Anti-HIV Agents 45[1] and Antitumor Agents 205.[2] Two New Sesquiterpenes, Leitneridanins A and B, and the Cytotoxic and Anti-HIV Principles from *Leitneria floridana*

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Two new sesquiterpenes, leitneridanin A (**1**) and leitneridanin B (**2**), and seven known compounds, lirioresinol B, (−)-pinoresinal, (+)-lariciresinol, quassimarin (**3**), simalikalactone D (**4**), 1-methoxycanthinone (**5**), and 5-methoxycanthinone (**6**), were isolated from *Leitneria floridana*. Their structures were identified on the basis of spectral data. In vitro biological evaluation showed that **5** is a potent anti-HIV agent (EC_{50} 0.26 μg/mL; TI >39) and that **3**−**6** suppressed the growth of a panel of human tumor cell lines (KB, A-549, HCT-8, CAKI-1, MCF-7, and SK-MEL-2). Compounds **3** and **4** were significantly active, with ED_{50} values in the range of 0.26−0.012 μg/mL.

Leitneria floridana Chapman, a rare tree or shrub restricted to scattered wet sites in the southern Atlantic and Gulf coastal plains of the United States, belongs to the monotypic family Leitneriaceae. It is also called corkwood for its very light wood, similar in texture to cork. It is used locally for making fish floats.[3] In the course of our continuing search for bioactive plant components, we found that a chloroform extract of this plant showed cytotoxicity in tumor cell lines, while the diethyl ether extract displayed anti-HIV activity. Bioassay-directed fractionation of these fractions led to the isolation and characterization of nine compounds: three lignans, lirioresinol B,[4] (−)-pinoresinal,[4] and (+)-lariciresinol,[5] and two simaroubolides, quassimarin (**3**),[6] and simalikalactone D (**4**),[7] from the chloroform extract; and two β-carbolines, 1-methoxycanthinone (**5**)[8] and its isomer 5-methoxycanthinone (**6**),[9] and two new sesquiterpenes, leitneridanin A (**1**) and leitneridanin B (**2**), from the diethyl ether extract. This report is the first phytochemical investigation of *L. floridana*. The discovery of simaroubolides in the plant family Leitneriaceae is significant, as this compound class, which has been isolated previously from the family Simaroubaceae, is not widely distributed. The structures of the new compounds were identified on the basis of their spectral data and comparison with authentic compounds reported in the literature.

Compound **1** was assigned the molecular formula $C_{15}H_{24}O_3$ by HRFABMS measurement at $[M + Na]^+$ (*m*/*z* 275.1582). The IR spectrum indicated the presence of a hydroxyl group (3340 cm^{-1}). The ^{13}C NMR spectrum showed signals for 15 carbons (Table 2), including four olefinic carbons (two methylenes at δ 108.8 and 113.5 and two quaternary carbons at δ 148.3 and 150.0), which indicated the presence of two double bonds. Three oxygenated carbons (two tertiary carbons at δ 69.5 and 75.0 and one quaternary carbon at δ 77.6) were also observed. The ^1H NMR spectrum contained two methyl groups at δ 0.77 and 1.77, respectively. From the ^1H and ^{13}C NMR data, **1** appeared to be a eudesmane sesquiterpene.[10] With two of the four degrees of unsaturation required by the molecular formula assigned to two double bonds, the two remaining degrees of unsaturation were ascribed to two carbocyclic systems. 2D NMR analysis was used to elucidate the relationships among the atoms. A C−HCOSY experiment provided an unambiguous assignment of the ^1H and ^{13}C NMR spectra of **1** (Table 2). In the DQF COSY spectrum of **1**, H-2 (δ 2.15, 1.82) was correlated with two oxygenated methine protons (δ 4.25, H-1, and 4.44, H-3). Additionally, H-7 (δ 2.47) was coupled to H-6 (δ 1.70) and H-8 (δ 1.46 and 1.66), and H-8 was coupled to H-9 (δ 1.68 and 1.85). Therefore, the connectivities from C-1 to C-3 and from C-6 to C-9 were determined. A correlation between H-12 (δ 1.77) and H-13 (δ 4.75) was also observed, which suggested the presence of an isopropenyl group. The structure of **1** and the positions of the functional groups on the skeleton were determined completely after the study of its HMBC spectrum and consideration of the isoprene rule. Significant long-range correlations of protons with their related car-

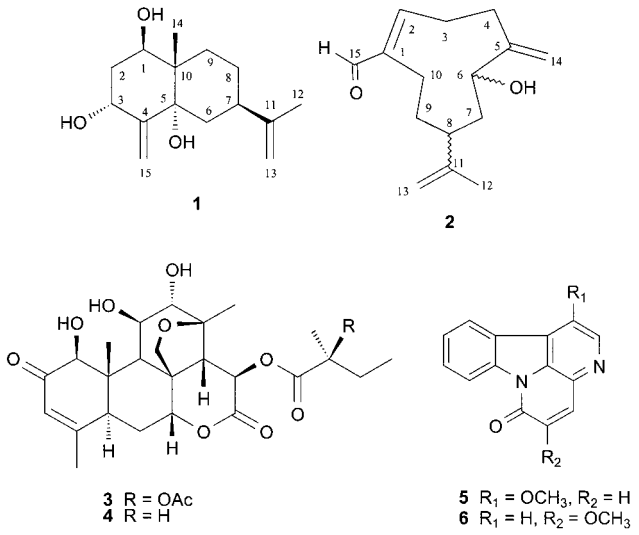

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Table 1. In Vitro Cytotoxicity Data of **3**−**6** Isolated from *L. floridana*

	cell line[a]/ED_{50} (μg/mL)[b]					
compound	KB	A549	HCT-8	CAKI-1	MCF-7	SK-MEL-2
3	0.06	0.03	0.012	0.05	0.006	0.05
4	0.018	0.04	0.013	0.26	0.014	0.13
5	3.6	4.3	3.6	3.6	13.5	>20 (46)[c]
6	2.9	3.1	2.5	4.4	5.0	17

[a] Human tumor cell lines: KB, epiderimoid carcinoma of the nasopharynx; A-549, lung carcinoma; HCT-8, ileocecal carcinoma; CAKI-1, renal cancer; MCF-7, breast cancer; SK-MEL-2, melanoma. [b] Cytotoxicity (ED_{50}) is the concentration of compound that caused a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay. [c] Bracketed value is the percentage of inhibition observed at the highest test concentration indicated.

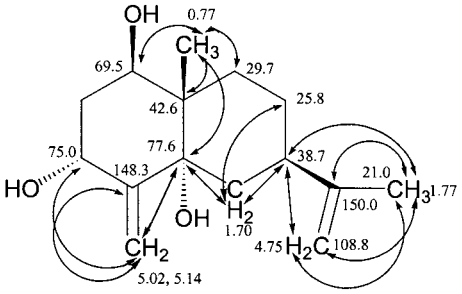

Figure 1. Significant long-range correlations observed in the HMBC spectrum of **1**.

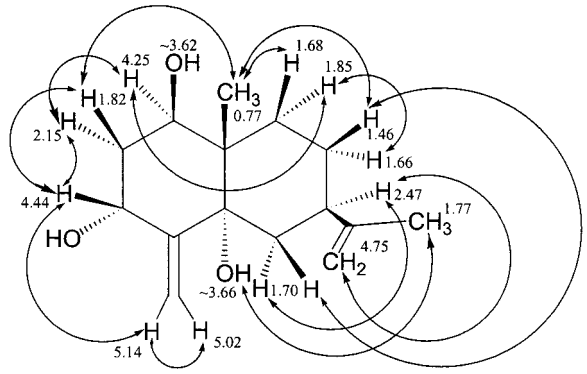

Figure 2. Significant correlations observed in the NOESY spectrum of **1**.

bons are shown in Figure 1. The relative stereochemistry was determined from NOESY experiment (Figure 2) and NOE difference experiments as well as comparison with reported data[11−13] for related compounds. In all naturally occurring eudesmanes whose configuration at C-7 is known, the isopropyl group is β-equatorial.[10] However, in the NOESY spectrum of **1**, H-7 (δ 2.47) did not show a correlation with H-9 (δ 1.85). Therefore, an NOE difference experiment was performed to provide additional data and clarify the structural assignment. When H-7 was saturated, two significant positive signals at δ 1.70 (H-6) and δ 4.75 (H-13) and one weak positive signal at δ 1.85 were observed. When H-9 (α-H, δ 1.85) was saturated, positive signals at δ 4.75 (H-1) and 2.47 (H-7) were observed, confirming the β-equatorial configuration of the isopropyl group. The spectral data of 5α-hydroxy-eudesma-4(15),11-diene and **1** [1β, 3α, 5α-trihydroxyeudesma-4(15),11-diene] are quite similar.[12]

The structure of **2** was also established from mass and ^{1}H and ^{13}C NMR spectral data. The molecular formula $C_{15}H_{22}O_2$ of **2** was assigned by HREIMS measurement at M^+ (*m*/*z* 234.1633). The IR spectrum suggested the presence of hydroxyl (3420 cm^{-1}) and carboxyl groups (1695 cm^{-1}). A maximum absorption in the UV spectrum at 232 nm ($CHCl_3$, log ϵ 1.70) suggested the presence of an α,β-unsaturated carboxyl group. The ^{1}H NMR spectrum contained one methyl group signal at δ 1.63, one oxygenated proton at δ 4.14, and five olefinic protons at δ 4.62, 4.68, 4.87, 5.09, and 6.53, respectively. A signal at δ 9.40 suggested the presence of an aldehyde group. The ^{13}C NMR spectrum showed signals for 15 carbons (Table 2). Two olefinic methylene carbon signals at δ 110.6 and 118.4, one olefinic methine carbon signal at δ 155.2, and three quaternary olefinic signals at δ 142.3, 147.4, and 148.1 indicated the presence of three double bonds, including two exomethylene groups. With one remaining degree of unsaturation (five in total from the molecular formula minus three double bonds and an aldehyde group) and the characteristics of the NMR spectra,[14,15] **2** was considered to be a monocyclic terpenoid. The proton and carbon signals were assigned from the HMQC spectrum (Table 2). One olefinic proton (δ 6.52, H-2) was correlated with a methylene group (δ 3.04, 2.36, H-3) in the COSY spectrum. These additional correlations were also found: one exocyclic methylene proton at δ 4.95 (H-14) with one methylene proton at δ 2.96 (H-4), the oxygenated proton at δ 4.13 (H-6) with the methylene group at δ 1.61 and 1.81 (H_2-7), the methane proton at δ 1.79 (H-8) with one methylene proton at δ 2.45 (H-9), and both methylene protons at 1.45 and 1.65 (H_2-9) with the methylene at δ 2.30 (H-10). The structure of **2** was determined as a 10-membered ring sesquiterpene, after analysis of its HMBC data together with consideration of the isoprene rule. Significant long-

Table 2. ^{1}H and ^{13}C NMR Data of **1** and **2** ($CDCl_3$)

	δ_H (J, Hz)		δ_C (DEPT)	
position	**1**	**2**	**1**	**2**
1	4.25 (dd, 4.9, 11.9)		69.5 (d)	142.3 (s)
2	1.82 (m), 2.15 (ddd, 14.0, 4.9, 2.1)	6.52 (dd, 12.0, 4.5)	38.1 (t)	155.2 (d)
3	4.44 (dd, 2.4, 3.8)	3.04 (m), 2.36 (m)	75.0 (d)	28.7 (t)
4		2.96 (m), 2.36 (m)	148.3 (s)	30.1 (t)
5			77.6 (s)	147.4 (s)
6	1.70 (d, 8.9)	4.13 (dd, 12.0, 5.0)	35.4 (t)	76.0 (d)
7	2.47 (m)	1.61 (m), 1.81 (m)	38.7 (d)	33.9 (t)
8	1.46 (m), 1.66 (m)	1.79 (m)	25.8 (t)	39.4 (d)
9	1.68 (m), 1.85 (m)	1.45 (m), 1.65 (m)	29.7 (t)	29.7 (t)
10		2.50 (ddd, 13.5, 5.0, 3.0)	42.7 (s)	21.1 (t)
11			150.0 (s)	148.1 (s)
12	1.77 (s)	1.62 (s)	21.0 (q)	19.5 (q)
13	4.75 (m)	4.62 (br s), 4.68 (br s)	108.8 (t)	110.6 (t)
14	0.77 (s)	4.86 (br s), 4.95 (br s)	12.7 (q)	118.4 (s)
15	5.02, 5.14 (d, 0.9)	9.40 (s)	113.8 (t)	195.1 (d)

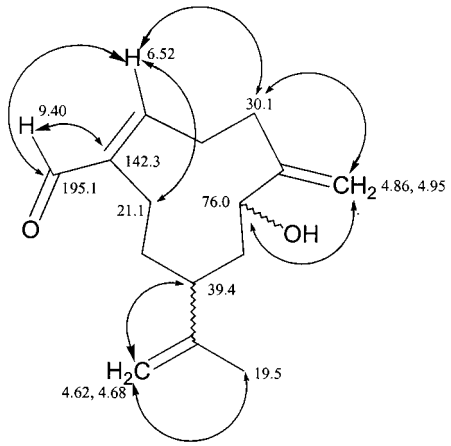

Figure 3. Significant long-range correlations observed in the HMBC spectrum of **2**.

range proton−carbon correlations are shown in Figure 3. Compound **2** was assigned as the germacrane-type sesquiterpenoid 6-hydroxy-5-methylene-8-isopropenylcyclodec-1-enecarbaldehyde. The stereochemistry of **2** has not yet been assigned, as no useful correlations were observed concerning the orientation of either H-6 or H-8 in NOESY or NOE difference experiments, perhaps due to flexibility of the large ring. In addition, the acetate derivative did not give a suitable crystal for X-ray analysis. Scarcity of sample has delayed the synthesis of additional other derivatives for crystallographic or NMR studies.

The new compounds **1** and **2** were inactive in both cytotoxic and anti-HIV evaluation; however, the following promising results were found with the known compounds. In an in vitro cytotoxicity assay, **3** and **4** suppressed KB, A549, HCT-8, CAKI-1, MCF-7, and SK-MEL-2 cell growth significantly; **5** inhibited KB, HCT-8, and CAKI-1 cell growth; and **6** inhibited KB, A549, and HCT-8 cell growth (Table 1). Previous literature has reported the cytotoxicity of **3** and **4** against murine P-388 leukemia and human KB cells (ED_{50} in KB cells at 10^{-2}−10^{-3} μg/mL),[6,16,17] and of **5** and **6** against guinea pig ear keratinocytes.[18]

In an in vitro anti-HIV assay, **5** suppressed HIV-infected H9 cell growth significantly and had an impressive therapeutic index (TI: IC_{50}/EC_{50}) of >391. The corresponding IC_{50} (concentration that inhibited uninfected H9 cell growth by 50%) and EC_{50} (concentration that inhibited viral replication by 50%) values were >100 and 0.256 μg/mL, respectively. This report is the first example of a β-carboline exhibiting anti-HIV activity.[1,19] Thus, this compound type might provide a useful lead for anti-AIDS drug development, and a structure−activity relationship study of β-carbolines is in progress.

Experimental Section

General Experimental Procedures. Melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO-CIP-1000 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1320 IR spectrophotometer. UV spectra were recorded on a UV-2101 PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 300 or 500 spectrometer. MS were recorded on a VG-70E double-focusing GC−MS spectrometer.

Plant Material. The aerial parts of *L. floridana* were collected in the North Carolina Botanical Garden, Chapel Hill, NC. The plant was identified by Professor Jim Massey, Department of Botany, University of North Carolina at Chapel Hill, where a voucher specimen is deposited.

Extraction and Isolation. The air-dried whole plant (0.5 kg) was extracted with MeOH at room temperature. After evaporating most of the solvent under reduced pressure, the remainder was stirred with water (10 times the volume). The aqueous solution was partitioned successively with hexane, Et_2O, $CHCl_3$, and EtOAc. The $CHCl_3$ extract was subjected to Si gel column chromatography and eluted with mixtures of $CHCl_3$ and MeOH of increasing polarities. The $CHCl_3$−MeOH (4:1) fraction was further separated by Si gel column chromatography eluting with gradient mixtures of CH_2Cl_2 and Me_2CO. A fraction eluted with CH_2Cl_2−Me_2CO (9:1) gave lirioresinol B[4] (11 mg) and (−)-pinoresinol[4] (19 mg), and a fraction eluted with CH_2Cl_2−Me_2CO (4:1) gave (+)-lariciresinol[5] (10 mg), quassimarin[6] (**3**, 11 mg), and simalikalactone D[7] (**4**, 9 mg). The Et_2O extract was also subjected to passage over a Si gel column eluting with mixtures of increasing polarities of $CHCl_3$ and MeOH. A fraction eluted with $CHCl_3$−MeOH (9:1) gave leitneridanin B (**2**, 9 mg) after repeated purification over Si gel using EtOAc−hexane (2:1) and CH_2Cl_2−Me_2CO (9:1) as eluents. The remaining eluents were combined and further separated over Sephadex LH-20, using EtOH and H_2O mixtures as eluents. Leitneridanin A (**1**, 10 mg) was obtained from the 80% EtOH fraction. The $CHCl_3$−MeOH (4:1) fraction gave 1-methoxycanthinone[8] (**5**, 17 mg) and 5-methoxycanthinone[9] (**6**, 22 mg) after repeated Si gel chromatography, using EtOAc−hexane (2:1) and CH_2Cl_2−Me_2CO (9:1) as eluents.

Leitneridanin A (1): light yellow solid; $[\alpha]^{21}_D$ +35° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3340 cm^{-1}; HRFABMS *m/z* $[M + Na]^+$ 275.1582 (calcd for $C_{15}H_{24}O_3$, 252.3528); EIMS *m/z* $[M - H_2O]^+$ 234, $[M - 2H_2O]^+$ 216, [216-Me] 201, 177, 111, 43, 28 (base).

Leitneridanin B (2): light yellow to brown gum, $[\alpha]^{21}_D$ −133° (*c* 0.3, MeOH), UV (EtOH) λ_{max} (log ϵ) 232 (1.70) nm; IR (KBr) ν_{max} 3420, 1695 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 500 MHz) data are shown in Table 2; HREIMS *m/z* 234.1633 (calcd for $C_{15}H_{22}O_2$, 234.3376); EIMS *m/z* $[M - CHO]^+$ 205, 190, 179, 165 (base).

Anti-HIV Assay. This assay was performed using methods as described previously in the literature.[20]

Cytotoxicity Assay. Compounds were tested according to procedures described in Rubinstein et al.[21] against epiderimoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), renal cancer (CAKI-1), breast cancer (MCF-7), and melanoma (SK-MEL-2) obtained from the Lineberger Comprehensive Cancer Center (UNC-CH). Samples were prescreened at 40, 4, 0.4, and 0.04, or 50, 5, 0.5, and 0.05 μg/mL, against selected HTCL with duplicate dose treatments and three-day exposure. Compounds **6** and **7** were further evaluated over dose-range based on these results with triplicate dose-treatments and three-day exposure. Variation of results between replicates varied no more than 5%.

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