Antineoplastic Agents. 489. Isolation and Structures of Meliastatins 1−5 and Related Euphane Triterpenes from the Tree *Melia dubia*[1]

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The bark of the giant neem tree *Melia dubia* was found to contain 11 euphane-type triterpenes. Five new compounds, meliastatins 1−5 (**1**−**5**), proved to inhibit growth of the P388 lymphocytic leukemia cell line (ED_{50} 1.7−5.6 μg/mL). Four of the others, the previously known methyl kulonate (**8**), kulinone (**9**), 16-hydroxybutyrospermol (**10**), and kulactone (**11**), were also found to inhibit (ED_{50} 2.5−6.2 μg/mL) the P388 cancer cell line. In addition, two new euphane triterpenes were isolated and named dubione A (**6**) and dubione B (**7**). Structures for each of the 11 euphane triterpenes were established by spectral techniques that included HRMS and 2D NMR.

A broad variety of species included in the plant family Meliaceae[2] contain constituents with potentially useful biological properties[3] such as the powerful insect antifeedant azadirachtin from *Azadirachta indica*, which is already produced commercially.[3c] The synthetic flavone, flavopiridol,[4a] which is currently in clinical development, was derived from rohitukine, an alkaloid that was isolated from the leaves and stems of *Amoora rohituka*[4b] and later from *Dysoxylum binectariferum* (Meliaceae). Our early evaluation of several Meliaceae species with respect to cancer cell growth inhibitory substances resulted in structure determinations for amoorastatin[5a] and aphanastatin.[5b] In the same period we undertook an investigation involving the genus *Melia*[6] that was concentrated on *Melia dubia*[7] Cav., first collected in the Philippines in 1974 as part of the U.S. National Cancer Institute natural products based anticancer drug discovery research. Alcohol extracts of the stem bark of *M. dubia* were found to significantly inhibit growth of the P388 lymphocytic leukemia (PS system) cell line and became the focus of the present investigation.

M. dubia is well known as a high tree in the Ghat forests of India and is generally termed the giant neem.[7] The tree enjoys a broad geographical distribution (e.g., in Indonesia and the Philippines) and is well known for its traditional medicinal properties and other biological activities such as insect or larval growth inhibition and as an antifeedant.[8,9] While little explored chemically, *M. dubia* has been shown to be a source of interesting tetranortriterpenoids.[10,11] In 1980 we obtained 19.5 kg of *M. dubia* collected in the Philippines and later proceeded with an 18 kg portion of this supply.

Results and Discussion

The stem bark of *M. dubia* Cav. was extracted using $CH_2Cl_2-CH_3OH$ (followed by dilution with H_2O). The CH_3OH-soluble fraction of the CH_2Cl_2 extract was successively partitioned between CH_3OH-H_2O (4:1 → 3:2 → 1:4) and hexane → CH_2Cl_2 → *n*-BuOH. The most encouraging PS activity (ED_{50} 2.3 μg/mL) was located in the hexane fraction. The latter was separated by bioassay (PS)-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography procedures and high-performance liquid chromatography (HPLC) to afford the new euphanes **1**−**7** as well as the known relatives **8**−**11**. The known constituents were identified as methyl kulonate (**8**), kulinone (**9**), 16-hydroxybutyrospermol (**10**), and kulactone (**11**) by comparison of physicochemical and spectral data with literature values.[12] The new constituents **1**−**5** were designated as meliastatins 1−5. Euphanes **1**−**5** and **8**−**11** were found to significantly inhibit growth of the P388 cancer cell line.

Meliastatin 1 (**1**) corresponded to molecular formula $C_{30}H_{48}O_3$, which was established by a molecular ion peak in the HREIMS. The IR spectrum exhibited bands at 3419, 1704, 1660, and 1623 cm^{-1}, characteristic of an alcohol, a ketone, and a double bond. A close inspection of the ^1H (Table 1) and ^{13}C NMR spectra (Table 4) by DEPT and $^1H-^{13}C$ correlation spectroscopy (COSY) experiments revealed the presence of one secondary methyl, seven tertiary methyls, seven sp^3-hybridized methylenes, five sp^3-methines including one hydroxymethine, five quaternary sp^3-carbons including one hydroxycarbon, 1,2-di- and trisubstituted double bonds, and one ketone. The $^1H-^1H$ COSY analysis of **1** led to four partial structural units, which were supported by HMBC correlations. The *E*-geometry of the Δ^{23}-double bond was deduced from a coupling constant ($J_{23,24}$ 15.6 Hz) corresponding to the olefinic protons. The connection of these four units and the remaining functional groups was determined on the basis of key HMBC correlations, and the planar structure of **1** was thereby elucidated. The structure was also supported by the MS fragment at *m/z* 311 $[a - H_2O]^+$, resulting from cleavage of the C-17−C-20 bond followed by dehydration.

The stereochemistry of **1** was deduced from NOESY experiments. The observation of NOE correlations from H-19 to H-29 and H-2β and from H-1α to H-5 indicated that the A-ring exists in a chair conformation with H-5 and H-19 in a *trans*-diaxial arrangement, implying the *trans* fusion of ring A to ring B. In addition, NOEs from H-5 to H-9 and H-6α and from H-29 to H-6β implied that the B-ring exists in a twist chair conformation with H-9 in an

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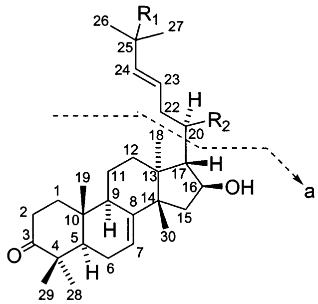
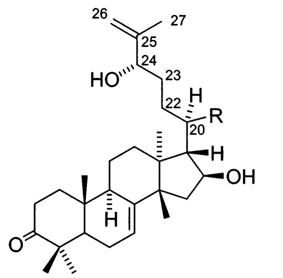


1, R_1 = OH, R_2 = CH_3

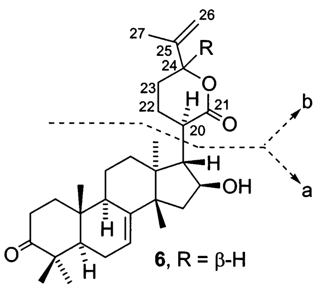
2, R_1 = OH, R_2 = CO_2CH_3

3, R_1 = OOH, R_2 = CO_2CH_3



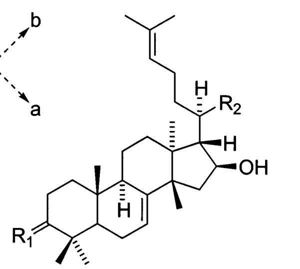
4, R = CO_2CH_3

5, R = CH_3



6, R = β-H

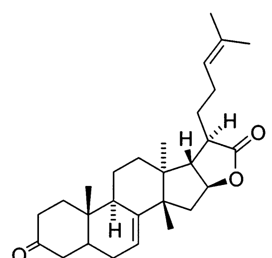
7, R = α-H



8, R_1 = O, R_2 = CO_2CH_3

9, R_1 = O, R_2 = CH_3

10, R_1 = α-H, β-OH, R_2 = CH_3



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axial (α) arrangement. Furthermore, the observation of NOEs from H-30 to H-11*β*, H-12*β*, and H-19 and from H-18 to H-9 indicated that the C-ring exists in a twist boat conformation with H-18 and H-30 in a *trans*-diaxial arrangement, suggesting the *trans* fusion of ring C to ring D. NOE correlations between H-18 and H-16 and between H-30 and H-17 showed that H-16 and H-17 are oriented *trans*-diaxially, and hence both have an *S*-configuration. The observation of NOEs from H-16 to H-21, from H-20 to H-18 and H-16, and from H-22α to H-12α indicated the configuration of C-20 to be *R*. In addition to these NOEs, those from H-20 to H-23 and H-22α and from H-22*β* to H-24 emphasized that the side chain exists in a conformation in which H-20 and H-23 are on the same side as H-16 and H-18 and on the side opposite to H-22*β* and H-24. The above evidence allowed the complete elucidation of the stereostructure and conformation of **1**.

Meliastatin 2 (**2**) was assigned a molecular formula that contained one carbon and two oxygen atoms more than that of **1**. The IR spectrum exhibited an absorption band (1730 cm^{-1}) for an ester, besides those of an alcohol, a ketone, and a double bond. The general features of its NMR spectra (Tables 1 and 4) closely resembled those of terpene **1** except that the proton and carbon signals of the C-21 methyl group were replaced by those of a methoxycarbonyl group (δ_H 3.68; δ_C 51.62 and 176.83), and the signals for H-17, H-20, H-22*β*, C-17, C-20, C-22, and C-23 appeared shifted relative to those of **1**. The planar structure deduced from the NMR spectral analysis was confirmed by analysis of $^1H-^1H$ COSY and HMBC correlations. On the basis of the observation of NOE data similar to those of **1**, the stereochemistry of **2** was expected to be the same.

Meliastatin 3 (**3**) was assigned a molecular formula that contained one oxygen atom more than that of **2**, as deduced from an $[M + Na]^+$ peak in HRESIMS. The general features of its IR and NMR spectra closely resembled those of **2** except for the chemical shifts of C-23−C-27 in the ^{13}C NMR spectrum (Table 4). In addition to the molecular formula, the appearance of the C-25 signal at δ_C 81.81 in **3** implied that the C-25 hydroxyl group was replaced by a hydroperoxy group.[13] The planar structure was supported by $^1H-^1H$ COSY and HMBC correlations. Since **3** exhibited NOE correlations similar to those of **2** in NOESY, the stereochemistry was considered to be the same as in terpene **2**.

Meliastatin 4 (**4**) exhibited the same molecular formula as **2**. The IR spectrum also exhibited absorption bands similar to those of terpene **2**. The general features of its NMR spectral data closely resembled those of terpene **2** except for the chemical shifts and splitting pattern of the proton (Table 2) and carbon signals of C-22−C-27 in the side chain (Table 4). In the NMR spectrum of **4**, the signals for an exomethylene (δ_H 4.86 and 4.94; δ_C 111.63 and 147.10), a hydroxymethine (δ_H 4.03; δ_C 75.79), and one methylene (δ_H 1.43 and 1.52; δ_C 32.24) were observed instead of the Δ^{23}-disubstituted double bond, the C-27 methyl group and the quaternary sp^3-carbon (C-25) bearing the hydroxy group in the side chain of **2**. The position of functional groups in the side chain of **4** was deduced from $^1H-^1H$ COSY (H-22α/H-23α and H-23α/H-24) and HMBC (H-26A/C-24 and H-26A/C-27) correlations. Thus the planar structure of **4** was elucidated.

On the basis of the similarity of the NOEs deduced for the protons in **4** to those of terpene **2**, the stereochemistry and conformation of the skeletons were considered to be the same in both. The stereochemistry of the side chain was established by a combination of observed coupling constants and NOESY correlations. The NOEs from H-20 to H-16, H-18, and H-22α and from H-22α to H-12α showed H-20 to have an α configuration (*R*). On the basis of the generalized Karplus relationship,[14,15] the observed coupling constants ($J_{20,22\alpha}$ 3.9 Hz, $J_{20,22\beta} = J_{22\alpha,23\beta} = J_{22\beta,23\alpha}$ 10.4 Hz, and $J_{22\beta,23\beta}$ 4.0 Hz) suggested that the dihedral angles for H-20/H-22α and H-22*β*/H-23*β* and each of H-20/H-22*β*, H-22α/H-23*β*, and H-22*β*/H-23α were approximately 56°, 55°, and 156°, respectively, implying that H-20 and H-22*β*, H-22α and H-23*β*, and H-22*β* and H-23α are nearly in an *anti* arrangement and H-20 and H-22α are in a *gauche* arrangement. Similarly, the coupling constants ($J_{24,23\alpha} = J_{24,23\beta}$ 6 Hz) suggested that the dihedral angles for H-24/H-23α and H-24/H-23*β* were approximately 144° and 31°, respectively. This evidence was supported by NOEs from H-20 to H-22α and H-23α and from H-23*β* to H-24. In addition to these data, NOEs from H-27 to H-23α, H-23*β*, and H-26A and from H-24 to H-26B pointed to the side chain of **4** as existing in a conformation with 20*R*,24*S*-configurations. Although H-22α and H-23α appeared as a multiplet, their coupling constants were estimated by a $^1H-^1H$ *J*-resolved spectrum, and the protons (H-22*β* and H-23*β*) coupling with them were clarified. On the basis of the above evidence, the stereochemistry and conformation of **4** were established.

Table 1. ^{1}H NMR Data (δ in $CDCl_3$) for Compounds **1**−**3**[a]

position	**1**	**2**	**3**
1α	1.45 (br td, 14.4, 3.9)	1.45 (br td, 14.6, 3.9)	1.45 (br td, 14.6, 3.9)
1β	1.98 (ddd, 14.4, 5.5, 3.2)	1.98 (ddd, 14.6, 5.7, 3.0)	1.98 (ddd, 14.6, 5.7, 3.0)
2α	2.25 (ddd, 14.4, 3.9, 3.2)	2.25 (ddd, 14.6, 3.9, 3.0)	2.25 (ddd, 14.6, 3.9, 3.0)
2β	2.76 (td, 14.4, 5.5)	2.76 (td, 14.6, 5.7)	2.76 (td, 1.46, 5.7)
5	1.72 (dd, 10.3, 7.3)	1.71 (dd, 10.3, 7.1)	1.71 (dd, 10.4, 7.0)
6α	2.11 (ddd, 14.0, 7.3, 3.2)	2.12 (ddd, 14.0, 7.1, 3.2)	2.12 (ddd, 14.0, 7.0, 3.2)
6β	2.10 (ddd, 14.0, 10.3, 3.2)	2.10 (ddd, 14.0, 10.3, 3.2)	2.10 (ddd, 14.0, 10.4, 3.2)
7	5.29 (br q, 3.2)	5.32 (br q, 3.2)	5.32 (br q, 3.2)
9	2.29 (m)	2.28 (m)	2.28 (m)
11α	1.60 (m)	1.59 (m)	1.61 (m)
11β	1.64 (m)	1.63 (m)	1.64 (m)
12α	1.63 (m)	1.60 (m)	1.59 (m)
12β	1.9 (br ddd, 13.0, 10.0, 4.0)	1.96 (br dd, 13.0, 10.0)	1.96 (br t, 13.0)
15α	2.10 (br ddq, 13.3, 8.5, 0.9)	2.06 (br ddq, 13.5, 8.5, 0.9)	2.06 (br ddq, 13.5, 8.5, 0.9)
15β	1.57 (br dd, 13.3, 0.9)	1.63 (br dd, 13.5, 0.9)	1.64 (br dd, 13.5, 0.9)
16	4.06 (br ddd, 8.5, 5.5, 0.9)	3.99 (br ddd, 8.5, 5.7, 0.9)	3.99 (br ddd, 8.5, 5.7, 0.9)
17	1.58 (dd, 9.8, 5.5)	2.11 (dd, 10.3, 5.7)	2.14 (dd, 11.0, 5.7)
18	0.85 (s)	0.86 (s)	0.87 (s)
19	1.020 (s)	1.02 (s)	1.03 (s)
20	1.68 (ddqd, 9.8, 9.2, 6.2, 3.2)	2.68 (td, 10.3, 4.1)	2.73 (ddd, 11.0, 10.0, 4.1)
21	1.019 (d, 6.2)		
22α	2.33 (br ddd, 13.3, 5.0, 3.2)	2.43 (dddd, 14.0, 6.4, 4.1, 1.2)	2.47 (dddd, 13.8, 4.1, 6.6, 1.2)
22β	1.76 (br ddd, 13.3, 9.2, 6.2)	2.30 (br ddd, 14.0, 10.3, 7.6)	2.30 (br ddd, 13.8, 10.0, 7.6)
23	5.59 (ddd, 15.6, 6.2, 5.0)	5.54 (ddd, 15.6, 7.6, 6.4)	5.64 (ddd, 15.8, 7.6, 6.6)
24	5.61 (br d, 15.6)	5.63 (br dt, 15.6, 1.2)	5.54 (br dt, 15.8, 1.2)
26	1.32 (s)	1.28 (s)	1.306 (s)
27	1.32 (s)	1.28 (s)	1.312 (s)
28	1.05 (s)	1.05 (s)	1.05 (s)
29	1.12 (s)	1.12 (s)	1.12 (s)
30	1.26 (br d, 0.9)	1.28 (s)	1.29 (br s)
21-OMe		3.68 (s)	3.68 (s)
16-OH	1.58 (br s)	1.57 (br s)	7.55 (br s)
25-OH	1.58 (br s)	not detected	not detected

[a] ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (J/Hz).

Meliastatins 5 (**5**) and 1 (**1**) were found to have the same molecular formula. Also the IR spectrum exhibited the same major absorption bands. The NMR signals of **5** (Tables 2 and 4) showed close correspondence with those of the **4** counterpart except that the proton and carbon signals of the C-20 methoxycarbonyl moiety in **4** were replaced by those from a methyl group (δ_H 1.054; δ_C 18.60). The signals for H-17, H-20, H-22β, C-17, C-20, and C-22 in **5** appeared shifted relative to those of **4**. The planar structure of **5** was deduced from analysis of the ^{1}H−^{1}H COSY and HMBC spectral correlations. On the basis of similarity of the NOEs analyzed for the skeletons of **5** and **4**, the stereochemistry was considered to be the same in each. The side chain protons of **5** showed the same NOE relationships as those of **4**. In addition, NOEs from H-21 to H-16 and H-17 were observed with **5**. These NOE data led to the configuration for C-20 and C-24 and to the conclusion that the conformation of the side chain was the same as that of **4**.

A molecular formula of $C_{30}H_{44}O_4$ was established for the new euphane triterpane termed dubione A (**6**) by a molecular ion peak in its HREIMS. The IR spectrum exhibited absorption bands typical of an alcohol, a ketone, a double bond, and an ester or lactone, as in **4**. The general features of its NMR spectral data closely resembled those of **4** except for the chemical shifts of H-24, H-23β, C-20, and C-23−C-25 in the side chain and the disappearance of the signals for the 24-OH and methyl ester (Tables 3 and 4). The absence of the 24-OH signal, the downfield shift of the H-24 signal by 0.76 ppm, relative to **4**, and the molecular formula of **6** suggested that it carried a six-membered lactone in the side chain. The structure of the side chain was confirmed by analysis of ^{1}H−^{1}H COSY and HMBC correlations. The planar structure thus deduced was supported by the MS fragments at m/z 329 $[a]^+$ and 139 $[b + H]^+$, resulting from cleavage of the C-17−C-20 bond.

The NOE data of the skeleton of **6** showed the stereochemistry to be the same as that of **1**−**4**. NOEs from H-20 to H-23α and from H-22β to H-24 in the side chain gave evidence for the six-membered lactone ring existing in a chair conformation with H-20 and H-23α, along with H-22β and H-24, respectively, in coaxial arrangements. In addition, NOEs from H-20 to H-16 and H-18 and from H-22β to H-17 showed that H-18, H-20, and H-23α are positioned on the same side, opposite the side on which H-17, H-22β, and H-24 appear, establishing 20*R*- and 24*R*-configurations. In addition to NOEs from H-20 to H-16 and H-18, an NOE from H-22α to H-12α and the $J_{17,20}$ value (11.6 Hz) suggested that the dihedral angle for H-17/H-20 is approximately 170−180°, indicating that H-17 and H-20 are almost in an *anti* arrangement. On the basis of the preceding evidence, the stereochemistry and conformation of **6** were deduced.

Dubione B (**7**) was found to have the same molecular formula as **6** as determined from the molecular ion peak in the HREIMS. The general features of its IR and NMR spectral data closely resembled those of **6** except for the chemical shifts of the carbon signals corresponding to C-20−C-24 in the side chain (Table 4). Also, the protons of the triterpene skeleton of **7** (Table 3) showed the same NOE data as those of triterpene **6**. These facts suggested **7** to be a side chain stereoisomer of **6**. NOEs from H-20 to H-16, H-18, H-22α, and H-24 in the side chain established that the six-membered lactone ring in the side chain is in a boat conformation, with H-20 and H-24 in a co-pseudoaxial arrangement with the C-20 and C-24 chiral centers bearing *R*- and *S*-configurations, respectively. In addition to NOEs from H-20 to H-16 and H-18 the presence of NOEs from

Table 2. ^{1}H NMR Data (δ in $CDCl_3$) for Compounds **4** and **5**[a]

position	4	5
1α	1.44 (br td, 14.5, 3.9)	1.45 (br td, 14.6, 3.9)
1β	1.98 (ddd, 14.5, 5.5, 3.0)	1.98 (ddd, 14.6, 5.7, 3.0)
2α	2.25 (ddd, 14.5, 3.9, 3.0)	2.25 (ddd, 14.6, 3.9, 3.0)
2β	2.76 (td, 14.5, 5.5)	2.76 (td, 14.6, 5.7)
5	1.708 (dd,10.4, 7.0)	1.71 (dd, 10.4, 7.0)
6α	2.10 (ddd, 14.0, 7.0, 3.2)	2.11 (ddd, 14.0, 7.0, 3.2)
6β	2.09 (ddd, 14.0, 10.4, 3.2)	2.09 (ddd, 14.0, 10.4, 3.2)
7	5.31 (br q, 3.2)	5.29 (q, 3.2)
9	2.28 (m)	2.29 (m)
11α	1.60 (m)	1.598 (m)
11β	1.605 (m)	1.607 (m)
12α	1.59 (m)	1.604 (m)
12β	1.94 (br dd, 13.0, 10.0)	1.90 (br dd,13.0, 10.0)
15α	2.06 (br ddq, 13.5, 8.0, 0.9)	2.10 (br ddq 13.5, 8.0, 0.9)
15β	1.613 (br dd, 13.5, 0.9)	1.564 (br dd, 13.5, 0.9)
16	3.99 (br ddd,8.0, 5.9, 0.9)	4.04 (br ddd, 8.0, 5.9, 0.9)
17	2.07 (dd, 10.4, 5.9)	1.559 (dd, 9.8, 5.9)
18	0.84 (s)	0.82 (s)
19	1.02 (s)	1.02 (s)
20	2.60 (td, 10.4, 3.9)	1.612 (m)
21		1.054 (d, 7.0)
22α	1.78 (m)	1.67 (m)
22β	1.51 (dtd, 14.6, 10.4, 4.0)	0.98 (m)
23α	1.43 (m)	1.43 (m)
23β	1.52 (br dddd, 15.5, 10.4, 6.0, 4.0)	1.68 (m)
24	4.03 (br t, 6.0)	4.03 (br t, 6.0)
26A	4.86 (quint, 1.6)	4.85 (quint, 1.6)
26B	4.94 (dquint, 1.6, 0.8)	4.94 (dquint, 1.6, 0.8)
27	1.706 (dd, 1.6, 0.8)	1.73 (dd, 1.6, 0.8)
28	1.04 (s)	1.046 (s)
29	1.12 (s)	1.12 (s)
30	1.27 (br d, 0.9)	1.25 (br d, 0.9)
21-OMe	3.72	
16-OH	1.56 br s	1.55 (br s)
24-OH	1.56 br s	1.55 (br s)

[a] ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (J/Hz).

Table 3. ^{1}H NMR Data (δ in $CDCl_3$) for Compounds **6** and **7**[a]

position	6	7
1α	1.44 (br td, 14.4, 3.9)	1.45 (br td, 14.4, 3.9)
β	1.99 (ddd, 14.4, 5.5, 3.0)	1.99 (ddd, 14.4, 5.5, 3.0)
2α	2.24 (ddd, 14.4, 3.9, 3.0)	2.25 (ddd, 14.4, 3.9, 3.0)
2β	2.76 (td, 14.4, 5.5)	2.77 (td, 14.4, 5.5)
5	1.71 (dd, 10.8, 6.8)	1.72 (dd, 10.8, 6.8)
6α	2.11 (ddd, 14.0, 6.8, 3.2)	2.113 (ddd, 14.0, 6.8, 3.2)
6β	2.10 (ddd, 14.0, 10.8, 3.2)	2.107 (ddd, 14.0, 10.8, 3.2)
7	5.38 (br q, 3.2)	5.37 (br q, 3.2)
9	2.22 (m)	2.26 (m)
11α	1.58 (m)	1.57 (m)
11β	1.66 (m)	1.67 (m)
12α	1.59 (m)	1.56 (m)
12β	1.92 (br dd, 13.0)	1.90 (br dd, 13.0)
15α	2.09 (br dd, 13.3, 8.6)	2.06 (br dd, 13.3, 8.5)
15β	1.76 (br d, 13.3)	1.76 (br d, 13.3)
16	4.20 (br dddd, 8.6, 4.8, 1.6, 1.0)	4.01 (br dd, 8.6, 4.8)
17	2.04 (dd, 11.6, 4.8)	2.09 (dd, 10.8, 4.8)
18	0.89 (s)	0.91 (s)
19	1.03 (s)	1.03 (s)
20	2.56 (td, 11.6, 6.4)	2.68 (td, 10.8, 8.2)
22α	2.22 (ddt, 14.4, 6.4, 4.3)	2.27 (dddd, 14.0, 10.2, 8.2, 6.9)
22β	1.65 (dtd, 14.4, 11.6, 4.3)	1.57(dtd, 14.0, 10.8, 4.0)
23α	1.80 (br dddd, 14.4, 11.6, 10.0, 4.3)	2.00 (ddt, 14.4, 10.2, 4.0)
23β	2.05 (ddt, 14.4, 5.0, 4.3)	1.804 (dddd, 14.4, 11.7, 10.8, 6.9)
24	4.79 (br dd, 10.0, 5.0)	4.75 (br dd, 11.7, 4.0)
26A	4.97 (br qd, 2.0, 1.0)	4.99 (br quint, 1.2)
26B	5.04 (br septet, 1.0)	5.09 (br septet, 1.2)
27	1.79 (br s)	1.806 (br s)
28	1.05 (s)	1.05 (s)
29	1.13 (s)	1.13 (s)
30	1.31 (br d, 0.9)	1.33 (br d, 0.9)
16-OH	4.55 (br s)	4.81 (br s)

[a] ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (J/Hz).

H-22α to H-12α and H-18 and from H-22β to H-12β and H-17 suggested that the lactone ring exists in a conformation in which the protons showing NOEs are close together. The stereostructure and conformation of **7** were elucidated by these interpretations of the spectral data.

All of the triterpenes except for **6** were evaluated for cancer cell growth inhibition against the P388 lymphocytic leukemia cell line. As shown in Table 5, all the compounds tested except for triterpene **7** exhibited significant inhibition of cancer cell growth. Meliastatins 1 (**1**) and 2 (**2**) as well as euphanes **8** and **9** were evaluated against a minipanel (Table 5) of human cancer cell lines and were also found to exhibit significant cancer cell growth inhibitory effects.

Experimental Section

General Experimental Procedures. The IR spectra were recorded using a Perkin-Elmer FT-IR spectrometer 1720X. Optical rotations were obtained with a JASCO ORD/UV-5 spectropolarimeter. Unless otherwise noted, 1D and 2D NMR spectra were recorded at 27 °C employing a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, with TMS as an internal reference. EIMS and ESIMS were determined using a Hitachi M-4000H and an Applied Biosystems Mariner mass spectrometer, respectively. Silica gel (mesh 230−400) was used for liquid chromatography. HPLC was performed with a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm × 20 mm i.d.). Analytical TLC proceeded with precoated Merck aluminum sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) and the solvent CH_2Cl_2−CH_3 OH (19:1). The TLC plates were viewed under UV and sprayed with 10% H_2SO_4 followed by heating.

Melia dubia **Cav. and Solvent Partition Sequence.**[16] The stem bark of *M. dubia* (18.1 kg) was collected in 1974 in the Phillippines by U.S. Department of Agriculture (USDA) botanists. The tree was identified by USDA specialists, and a voucher specimen is maintained by the USDA in Beltsville, MD. Extraction was accomplished with CH_2Cl_2−CH_3OH (1:1, 114 L), and H_2O was added to the extract to give a CH_2Cl_2 phase (625 g). A part of the CH_2Cl_2 extract (290 g) was successively partitioned between CH_3OH−H_2O (4:1) and hexane, CH_3OH−H_2O (3:2) and CH_2Cl_2, and CH_3OH−H_2O (1:4) and *n*-BuOH. Removal of solvents gave the hexane (94 g, PS, ED_{50} 2.3 μg/mL), CH_2Cl_2 (110 g, PS, ED_{50} 13 μg/mL), and *n*-BuOH (4 g, PS, ED_{50} >100 μg/mL) fractions.

Isolation of Euphane Triterpenes 1−11. The hexane fraction (94 g) was passed through a Sephadex LH-20 column, using CH_3OH−CH_2Cl_2 (1:1) as eluent. The fourth (Fr. 1, 45 g) and sixth fractions (Fr. 2, 16.2 g) were separately rechromatographed on a Sephadex LH-20 column, using hexane−toluene−CH_3OH (3:1:1) as eluent, and a silica gel column with a gradient of CH_2Cl_2−CH_3OH as eluent, respectively. The fourth fraction (Fr. 3, 11.3 g) from the column chromatography of Fr. 1 was chromatographed on a silica gel column with a gradient of acetone−CH_2Cl_2 as eluent. The two fractions (Fr. 4, 0.55 g; Fr. 5, 2.1 g) eluted with acetone−CH_2Cl_2 (3:97) were collected. Fr. 5 was further chromatographed on a silica gel column with a gradient of CH_2Cl_2−CH_3OH as eluent and then finally separated by HPLC (ODS) using CH_3OH−H_2O (9:1) as eluent to give **8** (369 mg) and **2** (30 mg). Fr. 4 was further chromatographed on a silica gel column with a gradient of CH_2Cl_2−CH_3OH as eluent to give **3** (4.3 mg), **9** (4.5 mg), and **11**

Table 4. ^{13}C NMR Data (δ in $CDCl_3$) for Compounds **1**−**7**[a]

position	1	2	3	4	5	6	7
1	38.5 (s)	38.5 (s)	38.5 (s)	38.5 (s)	38.5 (s)	38.5 (s)	38.5 (s)
2	35.1 (s)	34.9 (s)	34.9 (s)	34.9 (s)	34.9 (s)	34.9 (s)	34.9 (s)
3	216.8 (q)	216.7 (q)	216.7 (q)	216.7 (q)	216.8 (q)	216.7 (q)	216.7 (q)
4	47.9 (q)	47.9 (q)	47.9 (q)	47.9 (q)	47.9 (q)	47.9 (q)	47.9 (q)
5	52.4 (t)	52.4 (t)	52.4 (t)	52.4 (t)	52.4 (t)	52.4 (t)	52.4 (t)
6	24.4 (s)	24.4 (s)	24.4 (s)	24.4 (s)	24.4 (s)	24.3 (s)	24.4 (s)
7	118.2 (t)	118.6 (t)	118.7 (t)	118.6 (t)	118.1 (t)	118.8 (t)	118.7 (t)
8	145.0 (q)	114.6 (q)	144.5 (q)	144.6 (q)	145.2 (q)	144.6 (q)	144.5 (q)
9	47.9 (t)	47.9 (t)	47.9 (t)	47.9 (t)	47.9 (t)	48.1 (t)	48.1 (t)
10	34.9 (q)	35.1 (q)	35.1 (q)	35.1 (q)	35.6 (q)	35.0 (q)	35.1 (q)
11	18.2 (s)	18.0 (s)	18.0 (s)	18.0 (s)	18.2 (s)	18.3 (s)	18.2 (s)
12	33.2 (s)	33.1 (s)	33.0 (s)	33.0 (s)	33.2 (s)	33.8 (s)	33.5 (s)
13	45.4 (q)	45.5 (q)	45.5 (q)	45.5 (q)	45.4 (q)	46.2 (q)	45.9 (q)
14	49.9 (q)	49.9 (q)	49.9 (q)	49.8 (q)	49.9 (q)	49.4 (q)	49.9 (q)
15	45.7 (s)	44.5 (s)	44.6 (s)	44.7 (s)	45.7 (s)	44.1 (s)	43.9 (s)
16	77.9 (t)	76.4 (t)	77.2 (t)	77.1 (t)	78.1 (t)	77.6 (t)	77.6 (t)
17	62.1 (t)	58.2 (t)	57.9 (t)	58.9 (t)	62.6 (t)	58.8 (t)	58.2 (t)
18	23.6 (p)	23.7 (p)	23.6 (p)	23.5 (p)	23.5 (p)	23.0 (p)	23.2 (p)
19	12.8 (p)	12.8 (p)	12.8 (p)	12.8 (p)	12.8 (p)	12.8 (p)	12.8 (p)
20	34.3 (t)	47.9 (t)	48.1 (t)	47.5 (t)	34.2 (t)	44.4 (t)	41.9 (t)
21	18.7 (p)	176.8 (q)	177.0 (q)	177.5 (q)	18.6 (p)	176.3 (q)	178.1 (q)
22	38.0 (s)	34.0 (s)	34.2 (s)	27.3 (s)	30.9 (s)	25.3 (s)	22.9 (s)
23	125.4 (t)	123.0 (t)	127.7 (t)	32.2 (s)	32.0 (s)	27.4 (s)	26.1 (s)
24	139.7 (t)	140.8 (t)	136.4 (t)	75.8 (t)	76.4 (t)	83.8 (t)	80.0 (t)
25	70.7 (q)	70.6 (q)	81.8 (q)	147.1 (q)	147.6 (q)	142.7 (q)	141.6 (q)
26	29.9 (p)	29.8 (p)	24.4 (p)	111.6 (s)	111.3 (s)	113.0 (s)	113.6 (s)
27	30.0 (p)	29.9 (p)	24.2 (p)	17.3 (p)	17.4 (p)	18.0 (p)	18.1 (p)
28	24.5 (p)	24.5 (p)	24.5 (p)	24.5 (p)	24.5 (p)	24.5 (p)	24.5 (p)
29	21.6 (p)	21.6 (p)	21.6 (p)	21.6 (p)	21.6 (p)	21.6 (p)	21.6 (p)
30	27.9 (p)	27.8 (p)	27.8 (p)	27.9 (p)	27.8 (p)	28.0 (p)	27.4 (p)
21-OMe		51.6 (p)	51.8 (p)	51.8 (p)			

[a] Letters p, s, t, and q, in parentheses, indicate, respectively, primary, secondary, tertiary, and quaternary carbons, assigned by DEPT.

Table 5. Cancer Cell Growth Inhibition Evaluation of Euphane Triterpenes **1**−**5** and **7**−**11** against the P388 Lymphocytic Leukemia Cell Line and a Minipanel of Human Cancer Cell Lines[a,c]

compound	P388 ED_{50} (μg/mL)	HCL GI_{50} (μg/mL)
1	2.5	1.9−3.4
2	5.6	6.3−8.6
3	3.1	
4	2.0	
5	1.7	
7	20.5	
8	5.1	3.7−9.1
9	4.6	3.6−5.7
10	2.5	
11	6.2	
5-FU[b]	0.075	

[a] DMSO was used as vehicle in the tests of all compounds. [b] 5-FU was used as standard. [c] Breast MCF-7, CNS SF268, colon KM20L2, lung-NSC NCI-H460, pancreas-a BXPC-3, and prostate DU-145.

(4.2 mg). The ninth fraction (Fr. 6, 0.7 g) from chromatography of Fr. 1 was chromatographed on a silica gel column with a gradient of acetone−CH_2Cl_2 as eluent. The fraction (46 mg) eluted with acetone−CH_2Cl_2 (3:97) was subjected to HPLC (ODS) using CH_3OH−H_2O (9:1) as eluent to afford **4** (7.4 mg), **6** (0.8 mg), and **7** (1.1 mg). Fr. 2 was repeatedly chromatographed on a silica gel column with a gradient of CH_2Cl_2−CH_3OH as eluent. The fraction (0.46 g) eluted with CH_3OH−CH_2Cl_2 (1:99) was separated by HPLC (ODS) using CH_3OH as eluent, affording **10** (6.8 mg), **5** (43.4 mg), and **1** (30 mg).

Meliastatin 1 (1): colorless needles; mp 70−75 °C (acetone); $[\alpha]_D$ −16.7° (*c* 0.36, CH_3OH); IR ν_{max} (KBr)/cm^{-1} 3419 (OH), 1704 (C═O), 1660 (C═C, shoulder), 1623 (C═C); EIMS *m/z* (rel int) 456 $[M]^+$ (1%), 438 $[M - H_2O]^+$ (22), 424 (32), 423 (100), 405 (6), 311 $[a - H_2O]^+$ (6), 297 (9), 109 $[M - a - H_2O]^+$ (92); HREIMS *m/z* 456.3636 $[M]^+$ (calcd for $C_{30}H_{48}O_3$, 456.3601). The ^1H and ^{13}C NMR data are listed in Tables 1 and 4.

Meliastatin 2 (2): colorless oil; $[\alpha]_D$ −29.3° (*c* 0.16, EtOH); IR ν_{max} (liquid)/cm^{-1} 3449 (OH), 1730 (COOR), 1710 (C═O), 1660 (C═C), 1623 (C═C); CIMS *m/z* (rel int) 501 $[M + H]^+$ (17%), 483 $[M + H - H_2O]^+$ (24), 468 (80), 467 (100), 451 (54), 435 (4), 400 (24), 367 (17), 313 (25), 297 (46), 272 (81); HRCIMS *m/z* 501.3596 $[M + H]^+$ (calcd for $C_{31}H_{49}O_5$, 501.3577). The ^1H and ^{13}C NMR data are summarized in Tables 1 and 4.

Meliastatin 3 (3): colorless needles; mp 176−178 °C; $[\alpha]_D$ −17.8° (*c* 0.49, EtOH); IR ν_{max} (KBr)/cm^{-1} 3449 (OH), 1730 (COOR), 1713 (C═O), 1660 (C═C), 1623 (C═C); EIMS *m/z* (rel int) 498 $[M - H_2O]^+$ (6%), 482 (15), 467 (100), 451 (53), 435 (52), 425 (17), 400 (16), 367 (19), 313 (21), 311 $[a - H_2O]^+$ (17), 297 (85), 272 (87); ESIMS *m/z* (rel int) 539.2 $[M + Na]^+$ (100%), 523.3 $[M + Na - O]^+$ (6); HRESIMS *m/z* 539.3388 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_6Na$, 539.3349). Refer to Tables 1 and 4 for the ^1H and ^{13}C NMR data.

Meliastatin 4 (4): colorless needles; mp 88−92 °C (CH_3OH); $[\alpha]_D$ −7.8° (*c* 2.60, CH_3OH); IRν_{max} (KBr)/cm^{-1} 3492 (OH), 1733 (COOR), 1704 (C═O), 1652 (C═C), 1632 (C═C); EIMS *m/z* (rel int) 500 $[M]^+$ (11%), 483 $[M - OH]^+$ (58), 469 $[M - OMe]^+$ (100), 451 (30), 329 $[a]^+$ (6), 313 (17), 311 $[a^+ - H_2O]^+$ (10), 272 (6), 140 (18); HREIMS *m/z* 500.3512 $[M]^+$ (calcd for $C_{31}H_{48}O_5$, 500.3500). The ^1H and ^{13}C NMR data are given in Tables 2 and 4.

Meliastatin 5 (5): colorless needles; mp 78−82 °C (CH_3OH); $[\alpha]_D$ −40.0° (*c* 1.00, CH_3OH); IR ν_{max} (KBr)/cm^{-1} 3480 (OH), 1703 (C═O), 1653 (C═C), 1623 (C═C); EIMS *m/z* (rel int) 456 $[M]^+$ (43%), 438 $[M - H_2O]^+$ (9)$^+$, 424 (32), 423 (100), 405 (46), 311 $[a - H_2O]^+$ (11), 297 (12), 109 $[M - a - H_2O]^+$ (88); HREIMS *m/z* 456.3594 $[M]^+$ (calcd for $C_{30}H_{48}O_3$, 456.3601). The ^1H and ^{13}C NMR data appear in Tables 2 and 4.

Dubione A (6): colorless oil; $[\alpha]_D$ −65.3° (*c* 0.05, EtOH); IR ν_{max} (liquid)/cm^{-1} 3492 (OH), 1733 (COOR), 1704 (C═O), 1652 (C═C), 1632 (C═C); EIMS *m/z* (rel int) 468 $[M]^+$ (3%), 453 (29), 435 (100), 329 $[a]^+$ (20), 313 (67), 311 $[a - H_2O]^+$ (12), 295 (25), 272 (18), 231 (8), 140 $[b + H]^+$ (51), 122 $[b + H - H_2O]^+$ (82); HREIMS *m/z* 468.3242 $[M]^+$ (calcd for $C_{30}H_{44}O_4$, 468.3237). Tables 3 and 4 summarize the ^1H and ^{13}C NMR data.

Dubione B (7): colorless oil; $[\alpha]_D$ −53.1° (*c* 0.04, EtOH); IR ν_{max} (liquid)/cm^{-1} 3452 (OH), 1730 (lactone), 1710 (C═O), 1667 (C═C), 1623 (C═C); EIMS *m/z* (rel int) 468 $[M]^+$ (4%), 453 (34), 435 (100), 329 $[a]^+$ (16), 313 (66), 311 $[a - H_2O]^+$ (9), 295 (22), 272 (16), 231 (7), 140 $[b + H]^+$ (42), 122 $[b + H - H_2O]^+$ (61), 95 (14); HREIMS *m/z* 468.3234 [M]$^+$ (calcd for $C_{30}H_{44}O_4$, 468.3237). See Tables 3 and 4 for the ^{1}H and ^{13}C NMR data.

Methyl kulonate (8): colorless needles; mp 103−105 °C (acetone); $[\alpha]_D$ −20.0° (*c* 6.30, CH_3OH); IR ν_{max} (KBr)/cm^{-1} 3544 (OH), 1724 (COOR), 1704 (C═O), 1662 (C═C); HREIMS *m/z* 484.3585 [M]$^+$ (calcd for $C_{31}H_{48}O_4$, 484.3550). The physicochemical and ^{1}H NMR data were in accord with the published values.[12]

Kulinone (9): colorless needles; mp 130−132 °C (CH_3OH); $[\alpha]_D$ −26.6° (*c* 7.10, CH_3OH); IR ν_{max} (KBr)/cm^{-1} 3530 (OH), 1700 (C═O), 1652 (C═C); HREIMS *m/z* 440.3658 [M]$^+$ (calcd for $C_{30}H_{48}O_2$, 440.3651). The physicochemical and ^{1}H NMR data coincided with reported values.[12]

16-Hydroxybutyrospermol (10): colorless needles; mp 128−129 °C (CH_3OH); $[\alpha]_D$ +24.3° (*c* 4.50, CH_3OH); IR ν_{max} (KBr)/cm^{-1} 3424 (OH), 1648 (C═C); HREIMS *m/z* 442.3804 [M]$^+$ (calcd for $C_{30}H_{50}O_2$, 442.3804). The physicochemical and ^{1}H NMR data were consistent with the recorded data.[12]

Kulactone (11): colorless needles; mp 158−160 °C (CH_3-OH), $[\alpha]_D$ −40.0° (*c* 4.40, CH_3OH); IR ν_{max} (KBr)/cm^{-1} 1791 (γ-lactone), 1710 (C═O), 1647 (C═C); HREIMS *m/z* 452.3295 [M]$^+$ (calcd for $C_{30}H_{44}O_3$, 452.3289). The physicochemical and ^{1}H NMR data agreed with the literature.[12]

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Supporting Information Available: Figures and tables of ^{1}H−^{1}H COSY, NOESY, and HMBC correlations are available for compounds **1**−**7**. This material may be obtained free of charge via the Internet at htpp://pubs.acs.org.

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