Novel Antihyperglycemic Terpenoid-Quinones from *Pycnanthus angolensis*†

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Two new compounds, pycnanthuquinone A **(1)** and pycnanthuquinone B **(2)**, were isolated from leaves and stems of the African plant, *Pycnanthus angolensis* (Welw.) Warb (Myristicaceae), by bioassay-guided fractionation of an ethanolic extract using a diabetic mouse model. Pycnanthuquinones A and B are the first representatives of a novel terpenoid-type quinone skeleton, and both compounds possess significant antihyperglycemic activity.

Introduction

A multidisciplinary ethnomedical approach to discover drugs, combining aspects of ethnobotany, traditional medicine, and modern techniques of natural products chemistry to identify compounds as potential new therapeutic agents has been described.[1] In general, with this approach, plants of interest possess prior history of use in humans. Potential plant leads are derived from ethnobotanical and ethnomedical field research by a team of botanists and Western-trained physicians in collaboration with traditional healers. We recently described this approach as an alternative pathway to discover potentially new drugs for the treatment of Type 2 diabetes mellitus.[2] Plants were collected if they were found to be used by healers for the treatment of Type 2 diabetes mellitus or if they were used to treat a cluster of signs and symptoms that could occur in a Type 2 diabetic patient, which include fungal infections, impotency, pain and numbness of limbs, thirst, and chest pain.[3] The medicinal plant candidates that are generated through our ethnobotanical field research subsequently go through a comprehensive dereplication process.[4] The highest priority candidates then undergo in-house testing with the use of a *db*/*db* mouse model for Type 2 diabetes to direct the fractionation of extracts to pure compounds.[5]

This paradigm to discover potentially antihyperglycemic compounds from plants led us to the use and preparation of *Pycnanthus angolensis* (Welw.) Warb. (Myristicaceae), a tree that grows in West and Central Africa and has the common name "African nutmeg". This plant was recognized and subsequently selected because field research with traditional healers determined that its leaves, twigs, seed fat, and bark exudate are used to treat oral thrush, fungal skin infections, and shingles[6] and that its ground stembark is used as a mix with *Piper guineense* and water to produce a paste that is applied topically to treat headache, body aches, and chest pain.[7]

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(6) Based on Shaman Pharmaceuticals field research in collaboration with the Ewe culture in Ghana.

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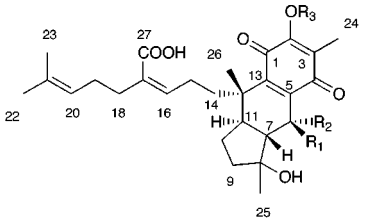
Oral thrush, fungal skin infections, body aches, and chest pain are all symptoms that can occur in a person with Type 2 diabetes mellitus.

A review of the literature on *P. angolensis* revealed the following medicinal uses. A hot water extract of the bark reportedly is used as a purgative and to treat leprosy in Guinea[8] and to combat sterility in women in Guinea-Bissau.[9] In parts of Western Africa, the wood *of P. angolensis* is made into chewing sticks for the treatment of toothache and various skin diseases.[10] Other reported ethnomedical indications in the literature include its use as an anthelmintic,[11] a treatment for cough and sore throat,[12] and for the treatment of rheumatism and hemorrhoids.[13] Despite these reports, the genus *Pycnanthus* has not been extensively investigated, and the only compounds reported include allantoin,[14] flavonoids,[15] kombic acid,[16] and dihydroguaiaretic acid.[17]

We now report the isolation and identification of two new compounds from the extracts of leaves and stems of *P. angolensis*, pycnanthuquinone A (**1**) and pycnanthuquinone B (**2**).[18] Elucidation of their structures reveals a novel terpenoid-quinone structure containing a fused 6,6,5-ring skeleton. Herein, we describe the isolation, structure elucidation, and antihyperglycemic activity of these new compounds. Compounds **1** and **2** are isomers and differ only at one stereocenter with inversion of an OH group at position C-6.



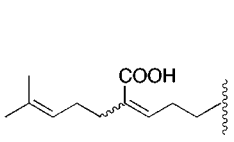
1 $R_1 = H; R_2 = OH; R_3 = H$
2 $R_1 = OH; R_2 = H; R_3 = H$
3 $R_1 = H; R_2 = OAc; R_3 = Ac$
4 $R_1 = OAc; R_2 = H; R_3 = H$

Results and Discussion

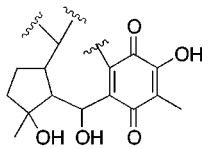
Pycnanthuquinone A **(1)** and pycnanthuquinone B **(2)** were isolated on a small scale from an ethanol extract of the dried leaves and stems of *P. angolensis* using in vivo-guided fractionation. The initial 95% ethanol extract was partitioned with hexane, and the hexane layer was extracted with 10% Na_2CO_3. After a dichloromethane wash, the basic aqueous extract was acidified and again extracted with dichloromethane. The organic layer was purified sequentially over two polyvinylstyrene-based resin (HP-20) columns, using 90% acetonitrile as the eluent for the first, and 90% methanol for the second. Final purification using reversed-phase HPLC afforded baseline separation of **1** and **2** in 0.002% and 0.0015% overall yields, respectively. Since multigram quantities of **1** and **2** were required for in-depth biological evaluation, a scale-up procedure was developed to improve the overall yield and provided for a safer process. This procedure, using 100 kg of dried leaves,[19] eliminated the use of hexanes, whereby extraction into ethyl acetate was followed by purification on a Sephadex LH-20 column, eluting with acetonitrile. Final separation was achieved with preparative HPLC using a reversed-phase polymeric C-18 (YMC) column to yield **1** and **2** as amorphous pale yellow powders. These compounds are sensitive to heat and light and were stored under nitrogen.

Pycnanthuquinone A **(1)** gave a molecular ion peak at *m/z* 472.2440 by HREIMS, indicating a molecular formula of $C_{27}H_{36}O_7$. The presence of a *p*-quinone ring in **1** was suggested from intense UV absorption at 270 and 398 nm, the strong IR absorption at 1634 cm^{-1}, the ^{13}C NMR signals at δ 186.5 and 184.2, and a strong $[M + 2H]^+$ peak in the FABMS.[20,21,22] The lack of a UV absorption band at 540−600 nm precluded an *o*-quinone ring system.[21] The ^1H and ^{13}C NMR data (Table 1) revealed that **1** contained three carbonyl groups and four carbon−carbon double bonds, thus requiring the presence of three rings to satisfy the unsaturation number implied by the molecular formula.

The ^1H NMR spectra of **1** revealed the presence of 5 methyl singlet peaks, and two isoprene unit olefinic signals at δ 5.04 (bt, H-20) and 5.69 (t, H-16). ^1H-^1H COSY data established spin systems from H-20 through H_2-18 and from H-16 through H_2-14. HMBC correlations from H-16 to C-18 and H_2-18 to C-16, along with correlations from H-16 and H_2-18 to a signal at δ 168.7 (C-27) and H-16 to one at 131.9 (C-17), revealed that these two spin systems flanked an α,β-unsaturated acid. HMBC correlations from H-20 to C-18, C-22, and C-23, and from H-19 to C-21, completed substructure **1a**.



1a



1b

Substructure **1b** required a fully substituted *p*-quinone ring and the presence of three OH groups. HMBC correlations were observed from a methyl singlet at δ 1.78 (H_3-24) to carbon signals at δ 153.5 (C-2), 115.6 (C-3), and 186.5 (C-4), and from an exchangeable proton signal

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Table 1. NMR Spectral Data of Pycnanthuquinone A (1) and Pycnanthuquinone B (2)[a]

	1			2		
position	^{13}C	^{1}H	COSY (600 MHz)	^{13}C	^{1}H	COSY (600 MHz)
1	184.2 (s)			183.9 (s)		
2	153.5 (s)			153.5 (s)		
3	115.6 (s)			115.9 (s)		
4	186.5 (s)			188.2 (s)		
5	143.7 (s)			148.1 (s)		
6	60.7 (d)	5.00 d (3.6)	H-7	64.4 (d)	4.82 d (8.0)	H-7
7	46.1 (d)	1.29 dd (14.0, 3.2)	H-6, H-11	51.0 (d)	1.42 dd (13.6, 8.8)	H-6, H-11
8	79.6 (s)			77.2 (s)		
9a	40.5 (t)	1.66[b]	H-9b	40.2 (t)	1.64[b]	
9b		1.50[b]	H-9a, H-10a, H-10b		1.62[b]	
10a	20.5 (t)	1.70[b]	H-9b, H-10b	20.6 (t)	1.58[b]	
10b		1.44[b]	H-9b, H-10a		1.31[b]	
11	37.6 (d)	2.68 dt (14.0, 8.2)	H-7, H-10	41.8 (d)	2.00[b]	H-7
12	41.2 (s)			40.3 (s)		
13	144.5 (s)			144.3 (s)		
14a	37.5 (t)	2.27 td (12.4, 4.8)	H-14b, H-15a, H-15b	37.0 (t)	2.29[b]	H-14b
14b		1.48[b]	H-14a, H-15a, H-15b		1.35[b]	H-14a
15a	25.0 (t)	2.42 t (7.6)	H-14a, H-14b, H-15b, H-16	24.5 (t)	2.29[b]	H-15b, H-16
15b		1.80[b]	H-14a, H-14b, H-15a, H-16		1.79[b]	H-15a, H-16
16	140.2 (d)	5.69 t (7.0)	H-15a, H-15b	140.4 (d)	5.62 bt (7.0)	H-15a, H-15b
17	131.9 (s)			131.8 (s)		
18	34.4 (t)	2.12[b]	H-19	34.4 (t)	2.05[b]	
19	27.3 (t)	2.02 bt (7.0)	H-18, H-20	27.3 (t)	1.97[b]	H-20
20	123.5 (d)	5.04 bt (7.0)	H-19, H-22, H-23	123.5 (d)	5.02 bt (7.2)	H-19, H-22
21	131.3 (s)			131.3 (s)		
22	25.5 (q)	1.63 s		25.5 (q)	1.62 s	
23	17.5 (q)	1.52 s		17.5 (q)	1.51 s	
24	7.9 (q)	1.78 s		7.9 (q)	1.76 s	
25	26.6 (q)	1.33 s		27.9 (q)	1.32 s	
26	19.9 (q)	1.08 s		20.6 (q)	1.16 s	
27	168.7 (s)			168.6 (s)		
2-OH		10.44 s				
-OH		12.31.bs, 5.14 bs				

[a] Spectral data recorded in DMSO-d_6 at 400 MHz for ^{1}H and at 100 MHz for ^{13}C; δ (ppm) (J = Hz). [b] Resonance multiplicity obscured by overlapping signals.

at δ 10.44 (C2-OH) to carbon signals at δ 184.2 (C-1), C-2, and C-3. Additional cross-peaks from a methyl singlet at δ 1.33 (H_3-25) to a quaternary alcohol signal at δ 79.6 (C-8), and to C-7 and C-9, were consistent with the presence of a five-membered ring system. Another spin system, H-6, H-7, H-11, H_2-10, H_2-9, was delineated from the COSY data, establishing connectivity between the three ring systems. Finally, HMBC cross-peaks from H-6 to a carbon resonating at δ 186.5 (C-4) and to one at δ 143.7 (C-5) linked the five-membered ring system to the quinone ring, thus completing substructure **1b**.

The two substructures were linked as follows: the remaining methyl group was placed at C-12 through HMBC correlations from a methyl singlet at δ 1.08 (H_3-26) to carbon signals at δ 41.2 (C-12) and 144.6 (C-13), and C-11 or C-14. Long-range C−H couplings from H-14b to C-11, C-12, C-13, and C-26 defined the remaining six-membered ring. Thus the side chain was connected through C-14 to C-12 and established the link of **1a** to **1b**, thereby completing the skeletal structure of pycnanthuquinone A (**1**). To solve any ambiguity due to proton signal overlap in DMSO-d_6, the δ 1.4−1.9 region was also examined with spectra taken in pyridine-d_5. In a COSY experiment the key correlation between H-10 (δ 2.10) and H-11 (δ 3.43) was observed.

Treatment of **1** with acetic anhydride−pyridine gave diacetate derivative **3**, M^+ m/z 556. In the ^{1}H NMR spectrum, seven methyl groups were observed as expected. Acetylation at C-6 (OH) caused the downfield shift of the methine proton at C-6 to δ 6.18 (d, J = 3.2 Hz).

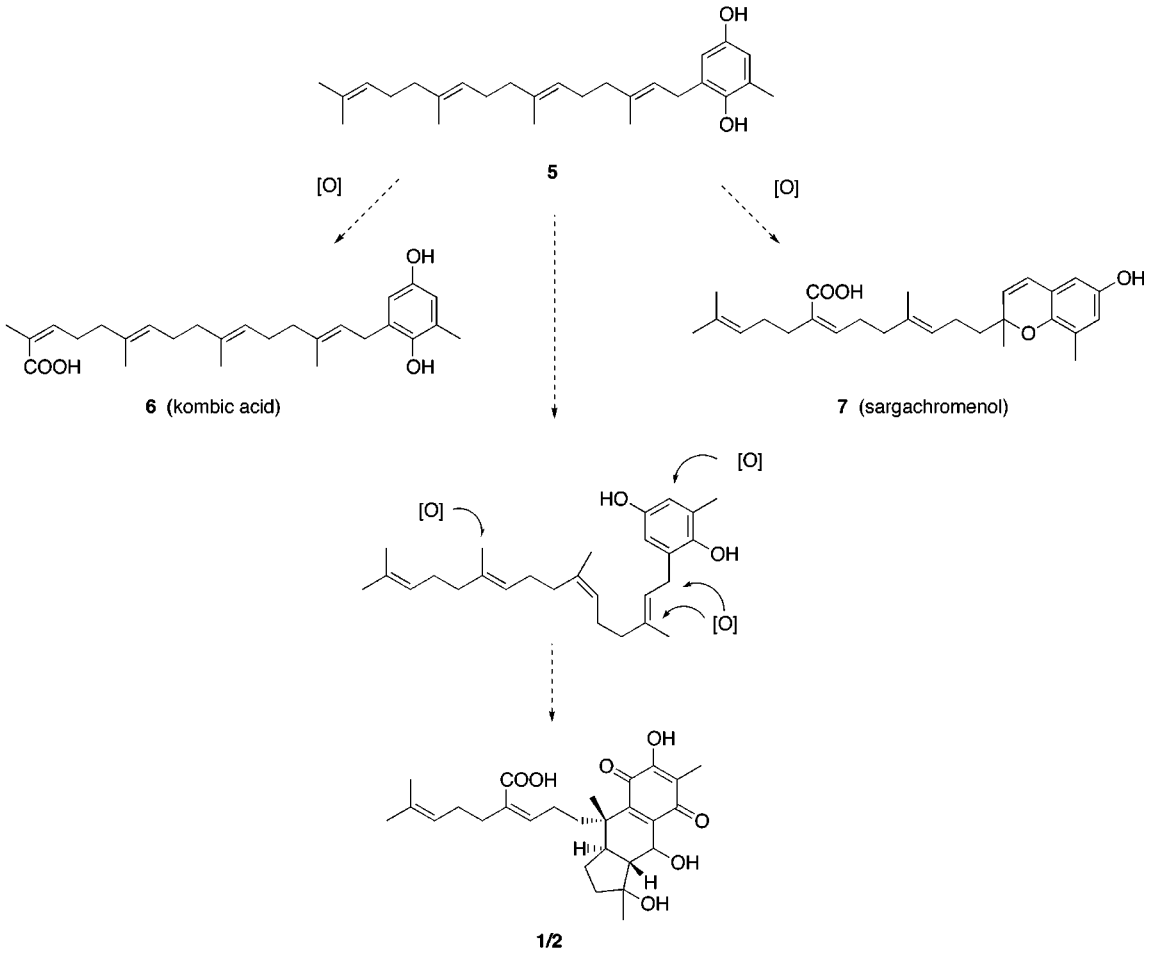
Pycnanthuquinone B **(2)** also gave a molecular ion peak at m/z 472.2435 by HREIMS, indicating that **2** was isomeric with **1**. The EIMS and ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. Significant differences were only observed in the carbon shifts assigned to C-5, C-6, C-7, C-8, and C-12 and the proton shift and coupling of H-6 (see Tables 1 and 2). Mass spectral and 2D NMR data revealed that **1** and **2** were indeed diastereomers. Complete HMBC correlations led to assignments given in Tables 1 and 2. Acetylation of **2** using identical

Table 2. HMBC Correlations of Pycnanthuquinone A (1)

proton	HMBC correlations for **1** in DMSO-d_6[a]	HMBC correlations for **1** in pyridine-d_5[b]
6	C-4, C-5, C-7, C-11	C-4, C-5, C-7, C-11, C-13
7	C-9, C-12, C-25	C-9, C-25
9a	C-7, C-11	C-7, C-8
9b	C-11	
10a	C-7, C-8, C-12	
10b	C-12	
11	C-7, C-14, C-26	C-6, C-7, C-10, C-12, C-26
14a	C-11, C-12, C-15, C-16, C-26	
14b	C-11, C-12, C-13, C-15, C-16, C-26	
15a	C-12, C-14, C-16, C-17	
15b	C-12, C-14, C-16, C-17	C-16, C-17
16	C-14, C-17, C-18, C-27	C-18, C-27
18	C-16, C-17, C-19, C-20, C-27	C-19, C-27
19	C-17, C-18, C-20, C-21	C-18, C-20, C-21
20	C-18, C-19, C-22, C-23	C-19, C-22, C-23
22	C-20, C-21, C-23	C-20, C-21, C-23
23	C-20, C-21, C-22	C-20, C-21, C-22
24	C-2, C-3, C-4	C-2, C-3
25	C-7, C-8, C-9	C-7, C-8, C-9
26	C-12, C-13, C-11 or C-14	C-12, C-13, C-14
2-OH	C-1, C-2, C-3	

[a] Recorded at 600 MHz. [b] Recorded at 400 MHz.

Scheme 1



conditions to those for **1** gave only the C-6 monoacetylated product: M^+, *m/z* 514. The H-6 proton was now shifted downfield to δ 6.06 (d, $J = 9.2$ Hz) coupled to H-7 (br d, $J = 9.2$ Hz). In **2**, C-6 resonated at δ 64.4, shifting to δ 67.3 upon acetylation (anticipated acetylation shifts at C-5 and C-7 were also seen). It is possible that the observed regioselectivity in the acetylation of **2** may be due to hydrolysis of any C-3 acetate upon workup.

Completion of stereochemical assignments in **1** and **2** were made on the basis of ROESY experiments. ROESY cross-peaks for **1** from H-16 to H_2-18 established a Δ16 *Z* geometry. A $^3J_{(H7-H11)}$ coupling of ~14 Hz in **1** and **2** required a *trans*-fused ring junction between the five- and six-membered rings. A coupling constant of 3.6 Hz between H-6 and H-7 in **1** indicated 6α-OH, while one of 8.0 Hz in **2** indicated 6β-OH. ROESY cross-peaks from H-7 to H_3-26, and from H-11 to H_2-14, led to the indicated stereochemistry at C-12 in **1**. Relative stereochemistry at C-8 could not be determined due to the lack of cross-peaks from H_3-25 to any other protons in **1** or in several derivatives that were synthesized.[23]

The mass spectral fragmentation data for both **1** and **2** in EI and FAB modes fully support the structures. Details are presented in the Supporting Information section.

The pycnanthuquinones may be considered as biogenetically derived through a series of oxidations and an elaborate ring forming process from a prenylated shikimate derivative **5** (Scheme 1). An oxidized derivative of **5**, kombic acid **(6)**, has been reported from the seed fat of *Pycnanthus kombo*.[16] We were unsuccessful at detecting **6** in *P. angolensis*; however, we did isolate and characterize sargachromenol **(7)**,[24] which is also presumably derived from **5**.

Pycnanthuquinones **1** and **2** were tested for antihyperglycemic activity in *db*/*db* mice by oral administration at 100 and 250 mg/kg. Blood glucose lowering effects were observed for pycnanthuquinone A (**1**), on days 1 and 2, and for pycnanthuquinone B (**2**), on day 2, at 100 and 250 mg/kg dose, respectively (Table 3). No effects on food intake or body weight were observed when compared to the vehicle control, except for **2** at the high dose.

Conclusion

Two novel quinones containing a unique terpenoid skeleton, pycnanthuquinone A **(1)** and pycnanthuquinone B **(2)**, have been isolated from *P. angolensis* using in vivo guided fractionation and characterized. Relative stereochemistry has been proposed for both compounds, with the exception of the quaternary stereocenter at C-8. Both compounds display significant antihyperglycemic activity when administered orally to *db*/*db* mice, a recognized animal model for Type 2 diabetes mellitus.

(23) Compounds **1** and **2** are amorphous solids. We attempted to crystallize **1** and **2** in many solvents but were unsuccessful. We also prepared various derivatives of **1** and **2** (for example, *p*-bromobenzoates) for NMR and X-ray crystallography studies but were unsuccessful in ascertaining the relative stereochemistry at C-8 using derivatives by NMR and were unsuccessful in obtaining crystals suitable for X-ray studies.

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Table 3. Effects of 1 and 2 Isolated from *P. angolensis* on Plasma Glucose, Body Weight, and Food Intake in *db/db* Mice[a]

treatment	dose, mg/kg	plasma glucose, % change[b] 3 h	27 h	ANOVA[c] 3 h	27 h	mean body weight, g/mouse 0 h	24 h	food intake, g/mouse/day
1	100	−13	−22	0.0144	0.0009	40.7 ± 0.9	40.7 ± 0.9	5.2
1	250	−14	−33	0.0104	<0.0001	41.0 ± 1.1	41.4 ± 1.2	5.2
2	100	−6	−12	0.1688	0.0623	40.8 ± 0.6	42.0 ± 0.8	6.0
2	250	−8	−58	0.1042	<0.0001	42.1 ± 0.7	41.9 ± 0.7	3.5
metformin[d]	250	−27	−27	<0.0001	<0.0001	42.3 ± 0.8	42.2 ± 0.8	4.5

[a] Mice ($n = 8$) were dosed at 0 and 24 h. Plasma glucose levels measured at 3 and 27 h postdose. [b] Compared with the vehicle control. [c] Analysis of variance (one factor). Vehicle used as a negative control. [d] The known antihyperglycemic agent metformin was used as a positive control. See ref 25.

Experimental Section

General Experimental Procedures. NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C, or on a 600 MHz spectrometer at Oregon State University (see Acknowledgments). H−C one-bond connectivities and carbon multiplicities were determined by HMQC and DEPT experiments. All solvents used were HPLC grade. TLC was performed on EM Science silica gel ($60F_{254}$) plates using a H_2SO_4/vanillin stain. Column chromatography was carried out using LH-20 from Pharmacia or HP-20 from Mitsubishi Chemical. A Primesphere C-18 (HC) 10 μm column (50 × 250 mm), a YMC-Pack polymeric C-18 10 μm column (50 × 250) with a guard column (30 × 50 mm), or a YMC-Pack polymeric C-18 10 μm column (20 × 250) was used for HPLC; this HPLC was equipped with diode array and light scattering detectors. The following solvents and HPLC conditions were used: A (2-propanol), B (acidified CH_3CN, 0.25 mL of glacial acetic acid to 1 L of CH_3CN), and C (acidified water at pH 4.5 ± 0.05, prepared by adding 10 μL of glacial acetic acid to 1 L of water); flow = 65 mL/min; solvent gradient = B/C (50:50) to B/C (70:30) in 10 min, B/C (70:30) for 10 min, A/B (50:50) for 5 min, B/C (50:50) for 5 min; monitoring wavelength $\lambda = 275$ nm, sample concentration = 450 mg/mL; injection volume = 500 μL.

Plant Material. The leaves and stems of *Pycnanthus angolensis* were collected in the village of Aburi, Ghana, and identified by D. K. Abbiw (Pharmahealth Centre Ltd. Ghana). Voucher specimens are deposited in the reference collection, Department of Ethnobotany and Conservation, Shaman Pharmaceuticals, Inc.

Optimized Extraction and Isolation. Dried ground leaves[19] of *P. angolensis* (5.0 kg) were placed in 80% ethanol (50 L) in a high-density polypropylene tank equipped with an air driven agitator (Grovhac Inc.). The extraction was performed at room temperature for 24 h while mixing vigorously. The extract was then filtered through Celite, the plant marc was soaked with 13 L of 80% EtOH, and then the marc extract was filtered through Celite. The combined ethanolic extracts were concentrated under reduced pressure at 40 °C to yield 559.0 g of extract (11.2%). A 292 g portion of the ethanol extract was subjected to liquid−liquid partition (H_2O:EtOAc, 1:1, 7 L), stirring with a mechanical stirrer for 30 min and then allowing the mixture to stand undisturbed for 1 h. The soluble material was transferred by decantation, and the layers were allowed to separate. The EtOAc layer was separated, and the aqueous layer was washed twice with 1 L portions of EtOAc. The EtOAc partitions were combined. The aqueous layer and the insoluble material were discarded. The EtOAc layer was transferred to a 10 L bottle equipped with a mechanical stirrer and 3 L of a 5% $NaHCO_3$ solution was added. The mixture was stirred for 5 min, transferred to a separatory funnel, and allowed to stand at room temperature overnight. Following separation, the EtOAc layer was extracted twice with 1 L portions of an aqueous 5% $NaHCO_3$ solution. The aqueous layers were combined, and the pH was adjusted to 4.2 by adding dropwise a solution of 10% aqueous H_2SO_4 while stirring in a container submerged in an ice−water bath. The acidified aqueous mixture was extracted with EtOAc (3 × 1.5 L). The combined EtOAc extracts were washed with water (2 × 400 mL), dried over Na_2SO_4, and concentrated under reduced pressure at 40 °C to yield 42.8 g (yield 1.63%) of extract. A 16.9 g portion of the EtOAc extract was dissolved in 100 mL of EtOAc and subjected to column chromatography on 1.9 L of LH-20 packed in CH_3CN (10 × 60 cm). The column was eluted with 25 L of CH_3CN at a flow rate of 45 mL/min. The entire CH_3CN eluent was then concentrated under reduced pressure at 40 °C in a rotary evaporator to yield 8.8 g (yield 0.85%) of an enriched fraction that contained **1** and **2**. Final purification by preparative HPLC using a Primesphere column (see General Experimental Procedures) gave 0.39 g (0.038%) of pycnanthuquinone A (**1**) and 0.43 g (0.042%) of pycnanthuquinone B (**2**).

Pycnanthuquinone A [(5*R,5a*R**,8a*R**,9*S**)-2,5,6-Trihydroxy-3,6,9-trimethyl-9-(4-carboxy-8-methylnona-3,7-dien-1-yl)cyclopenta[*g*]decalin-2,4a-diene-1,4-dione] (1):** TLC R_f 0.42 CH_2Cl_2:MeOH (9:1); $t_R = 15.4$ min (YMC-Pack, 20 × 250); UV (MeOH) λ_{max} 270 nm (log $\epsilon = 3.97$), λ_{max} 398 nm (log $\epsilon = 2.84$); see Table 1 for ^{1}H and ^{13}C NMR data in DMSO-d_6; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 5.89 (1H, t, J = 7.6, H-16), 5.66 (1H, d, $J = 3.6$, H-6), 5.23 (1H, tt, $J = 7.2$, 1.6, H-20), 3.43 (1H, m, H-11), 2.99 (1H, H-15a), 2.74 (1H, H-14a), 2.48 (3H, H-15b, H-18), 2.31 (2H, H-19), 2.10 (1H, H-10a), 2.09 (3H, s, H-24), 2.02 (1H, H-9a), 1.82 (1H, H-14b), 1.63 (4H, bs, H-10b, H-22), 1.62 (1H, H-9b), 1.56 (3H, bs, H-23), 1.54 (3H, s, H-25), 1.50 (1H, dd, J = 13.6, 3.6, H-7), 1.36 (3H, s, H-26); ^{13}C NMR (pyridine-d_5) δ 187.8 (C-4), 185.6 (C-1), 170.4 (C-27), 155.9 (C-2), 145.8 (C-13), 144.8 (C-5), 140.9 (C-16), 133.4 (C-17), 131.8 (C-21), 124.5 (C-20), 116.9 (C-3), 80.2 (C-8), 62.0 (C-6), 47.4 (C-7), 42.1 (C-12), 41.5 (C-9), 38.8 (C-11), 38.5 (C-14), 35.7 (C-18), 28.4 (C-19), 27.1 (C-25), 26.2 (C-15), 25.7 (C-22), 21.4 (C-10), 20.4 (C-26), 17.7 (C-23), 8.4 (C-24); HREIMS m/z 472.2440 [M^+], calcd for $C_{27}H_{36}O_7$ 472.2461.

Pycnanthuquinone B [(5*S,5a*R**,8a*R**,9*S**)-2,5,6-Trihydroxy-3,6,9-trimethyl-9-(4-carboxy-8-methylnona-3,7-diene-1-yl)cyclopenta[*g*]decalin-2,4a-diene-1,4-dione] (2):** TLC R_f 0.35 CH_2Cl_2:MeOH (9:1); $t_R = 13.6$ min (YMC-Pack, 20 × 250); UV (MeOH) λ_{max} 273 nm (log $\epsilon = 3.99$), λ_{max} 403 nm (log $\epsilon = 2.79$); see Table 1 for ^{1}H and ^{13}C NMR data; HREIMS m/z 472.2435 [M^+], calcd for $C_{27}H_{36}O_7$ 472.2461.

Pycnanthuquinone A diacetate (3): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.18 (1H, d, $J = 3.2$, H-6), 5.66 (1H, t, $J = 7.2$, H-16), 5.02 (1H, bt, $J = 7.2$, H-20), 2.65 (1H, m, H-11), 2.32 (3H, s, 2-OAc), 2.10 (2H, H-18ab), 1.99 (2H, H-19ab), 1.84 (3H, s, H-24 or 6-OAc), 1.83 (3H, s, H-24 or 6-OAc), 1.62 (3H, s, H-22), 1.51 (3H, s, H-23), 1.38 (1H, dd, $J = 13.6$, 2.8, H-7), 1.30 (3H, s, H-25), 1.08 (3H, s, H-26), signals for H-9, H-10, H-14, and H-15 obscured by signal overlap; ^{13}C NMR (DMSO-d_6) δ 185.1 (C-4), 180.3 (C-1), 168.8 (C-27 or 6-OAc), 168.7 (C-27 or 6-OAc), 167.8 (2-OAc), 149.1 (C-13), 148.9 (C-2), 141.6 (C-5), 140.2 (C-16), 131.8 (C-17), 131.8 (C-3), 131.3 (C-21), 123.5 (C-20), 77.9 (C-8), 61.0 (C-6), 46.5 (C-7), 42.0 (C-12), 41.8 (C-9), 38.1 (C-11), 37.5 (C-14), 34.3 (C-18), 27.2 (C-19), 25.4 (C-22), 24.9 (C-25), 24.8 (C-15), 21.4 (6-OAc), 20.0 (2-OAc), 19.9 (C-10), 19.3 (C-26), 17.5 (C-23), 8.9 (C-24).

Pycnanthuquinone B monoacetate (4): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.06 (1H, d, $J = 9.2$, H-6), 5.62 (1H, t, $J = 6.8$, H-16), 5.02 (1H, bt, $J = 7.2$, H-20), 2.33 (1H, H-14a), 2.31 (1H, H-15a), 2.16 (1H, H-11), 2.07 (2H, H-18ab), 1.97 (2H, H-19ab), 1.89 (3H, s, 6-OAc), 1.87 (1H, H-15b), 1.73 (3H, s, H-24), 1.62 (3H, s, H-22), 1.62 (3H, H-9ab, 10a), 1.61 (1H, dd, $J = 9.2$,

one *J* obscured, H-7), 1.51 (3H, s, H-23), 1.38 (2H, H-14b, 10b), 1.19 (3H, s, H-26), 1.16 (3H, s, H-25); ^{13}C NMR (DMSO-d_6) δ 186.1 (C-4), 183.3 (C-1), 169.3 (6-OAc), 168.6 (C-27), 153.3 (C-2), 147.0 (C-13), 143.8 (C-5), 140.2 (C-16), 132.0 (C-17), 131.3 (C-21), 123.5 (C-20), 116.1 (C-3), 76.7 (C-8), 67.3 (C-6), 49.1 (C-7), 42.1 (C-11), 40.4 (C-12), 40.2 (C-9), 36.9 (C-14), 34.4 (C-18), 27.3 (C-19), 26.6 (C-25), 25.4 (C-22), 24.4 (C-15), 21.2 (6-OAc), 20.5 (C-26), 20.4 (C-10), 17.5 (C-23), 7.9 (C-24).

Sargachromenol (7): UV (MeOH) λ_{max} 264 nm, 334 nm (lit.[24] λ_{max} 263 nm, 332 nm); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.71 (s, 6′-OH), 6.38 (1H, d, J = 2.8, H-7′), 6.28 (1H, d, J = 9.6, H-3′), 6.27 (1H, d, J = 2.4, H-5′), 5.76 (1H, t, J = 7.4, H-7), 5.64 (1H, d, J = 9.6, H-2′), 5.09 (1H, t, J = 7.2, H-11), 5.04 (1H, t, J = 7.2, H-3), 2.43 (2H, dd, J = 14.8, 7.2, H-8), 2.13 (2H, H-5), 2.04 (2H, H-12), 2.02 (3H, s, 8′-Me), 2.01 (2H, H-4), 1.98 (2H, dd, J = 14.8, 7.6, H-9), 1.61 (3H, s, H-1), 1.58 (2H, t, J = 8, H-13), 1.51 (3H, s, 2-Me), 1.50 (3H, s, 10-Me), 1.27 (3H, s, 1′-Me); ^{13}C NMR (DMSO-d_6) δ 168.9 (6-COOH), 150.3 (C-6′), 142.9 (C-9′), 140.2 (C-7), 134.0 (C-10), 131.9 (C-6), 131.2 (C-2), 130.5 (C-2′), 124.9 (C-8′), 124.3 (C-11), 123.5 (C-3), 122.8 (C-3′), 120.9 (C-4′), 116.8 (C-7′), 110.2 (C-5′), 77.1 (C-1′), 40.1 (C-13), 38.6 (C-9), 34.4 (C-5), 27.4 (C-4), 27.3 (C-8), 25.4 (C-1 and C-1′-Me), 22.1 (C-12), 17.5 (2-Me), 15.5 (10-Me), 15.2 (8′-Me); HREIMS *m/z* 424.2581 [M$^+$], calcd for $C_{27}H_{36}O_4$ 424.2614.

Antihyperglycemic Testing: *In Vivo* Studies Using *db/db* Mice. Genetically altered obese diabetic mice (designated C57BL/Ks-*db*/*db*) were purchased from the Jackson Laboratory (Bar Harbor, ME) and served as experimental animals. Male animals between the ages of 8−9 weeks were employed. Animals were housed (four mice/cage) under standard laboratory conditions at 22 °C with Purina rodent chow and water ad libitum. Prior to treatment, blood was collected from the tail vein of each animal. Mice that had plasma glucose levels between 350 and 600 mg/dL were used. Each treatment group consisted of eight mice that were distributed so that mean glucose levels were equivalent in each group at the start of the study. Mice were dosed orally by gavage once daily for 2 days with either vehicle, the experimental compound at 100 or 250 mg/kg, or metformin at 250 mg/kg qd (positive control). Metformin (1,1-dimethylbiguanide) was purchased from Sigma Chemical Co. (St. Louis, MO).[25] Compounds were delivered in an aqueous vehicle formulation consisting of 0.25% (w/v) carboxymethylcellulose, 1% (v/v) Tween 60, and 10% (v/v) DMSO in a volume of 10 mL/kg. Blood was sampled 3 h postdosing and analyzed for plasma glucose levels. Individual body weights and mean food consumption (each cage) were also measured after 24 h. Plasma glucose levels were determined colorimetrically using a glucose oxidase assay (Sigma Chemical Co.). Significant differences between groups (comparing compound-treated to vehicle-treated) were evaluated using analysis of variance.

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Supporting Information Available: Additional characterization data for **1** and **2**; ^{1}H NMR, COSY, ^{13}C NMR, and HMBC spectra for **1** and **2**; and a detailed interpretation of the mass spectral data . This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Metformin is commonly prescribed to treat Type II diabetic patients and is known as Glucophage.