Alvaradoins E−N, Antitumor and Cytotoxic Anthracenone *C*-Glycosides from the Leaves of *Alvaradoa haitiensis*

Sharnelle S. Phifer,[†] Dongho Lee,[†,‡] Eun-Kyoung Seo,[†,§] Nam-Cheol Kim,[†,⊥] Tyler N. Graf,[†] David J. Kroll,[†] Hernán A. Navarro,[†] Robert A. Izydore,[∥] Francisco Jiménez,[▽] Ricardo Garcia,[▽] William C. Rose,[○] Craig R. Fairchild,[○] Robert Wild,[○,#] Djaja D. Soejarto,[☆] Norman R. Farnsworth,[☆] A. Douglas Kinghorn,[☆,△] Nicholas H. Oberlies,*[,†] Monroe E. Wall,[†,◇] and Mansukh C. Wani[†]

Natural Products Laboratory, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709, Department of Chemistry, North Carolina Central University, Durham, North Carolina 27707, Jardin Botanico Nacional "Dr. Rafael Moscoso", Santo Domingo, Dominican Republic, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543, and Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

Received January 4, 2007

Bioactivity-directed fractionation of an extract of the leaves of *Alvaradoa haitiensis*, using the KB (human oral epidermoid carcinoma) cell line, led to the isolation and identification of 10 new anthracenone *C*-glycosides, alvaradoins E−N (**1**−**10**), along with the known compound chrysophanol (**11**). The cytotoxicity of all compounds was evaluated, and preliminary structure−activity relationships are suggested. The most potent compounds in the in vitro assays (**1** and **2**) were evaluated in vivo versus the P388 (murine lymphocytic leukemia) model, and alvaradoin E (**1**) showed antileukemic activity (125% T/C) at a dose of 0.2 mg kg^{-1} per injection when administered intraperitoneally.

The plant genus *Alvaradoa*, formerly classified in the family Simaroubaceae, was recently established in the family Picramniaceae and constitutes the subfamily Alvaradoideae.[1−3] Members of this genus can be found as shrubs or small- to medium-sized trees up to 15 m tall, and *Alvaradoa* is composed of seven species distributed throughout tropical America from southern Florida and Mexico through Central America and the West Indies to South America.[1,4] These species thrive in a variety of climates, being found at elevations ranging from sea level to above 1500 m.[4] The literature reports the isolation of bioactive compounds from only two species of *Alvaradoa*. Thus, an extract from the leaves of *A. amorphides*, which is used traditionally to treat skin rashes and head fever, yielded the compounds chaparrin, chrysophanol, and chrysophanein.[5,6] From the aerial parts of *A. jamaicensis*, the anthracenone *C*-glycosides alvaradoins A−D were isolated and tested for activity against *Mycobacterium tuberculosis*, but were inactive.[7] Anthracenone *C*-glycosides of a similar structural type have been isolated from the taxonomically related genus *Picramnia*, also in the Picramniaceae.[8−13] However, this class of bioactive plant secondary metabolites has not been explored extensively for potential anticancer activity.

As part of a collaborative research program to explore the plant kingdom for novel anticancer agents,[14] the chloroform-soluble extract of the leaves of *Alvaradoa haitiensis* Urb. (Picramniaceae) exhibited promising cytotoxicity against the KB (human oral epidermoid carcinoma) cell line. Using this assay to monitor subsequent bioactivity-directed fractionation studies, 10 new anthracenone *C*-glycosides were obtained (**1**−**10**), all of which displayed potent cytotoxic activities. The known compound chrysophanol (**11**) was also isolated. The structures of the new compounds **1**−**10** were elucidated via spectrometric and spectroscopic studies, including the determination of their absolute configurations by circular dichroism (CD). All compounds were evaluated in the KB assay, and preliminary structure−activity relationships (SAR) were developed from these data. The most potent compounds (**1** and **2**) were evaluated further in vivo in the P388 (murine lymphocytic leukemia) model.

Results and Discussion

Compounds **1**−**8** were isolated in the form of four pairs of diastereoisomers. These structurally related pairs (e.g., compounds **1** and **2**) were separated using normal-phase HPLC over a diol column (see Experimental Section). Hence, to simplify the discussion of the structure elucidation, the data for one compound, usually the *S* isomer, from each pair are described in detail. Then, the differences between it and its epimer are explained in the subsequent paragraphs.

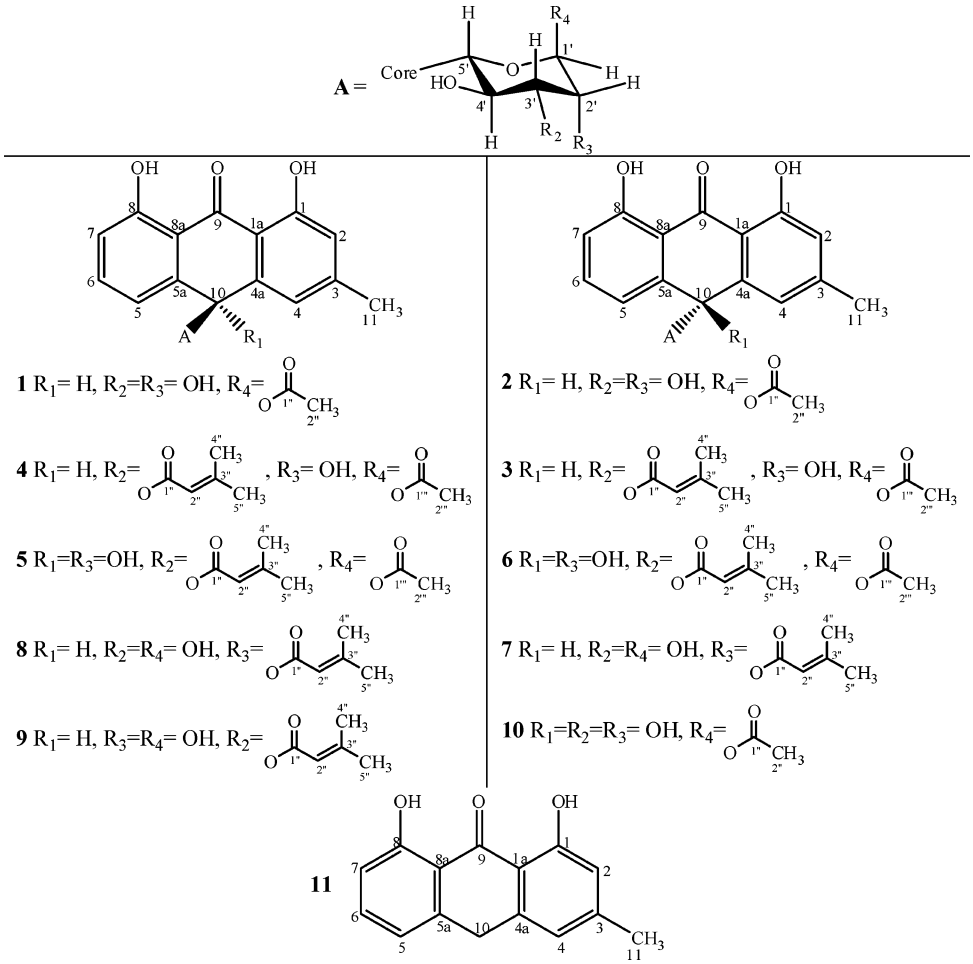
The HRFABMS of the potassium adduct of compound **1** yielded a molecular ion peak $[M + K]^+$ at *m*/*z* 469.0916, corresponding to the molecular formula $C_{22}H_{22}O_9$ and indicating an index of hydrogen deficiency of 12. Fundamental absorptions in the IR spectrum were observed at 3428, 1740, and 1719 cm^{-1}, suggestive of hydroxy and carbonyl moieties, and four bands in the UV spectrum (λ_{max} 202, 268, 297, and 358 nm) showed the presence of a highly conjugated system.

The ^{1}H and ^{13}C NMR data for compound **1** are given in Table 1. A ketone carbonyl was noted at δ_C 195.0 (C-9) in the ^{13}C NMR spectrum, and it was hydrogen-bonded to two nonequivalent phenolic hydroxy groups at C-1 and C-8, as evidenced by ^{1}H NMR resonances at δ_H 12.01 (OH-1) and 11.92 (OH-8), respectively. On the basis of HSQC data, five protonated aromatic carbons were determined (Table 1): two *meta*-coupled aromatic resonances at δ_H/δ_C 6.73/117.1 and 7.04/122.4 were assigned to H-2/C-2 and H-4/C-4, respectively, while three *ortho*-coupled aromatic resonances at δ_H/δ_C 6.85/116.4, 7.04/119.1, and 7.56/136.7 were assigned to

* To whom correspondence should be addressed. Tel: (919) 541-6958. Fax: (919) 541-6499. E-mail: oberlies@rti.org.
† Research Triangle Institute.
‡ Current address: Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, 136-705 Korea.
§ Current address: College of Pharmacy, Ewha Woman's University, Seoul 120-750, Korea.
⊥ Current address: Kraft Foods North America, Research and Development, 801 Waukegan Rd., Glenview, IL 60025.
∥ North Carolina Central University.
▽ Jardin Botanico Nacional.
○ Bristol-Myers Squibb Pharmaceutical Research Institute.
Current address: OSI Pharmaceuticals, Inc., 2860 Wilderness Place, Boulder, CO 80301.
☆ University of Illinois at Chicago.
△ Current address: College of Pharmacy, The Ohio State University, 500 W. 12th Ave., Columbus, OH 43210-1291.
◇ Deceased July 6, 2002.

10.1021/np070005a CCC: $37.00
Published on Web 06/07/2007

Chart 1



H-7/C-7, H-5/C-5, and H-6/C-6. A shielded resonance at δ_H/δ_C 2.38/22.2 (H_3-11/C-11) indicated a methyl group, which was attached to δ_C 148.0 (C-3) as a result of an HMBC correlation between H_3-11 and C-3. Resonances at δ_H/δ_C 4.66/44.0 (H-10) displayed HMBC correlations with carbons at δ_C 118.3 (C-1a), 116.1 (C-8a), 142.3 (C-4a), and 146.7 (C-5a), as well as with C-4 and C-5. From these data, two benzene rings, a nonaromatic ring, and a ketone carbonyl were characterized, all of which could be ascribed to an anthracenone skeleton, similar to alvaradoins A−D[7] and chrysophanol (**11**).[15,16]

The remaining resonances observed in the ^{1}H and ^{13}C NMR spectra of **1** were attributable to a glycosyl unit. The ^{1}H, ^{13}C, HSQC, and HMBC NMR spectroscopic data (Table 1 and Figure 1) displayed resonances characteristic for a glycosyl moiety containing five methine groups at δ_H/δ_C 3.84/81.3 (H-5′/C-5′), 3.59−3.62/68.2 (H-4′/C-4′), 3.65−3.69/73.1 (H-3′/C-3′), 3.65−3.69/70.6 (H-2′/C-2′), and 5.63/94.4 (H-1′/C-1′). This glycone was attached to the anthracenone core at C-10, as determined by HMBC correlations from H-10 to C-5′ and H-5′ to C-5a, as shown. H-1′ showed HMBC correlations to C-5′, C-3′, and a carbonyl at δ_C 168.3 (C-1″) of an acetoxy group, the methyl group of which (δ_H/δ_C 1.76/20.4; C-2″) showed HMBC correlations from H-2″ to C-1″. H-1′ was positioned equatorially and the acetoxy group axially on the basis of the extremely small coupling between H-2′ and H-1′ (J < 1 Hz), and this finding was supported by a strong ROESY correlation between these two hydrogens. H-5′ displayed a doublet of doublets splitting pattern in the ^{1}H NMR spectrum, and the J values indicated axial coupling to H-4′ (9.8 Hz), thereby placing the anthracenone core in the equatorial position (J = 2.4 Hz for coupling to H-10). Although the J values for the coupling of H-4′ to H-3′ could not be interpreted in the crowded, upfield region of the ^{1}H NMR spectrum of **1**, both were presumed to be in axial positions, the former due to the aforementioned diaxial coupling to H-5′ and the latter due to ROESY correlations between H-5′ and H-3′. In summation, due to the axial protons at C-4′ and C-3′ and equatorial protons at C-2′ and C-1′, the glycone moiety was determined to be a lyxopyranose unit containing an *O*-acetyl group at C-1′.

The absolute configuration of **1** at C-10 was determined using ROESY NMR and circular dichroism (CD) spectroscopic data. Key ROESY correlations were observed from H-10 to H-5′, H-4, and H-5, further supporting the connectivity of the glycone unit at C-10 (Table 1). Also, cross-peak correlations between H-5′ and H-5, and between H-4′ and H-4, indicated the *S* configuration for C-10 (Figure 2). This was supported by the CD spectrum, which showed a positive Cotton effect at 297 nm and negative Cotton effects at 323 and 268 nm, as reported for (10*S*)-aloin.[17,18] These results are analogous to those reported for sarcoside[12] and picramnioside B.[9]

The second $C_{22}H_{22}O_9$ isomer, compound **2**, displayed splitting patterns, chemical shifts, and coupling constants in the NMR spectra that were nearly identical to those of **1** (Table 1). Differences, however, were observed between these two compounds in their ROESY NMR spectra, specific rotation data, CD spectra, and melting points. The relative configuration was determined from observations in the ROESY spectrum from cross-peak correlations of H-5′ with H-4, and H-4′ with H-5, indicating the *R* configuration at C-10, analogous to mayoside[11] and picramnioside C.[9] Furthermore, **2** showed a negative Cotton effect at 299 nm and positive Cotton effects at 353 and 257 nm, as reported for (10*R*)-aloin,[17,18] supporting the opposite configuration of C-10 in compounds **1** and **2**. These results are analogous to those reported for similar pairs of diastereoisomers, such as aloins A and B,[18,19] 10-hydroxyaloins A and B,[20] and cascarosides A, B, C, and D.[17] Following the

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2** (acetone-d_6)

	1				**2**			
position	δ_H	δ_C	HMBC	ROESY	δ_H	δ_C	HMBC	ROESY
1		162.9				162.9		
1a		118.3				116.1		
2	6.73 (1H, s)	117.1	1, 4, CH_3-11	CH_3-11	6.70 (1H, s)	116.6	1, 1a, 4, CH_3-11	CH_3-11
3		148.0				148.7		
4	7.04 (1H, s overlap)	122.4	1a, 2, 10, CH_3-11	H-4′, OH-4′, H-10, CH_3-11	6.90 (1H, s)	120.2	1a, 10, CH_3-11	H-5′, H-10, CH_3-11
4a		142.3				146.7		
5	7.04 (1H, d overlap, 7.5)	119.1	7, 8a, 10	H-6, H-10, H-5′	7.18 (1H, d, 7.5)	121.2	8a, 10	H-4′, OH-4′
5a		146.7				142.4		
6	7.56 (1H, dd overlap, 8.0, 7.9)	136.7	5a, 8	H-5, H-7	7.53 (1H, dd overlap, 8.0, 7.9)	136.1	5a, 8	H-5, H-7
7	6.85 (1H, d, 8.4)	116.4	5, 8	H-6	6.88 (1H, d, 7.9)	116.8	5	H-6
8		163.2				163.1		
8a		116.1				118.3		
9		195.0				195.0		
10	4.66 (1H, d, 2.3)	44.0	4, 5, 5′, 4a, 5a, 1a, 8a	H-4, H-5, H-4′, H-5′	4.66 (1H, d, 2.3)	43.9	4, 5, 5′, 4a, 5a, 1a, 8a	H-5′
CH_3-11	2.38 (3H, s)	22.2	2, 3, 4	H-2, H-4	2.40 (3H, s)	22.0	2, 3, 4	H-2, H-4
1′	5.63 (1H, s)	94.4	3′, 5′, 1″	H-2′, OH-2′	5.62, s	94.4	3′, 5′, 1″	H-2′, OH-2′
2′	3.65-3.69 (2H, m)	70.6	4′	H-1′, OH-2′	3.66-3.70 (2H, m)	70.5	4′	H-1′
3′	3.65-3.69 (2H, m)	73.1	4′, 5′	OH-2′, OH-4′, H-5′	3.66-3.70 (2H, m)	73.1	4′	
4′	3.59-3.62 (1H, m)	68.2	3′	H-4, OH-4′, H-5′, H-10	3.59-3.61 (1H, m)	68.3	3′	OH-4′
5′	3.84 (1H, dd, 9.8, 2.4)	81.3	4a, 5a	H-3′, H-4′, H-5, H-10	3.86 (1H, dd, 9.9, 2.5)	81.4	5a	H-4
1″		168.3				168.3		
CH_3-2″	1.76 (1H, s)	20.4	1″		1.78 (1H, s)	20.4	1″	
OH-1	12.01 (1H, s)		1, 1a, 2		11.92 (1H, s)		1, 1a, 2,	
OH-8	11.92 (1H, s)		7, 8, 8a		12.04 (1H, s)		7, 8, 8a	
OH-2′	4.09 (1H, d, 3.7)		1′, 2′	H-1′, H-2′	4.08 (1H, d, 3.6)		1′, 2′	H-1′
OH-3′	3.97 (1H, d, 6.8)		3′		3.96 (1H, d, 6.9)		2′, 3′	
OH-4′	4.50 (1H, d, 4.8)		4′, 5′	H-4, H-3′, H-4′	4.43 (1H, d, 4.7)		4′, 5′	H-4′

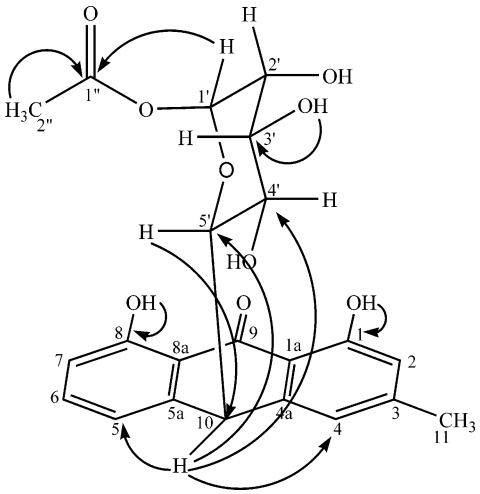


Figure 1. Key HMBC correlations for compound **1**.

nomenclature first established with a related species of *Alvaradoa* by Harding et al.,[7] compounds **1** and **2** were ascribed the trivial names alvaradoin E and alvaradoin F, respectively.

The HRFABMS of the potassium adduct of compound **4** yielded a molecular ion peak $[M + K]^+$ at *m*/*z* 551.1324 by HRFABMS, corresponding to a molecular formula of $C_{27}H_{28}O_{10}$ and indicating an index of hydrogen deficiency of 14. As with **1** and **2**, absorptions in the IR spectrum were observed at 3414, 1741, 1725, and 1638 cm^{-1}, suggestive of hydroxy and carbonyl moieties, and four bands in the UV spectrum (λ_{max} 201, 268, 296, and 359 nm) indicated a highly conjugated system.

The ^{1}H and ^{13}C NMR data for **4** (Tables 2 and 3) displayed chemical shifts and splitting patterns that were similar to those observed with **1** and **2**. The hydrogen deficiency increased by two and the molecular formula by C_5H_6O over the aforementioned compounds, and this could be rationalized via the addition of a 3-methylbut-2-enoyl (senecioyl) moiety to the glycone. This side chain was characterized via a series of resonances (H-2″, H-4″, and H-5″ in Table 2 and C-1″ to C-5″ in Table 3), and this included discerning which methyl group was *cis* (H-5″/C-5″) versus *trans* (H-4″/C-4″) to proton H-2″, on the basis of chemical shift differences[21] and a strong ROESY correlation between H-2″ and H-5″; these findings are opposite of those reported for a similar series of compounds.[7] The point of attachment for the glycone to the anthracenone core was analogous to what was observed with **1** and **2**, via resonances at δ_H/δ_C 3.99/81.6 (H-5′/C-5′) that displayed axial coupling to H-4′ (*J* value of 9.9 Hz), and was supported by an HMBC correlation of H-5′ to C-4a. Similarly, the acetoxy group (H-1‴/C-1‴ and H-2‴/C-2‴) was connected to position C-1′, as in both **1** and **2**, via an HMBC correlation from H-1′ to C-1‴. Likewise, hydroxy groups were observed at C-4′ and C-2′, again on the basis of HMBC correlations. Finally, the chemical shifts of H-3′/C-3′ shifted downfield as compared to **1** and **2**, especially apparent in the ^{1}H NMR data (Table 2), due to attachment of the 3-methylbut-2-enoyl moiety, and this was substantiated by an HMBC correlation from H-3′ to C-1″. Analogous to **1**, the absolute configuration of **4** was determined to be 10*S* using ROESY and CD data. Key ROESY signals included correlations of H-5′ with H-5, and H-4′ with H-4, and the CD spectrum revealed a positive Cotton effect at 296 nm and negative Cotton effects at 322 and 268 nm.

For the second $C_{27}H_{28}O_{10}$ isomer, **3**, the chemical shifts, splitting patterns, and coupling constants were similar to those observed in **4**, but the compounds differed in their specific rotations, CD spectra, and melting points. The absolute configuration at C-10 was determined to be 10*R* from the CD spectrum, in which a negative Cotton effect at 296 nm and positive Cotton effects at 351 and 258

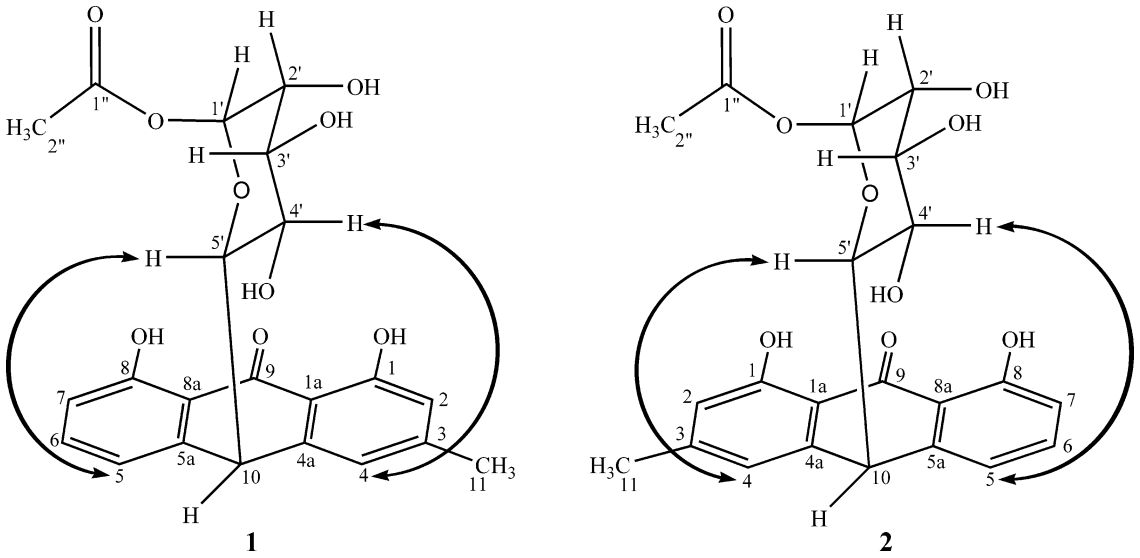


Figure 2. Key ROESY (↔) correlations of compounds **1** and **2**.

Table 2. ^{1}H NMR Data of Compounds **3**−**10** (acetone-d_6)

position	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
H-2	6.72 (s)	6.73 (s)	6.80 (s)	6.75 (s)	6.70 (s)	6.76 (s)	6.71 (s)	6.73 (s)
H-4	6.96 (s)	7.01 (s)	7.28 (s)	7.36 (s)	6.91 (s overlap)	7.03 (s)	6.99 (s)	7.38 (s)
H-5	7.16 (d, 7.5)	7.07 (d, 7.5)	7.50 (d, 7.6)	7.41 (dd, 7.6, 1.0)	7.15 (d, 7.4)	7.04 (d, 7.6)	7.06 (d, 7.4)	7.41, (dd, 7.8, 0.9)
H-6	7.58 (d, 7.7)	7.56 (dd, 8.1, 7.2)	7.62 (brt, 8.1, 7.8)	7.63 (t, 8.0)	7.57 (t, 7.9)	7.55 (t, 8.1, 7.8)	7.53 (t, 7.9)	7.62, (t, 7.8, 8.1)
H-7	6.89 (d, 8.3)	6.85 (d, 8.1)	6.90 (d, 8.3)	6.95 (dd, 8.3, 0.8)	6.91 (d overlap, 7.9)	6.84 (d, 8.4)	6.83 (d, 7.6)	6.93, (dd, 8.4, 0.9)
H-10	4.71 (brs)	4.69 (s)			4.68 (brs)	4.68 (brs)	4.70 (d, 2.1)	
CH_3-11	2.41 (s)	2.38 (s)	2.43 (s)	2.43 (s)	2.39 (s)	2.43 (s)	2.38 (s)	2.41 (s)
H-1′	5.62 (dd, 5.5, 1.6)	5.63(d, 4.8)	5.57 (brs)	5.56 (d, 1.8)	4.81 (brd, 6.8)	4.81 (brd, 4.2)	4.81 (m)	5.55 (d, 1.5)
H-2′	3.87 (m)	3.84-3.92 (m)	3.84 (m)	3.84 (m)	4.88 (m)	4.89 (m)	3.81 (m)	3.63 (overlap)
H-3′	4.98 (dd, 8.8, 3.6)	4.96 (dd, 9.6, 3.3)	4.96 (dd, 9.1, 3.2)	4.97 (dd, 9.1, 3.3)	3.96 (m)	3.97 (m)	5.07 (dd, 9.7, 3.1)	3.69 (overlap)
H-4′	3.87 (m)	3.84-3.92 (m)	3.95 (brt, 9.3, 9.2)	3.93 (m)	3.65 (brt, 9.7, 9.5)	3.70 (brt, 9.6)	3.89 (m)	3.63 (overlap)
H-5′	4.01 (dd,)	3.99 (dd, 9.9, 2.1)	3.72 (d, 9.7)	3.74 (d, 9.6)	4.05 (dd, 9.9, 2.3)	4.05 (dd, 9.8, 2.1)	4.15 (dd, 9.9, 2.3)	3.57 (overlap)
H-2″	5.67 (dd, 2.8, 1.4)	5.66 (dd, 2.4, 1.3)	5.62 (s)	5.62 (m)	5.32 (brs)	5.33 (brs)	5.66 (m)	
CH_3-4″	2.14 (s)	2.12 (s)	2.12 (s)	2.11 (d, 1.1)	1.90 (s)	1.89 (s)	2.13 (d, 1.2)	
CH_3-5″	1.89 (s)	1.88 (s)	1.89 (s)	1.88 (d 1.2)	1.84 (s)	1.85 (s)	1.88 (d, 1.2)	
CH_3-2‴	1.83 (s)	1.80 (s)	1.79 (s)	1.81 (s)				1.75 (s)
OH-1	11.95 (s)	12.00 (s)	11.93 (s overlap)	11.85 (s)	11.85 (s)	11.86 (s)	11.95 (s)	11.84 (s)
OH-8	12.06 (s)	11.91 (s)	11.92 (s overlap)	12.04 (s)	11.95 (s)	11.95 (s)	11.85 (s)	12.03 (s)
OH-10			6.41 (s)	6.40 (brs)				6.54 (s)
OH-1′					5.74 (d, 4.4)	5.70 (d, 4.5)	5.44 (d, 4.3)	
OH-2′	4.47 (d, 4.0)	4.42 (d, 4.5)	4.46 (brs)	4.40 (d, 4.5)			3.85 (d, 4.4)	4.08 (d, 3.6)
OH-3′					4.14 (brs)	4.12 (brs)		4.05 (d, 6.6)
OH-4′	4.83 (d, 6.0)	4.81 (d, 5.7)	5.73 (brs)	5.63 (brs)	4.40 (brs)	4.44 (brs)	4.54 (d, 6.1)	5.65 (d, 2.1)

nm were observed, indicating a configuration that was analogous to **2**. This pair was ascribed the trivial names alvaradoin G (**3**) and alvaradoin H (**4**).[22]

The HRMS of the sodium adduct of compound **5** displayed a molecular ion peak $[M + Na]^+$ at *m*/*z* 551.1525, corresponding to the molecular formula $C_{27}H_{28}O_{11}$. Relative to compound **4**, these data indicated the addition of a single oxygen atom. The NMR data of **4** and **5** were also quite similar (Tables 2 and 3), although the latter displayed an oxygenated quaternary carbon at δ_C 76.3 (C-10). The amalgamation of these data demonstrated that compound **5** is representative of the C-10-hydroxylated analogue of compound **4**, and HMBC correlations of δ_H 6.41 (O*H*-10) with both C-10 and C-5′ (δ_C 79.8) supported this structural assignment. The relative configuration of **5** at C-10 was determined by ROESY experiments, which displayed correlations between H-5′ and H-5 and between H-4′ and H-4, indicating a configuration similar to **4**, although this was now designated as *R* due to the priority of the hydroxy moiety. Similarly, the CD spectrum of **5** was nearly identical to that of **4**, with a positive Cotton effect at 301 nm and negative Cotton effects at 332 and 268 nm.

The other $C_{27}H_{28}O_{11}$ isomer (compound **6**) had NMR data that were similar to compound **5** (Tables 2 and 3), although clear differences were noted in their specific rotation, CD, and melting point data. Akin to the discussion above, these data suggested that **6** is the hydroxylated analogue of **3**, and this finding was supported by HMBC correlations that demonstrated OH-10 is attached to

Table 3. ^{13}C NMR Data of Compounds **3**−**10** (acetone-d_6)[a]

carbon	3	4	5	6	7	8	9	10
C-1	163.4	162.8	163.2	162.9	162.7	163.2	162.5	162.8
C-1a	116.3	116.4	114.9	114.9	116.2	116.4	116.5	114.9
C-2	117.1	117.2	118.1	117.7	116.5	116.9	116.9	117.6
C-3	148.2	148.0	148.1	148.8[b]	148.7	147.8	147.8	148.8
C-4	120.5	122.2	120.0	118.5	120.7	122.5	122.4	118.4
C-4a	146.5	142.2	145.8	148.9[b]	146.9	143.2	143.2	149.1
C-5	121.2	119.2	117.7	118.8	121.2	119.6	119.6	118.8
C-5a	142.4	146.3	149.0	145.8	143.3	147.1	147.1	146.1
C-6	136.3	136.7	136.9	136.3	135.9	136.9	136.9	136.2
C-7	116.7	116.5	117.2	118.0	116.6	116.3	116.2	117.8
C-8	163.3	163.2	162.6	162.9	162.9	162.6	163.1	162.9
C-8a	118.4	117.1	117.1	116.9	118.5	118.3	118.3	116.9
C-9	193.1	194.9	194.0	193.9	195.1	195.2	195.2	193.9
C-10	44.2	44.0	76.3	76.2	44.6	44.6	44.4	76.3
CH_3-11	22.3	22.1	22.4	22.2	22.1	22.2	22.2	22.2
C-1′	94.6	94.4	94.1	94.0	92.9	93.0	95.7	93.9
C-2′	68.7	68.5	68.2	68.1	73.3	73.4	70.5	70.2
C-3′	74.9	74.8	74.5	74.4	70.5	70.6	75.0	72.7
C-4′	65.9	65.6	67.3	67.3	69.1	69.1	66.2	70.0
C-5′	81.9	81.6	79.8	79.9	79.2	79.2	79.4	79.5
C-1″	166.5	166.0	166.2	166.2	166.0	166.1	166.5	
C-2″	116.9	116.7	116.6	116.6	116.8	116.9	117.1	
C-3″	157.7	157.8	158.0	157.9	157.0	156.9	157.2	
CH_3-4″	20.2	20.1	20.1	20.1	20.3	20.3	20.0	
CH_3-5″	27.3	27.2	27.2	27.2	27.3	27.4	27.2	
C-1‴	168.4	168.0	168.2	168.1				168.1
CH_3-2‴	20.6	20.4	20.4	20.3				20.3

[a] Data are based on DEPT, HSQC, and HMBC experiments. [b]Signals may be interchanged.

C-10. In the ROESY spectrum, cross-peak correlations were observed between H-5′ and H-4 and between H-4′ and H-5, which indicated a configuration similar to compound **3** that is indicated as 10*S*, again due to the priority of the hydroxy moiety. The CD spectrum revealed a negative Cotton effect at 299 nm and positive Cotton effects at 353 and 260 nm, supporting the opposite configuration at C-10 for these anthracenone *C*-glycosides, and accordingly, this pair of compounds were ascribed the trivial names alvaradoin I (**5**) and alvaradoin J (**6**).

The HRMS of the sodium adducts of compounds **7**−**9** were similar, having peaks for $[M + Na]^+$ at *m*/*z* 493.1475, 493.1452, and 493.1461, respectively, which corresponded to a molecular formula of $C_{25}H_{26}O_9$ for each compound. The spectroscopic data were consistent with those observed for the aforementioned compounds (Tables 2 and 3), indicating a very similar ring system and substitution patterns. Compared to compounds **5** and **6**, these compounds did not appear to have a C-10 hydroxy moiety, as evidenced by the ^{13}C NMR data for C-10, which was δ_C 44.6 for both **7** and **8** and δ_C 44.9 for **9**. Also in relation to compounds **5** and **6**, the 3-methylbut-2-enoyl moiety was located at C-2′ of the glycone in compounds **7** and **8**, as supported by HMBC correlations between H-2′ and C-1″. For compound **9** the 3-methylbut-2-enoyl moiety was placed at C-3′ of the glycone, as corroborated by the HMBC correlation between δ_H 5.07 (H-3′) and δ_C 166.5 (C-1″). Resonances for an acetate moiety in the glycone were not apparent in any of these three compounds, and instead a hydroxy group was observed at position C-1′, as noted by HSQC data for compounds **7**−**9** via resonances at δ_H/δ_C 4.81/92.9, 4.81/93.0, and 4.81/95.7, respectively. Compounds **7** and **8** were nearly identical, on the basis of their 1-D NMR data (Tables 2 and 3), but differed in their relative configuration. As indicated by the ROESY data, cross-peak correlations between H-5′ and H-5 and between H-4′ and H-4 indicated the *S* configuration at C-10 for **8**, while cross-peak correlations between H-5′ and H-4 and between H-4′ and H-5 indicated the *R* configuration at C-10 for **7**. Although the 1-D NMR data between compounds **8** and **9** were somewhat different, they displayed similar ROESY correlations, indicating also an *S* configuration at C-10 in compound **9**. The CD data exhibited Cotton effects similar to **1** for both compounds **8** and **9** and Cotton effects similar to **2** for compound **7** (see Experimental Section). Although the 10*R* analogue of compound **9** appeared to be present, not enough material was isolated for thorough structure elucidation studies. This trio of isomers were ascribed the trivial names alvaradoin K (**7**), alvaradoin L (**8**), and alvaradoin M (**9**).

Table 4. Cytotoxicity of Compounds Isolated from *A. haitiensis* against the KB Cell Line

compound	KB[a]
1	0.050 ± 0.019
2	0.065 ± 0.026
3	0.65 ± 0.15
4	1.07 ± 0.43
5	12.5 ± 3.03
6	15.9 ± 3.41
7	0.27 ± 0.072
8	0.59 ± 0.10
9	0.38 ± 0.043
10	2.94 ± 1.30
11	>20
camptothecin[b]	0.0036 ± 0.0029

[a] Human oral epidermoid carcinoma. Results are expressed as EC_{50} values in μM (see Experimental Section). Mean ± SEM determined from three separate experiments. [b]Positive control as typical average value.

The HRMS of the sodium adduct of compound **10** revealed a peak corresponding to $[M + Na]^+$ at *m*/*z* 469.1097, suggesting a molecular formula of $C_{22}H_{22}O_{10}$. On the basis of spectroscopic properties, especially in the ^1H and ^{13}C NMR spectra (Tables 2 and 3), this compound was similar to compound **6**, including the presence of a hydroxy moiety at C-10 (δ_C 76.3). The major difference between these compounds was the replacement of the 3-methylbut-2-enoyl moiety at position C-3′ in **6** with a hydroxy group in **10**, as supported by HMBC data from δ_H 4.05 (OH-3′) to δ_C 72.7 (C-3′). Using CD data, the absolute configuration of **10** was found to be 10*S*, again analogous to compound **6**. This compound was ascribed the trivial name alvaradoin N, and, as with compound **9**, despite considerable effort, it was not feasible to isolate the 10*R* analogue of **10**.

Compounds **1**−**11** were tested for activity in the KB cell line (human oral epidermoid carcinoma; Table 4). Compounds **1** and **2** were the most cytotoxic, having EC_{50} values that were only an order of magnitude less active than the positive control, camptothecin. In compounds **3** and **4**, where the hydroxy at R_2 of the glycoside of **1** and **2** has been esterified as a 3-methylbut-2-enoyl moiety, the EC_{50} values rose by another order of magnitude. Analogous results were observed in compounds **7**−**9**, where an identical conversion was placed at R_3 (**7** and **8**) or R_2 (**9**) of the glycoside and the R_4 acetate was converted to a hydroxy moiety. In compounds **5**, **6**, and **10**, where a hydroxy was inserted at C-10 of the anthracenone, cytotoxicity was reduced substantially. Chrysophanol (**11**), which represents the core anthracenone aglycone of **1**−**10**, was completely inactive, suggesting that a combination of the anthracenone and glycoside units is essential for cytotoxic activity. Four structurally related compounds (alvaradoins A−D) have been described from a related species of *Alvaradoa*, and although the authors did not report cytotoxicity test results, these compounds were reported as inactive against *Mycobacterium tuberculosis* when tested at 12.5 μg/mL.[7]

Compounds **1** and **2** exhibited sufficient cytotoxicity to justify further evaluation using the in vivo P388 murine lymphocytic leukemia model.[23] Modest in vivo activity against intraperitoneal (ip) implanted P388 xenografts (T/C of 125%) was observed with compound **1** when mice were injected via the ip route daily for 5 days per week at an optimal dose of 0.2 mg kg^{-1} body weight per injection. Compound **2** was evaluated in the same model, but displayed less activity (115% T/C), even when delivered at its maximally tolerated dose of 0.4 mg kg^{-1} per injection using the same model and dosing schedule. This suggests that compound **1**

possesses the preferred stereochemistry, and as such, **1** is under consideration for further evaluation, possibly via derivatization and/or analogue development of the lead pharmacophore. Moreover, **1** and **2** were evaluated in a similar P388 model, where both the cancer cells and the treatment were delivered intravenously. In this more rigorous model of chemotherapy, both compounds were found to be inactive, suggesting metabolic degradation or other issues that influence drug distribution or accessibility of leukemia cells in bone marrow. These latter findings are consistent with those reported by our group earlier,[24] whereby in vivo activity for **1** and **2** was noted in the hollow fiber assay only when the cancer cells were implanted ip.

Experimental Section

General Experimental Procedures. Melting points were measured on either a Kofler hot-stage apparatus or a Mel-Temp II digital thermometer melting point apparatus and are uncorrected. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter at 25 °C, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. CD spectra were obtained from an Aviv stopped flow model 202 spectrometer. UV spectra were recorded on a Varian Cary 3G UV−visible spectrophotometer, and IR (NaCl or KBr pellet) spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. All NMR experiments were performed using a Bruker AMX-500 spectrometer with TMS as an internal standard in acetone-d_6, except for compound **11**, for which $CDCl_3$ was utilized. EIMS and ESIMS were recorded on HP5989A and Finnigan LCQ instruments, respectively. HRMS measurements were obtained via direct-insertion probe EI or fast-atom bombardment on a VG70S magnetic sector instrument (Micromass, Beverly, MA) or by an Applied Biosystems (Framingham, MA) TOF/TOF mass spectrometer, equipped with a Nd:YAG laser operating at 355 nm and 200 Hz. The latter instrument was operated in the reflectron mode, and the matrix employed was 2,5-dihydroxybenzoic acid prepared at a concentration of 9 mg/mL in 70:30 (v/v) acetonitrile−0.1% trifluoroacetic acid. Column chromatography was carried out on Si gel 60 (230−400 mesh, Merck, Darmstadt, Germany). Fractions were monitored by TLC (silica gel 60 F254 plates, 0.25 mm thickness) visualized with UV light (254 and 365 nm) and with 1% H_2SO_4 in EtOH. Preparative HPLC was carried out via a Varian Prostar 210 pump system using either Diol NP or ODS-A (both 250 × 25 mm, i.d., 5 μm; YMC, Wilmington, NC) columns. The peaks were detected using a Prostar 330 PDA detector, and data were recorded by the Star Chromatography Workstation version 5.51 software system. Empirically, flow rates of either 7 or 10 mL/min were utilized on a case-by-case basis. The percent yields of isolated compounds were calculated on the basis of the dry weight of the plant material (w/w).

Plant Material. Leaves of *Alvaradoa haitiensis* Urb. (Picramniaceae) were collected under a Consultant Agreement in February 1996 by F.J. and R.G. at Cordillera Central, San Cristobal Province, Dominican Republic, and dried. Voucher specimens (2047 and 7357) have been deposited at the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The dried and ground leaves (5408 g) of *A. haitiensis* were extracted with MeOH (6 L × 2) for 24 h at rt and concentrated in vacuo. The concentrated MeOH solution was diluted with H_2O to give a MeOH−H_2O (9:1) solution (2 L), defatted with hexane (2 L × 2), and then concentrated in vacuo. The aqueous MeOH fraction was dissolved in $CHCl_3$−MeOH (4:1, 1 L) and partitioned further with H_2O (1 L × 2). The organic fraction was washed using 1% saline solution until there was no evidence of tannins[25] and concentrated in vacuo to afford 169 g of a crude extract with a high degree of cytotoxicity (1% survival of KB cells tested at a concentration of 20 μg/mL). This extract was purified by low-pressure column chromatography with Si gel (1300 g) using gradient mixtures of 50 → 100% $CHCl_3$ in hexane, then 0 → 10% MeOH in $CHCl_3$, resulting in 17 pooled fractions (F01−F17). Of these, F07−F14 showed significant inhibition of KB cancer cells (<10% survival at 2 μg/mL). A precipitate from F07 and F08 [eluted with $CHCl_3$−MeOH (99:1); <10% survival at 2 μg/mL] was recrystallized from $CHCl_3$−MeOH (4:1) to yield 1.6 g of solid, which contained an approximate 3:2 mixture of compounds **3** and **4**, according to the ^{1}H NMR spectrum. These compounds were separated by gradient normal-phase HPLC with a YMC-Diol NP column under the following conditions: A:B (A = 19:1 $CHCl_3$−2-propanol; B = hexane), 33:67 → 40:60 for 28 min, then isocratic (40:60 for 17 min) to yield pure compounds **3** and **4**.

F09 [eluted with $CHCl_3$−MeOH (98.5:1.5); KB, 5% survival at 2 μg/mL] was subjected to Si gel chromatography using a gradient of hexane−acetone (100:0 → 0:100) to afford 14 fractions (F15−F28). F26 [eluted with hexane−acetone (3:2); 3% survival at 0.2 μg/mL] was purified by reversed-phase preparative HPLC using a YMC ODS-A C_{18} column under the following conditions: MeCN−H_2O, 45:55 → 60:40 for 45 min, then 60:40 → 100:0 for 5 min, resulting in four impure fractions (F29−F32). Further purification of F29 was achieved using normal-phase preparative HPLC using a YMC-Diol column with a solvent system A:B (A = 19:1 $CHCl_3$−2-propanol; B = hexane), 65:35 → 90:10 for 50 min, to yield pure compounds **7** and **8**. In turn, F30 was purified using the same HPLC column with solvent system of A:B (A = 9:1 $CHCl_3$−2-propanol; B = hexane), 30:70 → 40:60 for 60 min, to yield pure compounds **5**, **6**, and **9**.

F13 [eluted with $CHCl_3$−MeOH (19:1); KB, <5% survival at 2 μg/mL] was subjected to Si gel chromatography using hexane−acetone (90:10 → 0:100, gradient mixtures), resulting in 11 fractions (F33−F43). A precipitate formed from F38 [eluted with hexane−acetone (55:45); >5% survival at 2 μg/mL] to give a yellow solid (1.2 g). The solid was purified via preparative HPLC using a YMC-Diol NP with MeOH−$CHCl_3$ (2:98 → 6:94 for 40 min), affording pure compounds **1**, **2**, and **10**.

Alvaradoin E [(10*S*)-*C*-(1-*O*-acetyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 1]: yellow solid (219.5 mg, yield 0.0041% w/w); t_R 14.12 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 13.32 min in 2:98 → 4:96 MeOH−$CHCl_3$ over 20 min, with ODS A and Diol NP columns, respectively; mp 194−196 °C; $[\alpha]_D$ −16.8 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 358 (3.97), 297 (3.85), 268 (3.79), 202 (4.43) nm; CD (MeOH) [θ] (nm) -5.87×10^{-6} (323), $+8.66 \times 10^{-6}$ (297), -5.40×10^{-6} (268); IR (KBr) ν_{max} 3428, 2921, 1740, 1636, 1618, 1457, 1294, 1234, 1139, 1021, 959, 806, 761 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HMBC data, see Table 1; ESIMS *m/z* 453 $[M + Na]^+$; HRFABMS *m/z* 469.0916 $[M + K]^+$ (calcd for $C_{22}H_{22}O_9K$, 469.0901).

Alvaradoin F [(10*R*)-*C*-(1-*O*-acetyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 2]: yellow solid (93.5 mg, yield 0.0017% w/w); t_R 13.95 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 14.39 min in 2:98 → 4:96 MeOH−$CHCl_3$ over 20 min, with ODS A and Diol NP columns, respectively; mp 210−213 °C; $[\alpha]_D$ −107.7 (*c* 0.05, methanol); UV (MeOH) λ_{max} (log ϵ) 357 (4.03), 296 (3.94), 268 (3.89), 203 (4.46) nm; CD (MeOH) [θ] (nm) $+4.73 \times 10^{-6}$ (353), -2.44×10^{-7} (299), $+2.63 \times 10^{-6}$ (257); IR (KBr) ν_{max} 3423, 2924, 1750, 1635, 1617, 1457, 1293, 1230, 1139, 1021, 961, 800, 770 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HMBC data, see Table 1; ESIMS *m/z* 453 $[M + Na]^+$; HRFABMS *m/z* 469.0916 $[M + K]^+$ (calcd for $C_{22}H_{22}O_9K$, 469.0901).

Alvaradoin G [(10*R*)-*C*-(1-*O*-acetyl-3-*O*-senecioyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 3]: brown solid (6.9 mg, yield 0.00013% w/w); t_R 16.64 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 17.11 min in 38:62 A:B (A = 19:1 $CHCl_3$−2-propanol, B = hexane) over 30 min, with ODS A and Diol NP columns, respectively; mp 197−200 °C; $[\alpha]_D$ −85.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 360 (3.99), 295 (3.92), 268 (3.94), 205 (4.67) nm; CD (MeOH) [θ] (nm) $+3.11 \times 10^{-6}$ (351), -2.03×10^{-7} (296), $+1.62 \times 10^{-6}$ (258); IR (NaCl) ν_{max} 3446, 2990, 1759, 1693, 1616, 1596, 1286, 1220, 1127, 1018, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-3, CH_3-11, C-4; H-4/C-2, C-10, CH_3-11; H-5/C-6, C-10, C-7; H-6/C-5a, C-8; H-7/C-5; H-10/C-4a, C-5a, C-4, C-5, C-1a, C-8a, C-1′; CH_3-11/C-2, C-3, C-4; H-5′/C-4′, C-4a, C-5a; H-4′/C-3′; H-3′/C-4′, C-1″; H-2′/C-3′; H-1′/C-5′, C-3′, C-2′, C-1‴; H-2″/C-1″, C-4″, C-5″; CH_3-4″/C-2″, C-3″, C-5″; CH_3-5″/C-2″, C-3″, C-4″; CH_3-2‴/C-1‴; OH-1/C-1, C-1a; OH-8/C-8, C-8a; OH-4′/C-5′, C-4′; OH-2′/C-3′, C-2′, C-1′; EIMS *m/z* 512 $[M]^+$ (2), 452 (1), 352 (5) 280 (5), 265 (6), 240 (100), 165 (9); HRFABMS *m/z* 551.1336 $[M + K]^+$ (calcd for $C_{27}H_{28}O_{10}K$, 551.1319).

Alvaradoin H [(10*S*)-*C*-(1-*O*-acetyl-3-*O*-senecioyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 4]: yellow solid (12 mg, yield 0.00022% w/w); t_R 16.72 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 15.45 min in 38:62 A:B (A = 19:1 $CHCl_3$−2-propanol, B = hexane) over 30 min, with ODS A and Diol NP columns, respectively; mp 240−243 °C; $[\alpha]_D$ −26.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 358 (4.06), 297 (3.98), 268 (3.98), 207 (4.77)

nm; CD (MeOH) [θ] (nm) -5.18×10^{-6} (322), $+3.46 \times 10^{-6}$ (296), -6.00×10^{-6} (268); IR (NaCl) ν_{max} 3460, 3026, 2977, 1747, 1693, 1617, 1598, 1294, 1228, 1156, 1022, 752 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH_3-11, C-4; H-4/C-2, C-10, CH_3-11; H-5/C-10; H-6/C-5a, C-8; H-7/C-5, C-8; H-10/C-4a, C-5a, C-4, C-5, C-1a, C-8a, C-5′; CH_3-11/C-2, C-3, C-4; H-5′/C-4′, C-4a; H-4′/C-3′; H-3′/C-4′; H-2′/C-3′; H-1′/C-5′, C-3′, C-2′, C-1‴; H-2″/C-1″, C-4″, C-5″; CH_3-4″/C-2″, C-3″, C-5″; CH_3-5″/C-2″, C-3″, C-4″; CH_3-2‴/C-1‴; OH-1/C-1, C-1a, C-2; OH-8/C-8, C-8a; OH-4′/C-5′, C-4′; OH-2′/C-2′, C-1′; EIMS *m*/*z* 512 [M]$^+$ (1), 452 (2), 352 (5), 280 (34), 265 (45), 240 (100), 165 (18); HRFABMS *m*/*z* 551.1324 [M + K]$^+$ (calcd for $C_{27}H_{28}O_{10}K$, 551.1319).

Alvaradoin I [(10*R*)-*C*-(1-*O*-acetyl-3-*O*-senecioyl)-β-L-lyxopyranosyl-1,8,10-trihydroxy-3-methylanthracen-9-one, 5]: yellow solid (6.0 mg, yield 0.00011% w/w); t_R 13.24 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 9.92 min in 20:80 → 60:40 A:B (A = 9:1 $CHCl_3$−2-propanol, B = hexane) over 10 min, with ODS A and Diol NP columns, respectively; mp 148−149 °C; [α]$_D$ −11.7 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 369 (3.89), 300 (3.71), 213 (4.50) nm; CD (MeOH) [θ] (nm) -1.73×10^6 (332), $+7.61 \times 10^6$ (301), -8.98×10^6 (268); IR (NaCl) ν_{max} 3394, 3016, 2980, 2917, 1719, 1642, 1220, 1142, 757 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH_3-11, C-4; H-4/C-1a, C-2, C-10, CH_3-11; H-5/C-7, C-8a, C-10; H-6/C-5a, C-8; H-7/C-5, C-8a; CH_3-11/C-2, C-3, C-4; H-1′/C-4a, C-5a, C-10, C-4′, C-3′, C-1′; H-4′/C-3′; H-3′/C-4′, C-2′, C-1″; H-1′/C-5′, C-3′, C-5′; H-2″/C-4″, C-5″; CH_3-4″/C-2″, C-3″, C-5″; CH_3-5″/C-2″, C-3″, C-4″; CH_3-2‴/C-1‴; OH-1/C-1, C-1a; OH-8/C-7, C-8, C-8a; OH-2′/C-2′, C-1′; EIMS *m*/*z* 528 [M]$^+$ (3), 468 (1), 315 (3), 298 (8), 273 (14), 256 (100), 240 (2), 213 (24), 83 (53); HRMALDITOFMS *m*/*z* 551.1518 [M + Na]$^+$ (calcd for $C_{27}H_{28}O_{11}Na$, 551.1529).

Alvaradoin J [(10*S*)-*C*-(1-*O*-acetyl-3-*O*-senecioyl)-β-L-lyxopyranosyl-1,8,10-trihydroxy-3-methylanthracen-9-one, 6]: yellow solid (10.4 mg, yield 0.00019% w/w); t_R 13.27 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 10.39 min in 20:80 → 60:40 A:B (A = 9:1 $CHCl_3$−2-propanol, B = hexane) over 10 min, with ODS A and Diol NP columns, respectively; mp 138−139 °C; [α]$_D$ −56.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 369 (3.88), 300 (3.68), 213 (4.48) nm; CD (MeOH) [θ] (nm) $+2.10 \times 10^6$ (353), -1.40×10^7 (299), $+4.29 \times 10^6$ (260); IR (NaCl) ν_{max} 3417, 3021, 2980, 2923, 1723, 1604, 1287, 1217, 1143, 754; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH_3-11, C-4; H-4/C-1a, C-2, C-3, C-10, CH_3-11; H-5/C-6, C-7, C-8a, C-10; H-6/C-5a, C-8; H-7/C-5, C-8, C-8a; CH_3-11/C-2, C-3, C-4; H-5′/C-4a, C-5a, C-10, C-4′, C-3′, C-1′; H-4′/C-3′; H-3′/C-4′, C-1″; H-1′/C-5′, C-3′, C-2′, C-1‴; H-2‴/C-4″, C-5″; CH_3-4″/C-2″, C-3″, C-5″; CH_3-5″/C-2″, C-3″, C-4″; CH_3-2‴/C-1‴; OH-1/C-1, C-1a, C-2; OH-8/C-7, C-8; OH-10/C-4a, C-10, C-5′; OH-2′/C-2′, C-1′; EIMS *m*/*z* 528 [M]$^+$ (2), 468 (1), 315 (2), 298 (2), 285 (3), 273 (15), 256 (100), 240 (3), 213 (23), 83 (48); HRMALDITOFMS *m*/*z* 551.1525 [M + Na]$^+$ (calcd for $C_{27}H_{28}O_{11}Na$, 551.1529).

Alvaradoin K [(10*R*)-*C*-(2-*O*-senecioyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 7]: yellow solid (14 mg, yield 0.00026% w/w); t_R 17.56 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 10.95 min in 80:20 → 95:5 A:B (A = 19:1 $CHCl_3$−2-propanol, B = hexane) over 15 min, with ODS A and Diol NP columns, respectively; mp 143−144 °C; [α]$_D$ −65.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 359 (3.87), 297 (3.77), 210 (4.42) nm; CD (MeOH) [θ] (nm) $+1.47 \times 10^6$ (351), -8.51×10^5 (298); IR (NaCl) ν_{max} 3451, 3020, 2975, 2936, 1690, 1603, 1291, 1229, 1074, 754 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/C-1, CH_3-11, C-4; H-4/C-10, CH_3-11; H-5/C-6, C-10; H-6/C-5a, C-8; H-7/C-5; H-10/C-4a, C-5a, C-1a, C-8a, C-5′, C-4′; CH_3-11/C-2, C-3, C-4; H-5′/C-10, C-4′, C-3′, C-1′; H-4′/C-10, C-5′, C-3′; H-2′/C-4′, C-3′, C-1″; H-1′/C-5′, C-3′, C-2′; H-2″/C-4″, C-5″; CH_3-4″/C-2″, C-3″, C-5″; CH_3-5″/C-2″, C-3″, C-4″; OH-1/C-1, C-1a, C-2; OH-8/C-8, C-8a; OH-1′/C-2′, C-1′; ESIMS *m*/*z* 493 [M + Na]$^+$; HRMALDITOFMS *m*/*z* 493.1475 [M + Na]$^+$ (calcd for $C_{25}H_{26}O_9Na$, 493.1474).

Alvaradoin L [(10*S*)-*C*-(2-*O*-senecioyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 8]: yellow solid (14 mg, yield 0.00026% w/w); t_R 15.91 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 11.63 min in 80:20 → 95:5 A:B (A = 19:1 $CHCl_3$−2-propanol, B = hexane) over 10 min, with ODS A and Diol NP columns, respectively; mp 141−142 °C; [α]$_D$ −26.7 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 360 (3.92), 298 (3.82), 210 (4.47) nm; CD (MeOH) [θ] (nm) -3.19×10^6 (327), $+3.64 \times 10^6$ (298), -4.90×10^6 (268); IR (NaCl) ν_{max} 3436, 3020, 2975, 2924, 1691, 1603, 1291, 1231, 1079, 755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH_3-11, C-4; H-4/C-1a, C-10, CH_3-11; H-5/C-7, C-8a; H-6/C-5a, C-8; H-7/C-5, C-8; H-10/C-4a, C-5a, C-4, C-1a, C-8a, C-5′, C-4′; CH_3-11/C-2, C-3, C-4; H-5′/C-4a, C-5a, C-10, C-4′; H-2′/C-4′, C-1″; H-1′/C-5′, C-3′, C-2′; H-2″/C-1″, C-4″, C-5″; CH_3-4″/C-2″, C-3″, C-5″; CH_3-5″/C-2″, C-3″, C-4″; OH-1/C-1, C-1a; OH-8/C-7, C-8, C-8a; OH-4′/C-5′; OH-3′/C-4′, C-3′; OH-1′/C-2′, C-1′; ESIMS *m*/*z* 493 [M + Na]$^+$; HRMALDITOFMS *m*/*z* 493.1452 [M + Na]$^+$ (calcd for $C_{25}H_{26}O_9Na$, 493.1474).

Alvaradoin M [(10*S*)-*C*-(3-*O*-senecioyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 9]: yellow solid (6.8 mg, yield 0.00013% w/w); t_R 15.91 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 11.91 min in 20:80 → 60:40 A:B (A = 9:1 $CHCl_3$−2-propanol, B = hexane) over 15 min, with ODS A and Diol NP columns, respectively; mp 153−154 °C; [α]$_D$ −32.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 360 (3.86), 297 (3.74), 212 (4.42) nm; CD (MeOH) [θ] (nm) -1.58×10^6 (322), $+4.97 \times 10^5$ (301), -2.65×10^6 (268); IR (NaCl) ν_{max} 3452, 3019, 2974, 2926, 1699, 1603, 1292, 1230, 1079, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/C-1, CH_3-11, C-4; H-4/C-1a, C-10, CH_3-11; H-5/C-7, C-8a, C-10; H-6/C-5a, C-8; H-7/C-5; H-10/C-4a, C-5a, C-4, C-5, C-1a, C-5′; CH_3-11/C-2, C-3, C-4; H-5′/C-4a; H-3′/C-4′; H-2″/C-4″, C-5″; CH_3-4″/C-2″, C-3″, C-5″; CH_3-5″/C-2″, C-3″, C-4″; OH-1/C-1a; OH-8/C-7, C-8a; OH-4′/C-5′; OH-1′/C-2′, C-1′; ESIMS *m*/*z* 493 [M + Na]$^+$; HRMALDITOFMS *m*/*z* 493.1461 [M + Na]$^+$ (calcd for $C_{25}H_{26}O_9Na$, 493.1474).

Alvaradoin N [(10*S*)-*C*-(1-*O*-acetyl)-β-L-lyxopyranosyl-1,8,10-trihydroxy-3-methylanthracen-9-one, 10]: yellow solid (6.1 mg, yield 0.00011% w/w); t_R 9.64 min in 50:50 → 100:0 MeOH−H_2O over 25 min and t_R 17.58 min in 2:98 → 4:96 MeOH−$CHCl_3$ over 20 min, with ODS A and Diol NP columns, respectively; mp 139−140 °C; [α]$_D$ −56.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 368 (3.70), 301 (3.54), 211 (4.13) nm; CD (MeOH) [θ] (nm) $+4.61 \times 10^6$ (351), -4.72×10^8 (299), $+1.51 \times 10^8$ (259); IR (NaCl) ν_{max} 3400, 2925, 2854, 1743, 1628, 1607, 1285, 1219, 1071, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HMBC H-2/CH_3-11, C-4; H-4/C-10, CH_3-11; H-5/C-10; H-6/C-5a, C-8; H-7/C-5; CH_3-11/C-2, C-3, C-4; H-5′/C-5a, C-10, C-4′, C-3′; H-4′/C-5′, C-3′; H-3′/C-4′; H-2′/C-3′; H-1′/C-5′, C-3′, C-1″; H-2″/C-1″; OH-1/C-1, C-2; OH-8/C-7, C-8; OH-10/C-4a, C-10, C-5′; OH-3′/C-3′, C-2′; OH-2′/C-3′, C-2′, C-1′; ESIMS *m*/*z* 445 [M − 1]$^+$; HRMALDITOFMS *m*/*z* 469.1097 [M + Na]$^+$ (calcd for $C_{22}H_{22}O_{10}Na$, 469.1111).

Chrysophanol (11): physical data were comparable to literature values.[15,16]

KB Cytotoxicity Assay. The extract, fractions, and pure compounds were tested in a human oral epidermoid carcinoma (KB) cell line using established protocols as described previously.[26]

In Vivo Evaluation of Compounds 1 and 2. Compounds **1** and **2** were evaluated in an in vivo test system using the murine P-388 lymphocytic leukemia model as described previously.[23]

Acknowledgment. This paper is dedicated to the memory of Dr. Monroe E. Wall (deceased July 6, 2002). This investigation was supported by grant U19-CA52956 from the National Cancer Institute, NIH, Bethesda, MD. N.H.O. and D.L. acknowledge partial support via a Research Scholar Grant from the American Cancer Society (RSG-02-024-01-CDD). We thank Snr. M. Mejía (Director of the Botanical Garden in Santo Domingo) for facilitating the collection through the signing of the Memorandum of Agreement between University of Illinois at Chicago and the Jardin Botanico Nacional of Santo Domingo. HRMS data were acquired by the Mass Spectrometry Research Group at Research Triangle Institute and Dr. M. Walla from the Department Chemistry and Biochemistry at University of South Carolina. Dr. J. Burgess of Research Triangle Institute performed NMR experiments. We thank Ms. K. Dau for technical assistance.

References and Notes

(1) Fernando, E. S.; Quinn, C. J. *Taxon* **1995**, *44*, 177−181.
(2) Fernando, E. S.; Gadek, P. A.; Quinn, C. J. *Am. J. Bot.* **1995**, *82*, 92−103.
(3) Jacobs, H. *Biochem. Syst. Ecol.* **2003**, *31*, 773−783.
(4) Cronquist, A. *Studies in the Simaroubaceae-IV. Resume of the American Genera*; New York Botanical Garden Press: Bronx, NY, 1944; pp 128−147.

(5) Soto de Villatoro, B.; Gonzalez, F. G.; Polonsky, J.; Baskevitch-Varon, Z. *Phytochemistry* **1974**, *13*, 2018−2019.
(6) Heywood, V. H.; Moore, D. M.; Stearn, W. T.; Richardson, I. B. K. *Flowering Plants of the World*; Oxford University Press: New York, 1993; pp 199−200.
(7) Harding, W. W.; Henry, G. E.; Lewis, P. A.; Jacobs, H.; McLean, S.; Reynolds, W. F. *J. Nat. Prod.* **1999**, *62*, 98−101.
(8) Rodriguez-Gamboa, T.; Fernandes, J. B.; Fo, E. R.; Da Silva, M. F. D. G. F.; Vieira, P. C.; Castro, O. *Phytochemistry* **1999**, *51*, 583−586.
(9) Solis, P. N.; Ravelo, A. G.; Gonzalez, A. G.; Gupta, M. P.; Phillipson, J. D. *Phytochemistry* **1995**, *38*, 477−480.
(10) Hernandez-Medel, M. D. R.; Garcia-Salomones, I.; Santillan, R.; Trigos, A. *Phytochemistry* **1996**, *49*, 2599−2601.
(11) Hernandez-Medel, M. D. R.; Lopez-Marquez, O.; Santillan, R.; Trigos, A. *Phytochemistry* **1996**, *43*, 279−281.
(12) Hernandez-Medel, M. D. R.; Ramirez-Corzas, C. O.; Rivera-Dominguez, M. N.; Ramirez-Mendez, J.; Santillan, R.; Rojas-Lima, S. *Phytochemistry* **1999**, *50*, 1379−1383.
(13) Diaz, F.; Chai, H. B.; Mi, Q.; Su, B. N.; Vigo, J. S.; Graham, J. G.; Cabieses, F.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2004**, *67*, 352−356.
(14) Kinghorn, A. D.; Farnsworth, N. R.; Soejarto, D. D.; Cordell, G. A.; Swanson, S. M.; Pezzuto, J. M.; Wani, M. C.; Wall, M. E.; Oberlies, N. H.; Kroll, D. J.; Kramer, R. A.; Rose, W. C.; Vite, G. D.; Fairchild, C. R.; Peterson, R. W.; Wild, R. *Pharm. Biol.* **2003**, *41* (Suppl.), 53−67.
(15) Arnone, A.; Fronza, G.; Mondelli, R.; Pyrek, J. S. *J. Magn. Reson.* **1977**, *28*, 69−79.
(16) Danielson, K.; Akses, D. W. *Magn. Reson. Chem.* **1992**, *30*, 359−363.
(17) Manitto, P.; Monti, D.; Speranza, G.; Mulinacci, N.; Vincieri, F. F.; Griffini, A.; Pifferi, G. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1577−1580.
(18) Manitto, P.; Monti, D.; Speranza, G. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1297−1300.
(19) Rauwald, H. W.; Lohse, K.; Bats, J. W. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1528−1529.
(20) Rauwald, H. W.; Lohse, K.; Bats, J. W. *Z. Naturforsch. B* **1991**, *46*, 551−557.
(21) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; Wiley: New York, 1991; pp x, 419.
(22) Although this nomenclature may seem opposite of what was proposed for compounds **1** and **2**, it is keeping with a patent application that has been filed on these compounds: Wall, M. E.; Wani, M. C.; Phifer, S. S.; Lee, D.; Seo, E. K. PCT Int. Appl. WO 2006002233 A2, January 5, 2006.
(23) Rose, W. C.; Schurig, J. E.; Meeker, J. B. *Anticancer Res.* **1988**, *8*, 355−367.
(24) Mi, Q.; Lantvit, D.; Reyes-Lim, E.; Chai, H.; Phifer, S. S.; Wani, M. C.; Wall, M. E.; Tan, G. T.; Cordell, G. A.; Farnsworth, N. R.; Kinghorn, A. D.; Pezzuto, J. M. *Anticancer Res.* **2005**, *25*, 779−787.
(25) Wall, M. E.; Wani, M. C.; Brown, D. M.; Fullas, F.; Oswald, J. B.; Josephson, F. F.; Thornton, N. M.; Pezzuto, J. M.; Beecher, C. W. W.; Farnsworth, N. R.; Cordell, G. A.; Kinghorn, A. D. *Phytomedicine* **1996**, *3*, 281−285.
(26) Oberlies, N. H.; Burgess, J. P.; Navarro, H. A.; Pinos, R. E.; Soejarto, D. D.; Farnsworth, N. R.; Kinghorn, A. D.; Wani, M. C.; Wall, M. E. *J. Nat. Prod.* **2001**, *64*, 497−501.

NP070005A