity than **1** with a good selectivity index. Isoiguesterin (**1**) and 20-*epi*-isoiguesterinol (**2**) both had moderate activity against the W2 clone in comparison to

Table 1. Antiplasmodial Activity for Compounds **1** and **2** against D6 and W2 Clones of *Plasmodium falciparum* (IC_{50} in ng/mL)

	D6 clone		W2 clone		VERO[a]
compound	IC_{50}	SI[b]	IC_{50}	SI[b]	IC_{50}
isoiguesterin (**1**)	200	3.4	170	3.9	670
chloroquin	5.5		110		
astemisinin	9.0		9.0		
20-*epi*-isoiguesterinol (**2**)	68	31	68	31	2100
chloroquine	7		115		
artemisinin	3.6		1.8		

[a] VERO = monkey kidney cell line. [b] SI (selectivity Index) = IC_{50} (VERO cells)/IC_{50} (*P. falciparum* clone).

Table 2. Antileishmanial Activity for Compounds **1** and **2** against *Leishmania donovanii* (IC_{50} in μg/mL)

compound	test 1 IC_{50}	test 2 IC_{50}	mean IC_{50}	IC_{90}	VERO IC_{50}	selectivity index[a]
isoiguesterin (**1**)	0.036	0.03	0.032	0.055	1.6	50
20-*epi*-isoiguesterinol (**2**)	0.027		0.027	0.057	2.1	31
amphotericin B	0.17	0.05	0.11	0.23	6.5	59

[a] Selectivity index = IC_{50} for VERO cells/mean IC_{50}.

chloroquine and artemisinin, with **2** again having a better selectivity index.

In the antileishmanial activity assay, the two compounds were tested against *Leishmania donovani*, a species known to cause visceral leishmaniasis (Table 2). Both isoiguesterin (**1**) and 20-*epi*-isoiguesterinol (**2**) were an order of magnitude more active, with comparable selectivity indices, than amphotericin B, a current treatment for leishmaniasis. Thus, isoiguesterin (**1**) and 20-*epi*-isoiguesterinol (**2**) show great potential for future drug development against *Leishmania*.

Experimental Section

General Experimental Procedures. UV spectra were obtained on an Agilent 8453 UV−vis system with UV−visible Chemstation software. FTIR spectra were obtained on a Nicolet Nexus 670 FT-IR spectrometer as films on a NaCl disk. NMR spectra were obtained in $CDCl_3$ on either a Varian Mercury 300 MHz spectrometer equipped with a Sun Microsystems Ultra 5 processor and VNMR version 5.1b software or a Varian Inova 400 MHz spectrometer with a Sun Microsystems Ultra 1 processor and VNMR version 5.1c software. The Virginia Commonwealth University Mass Spectrometry Center provided the high-resolution electrospray mass spectra in positive ion mode, which were obtained on a Micromass Q-TOF 2 quadrupole/time-of-flight mass spectrometer calibrated with polyalanine. Samples were electrosprayed from CH_2Cl_2/EtOAc/MeOH (3:3:1). Solvents were reagent grade and used as purchased. Column chromatography employed 60−200 mesh silica gel (J. T. Baker). Preparative thin-layer chromatography was carried out on J. T. Baker Si250F silica gel TLC plates. The compounds were visualized by short (λ = 254 nm) and long (λ = 366 nm) wave ultraviolet light and by spraying with 1% vanillin in ethanol/concentrated H_2SO_4 (4:1), followed by heating at 100 °C for 5 min. Initial screens for activity against *Leishmania* were conducted at Walter Reed Army Institute of Research. Cytotoxicity, antiprotozoal, antimicrobial, and antileishmanial testing was conducted at the National Center for Natural Products Research, College of Pharmacy, University of Mississippi, using procedures described in ref 7.

Plant Materials. Dried roots of *Salacia madagascariensis* (Lam.) DC. (PR-80767) collected in Tanzania in March 1974 were supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, MD, where voucher specimens are preserved.

Extraction and Isolation. The dried, ground roots of *S. madagascariensis* (2 kg) were extracted twice with petroleum ether (8.0 L) for 20 h. The petroleum ether was removed in vacuo to give 4.66 g of dark red, tar-like solid. The residue was subjected to silica gel column chromatography eluted with 0−5% EtOAc in CH_2Cl_2, followed by 5−10% MeOH in CH_2Cl_2. Twenty-eight fractions were collected and combined into nine fractions (A1−A9) based on TLC analysis.

Fraction A8 (1.36 g) was subjected to silica gel column chromatography eluting with 0−10% MeOH in CH_2Cl_2, collecting 130 fractions, which were recombined into 13 fractions, B1−B13. Preparative TLC of B7 (0.040 g) on silica gel developed 3× in 30% EtOAc in CH_2Cl_2 yielded 20-*epi*-isoiguesterinol (**2**) (9 mg). Preparative TLC of B9 (0.036 g) on silica gel developed 3× in 30% EtOAc in CH_2Cl_2 yielded additional **2** (6 mg). Preparative TLC of B6 (0.078 g) on silica gel developed 5× in 20% EtOAc in CH_2Cl_2 followed by preparative TLC of the major orange band on silica gel developed 3× in 10% MeOH in toluene yielded **2** (15 mg). Additional quantities of 20-*epi*-isoiguesterinol (**2**) were isolated from fraction B5 (0.197 g) following column chromatographic separation on silica gel using 10−50% EtOAc in CH_2Cl_2. The 100 fractions were combined into five fractions: C1−C5. Preparative TLC of fraction C5 on silica gel, developed 3× in 10% MeOH in toluene, yielded 9 mg of 20-*epi*-isoiguesterinol (**2**).

20-*epi*-Isoiguesterinol (2): amorphous orange solid (39 mg); UV (EtOH) λ_{max} (log ϵ) 422 (3.76) nm; IR (film), ν_{max} 3373 (OH), 2936, 1593 (C=O), 1513, 1441, 1378 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.03 (1H, dd, J = 1.2, 6.7 Hz, H-6), 6.98 (1H, s, 3-OH), 6.53 (1H, d, J = 1.2 Hz, H-1), 6.27 (1H, d, J = 6.7 Hz, 7-H), 3.40 (2H, d, J = 5.7 Hz, 29-H_2), 2.22 (3H, s, 23-H_3), 2.18 (2H, m, 11-H_2), 1.93 (1H, m, 22-H), 1.77 (3H, m, 12-H_2, 20-H), 1.46−1.61 (7H, m, 15-H_2, 16-H_2, 18-H, 19-H_2), 1.48 (3H, s, 25-H_3), 1.30 (3H, s, 26-H_3), 1.18 (2H, m, 21-H_2), 1.09 (3H, s, 28-H_3), 1.06 (1H, m, 22-H), 0.82 (3H, s, 27-H_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.1 (C-2), 170.1 (C-8), 164.8 (C-10), 145.9 (C-3), 134.0 (C-6), 127.3 (C-5), 119.5 (C-1), 118.0 (C-4, C-7), 69.6 (C-29), 45.0 (C-14), 43.2 (C-18), 43.0 (C-9), 40.7 (C-13), 38.9 (C-25), 36.5 (C-16/C-22), 36.2 (C-16/C-22), 35.7 (C-20), 33.0 (C-11), 31.6 (C-17), 31.4 (C-28), 30.0 (C-12), 28.6 (C-15), 24.8 (C-19/C-21), 24.7 (C-19/C− 21), 21.6 (C-26), 21.4 (C-27), 10.4 (C-23); HRESIMS *m/z* 423.2992 $[M + H]^+$, (calcd for $C_{28}H_{39}O_3$, 423.2899).

Formation of Dihydro-20-*epi*-Isoiguesterinol Triacetate (3). 20-*Epi*-isoiguesterinol (**2**) (4.4 mg) was dissolved in 100% EtOH (2.0 mL) and $NaBH_4$ (5 mg) was added, producing an immediate loss of the orange color. After stirring under N_2 for 1 h, the reaction was quenched by adding glacial HOAc, dropwise. The solvent was evaporated in vacuo. The residue was dissolved in pyridine (1 mL), and acetic anhydride (1 mL) was added. After stirring under nitrogen for 16 h, the mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and washed three times with 1 M HCl (5 mL), twice with 5% $NaHCO_3$ (5 mL), and once with saturated NaCl (5 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. Preparative TLC on silica gel developed with 5% EtOAc in CH_2Cl_2 gave **3**.

Dihydro-20-*epi*-Isoiguesterinol Triacetate (3): amorphous pale yellow solid (1.7 mg); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.01 (1H, s, H-1), 5.76 (1H, dd, J = 6, 2.1 6 Hz, H-7), 3.81 (2H, d, J = 6.3 Hz, H-29), 3.35 (1H, dd, J = 21, 6 Hz, H-6b), 3.08 (1H, dd, J = 21, 2.1 Hz, H-6a), 2.31 (3H, s, C-2 OCOCH_3), 2.27 (3H, s, C-3 OCOCH_3), 2.07 (3H, s, H-23), 2.06 (3H, s, C-29 OCOCH_3), 1.36 (3H, s, H-25), 1.25 (3H, s, H-26), 1.06 (3H, s, H-28), 0.84 (3H, s, H-27); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.6 (C-20 O*C*OCH$_3$), 169.0 (C-3 O*C*OCH$_3$), 168.7 (C-2 O*C*OCH$_3$), 149.4 (C, C-8), 147.9 (C, C-10), 140.8 (C, C-2), 138.2 (C, C-3), 131.9 (C, C-5), 128.1 (C, C-4), 117.1 (CH, C-7), 116.9 (CH, C-1), 70.9 (CH_2, C-29), 43.8 (CH, C-14), 43.7 (CH, C-18), 38.9 (C, C-13), 37.4 (C, C-9), 37.0 (CH_2, C-16), 36.1 (CH_2, C-22), 34.8 (CH_3, C-25), 32.6 (CH, C-20), 31.6 (CH_3, C-28), 30.7 (CH, C-17), 29.9 (CH_2, C-12), 29.0 (CH_2, C-15), 28.3 (CH_2, C-6), 25.2 (CH_2, C-21), 24.7 (CH_2, C-19), 23.0 (CH_3, C-26), 21.5 (CH_3, C-27), 21.2 (C-29 OCO*C*H$_3$), 20.9 (C-3 OCO*C*H$_3$), 20.7 (C-2 OCO*C*H$_3$), 12.8 (CH_3, C-23).

Isolation of Isoiguesterinol (4). The dried ground roots of *S. madagascariensis* (2 kg) were extracted at RT with petroleum ether (6.0 L, 5×), followed by percolation with petroleum ether (Soxhlet) (8.0 L) for 24 h. After cooling, the extract contained a solid that was removed by filtration. The residue from the Soxhlet extraction (3.4 g) was subjected to silica gel column chromatography, eluting with 0−100% EtOAc in CH_2Cl_2. Twenty fractions were collected. Preparative TLC of combined fractions 18−20 on silica gel, developed 3× using 30% EtOAc in CH_2Cl_2, gave 20-*epi*-isoiguesterinol (**2**) containing a single impurity. Preparative TLC on silica gel developed 2× using 12% MeOH in toluene followed by preparative TLC on silica gel developed 3× using 30% EtOAc in CH_2Cl_2 gave 20-*epi*-isoiguesterinol (**2**) and isoiguesterinol (**4**).

Isoiguesterinol (4): amorphous orange solid (2.4 mg); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.03 (1H, dd, J = 6.9, 1.2 Hz, H-6), 6.97 (1H, s, C-3 OH), 6.53 (1H, d, J = 1.2 Hz, H-1), 6.37 (1H, d, J = 6.9 Hz, H-7), 3.47 (1H, dd, J = 10, 6 Hz, H-29a), 3.40 (1H, dd, J = 10, 6 Hz, H-29b), 2.21 (3H, s, H-23), 1.44 (3H, s, H-25), 1.34 (3H, s, H-26), 1.19 (3H, s, H-28), 0.72 (3H, s, H-27).

Isolation of 6-Oxoisoiguesterin (5). The filtered solid from the Soxhlet extraction was washed with petroleum ether and then acetone until the filtrate was colorless. The filtrates were combined and the solvent was removed under reduced pressure. The residue was dissolved in toluene and hexanes were added until a precipitate formed. After cooling in the freezer, additional solids were removed by filtration. The filtrate was concentrated in vacuo and the residue (0.364 g) was subjected to silica gel column chromatography, eluted with 10−75% EtOAc in CH_2Cl_2. The 18 fractions were recombined into six fractions, D1−D6. Preparative TLC of D2 on silica gel developed 2× using 15% EtOAc in CH_2Cl_2 followed by preparative TLC on silica gel developed 3× in 5% MeOH in toluene gave 6-oxoisoiguesterin (**5**).

6-Oxoisoiguesterin (5): amorphous pale yellow solid (3.2 mg); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.91 (1H, s, 1-H), 6.23 (1H, s, 7-H), 4.56 (2H, s, 29-H_2), 2.66 (3-H, s, 23-H_3), 1.54 (3H, s, 25-H_3), 1.31 (3H, s, 26-H_3), 1.15 (3H, s, 28-H_3), 0.71 (3H, s, 27-H_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 187.7 (C-6), 172.1 (C-8), 151.9 (C-10), 148.2 (C-20), 147.7 (C-2), 140.3 (C-3), 125.6 (C-1), 125.1 (C-4), 122.6 (C-5), 108.8 (C-7), 106.5 (C-29), 44.8 (C-18), 44.4 (C-9), 40.8 (C-13), 40.1 (C-14), 38.4 (C-25), 36.8 (C-16/C-22), 36.0 (C-16/C-22), 34.4 (C-11), 31.6 (C-17), 31.1 (C-28), 30.5 (C-19/C-21), 30.3 (C-19/C-21), 29.9 (C-12), 28.2 (C-15), 20.4 (C-26), 19.6 (C-27), 13.6 (C-23); HRESIMS *m/z* 421.2557 $[M + H]^+$ (calcd for $C_{28}H_{37}O_3$, 421.2743).

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References and Notes

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