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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · MAY 2005

Impact Factor: 3.8 · DOI: 10.1021/np0496688 · Source: PubMed

Constituents of the Leaves and Twigs of *Calyptranthes pallens* Collected from an Experimental Plot in Southern Florida

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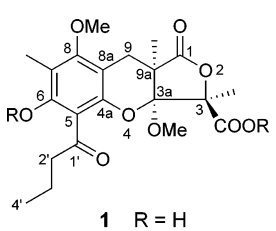
Received October 14, 2004

Fractionation of the chloroform-soluble extract of the leaves and twigs of *Calyptranthes pallens*, collected from an experimental plot in a hardwood forest of southern Florida, using a hormone-dependent human prostate carcinoma (LNCaP) tumor cell line, led to the isolation of a phloroglucinol derivative with a novel carbon skeleton, pallenic acid (**1**), and a new triterpenoid, 3β-hydroxy-18α,19α-urs-20-en-28-oic acid (**2**). The known compound methylene-bis-aspidinol (**3**) was selectively active against the human oral epidermoid carcinoma (KB) cell line. Several known compounds of the ellagic acid, lignan, phloroglucinol, sterol, and triterpene types were also obtained in the present investigation.

A plot-based collection was carried out in three locations of tropical hardwood forests in Southern Florida known as "hammocks", as an approach to the selection and recollection of bioactive plants representing wide biodiversity for laboratory study.[1−4] As part of our program to discover novel anticancer agents from plants,[5] one of the plants selected for study from these plots was *Calyptranthes pallens* Griseb. The genus *Calyptranthes*, with about 100 species distributed from Mexico to Uruguay, belongs to the family Myrtaceae.[6] Two species in this genus, *C. aromatica* St. Hil. and *C. schiedeana* Beng., are used as spices in Brazil and Mexico, respectively.[7] Most of the phytochemical work performed on the genus *Calyptranthes* has been on the characterization of the essential oils from the leaf extracts.[8−10] *C. pallens*, known as "pale lidflower" or "spicewood", is a rare species, native to the Southern Florida coastal hammocks and the Keys,[11] with no previous reports on either its biological activities or phytochemistry. This species is a small tree characterized by a smooth gray bark and branchlets produced in pairs at each leaf node. The most distinguishing characters of this species are its petal-less flowers in large clusters and reddish trichomes that cover the twigs, flowers, and lower leaf surface.[11]

The chloroform-soluble extract of a mixture of the leaves and twigs of *C. pallens* exhibited selective cytotoxic activity against a hormone-dependent human prostate carcinoma (LNCaP, ED_{50} 6.0 μg/mL) cell line. Therefore, the extract was submitted to bioassay-guided fractionation testing against this same cell line, leading to the isolation of a new compound based on a novel carbon skeleton, pallenic acid (**1**), a new triterpene, 3β-hydroxy-18α,19α-urs-20-en-28-oic acid (**2**), and 11 known compounds.

Activity-guided fractionation of *C. pallens* yielded five phloroglucinol derivatives, pallenic acid (**1**), 1-(2,6-dihydroxy-4-methoxy-3-methylphenyl)ethanone,[12] aspidinol,[13] methylene-bis-aspidinol (**3**),[14] and tetracosyl ferulate,[15] a lignan, isoguaiacin,[16] two triterpenes, asiatic acid[17] and another new compound, 3β-hydroxy-18α,19α-urs-20-en-28-oic acid (**2**), two sterols, β-sitosterol[18] and β-sitosterol 3-*O*-β-D-glucopyranoside,[19] and three ellagic acid derivatives, 4,3′-di-*O*-methylellagic acid,[20] 3,4-methylenedioxy-3′-*O*-methylellagic acid,[21] and pteleoellagic acid.[22] The structures of the known compounds were identified by comparison of their spectroscopic data with those values reported previously. ^{1}H and ^{13}C NMR data are included in the Experimental Section for compound **3**, 1-(2,6-dihydroxy-4-methoxy-3-methylphenyl)ethanone, and aspidinol.



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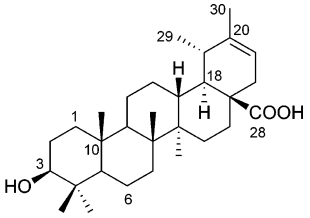
1a R = Me



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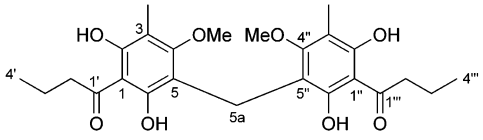

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Compound **1**, isolated as a white solid, mp 120−121 °C and $[\alpha]^{20}_D$ +17° (MeOH), exhibited a molecular ion peak at *m/z* 422 in the EIMS, and the HREIMS {$[M]^+$ *m/z* 422.1566} confirmed a molecular formula of $C_{21}H_{20}O_9$ (calcd 422.1577), indicating 12 degrees of unsaturation. The IR spectrum showed a broad band at 3324 cm^{-1}, typical of hydroxyl group(s), and absorptions at 1770 and 1710 cm^{-1}, supportive of a carboxylic acid group and a lactone carbonyl, respectively. Observed in the ^{1}H NMR spectrum (300 MHz, MeOH-d_4) were signals for two methoxy groups at δ 3.74 (3H, OCH_3-8) and 3.67 (3H, OCH_3-3a), three methyl singlets at δ 2.05 (3H, CH_3-7), 1.73 (3H, CH_3-3), and 1.48 (3H, CH_3-9a), one methyl triplet at δ 0.92 (3H, H-4′), two methylenes seen as a doublet at δ 3.22 (2H, H-2′) and a multiplet at δ 1.70 (2H, H-3′), respectively, and two geminal-coupled protons of a third methylene at δ 3.14 (1H, J = 15.3 Hz, H_a-9) and 2.59 (1H, J = 15.3 Hz, H_b-9). In

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10.1021/np0496688 CCC: $30.25
Published on Web 03/15/2005

addition to the above-mentioned signals, the ^{13}C and DEPT-135 NMR spectra indicated the presence of 12 quaternary carbons in the molecule of **1**. Comparison of these 1D NMR data with those of 1-(2,6-dihydroxy-4-methoxy-3-methylphenyl)ethanone, aspidinol, and methylene-bis-aspidinol (**3**) suggested the presence of an aspidinol (1-[2,6-dihydroxy-4-methoxy-3-methylphenyl]propanone) moiety in the structure of compound **1**. This was confirmed by the observed $^1H-^1H$ COSY correlations from H-3′ to both H-2′ and CH_3-4′ and the HMBC correlations from H-3′ to C-1′, C-2′, and CH_3-4′, from the protons of CH_3-4′ to C-2′ and C-3′, from CH_3-7 to C-8, C-7, and C-6, from OCH_3-8 to C-8, and from both H_a-9 and H_b-9 to C-4a, C-8a, and C-8. In addition to the 13 carbons of the aspidinol structural unit of **1**, the remaining nine carbons comprised two methyl groups at δ 22.4 and 23.0, one methoxy group at δ 53.4, one aliphatic quaternary carbon at δ 48.4, two oxygenated quaternary carbons at δ 92.2 and 110.1, and two carbonyl carbons at δ 173.2 and 178.7. In the HMBC spectrum of **1**, correlations were observed from both Ha-9 and Hb-9 to C-1, C-9a, C-3a, and CH_3-9a, from the proton signal of CH_3-9a to C-1, C-9a, C-3a, and C-9, from the aliphatic methoxy signal at δ 3.67 (OMe-3a) to the doubly oxygenated carbon at δ 110.1 (C-3a), and from the methyl singlet at δ 1.73 (CH_3-3) to C-3, C-3a, and a carbonyl carbon at δ 173.2 (COOH-3). From these correlations, in combination with the unsaturation value of compound **1**, the most probable structure for this novel isolate was proposed as shown. To confirm the structure, compound **1** was methylated overnight using freshly prepared CH_2N_2. One aromatic methoxy and one ester methoxy group for **1** would be expected to be formed additionally by methylation. The EIMS of the methylation product **1a** exhibited a molecular ion peak at *m/z* 450 $[M]^+$, and the HREIMS data at *m/z* 450.1886 suggested a molecular formula of $C_{23}H_{30}O_9$. This confirmed the presence of two additional methoxy groups in the methylation product compared with the isolate. The ^1H NMR spectrum of **1a** showed a total of four resonances in the methoxy region at δ 3.81, 3.74, 3.67, and 3.56, one aromatic methoxy (δ 3.74, 3H, s, OCH_3-6) and one ester methoxy (δ 3.81, 3H, s, $COOCH_3$-3) more than those displayed in the ^1H NMR spectrum of **1**. Accordingly, the structure of the original compound was determined as **1**. The assignments for both compounds (**1** and **1a**) were based on the analysis of the observed correlations in their 2D NMR spectra. The relative stereochemistry of compound **1** was deduced from the NOESY correlations from CH_3-9a to both CH_3-3 and the methoxy signal at C-3a. Similar correlations were also obtained in the NOESY spectrum of the methylation product, **1a**. On the basis of the above-described spectral and chemical evidence, the structure of **1** was proposed as shown, with this compound assigned the trivial name pallenic acid.

Compound **2** was isolated as a white amorphous solid, mp 250−253 °C, and $[\alpha]^{20}_D$ −67° (MeOH). It exhibited a molecular ion at *m/z* 456 $[M]^+$ in the EIMS and in the HREIMS at *m/z* 456.3563 (calcd 456.3604), supporting a molecular formula of $C_{30}H_{48}O_3$. The IR spectrum showed a broad peak at 3546 cm^{-1}, typical of a hydroxyl group, and an absorption at 1764 cm^{-1}, characteristic of a carboxylic acid. The ^1H NMR (360 MHz, pyridine-d_5) spectrum exhibited a distinctive triterpenoid profile, with a downfield doublet at δ 5.49 typical of a double bond, a methine proton triplet at δ 3.49, and six singlets and one doublet accounting for seven methyl groups. The ^{13}C NMR spectrum showed resonances for 30 carbons that were differentiated by a DEPT-135 NMR experiment as seven methyls, nine

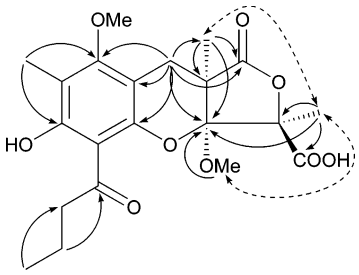

Figure 1. Selected HMBC (H → C) and NOESY (H <- - -> H) correlations of pallenic acid (**1**).

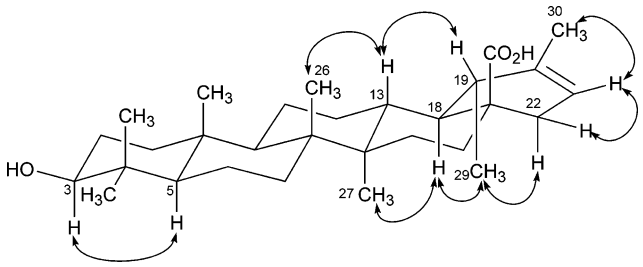

Figure 2. Selected NOESY correlations of 3-β-hydroxy-18α,19α-urs-20-en-28-oic-acid (**2**).

methylenes, seven methines, and seven quaternary carbons. The most downfield signals corresponded to a carbonyl at δ 178.2 (C-28), two olefinic carbons at δ 143.1 (C-20) and 117.9 (C-21), and an oxygenated carbon at δ 78.1 (C-3). The assignments of the ^1H NMR and ^{13}C NMR spectra for these groups were carried out by a combination of 2D HMBC, HMQC, and COSY NMR experiments and permitted the carbon skeleton of **2** to be determined as ursane, the same as the previously mentioned asiatic acid.[21] In the HMQC spectrum, the proton at δ 3.49 (H-3) was correlated with the oxygenated carbon at δ 78.1 (C-3). The cross-peaks between H-3 and carbons of the methyl groups at C-23 (δ 16.5) and C-24 (δ 28.3) in the HMBC spectrum confirmed the position of the hydroxyl group at C-3. The most relevant correlations in the HMBC spectrum were seen between H-21 (δ 5.49) and C-30 (δ 22.3), C-19 (δ 37.8), and C-17 (δ 49.1). Similarly, there were cross-peaks of CH_3-30 (δ 1.74) with C-21 (δ 117.9) and C-20 (δ 143.1), as well as a correlation between CH_3-29 (δ 1.13) and C-20 (δ 143.1). These connectivities suggested the presence of a double bond between C-20 and C-21. Correlations from H-13 to both H-19 and CH_3-26 and from H-18 to both CH_3-27 and CH_3-29 were observed in the NOESY spectrum of **2** (Figure 2). These key correlations indicated the relative configuration for H-13, H-18, and H-19 to be β, α, and β, respectively. On the basis of the interpretation of all of the above data, the structure of **2** was assigned as 3β-hydroxy-18α,19α-urs-20-en-28-oic acid.

Of the isolates from *C. pallens*, isoguaiacin and the new triterpene 3β-hydroxy-18α,19α-urs-20-en-28-oic acid (**2**) exhibited marginal cytotoxic activity against several human cancer cell lines (Table 1). The known compound aspidinol (**3**) exhibited selective cytotoxicity for the human oral epidermoid carcinoma (KB cell line), of the cell lines in which it was evaluated.[22] The remaining compounds were inactive in this tumor cell panel. It is possible that the activity of the crude chloroform-soluble extract (Experimental Section) was due to the synergistic effect of the mixtures of the chemical constituents of *C. pallens*, since the cytotoxicities of the fractions and the pure compounds obtained in the present study were somewhat weaker than this extract.

Table 1. Cytotoxicity of Selected Constituents of *Calyptranthes pallens* Leaves and Twigs

	cell line[a]					
compound	Lu1	LNCaP	MCF-7	KB	hTERT-RPE1	HUVEC
2	12.9	7.3	9.7	>20	NT[b]	10.4
3	19.4	6.6	7.3	2.7	NT[b]	6.1
isoguaiacin	15.7	11.8	12.4	6.4	>20	14.7

[a] Cell lines: Lu1 = human lung carcinoma; LNCaP = hormone-dependent human prostate carcinoma; MCF-7 = human breast carcinoma; KB = human epidermoid carcinoma in the mouth; hTERT-RPEI = human telomerase reverse transcriptase-retinal pigment epithelial; HUVEC = human umbilical vein endothelial. Results are expressed as ED_{50} values (μg/mL), with testing performed according to established protocols.[23] [b]Not tested.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. Circular dichroism (CD) measurements were performed using a JASCO 600 CD spectrometer. IR spectra were recorded using a JASCO FT/IR-410 Fourier transform infrared spectrometer. NMR spectra were recorded with TMS as internal standard, using either a Bruker Avance DPX-300 NMR (300 MHz), DPX-360 NMR (360 MHz), or DRX-500 (500 MHz) NMR spectrometer. HREIMS and LREIMS were recorded on a Finnigan-MAT 95 mass spectrometer (70 eV), while LRFABMS were recorded on a VG70E-HF mass spectrometer. Column chromatography was carried out on silica gel 60 (Merck, Darmstadt, Germany; 70−230 mesh), octadecyl (C_{18})-derivatized reversed-phase silica gel (Sigma-Aldrich, St. Louis, MO, 230−400 mesh), and Sephadex LH-20 (Amersham Biosciences, Ltd, Amersham, Buckinghamshire, U.K.). Preparative TLC was performed on silica gel 60 F254 glass plates, 1 mm thick (Merck, Darmstadt, Germany). Analytical TLC was performed on precoated, 0.25 mm thick, silica gel 60 F254 aluminum plates. Fractions were monitored by TLC with visualization under UV light (254 and 365 nm) and by dipping the plates into a solution of 1% vanillin/sulfuric acid and heating (110 °C).

Plant Material. The leaves and twigs of *C. pallens* were collected from an experimental plot established in Matheson Hammock, Dade County, FL, in spring 2000 under permit No. 0014 (1999−2003) from the Natural Areas Management, Miami-Dade County Park and Recreation Department. Voucher specimens representing this collection have been deposited at the Fairchild Tropical Garden Herbarium, Coral Gables, FL, and at the Field Museum of Natural History, Chicago, IL, under accession numbers TL-47 and F-2251611, respectively.

Extraction and Isolation. An air-dried mixture of the leaves and twigs of *C. pallens* (3 kg) was milled and extracted with MeOH (6 L × 3) in a percolator at room temperature. The MeOH solutions were combined, filtered, and evaporated under a vacuum. The dried MeOH extract was dissolved in 1 L of a mixture of MeOH−H_2O (9:1) and then partitioned with petroleum ether (500 mL × 6) to afford a dried petroleum ether extract (130 g). The aqueous MeOH solution was then partitioned with $CHCl_3$ (500 mL × 5). The combined organic layer was washed using 1% saline and concentrated under vacuum to yield a $CHCl_3$ extract (160 g), which showed cytotoxicity against the LNCaP cell line with an ED_{50} value of 6.0 μg/mL. The $CHCl_3$-soluble extract (160 g) was subjected to fractionation by silica gel open column chromatography eluted with gradient mixtures of petroleum ether−EtOAc (9:1) to EtOAc−MeOH (1:1), resulting in 11 pooled fractions. Fractions 4−7 were active when tested against the LNCaP cell line (ED_{50} 8.9, 11.4, 14.3, and 10.5 μg/mL, respectively). Fraction 4 (3.0 g) was fractionated using reversed-phase (C_{18}) silica gel low-pressure column chromatography and eluted with a gradient mixture of H_2O−MeOH (3:7 to 100% MeOH), leading to eight subfractions (F00401−F00408), which were evaluated in the LNCaP cell line. One of the active subfractions, F00403 (ED_{50} 9.0 μg/mL; 267 mg), was purified over a reversed-phase (C_{18}) silica gel low-pressure column, eluted with an isocratic system using MeOH−H_2O (6:4), to yield 1-(2,6-dihydroxy-4-methoxy-3-methylphenyl)ethanone (4.2 mg), aspidinol (7.3 mg), and isoguaiacin (5.1 mg). Another of the active subfractions (F00405, ED_{50} 6.7 μg/mL; 209.8 mg) was chromatographed by normal-phase HPLC (UV at 340 nm), eluted with hexane−acetone (8:2) at a flow rate 8.0 mL/min, which afforded methylene-bis-aspidinol (**3**, t_R 6.0 min) (6.4 mg)and tetracosyl ferulate (t_R 6.7 min), mp 89 °C (2.2 mg). Fraction 5 (12.3 g) was chromatographed using a reversed-phase (C_{18}) silica gel low-pressure column, resulting in nine subfractions. Subsequently, asiatic acid (3.4 mg), mp >300 °C (lit.[17] mp 300−305 °C), was purified by preparative TLC as a white solid, using $CHCl_3$−MeOH (95:5) for elution, and 3β-hydroxy-18α,19α-urs-20-en-28-oic acid (**2**, 8.3 mg) was purified using Sephadex LH-20 eluted with MeOH. β-Sitosterol (12.1 mg), mp 140−141 °C (lit.[18] mp 139−145 °C), $[\alpha]^{20}_D$ −36° (*c* 0.1, MeOH) {lit.[24] $[\alpha]^{20}_D$ −37° (*c* 0.1, MeOH)}, was precipitated as a white solid from the last subfraction (1.09 g) from fraction 5 (12.3 g), and β-sitosterol 3-*O*-β-D-glucopyranoside (16.3 mg), mp >300 °C (lit.[19] mp 295−298 °C), $[\alpha]^{20}_D$ −46° (*c* 0.1, MeOH) {lit.[25] $[\alpha]^{20}_D$ −36° (*c* 0.1, MeOH)}, was precipitated from fraction 9 (11.3 g). Fraction 7 (16.4 g) was chromatographed using a vacuum-liquid column (VLC), eluted in a gradient mixture of increasing polarity of hexane−EtOAc−MeOH, resulting in 10 final pooled subfractions. Subsequently, the first subfraction (211 mg) was chromatographed over Sephadex LH-20, to afford 4,3′-di-*O*-methylellagic acid (1.3 mg), mp >300 °C (lit.[20] 302−304 °C), 4-methylenedioxy-3′-*O*-methylellagic acid, mp >300 °C (lit.[21] 298−300 °C) (9.3 mg), and pteleoellagic acid (7.3 mg), mp 240−242 °C (lit.[21] 297−299 °C). Fraction 10 (398 mg) was subjected to HPLC using a reversed-phase column monitored by UV at 321 and 230 nm. The solvent system used was a gradient mixture of 0.1% acetic acid in HPLC water and acetonitrile with a flow rate of 10.0 mL/min. A peak with a retention time of 12.0 min was collected and subsequently re-purified with HPLC using a gel permeation column eluted with 100% methanol (flow rate = 8.0 mL/min), to yield pallenic acid (**1**, 21.6 mg), t_R 28.0 min.

Pallenic acid {(*rel*-3*R*,3a*S*,9a*R*)-3,3a,9,9a-tetrahydro-6-hydroxy-3a,8-dimethoxy-3,7,9a-trimethyl-1-oxo-5-(1-oxobutyl)-1*H*-furo-[3,4-*b*]-1-benzopyran-3-carboxylic acid} (1): white solid, mp 120−121 °C; $[\alpha]^{20}_D$ +17° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 373 (4.21), 215 (3.12) nm; IR (film) ν_{max} 3324, 1770, 1710, 1638, 1156 cm^{-1}; ^{1}H NMR (300 MHz, MeOH-d_4) δ 3.74 (3H, s, OCH_3-8), 3.67 (3H, s, OCH_3-3a), 3.22 (2H, d, J = 7.0 Hz, H-2′), 3.14 (1H, d, J = 15.3 Hz, Ha-9), 2.59 (1H, d, J = 15.3 Hz, Hb-9), 2.05 (3H, s, CH_3-7), 1.73 (3H, s, CH_3-3), 1.70 (2H, m, H-3′), 1.48 (3H, s, CH_3-9a), 0.92 (3H, t, J = 7.4 Hz, H-4′); ^{13}C NMR (75 MHz, MeOH-d_4) δ 208.7 (C-1′), 178.7 (C-1), 173.2 (COOH-3), 162.3 (C-6), 162.1 (C-8), 154.0 (C-4a), 114.2 (C-7), 110.3 (C-5), 110.1 (C-3a), 107.7 (C-8a), 92.2 (C-3), 61.2 (OCH_3-8), 53.4 (OCH_3-3a), 48.4 (C-9a), 47.1 (C-2′), 28.6 (C-9), 23.0 (CH_3-3), 22.4 (CH_3-9a), 19.1 (C-3′), 14.0 (CH_3-4′), 8.65 (CH_3-7); EIMS *m/z* 422 ([M]$^+$, 15), 355 (1), 251 (15), 207 (14), 181 (12), 85 (19), 55 (20), 43 (63); HREIMS *m/z* 422.1566 (calcd for $C_{21}H_{20}O_9$, 422.1577).

6-*O*-Methylpallenic acid methyl ester (1a): white solid, mp 63−65 °C; $[\alpha]^{20}_D$ +16° (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 372.5 (3.44) nm; IR (film) ν_{max} 1765, 1652, 1093 cm^{-1}; ^{1}H NMR (360 MHz, MeOH-d_4) δ 3.81 (3H, s, COO*CH*$_3$-3), 3.74 (3H, s, OCH_3-6), 3.67 (3H, s, OCH_3-8), 3.56 (3H, s, OCH_3-3a), 3.19 (1H, d, J = 15.1 Hz, Ha-9), 2.71 (1H, J = 15.1 Hz, Hb-9), 2.63 (2H, d, J = 7.2 Hz, H-2′), 2.13 (3H, s, CH_3-7), 1.76 (3H, s, CH_3-9a), 1.66 (2H, q, J = 7.4 Hz, H-3′), 1.51 (3H, s, CH_3-3), 0.96 (3H, t, J = 7.4 Hz, H-4′); ^{13}C NMR (90 MHz, MeOH-d_4) δ 205.1 (C-1′), 177.1 (C-1), 170.6 (*C*OOCH$_3$-3), 158.9 (C-6), 156.6 (C-8), 147.3 (C-4a), 122.6 (C-7), 120.4 (C-5), 111.5 (C-3a), 106.8 (C-8a), 91.1 (C-3), 62.9 (OCH_3-8), 60.9 (OCH_3-6), 53.6 (COO*CH*$_3$-3), 53.1 (OCH_3-3a), 48.3 (C-2′), 47.9 (C-9a), 28.4 (C-9), 23.2 (CH_3-3), 21.9 (CH_3-9a), 18.1 (C-3′), 14.0 (C-4′), 9.2 (CH_3-7); EIMS *m/z* 450 ([M]$^+$, 25), 407 (20), 319 (22), 305 (10); HREIMS *m/z* 450.1886 (calcd for $C_{23}H_{30}O_9$, 450.1890).

3β-Hydroxy-18α,19α-urs-20-en-28-oic acid (2): white amorphous solid; mp 250−253 °C; $[\alpha]^{20}_D$ −67° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (3.11) nm; IR (film) ν_{max} 3546, 2967, 1764 cm^{-1}; ^{1}H NMR (360 MHz, pyridine-d_5) δ 5.49 (1H, d, J = 6.8 Hz, H-21), 3.49 (1H, t, J = 7.8 Hz, H-3), 2.87 (1H, m, H-13), 2.64 (2H, dd, J = 7.0, 15.3 Hz, H_b-22), 2.47 (1H, t, H-19), 2.36 (1H, dd, J = 10.1 Hz, H_b-12), 2.04 (1H, m, H_a-22), 1.91 (2H, m, H-2), 1.88 (2H, m, H-15), 1.81 (2H, H-16), 1.74 (3H, s, CH_3-30), 1.71 (2H, m, H-1), 1.58 (2H, m, H-6), 1.55 (1H, d, J = 10.0 Hz, H_a-12), 1.40 (2H, m, H-7), 1.36 (2H, m, H-11), 1.34 (1H, m, H-9), 1.31 (1H, m, H-18), 1.25 (3H, s, CH_3-24), 1.13 (3H, d, J = 6.5 Hz, CH_3-29), 1.08 (3H, s, CH_3-26), 1.06 (3H, s, CH_3-27), 1.04 (3H, s, CH_3-23), 0.88 (3H, s, CH_3-25); ^{13}C NMR (90 MHz, pyridine-d_5) δ 178.2 (C-28), 143.1 (C-20), 117.9 (C-21), 78.1 (C-3), 55.9 (C-5), 51.1 (C-9), 49.3 (C-18), 49.1 (C-17), 42.3 (C-14), 41.2 (C-8), 39.5 (C-13), 39.4 (C-1), 39.3 (C-4), 38.5 (C-22), 37.8 (C-19), 37.5 (C-10), 34.7 (C-7), 33.7 (C-12), 29.6 (C-16), 28.3 (C-24), 27.9 (C-15), 27.1 (C-2), 23.7 (C-29), 22.3 (C-30), 21.9 (C-11), 18.7 (C-6), 16.7 (C-25), 16.5 (C-23), 16.4 (C-26), 15.1 (C-27); EIMS *m/z* 456 ([M]$^+$, 34) 438 (37), 411 (18), 395 (32), 247 (33), 207 (42); HREIMS *m/z* 456.3563 (calcd for $C_{30}H_{48}O_3$, 456.3604).

Methylene-bis-aspidinol (3): yellow needles; mp 186−187 °C (lit.[14] mp 187−190 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 (1H, s, H-5), 3.82 (3H, s, OCH_3-4), 3.06 (2H, t, J = 6.0 Hz, H-2′), 2.01 (3H, s, CH_3-3), 1.73 (2H, q, J = 6.0 Hz, H-3′), 0.99 (3H, t, J = 6.0 Hz, CH_3-4′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.2 (C-1′), 163.1 (C-2), 161.2 (C-4), 159.7 (C-6), 104.6 (C-3), 103.3 (C-1), 91.4 (C-5), 55.6 (OCH_3-4), 46.1 (C-2′), 18.1 (C-3′), 14.1 (CH_3-4′), 7.1 (CH_3-3). Compound **3** exhibited UV, IR, and EIMS data comparable to published values.[14]

1-(2,6-Dihydroxy-4-methoxy-3-methylphenyl)ethanone: yellow needles; mp 199−201 °C (lit.[13] mp 200−201 °C); UV (EtOH) λ_{max} (log ϵ) 334 (3.73), 286 (3.72) nm; IR (film) ν_{max} 3322, 1661, 1421, 1130 cm^{-1}; ^{1}H NMR (300 MHz, MeOH-d_4) δ 6.00 (1H, s, H-5), 3.81 (3H, s, OCH_3-4), 2.62 (3H, s, CH_3-2′), 1.91 (3H, s, CH_3-3); ^{13}C NMR (75 MHz, MeOH-d_4) δ 205.2 (C-1′), 165.3 (C-2), 163.7 (C-4), 162.4 (C-6), 106.1 (C-3), 104.5 (C-1), 90.9 (C-5), 55.9 (OCH_3-4), 33.1 (CH_3-2′), 7.2 (CH_3-3); EIMS *m/z* 196 ([M]$^+$, 100) 180 (25), 156 (15).

Aspidinol: yellow needles; mp 144−145 °C (lit.[17] mp 142−143 °C); UV (EtOH) λ_{max} (log ϵ) 287 (3.73), 211 (3.21) nm; IR (film) ν_{max} 3297, 2960, 2353, 1611, 1409, 1139 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 (1H, s, H-5), 3.82 (3H, s, OCH_3-4), 3.06 (2H, t, J = 6.0 Hz, H-2′), 2.01 (3H, s, CH_3-3), 1.73 (2H, q, J = 6.0 Hz, H-3′), 0.99 (3H, t, J = 6.0 Hz, CH_3-4′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.2 (C-1′), 163.1 (C-2), 161.2 (C-4), 159.7 (C-6), 104.6 (C-3), 103.3 (C-1), 91.4 (C-5), 55.6 (OCH_3-4), 46.1 (C-2′), 18.1 (C-3′), 14.1 (CH_3-4′), 7.1 (CH_3-3); ESIMS *m/z* 223 ([M − H]$^-$, 100), 207 (30), 179 (15), 151 (10).

Isoguaiacin: white solid; mp 195−197 °C (lit.[19] mp 198−200 °C); $[\alpha]^{20}_D$ −14° (*c* 0.1, MeOH); CD (MeOH) $\Delta\epsilon_{226}$ −3.08, $\Delta\epsilon_{257}$ −0.96, $\Delta\epsilon_{278}$ −6.33, $\Delta\epsilon_{294}$ +6.97. This compound exhibited spectroscopic (UV, IR, ^{1}H NMR, ^{13}C NMR, and EIMS) data comparable to published values.[19]

Bioassay Evaluation Procedures. The cytotoxic activity of extracts, chromatographic fractions, and pure compounds was evaluated against a panel of human cancer cell lines, according to established protocols.[23] The criterion for sample activity for extracts and fractions was established at ED_{50} values ≤20 μg/mL. Pure compounds were considered active at ED_{50} values of ≤5 μg/mL and having marginal activity with ED_{50} values between 6 and 10 μg/mL.

Acknowledgment. This investigation was supported by grant U19-CA-52956, funded by the National Cancer Institute, NIH, Bethesda, MD. T.L.-E. acknowledges the Colombian Institute for Science and Technology Development "Francisco Jose de Caldas" (COLCIENCIAS) (Bogotá, Colombia) and the Fulbright Commission for a Doctoral Fellowship. We thank the staff of Fairchild Tropical Garden Research Center and the staff from Metro-Dade County Park and Recreation Department, Miami, FL, for making possible the plant collection under permit No. 0014 (1999−2003). We are grateful to Drs. J. L. Anderson and Dr. Y. Wang for the mass spectral data and the Nuclear Magnetic Resonance Laboratory of the Research Resources Center, University of Illinois at Chicago, for the provision of NMR facilities used in this investigation.

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NP0496688