Quassinoids from *Eurycoma longifolia*

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Received August 6, 2009

Ten new structurally diverse quassinoids (**1**−**10**) and 14 known compounds were isolated from the stems of *Eurycoma longifolia*. The new compounds were two eurycomanone-type C_{20} quassinoids (**1**, **2**), one klaineanone-type C_{20} quassinoid (**3**), one C_{19} quassinoid (**4**) with a 1,2-*seco*-1-nor-6(5→10)-*abeo*-picrasan-2,5-olide skeleton, and six eurycomalactone-type C_{19} quassinoids (**5**−**10**). Compounds **5** and **6** both possessed a 3,4-epoxy group observed for the first time in eurycomalactones. Compound **1** had an α-oriented OH group at C-14 that had not been reported previously in eurycomanone-type quassinoids. All of the isolates were evaluated for cytotoxicity toward the highly metastatic HT-1080 human fibrosarcoma cell line, and compounds **11**, **23**, and **24** showed potent cytotoxicity (IC_{50} values 0.93−1.1 μM).

Eurycoma longifolia Jack (Simaroubaceae) is a shrub tree endemic to many countries of Southeast Asia. It is known locally as "Cay ba binh" in Vietnam, "pasak bumi" in Indonesia, and "tongkat ali" in Malaysia and is used as an aphrodisiac tonic, as an antimalarial drug, and as a health supplement.[1] The roots, stems, and bark of this plant are used as traditional medicines in different ways for alleviation of various illnesses. The plant is reported to possess anxiolytic properties,[2] to increase muscle strength,[3] and to have antiplasmodial[4] and cytotoxic properties.[5] As a part of our continued studies of medicinal plants from Southeast Asia, we screened some Vietnamese medicinal plants for their antiproliferative activity against a panel of six cancer cell lines.[6] Among the 77 plants screened, an extract of *E. longifolia* displayed cytotoxic activity against the highly metastatic HT-1080 human fibrosarcoma cell line (IC_{50} 15.8 μg/mL). Therefore, we carried out a detailed phytochemical investigation on this plant, which led to the isolation of 24 quassinoids including 10 new ones. We herein report structures of the new compounds together with the cytotoxicity of all of the isolates against the HT-1080 cell line.

Results and Discussion

Stems of *E. longifolia* were extracted with 70% ethanol under sonication. The 70% ethanol extract was fractionated into ethyl acetate-, *n*-butanol-, and water-soluble fractions. The EtOAc-soluble fraction showed the most potent cytotoxicity, with IC_{50} = 7.9 μg/mL against HT-1080 cells. Thus, the ethyl acetate-soluble fraction was separated by a combination of silica gel column chromatography (CC) and preparative TLC techniques to give 10 new quassinoids (**1**−**10**) together with 14 known ones: eurycomalactone (**11**),[7] 5,6-dehydroeurycomalactone (**12**),[7] 11-dehydroklaineanone (**13**),[7] 6α-hydroxyeurycomalactone (**14**),[8] 7α-hydroxyeurycomalactone (**15**),[9] laurycolactone A (**16**),[10] laurycolactone B (**17**),[10] 12-*epi*-11-dehydroklaineanone (**18**),[11] eurycomalide A (**19**),[12] eurycomalide B (**20**),[13] eurycolactone E (**21**),[14] longilactone (**22**),[15] 14,15β-dihydroxyklaineanone (**23**),[15] and 13,21-dihydroeurycomanone (**24**).[15]

Compound **1** showed a molecular-related ion at *m*/*z* 433.1466 in the HRFABMS, corresponding to the molecular formula $C_{20}H_{26}O_9$. The ^{1}H NMR spectrum of **1** (Table 1) showed signals due to an olefin (δ_H 6.11), four oxymethines (δ_H 5.08, 5.01, 4.46, 4.17), an oxymethylene (δ_H 4.80, 3.92), three methines (δ_H 3.79, 3.02, 2.90), a methylene (δ_H 2.28, 2.08), two tertiary methyls (δ_H 1.77, 1.48), a secondary methyl (δ_H 1.33), and three hydroxyl protons (δ_H 8.94, 8.29, 5.66). The ^{13}C NMR spectrum of **1** (Table 2) revealed 20 signals including those of a ketone carbonyl carbon (δ_C 197.4), a pair of olefinic carbons (δ_C 161.5, 125.9), a lactone carbonyl carbon (δ_C 172.3), a hemiacetal carbon (δ_C 110.4), and six oxygen-substituted carbons (δ_C 84.3, 81.3, 78.3, 73.8, 72.9, 71.4). These data closely resembled those of 13,21-dihydroeurycomanone (**24**), isolated from the same extract, except for the higher-field shift of H_3-21 (δ_H 1.33) and lower-field shift of H-13 (δ_H 3.02) compared to those in **24** (H_3-21: δ_H 1.84; H-13: δ_H 2.85). Thus, compound **1** was considered to be a stereoisomer of **24**, which was supported by the COSY, HMQC, HMBC, and COLOC data (Figure 1). The relative configuration of **1** was assigned on the basis of the NOESY correlations (Figure 1). Correlations of H-5 with H-1 and H-9 and of H_3-19 with H-6β and H-20 at δ_H 3.92 indicated that **1** has the same configuration as that of **24** on rings A and B, while the NOESY correlations of H-13 with H-12 and H-20 at δ_H 4.80 indicated that both H-12 and H-13 had β-orientations. Moreover, the NOESY correlation H_3-21/H-15 indicated H-15 to be α-equatorial. Finally, NOESY correlations of 14-OH with H-9 and H_3-21 indicated that the 14-OH group had α-axial orientation. Thus, compound **1** was concluded to be 14-*epi*-13,21-dihydroeurycomanone. To the best of our knowledge, this is the first naturally occurring quassinoid with a 14α-substituent.

Compound **2** had an ^{1}H NMR spectrum (Table 1) similar to that of 13,21-dihydroeurycomanone (**24**), except for the presence of two acetyl groups and the lower-field shifts of H-12 (δ_H 5.57) and H-15 (δ_H 6.69) compared to those in **24** (H-12: δ_H 4.13; H-15: δ_H 5.61). Thus, compound **2** was concluded to be 12,15-*O,O*-diacetyl-13,21-dihydroeurycomanone.

HREIMS of compound **3** indicated the molecular formula $C_{20}H_{28}O_9$, one oxygen atom more than that of 14,15β-dihydroxyklaineanone (**23**). The ^{1}H and ^{13}C NMR data (Tables 1 and 2), including analysis by COSY and HMQC spectra, were similar to those of **23** except for an oxygenated methine (δ_H 4.22, δ_C 66.4) instead of a methylene (δ_H 2.14, 2.22, δ_C 26.8) ascribed to C-6 in **23**. Therefore, **3** had an OH group at C-6, which was confirmed by the HMBC correlations. The 6-OH group was determined to be α-equatorial based on the NOESY correlations H_3-19/H-6 and H_3-20/H-6. Thus, compound **3** was concluded to be 6α,14,15β-trihydroxyklaineanone.

The ^{1}H and ^{13}C NMR spectra of compound **4** were similar to those of **24**, but the signals for C-1 to C-5 differed and the signal ascribable to C-1 was missing. Thus, **4** was considered to have a 1,2-*seco*-1-nor-6(5→10)-*abeo*-picrasan-2,5-olide skeleton similar to ailanquassin A and cedronolactone B, which were reported as constituents of *Simaba cedron* (Simaroubaceae).[16] This conclusion was supported by the COSY, HMQC, and HMBC spectra (Figure 2). The relative configuration (C-6 to C-20) was determined by

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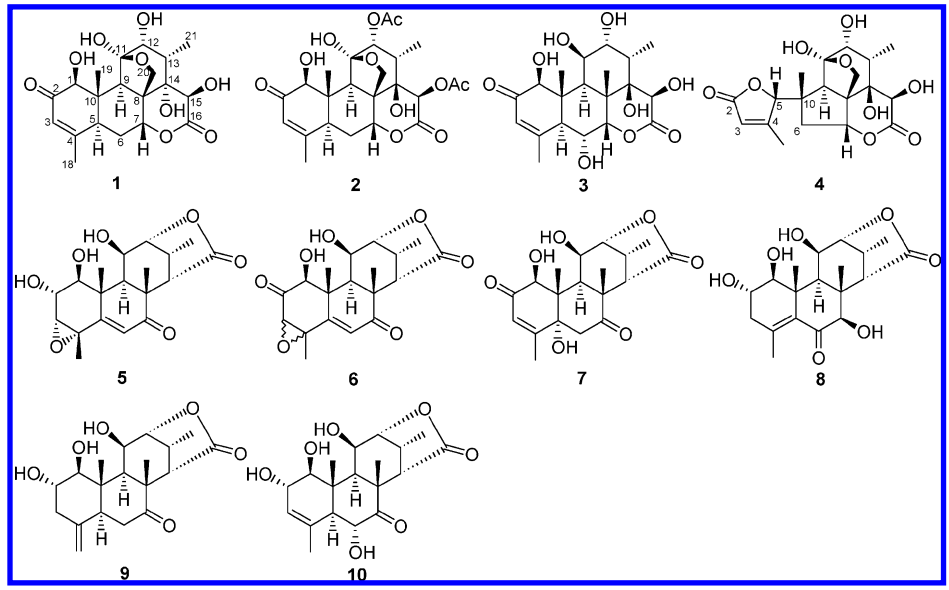
10.1021/np900486f CCC: $40.75
Published on Web 11/17/2009

Chart 1



the NOE difference experiment (Figure 2). On the other hand, the configuration at C-5 was determined by comparing the chemical shift values around C-5 with those of ailanquassin A and cedronolactone B. The ^{1}H NMR data of H_2-6 and H_3-18 in ailanquassin A (H_2-6: δ_H 2.93, 2.32; H_3-18: δ_H 2.49) and cedronolactone B (H_2-6: δ_H 2.54, 2.31; H_3-18: δ_H 2.05) had the characteristic difference.[16] The signals of H_2-6 (δ_H 3.03, 2.40) and H_3-18 (δ_H 2.51) of **4** had chemical shifts the same as those of ailanquassin A, but different from those of cedronolactone B. Thus, compound **4** was determined to have the same C-5 configuration as ailanquassin A.

Compound **5** had a molecular-related ion at *m/z* 387.1440 in the HRFABMS, corresponding to the molecular formula $C_{19}H_{24}O_7$. The ^{1}H NMR spectrum of **5** (Table 1) showed signals due to an olefinic (δ_H 6.25), five oxymethine (δ_H 5.25, 4.22, 3.95, 3.35, 3.22), three methine (δ_H 3.03, 2.88, 1.91), three tertiary methyl (δ_H 1.55, 1.53, 1.52), and a secondary methyl (δ_H 1.10). The ^{13}C NMR spectrum of **5** (Table 2) revealed 19 signals including those for a ketone carbonyl (δ_C 200.9), a pair of olefinic carbons (δ_C 164.5, 127.7), a γ-lactone carbonyl carbon (δ_C 179.1), and six oxygen-substituted carbons (δ_C 85.9, 79.1, 71.9, 70.0, 64.3, 59.3). These data closely resembled those of eurycomalide B (**20**), except for the higher-field shift of the signals of the tertiary methyl protons (**5**: δ_H 1.55; **20**: δ_H 1.90) and the signals due to an oxymethine (δ_H 3.35, δ_C 64.3) and an oxygenated quaternary carbon (δ_C 59.3) instead of those of an olefin (δ_H 5.96; δ_C 137.0, 132.7) in **20**. These data, together with the molecular formula $C_{19}H_{24}O_7$, suggested that **5** had an epoxide instead of an olefin at C-3(4) as in **20**. The COSY, HMQC, and HMBC spectra in $CDCl_3$ (Figure 3) indicated the epoxide ring to be at C-1(2), C-2(3), or C-3(4). In DMSO-d_6, COSY correlations of the OH protons at δ_H 5.29, 3.32, and 5.10 with H-1 (δ_H 2.98), H-2 (δ_H 3.78), and H-11 (δ_H 5.16), respectively, indicated C-1, C-2, and C-11 to have OH groups. Thus, the epoxide ring was concluded to be at C-3(4). NOESY correlations and coupling constants confirmed the relative configuration of **5** to be the same as that of **20** (Figure 3). Regarding the configuration of the epoxide ring, the small coupling constant (2.4 Hz) between H-2 and H-3 indicated them to be *cis*; that is, the epoxide ring has an α-orientation. Thus, compound **5** was determined to be 3α,4α-epoxyeurycomalide B, the first example of a eurycomalactone-type quassinoid with a 3,4-epoxy moiety.

The ^{1}H and ^{13}C NMR spectra of compound **6** (Tables 1 and 2) were similar to those of **5**, but they suggested the replacement of an oxymethine (δ_H 3.95, δ_C 71.9) in **5** by a ketone carbonyl carbon (δ_C 204.9). This was confirmed by the COSY, HMQC, and HMBC spectra. The relative configuration of **6**, except for the epoxide ring, was determined by the NOESY experiment and the coupling pattern in the ^{1}H NMR spectrum. The configuration of the epoxide ring, however, could not be determined due to the meager amount isolated. Thus, compound **6** was concluded to be a 3ξ,4ξ-epoxy-5,6-dehydroeurycomalactone.

The HREIMS of compound **7** indicated the molecular formula $C_{19}H_{24}O_7$. The ^{1}H and ^{13}C NMR spectra resembled those of eurycomalactone (**11**), except for the appearance of an oxygenated quaternary carbon (δ_C 77.9) instead of the methine (δ_H 2.86, δ_C 49.3) ascribed to C-5. Thus, compound **7** was considered to be a 5-OH derivative of **11**, which was confirmed by the COSY, HMQC, and HMBC spectra. The 5-OH group was determined to be α-axial, as H-6_{ax} showed NOE correlations with H_3-18, H_3-19, and H_3-20, while H-6_{eq} showed correlations only with H_3-18. Thus, compound **7** was determined to be 5α-hydroxyeurycomalactone.

The molecular formula of compound **8** was $C_{19}H_{26}O_7$ (HREIMS). The ^{1}H and ^{13}C NMR signals closely matched those of **11** and indicated the presence of a lactone carbonyl (δ_C 177.8), three tertiary methyls (δ_H 1.64, 1.66, 1.67; δ_C 20.2, 20.4, 20.5), and one secondary methyl (δ_H 1.06, δ_C 16.8). However, **8** lacked one of the ketone carbonyls and possessed five oxygenated carbons, instead of three as in **11**. Analysis of the COSY and HMQC spectra (Figure 4) led to the partial connectivities $C_1-C_2-C_3$ and $C_9-C_{11}-C_{12}-C_{13}(C_{21})-C_{14}$. The connectivities from C_9 to C_{14} were similar to those of **11**, suggesting **8** to have the same ring C system as in **11**. Therefore, **8** and **11** had different substituents on rings A and B. The connectivity between $C_1-C_2-C_3$ and the HMBC correlations of H_3-18 with C-3, C-4, and C-5 and of H_3-19 with C-1, C-5, and C-10 established the structure of ring A (Figure 4). The HMBC correlations of H_3-20 with C-7, C-8, and C-9 led to the construction of ring B having an OH at C-7. The remaining carbonyl carbon was thus assigned to C-6, resulting in the planar structure shown in Figure 4. The relative configuration was determined on the basis of the NOESY correlations and coupling constants. The correlation of H-7 with H-9 suggested that the 7-OH group is β-equatorial. Thus, compound **8** was concluded to be 5-dehydro-3-hydro-7β-hydroxy-6-oxoeurycolactone E.

The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of compound **9** resembled those of eurycolactone E (**21**) for rings B, C, and D. However, they differed in the presence of an *exo*-olefin (C-4: δ_C 145.2; C-18: δ_H 4.62, 4.92, δ_C 108.7) and a methylene (C-3: δ_H 2.34, 2.82, δ_C 43.62) instead of the *endo*-olefin (C-3: δ_H 5.75, δ_C 126.8; C-4: δ_C 133.7) and a methyl (C-18: δ_H 1.54, δ_C 20.4) in **21**. The *exo*-olefin was determined to be at C-4(18) by the HMBC

Table 1. ^{1}H NMR Data (δ) of Compounds **1**−**10**

position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[b]	**6**[c]	**7**[c]	**8**[a]	**9**[a]	**10**[c]
1	4.46 s	4.17 s	4.00 s		3.22 d (8.3)	4.49 d (2.9)	4.57 brs	3.87 d (9.5)	3.73 d (8.3)	3.62 d (6.6)
2					3.95 dd (8.3, 2.4)			4.17 m	4.14 ddd (12.2, 8.3, 5.9)	4.15 m
3	6.11 brs	6.19 brs	6.03 brs	5.96 brs	3.35 d (2.4)	3.64 s	6.04 d (1.5)	2.28 dd (18.0, 10.0)	2.34 dd (12.7, 12.2)	5.51 m
								2.53 dd (18.0, 6.1)	2.82 dd (12.7, 5.9)	
5	2.90 brd (15.1)	3.08 brd (12.5)	2.94 brd (11.2)	5.10 s					2.26 brd (13.7)	2.27 brd (12.0)
6α	2.08 ddd (15.1, 12.7, 4.2)	2.06 dd (14.9, 12.5)		3.03 d (16.1)	6.25 s	6.41 s	2.75 d (15.6)		2.42 dd (15.4, 3.7)	
6β	2.28 brd (12.7)	2.34 brd (14.9)	4.22 dd (11.2, 2.4)	2.40 dd (16.1, 4.6)			3.11 d (15.6)		3.13 dd (15.4, 13.7)	4.50 dd (12.0, 2.7)
7	5.08 d (4.2)	5.19 brs	4.44 d (2.4)	5.09 d (4.6)				4.79 s		
9	3.79 s	3.20 s	2.12 d (2.7)	3.57 s	1.91 d (3.4)	2.35 d (3.7)	3.02 d (3.4)	2.25 brs	2.05 d (3.4)	1.65 d (3.2)
11			5.10 dd (3.2, 2.7)		5.25 dd (5.1, 3.4)	4.81 dd (5.9, 4.9)	4.71 ddd (5.6, 4.6, 3.4)	5.28 m	5.34 ddd (5.4, 4.9, 3.4)	4.85 m
12	4.17 dd (4.4, 4.2)	5.57 d (4.2)	3.64 dd (3.2, 2.9)	4.15 dd (4.4, 4.1)	4.22 dd (5.1, 1.5)	4.35 d (4.9)	4.39 d (4.6)	4.63 d (4.9)	4.52 d (4.9)	4.34 dd (4.2, 1.4)
13	3.02 td (6.8, 4.2)	2.93 qd (7.3, 4.2)	2.37 qd (7.3, 2.9)	2.75 m	3.03 q (6.8)	2.91 q (7.1)	2.907 q (7.1)	3.21 q (6.8)	3.14 q (7.1)	2.91 q (6.8)
14					2.88 d (1.5)	2.99 brs	2.908 s	3.03 s	3.27 brs	2.92 d (1.4)
15	4.97 brs	6.69 s	5.45 s	5.56 br s						
18	1.77 s	1.83 brs	2.29 brs	2.51 br s	1.55 s	1.68 s	2.07 d (1.5)	1.64 s	4.62 brs	1.96 d (1.7)
									4.92 brs	
19	1.48 s	1.53 s	1.26 s	1.59 s	1.52 s	1.33 s	1.32 s	1.67 s	1.52 s	1.64 s
20	3.92 d (9.8)	4.05 d (9.8)	1.48 s	4.76 d (9.3)	1.53 s	1.52 s	1.58 s	1.66 s	1.70 s	1.43 s
	4.80 d (9.8)	4.55 d (9.8)		3.95 d (9.3)						
21	1.33 d (6.8)	1.28 d (7.3)	1.20 d (7.3)	1.77 d (7.3)	1.10 d (6.8)	1.17 d (7.1)	1.16 d (7.1)	1.06 d (6.8)	1.04 d (7.1)	1.17 d (6.8)
1-OH						4.14 d (2.9)	4.11 brs			
6-OH										3.98 d (2.7)
11-OH						2.65 d (5.9)	3.15 d (5.6)	5.98 d (5.4)	5.92 d (5.4)	3.28 d (5.4)
12-OH	8.29 d (4.4)			7.48 brs						
14-OH	5.66 s									
15-OH	8.94 d (4.6)			7.92 brs						
Ac		2.13[d] s								
Ac		2.29[d] s								

[a] Measured in pyridine-d_5. [b] Methanol-d_4. [c] Chloroform-d_1. [d] Signals may be interchanged.

Table 2. ^{13}C NMR Data (δ) of Compounds **1** and **3**−**9**

position	**1**[a]	**3**[b]	**4**[a]	**5**[b]	**6**[c]	**7**[c]	**8**[a]	**9**[a]
1	84.3	86.1		79.1	76.7	76.7	80.3	82.5
2	197.4	200.8	172.7	71.9	204.9	198.2	66.7	72.6
3	125.9	127.3	119.0	64.3	61.8	124.9	40.7	43.6
4	161.5	169.3	170.1	59.3	63.9	161.1	133.8	145.2
5	42.7	51.7	92.2	164.5	158.4	77.9	139.5	50.7
6	25.2	66.4	46.1	127.7	128.7	44.1	206.1	37.4
7	72.9	85.9	82.2	200.9	197.8	206.0	81.8	207.9
8	50.8	44.9	63.3	48.7	47.6	51.1	45.1	43.6
9	46.4	43.4	46.9	46.7	46.7	39.2	48.9	50.9
10	45.6	49.6	47.1	46.3	50.0	49.2	47.9	51.8
11	110.4	73.8	111.1	70.0	69.3	70.3	69.6	70.1
12	81.3	77.9	81.5	85.9	83.3	83.4	85.1	84.1
13	32.7	36.0	43.5	32.9	31.8	32.5	32.7	32.7
14	78.3	78.1	75.4	53.5	52.8	52.9	56.1	53.7
15	73.8	71.1	71.1	179.1	176.1	176.6	177.8	176.8
16	172.3	176.6	174.5					
18	22.5	26.2	16.2	21.6	20.5	19.8	20.2	108.7
19	9.4	13.0	18.4	15.1	17.2	16.4	20.5	23.6
20	71.4	17.5	68.7	23.3	23.0	22.7	20.4	12.8
21	10.3	13.2	13.1	16.9	16.5	16.7	16.8	16.8

[a] Measured in pyridine-d_5. [b] Methanol-d_4. [c] Chloroform-d_1.

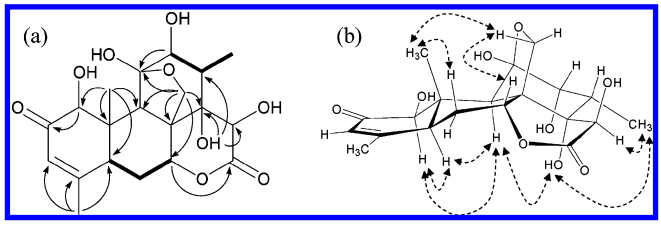


Figure 1. Selected HMBC and COLOC correlations (arrows), COSY connectivities (bold lines) (a), and key NOESY correlations (dotted arrows) (b) for **1**.

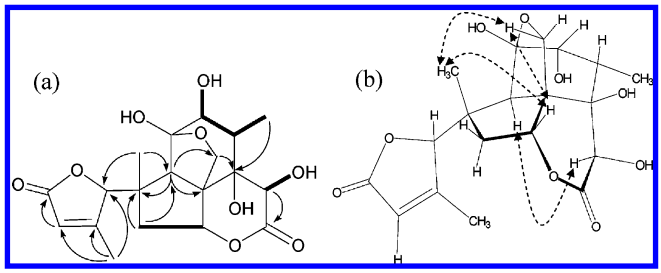


Figure 2. Selected HMBC correlations (arrows), COSY connectivities (bold lines) (a), and key NOE (dotted arrows) (b) for **4**.

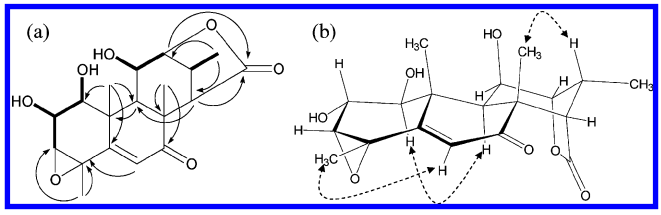


Figure 3. Selected HMBC correlations (arrows), COSY connectivities (bold lines) (a), and key NOESY correlations (dotted arrows) (b) for **5**.

correlations of H_2-18 with C-3 and C-5. Thus, compound **9** was concluded to be the Δ4(18)-isomer of eurycolactone E.

Compound **10** had the molecular formula $C_{19}H_{26}O_7$. The ^1H NMR spectrum of **10** resembled that of eurycolactone E (**21**) with a difference attributed to the presence of an oxygenated methine (δ_H 4.50) instead of the C-6 methylene (δ_H 2.69, 2.61) in **21**. Therefore, an additional OH group at C-6 was assumed, which was confirmed by the COSY spectrum. The OH group was determined to be α-equatorial on the basis of the NOESY correlations H_3-19/H-6

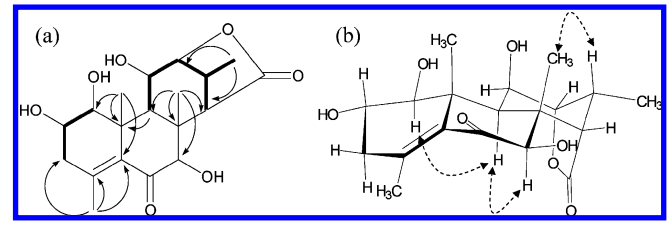


Figure 4. Selected HMBC correlations (arrows), COSY connectivities (bold lines) (a), and key NOESY correlations (dotted arrows) (b) for **8**.

Table 3. Cytotoxicity of *Eurycoma longifolia* Quassinoids toward HT-1080 Human Fibrosarcoma Cells

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	90	**14**	5.0
2	>100	**15**	6.2
3	90	**16**	>100
4	>100	**17**	>100
5	>100	**18**	>100
6	>100	**19**	90
7	8.8	**20**	39
8	>100	**21**	7.3
9	45	**22**	19
10	65	**23**	1.1
11	0.98	**24**	0.93
12	5.3	5-fluorouracil	5.2
13	7.5	doxorubicin	0.53

and H_3-20/H-6. Thus, compound **10** was concluded to be 6α-hydroxyeurycolactone E.

Absolute configurations of the isolated compounds were not determined, but structures are drawn with the 1*S* absolute configuration by biosynthetic analogy with known quassinoids.[17]

The isolated compounds were all tested for cytotoxic activity against the HT-1080 cell line[18] (Table 3). Clinically used anticancer agents, 5-fluorouracil[19] and doxorubicin,[19] were used as positive controls. In general, eurycomalactones having an α,β-unsaturated ketone carbonyl at C-2 were cytotoxic, and compounds **11**, **23**, and **24** exhibited the greatest cytotoxicity with IC_{50} values ranging from 0.93−1.1 μM, more than the positive control 5-fluorouracil (IC_{50}, 5.2 μM).

In conclusion, *E. longifolia* extract showed significant cytotoxicity against the metastatic human HT-1080 fibrosarcoma cell line. Investigation of the cytotoxic EtOAc fraction led to the isolation of 10 new quassinoids (**1**−**10**). Compound **1** represents a eurycomanone-type C_{20} quassinoid with an unusual 14α-OH group. Compounds **5** and **6** represent the first examples of eurycomalactone-type C_{19} quassinoids having a 3,4-epoxy functionality. Compounds **7**−**10** represent eurycomalactone-type C_{19} quassinoids. Compound **2** is a diacetoxy derivative of eurycomanone. Compound **3** represents a klaineanone-type C_{20} quassinoid, and **4** represents a C_{19} quassinoid with a 1,2-*seco*-1-nor-6(5→10)-*abeo*-picrasan-2,5-olide skeleton. Compounds **11**, **23**, and **24** were the most cytotoxic, of the compounds isolated, against the HT-1080 human fibrosarcoma cell line.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured in $CHCl_3$ with a Shimadzu IR-408 spectrophotometer. NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as δ values. MS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as matrix. Column chromatography (CC) was performed with silica gel 60N (spherical, neutral, 63-210 mesh; Kanto Chemical Co., Inc., Tokyo, Japan) and LiChroprep RP-18 (40−63 mesh; Merck, Darmstadt, Germany). MPLC was performed with an Ultra Pack and Pump560 (Yamazen Co., Ltd., Osaka, Japan).

Analytical and preparative TLC was carried out on precoated silica gel $60F_{254}$ and RP-$18F_{254}$ plates (0.25 or 0.50 mm thickness; Merck). 5-Fluorouracil was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), and doxorubicin hydrochloride was from Kyowa Hakko Co. Ltd. (Tokyo, Japan). α-Modified minimum essential medium (MEMα) was obtained from Wako Pure Chemicals Ind., Ltd. (Osaka, Japan). Sodium bicarbonate was purchased from Nacalai Tesque Inc. (Kyoto, Japan), and fetal bovine serum (FBS) was from Gibco BRL Products (Gaithersburg, MD). Antibiotic antimycotic solution and 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) were from Sigma-Aldrich Inc. (St. Louis, MO). Cell culture flasks and 96-well plates were from Corning Inc. (Corning, NY).

Plant Material. Stems of *Eurycoma longifolia* Jack were collected at Phu Quoc Island, Vietnam, in June 2007. The plant was identified by Dr. Hoang Viet (Department of Botany, University of Natural Sciences, National University-Hochminh City). A voucher specimen (TMPW 26525) was deposited at the Museum for Materia Medica, Institute of Natural Medicine, University of Toyama, Japan.

Extraction and Isolation. Air-dried stems of *E. longifolia* (2.5 kg) were cut into small pieces and extracted with 70% EtOH (20 L, room temperature, 1.5 h sonication, 3 times) to afford a 70% EtOH extract (133 g). The extract (120 g) was dissolved in water and partitioned with EtOAc and with *n*-BuOH, successively, to yield EtOAc-soluble (23.2 g), BuOH-soluble (15.5 g), and H_2O-soluble (70.9 g) fractions. The EtOAc-soluble fraction (23.2 g) was subjected to silica gel CC (55 mm × 300 mm) with a MeOH−CH_2Cl_2 gradient system [CH_2Cl_2 (4 L) → 0.5% MeOH−CH_2Cl_2 (2 L) → 1% MeOH (2 L) → 2% MeOH (2 L) → 4% MeOH (2 L) → 8% MeOH (2 L) → 16% MeOH (2 L) → 30% MeOH (2 L) → 100% MeOH (2 L); collection volume 500 mL] to yield 40 fractions.

A part of fraction 18 (766 mg), eluted with 2% MeOH−CH_2Cl_2, was subjected to MPLC on silica gel (37 mm × 300 mm) with an EtOAc−*n*-hexane gradient system (0:1 → 3:7 → 1:1 → 4:1 → 1:0; each 1 L) to yield 16 subfractions. Subfraction 8 (44.0 mg) was applied to normal-phase preparative TLC with EtOAc−CH_2Cl_2 (2:23) to yield 6α-hydroxyeurycomalactone (**14**, 2.8 mg). Subfraction 9 (66.6 mg) was purified by normal-phase preparative TLC with MeOH−CH_2Cl_2 (1:19), followed by MeOH−benzene (1:24) and EtOAc−CH_2Cl_2 (1:9), to yield **6** (1.0 mg) and 5,6-dehydroeurycomalactone (**12**, 0.9 mg).

Fraction 19 (667 mg), eluted with 2% MeOH−CH_2Cl_2, was further subjected to MPLC on silica gel (37 mm × 300 mm) with an EtOAc−*n*-hexane gradient system (0:1 → 1:4 → 3:7 → 1:1 → 7:3 → 1:0; each 1 L) to yield 14 subfractions. Subfraction 10 (128 mg) was applied to MPLC on silica gel (11 mm × 300 mm) with a MeOH−benzene gradient system (0:1 → 1:99 → 1:49 → 1:19 → 1:4; each 500 mL), followed by reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (1:1:3), to afford 7α-hydroxyeurycomalactone (**15**, 1.5 mg) and laurycolactone B (**17**, 1.9 mg). Subfraction 11 (110 mg) gave eurycomalactone (**11**, 54.0 mg) by reprecipitation from CH_2Cl_2.

Fraction 20 (1.20 g), eluted with 2% MeOH−CH_2Cl_2, was subjected to MPLC on silica gel (37 mm × 300 mm) with an EtOAc−*n*-hexane gradient system [0:1 (1 L) → 2:3 (500 mL) → 4:1 (1 L) → 1:0 (500 mL)], followed by MeOH−EtOAc (1:9 → 1:4; each 500 mL), to yield 13 subfractions. Subfraction 6 (209 mg) was further purified by MPLC on silica gel (11 mm × 300 mm) with an acetone−*n*-hexane gradient system (0:1 → 1:4 → 3:7 → 1:1; each 400 mL), followed by reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (2:2:1) and then normal-phase preparative TLC with acetone−toluene (1:4), to afford laurycolactone A (**16**, 2.3 mg).

Fractions 22 (670 mg) and 23 (1.10 g), eluted with 4% MeOH−CH_2Cl_2, were subjected to MPLC on silica gel (37 mm × 300 mm) with an acetone−CH_2Cl_2 gradient system (1:9 → 1:4 → 3:7; each 1 L) to yield 11 subfractions. Subfraction 2 was further purified by MPLC on silica gel (37 mm × 300 mm) with a MeOH−$CHCl_3$ gradient system (0:1 → 1:199 → 1:99 → 1:49 → 1:19 → 1:4; each 1 L), followed by reversed-phase preparative TLC with MeOH−H_2O (4:1) and then normal-phase preparative TLC with EtOAc−CH_2Cl_2 (3:2), to yield **7** (3.3 mg). Subfraction 3 (78.5 mg) was separated by successive reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (2:2:1) and with MeOH−CH_3CN−H_2O (1:1:3) to yield **2** (1.2 mg). Subfraction 7 (151 mg) was further purified by MPLC on silica gel (11 mm × 300 mm) with an acetone−CH_2Cl_2 gradient system (0:1 → 1:4 → 2:3; each 200 mL), followed by reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (2:2:1), to yield 11-dehydroklaineanone (**13**, 5.8 mg). Subfraction 9 (61.7 mg) was separated by successive reversed-phase preparative TLC with MeOH−H_2O (9:1) and with MeOH−H_2O (1:1) to afford 12-*epi*-11-dehydroklaineanone (**18**, 1.3 mg).

A part (592 mg) of combined fractions 24 and 25 (4% and 8% MeOH−CH_2Cl_2 eluate) was subjected to MPLC on silica gel (37 mm × 300 mm) with a MeOH−$CHCl_3$ gradient system (0:1 → 1:99 → 1:49 → 1:19 → 3:7; each 1 L) to yield 11 subfractions. Subfraction 5 (43.4 mg) was successively applied to reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (2:2:1), normal-phase preparative TLC with acetone−CH_2Cl_2 (1:4), and reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (1:1:3) to afford **8** (0.8 mg), **9** (2.3 mg), and longilactone (**22**, 2.4 mg). Subfraction 6 (220 mg) was further purified to MPLC on silica gel (11 mm × 300 mm) with a MeOH−$CHCl_3$ gradient system (0:1 → 1:99 → 1:49 → 1:19 → 3:7; each 200 mL), followed by reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (1:1:2), normal-phase preparative TLC with EtOAc−CH_2Cl_2 (3:2), and reversed-phase preparative TLC with MeOH−H_2O (1:1), to afford **10** (1.1 mg) and eurycolactone E (**21**, 28.5 mg). Subfraction 7 (30.8 mg) was applied to reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (1:1:2), followed by normal-phase preparative TLC with EtOAc−CH_2Cl_2 (3:2), to afford eurycomalide A (**19**, 5.3 mg). Subfraction 8 (51.6 mg) was successively subjected to reversed-phase preparative TLC with MeOH−CH_3CN−H_2O (1:1:2) and then normal-phase preparative TLC with EtOAc−CH_2Cl_2 (3:2) to yield eurycomalide B (**20**, 2.0 mg).

Fraction 26 (150 mg), eluted with 8% MeOH−CH_2Cl_2, was successively subjected to normal-phase preparative TLC with MeOH−CH_2Cl_2 (1:9), reversed-phase preparative TLC with MeOH−H_2O (1:1), and normal-phase preparative TLC with MeOH−benzene (3:17) to afford 14,15β-dihydroxyklaineanone (**23**, 23.9 mg).

Fraction 27 (282 mg), eluted with 8% MeOH−CH_2Cl_2, was subjected to reversed-phase preparative TLC with MeOH−H_2O (3:7), followed by normal-phase preparative TLC with MeOH−CH_2Cl_2 (1:9), to afford **1** (3.2 mg), **5** (1.8 mg), and 13,21-dihydroeurycomanone (**24**, 15.5 mg).

Fraction 28 (548 mg), eluted with 8% MeOH−CH_2Cl_2, was subjected to MPLC on silica gel (37 mm × 300 mm) with a MeOH−benzene gradient system (1:9 → 1:4) to yield seven subfractions. Subfraction 4 (68.5 mg) was further purified by MPLC on ODS silica gel (37 mm × 300 mm) with a MeOH−H_2O gradient system (0:1 → 3:7 → 2:3 → 1:1 → 3:2 → 7:3 → 4:1 → 9:1 → 1:0; each 500 mL), followed by normal-phase preparative TLC with MeOH−CH_2Cl_2 (13:87), to afford **3** (1.0 mg). Subfraction 5 (77.8 mg) was further purified by MPLC on ODS silica gel (26 mm × 300 mm) with a MeOH−H_2O gradient system (3:7 → 1:1 → 4:1 → 1:0; each 500 mL), followed by normal-phase preparative TLC with MeOH−CH_2Cl_2 (1:9), to afford **4** (4.1 mg).

Compound 1: colorless, amorphous solid; $[\alpha]^{25}_D$ +57 (*c* 0.05, MeOH); ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; FABMS *m/z* 411 $[M + H]^+$, 393 $[M + H - H_2O]^+$; HRFABMS *m/z* 433.1466 (calcd for $C_{20}H_{26}O_9Na$ $[M + Na]^+$, 433.1475).

Compound 2: colorless, amorphous solid; $[\alpha]^{22}_D$ −33 (*c* 0.06, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1735, 1678, 1598; ^{1}H NMR, Table 1; EIMS *m/z* 494 $[M]^+$, 476 $[M - H_2O]^+$, 435 $[M - Ac]^+$; HREIMS *m/z* 494.1801 (calcd for $C_{24}H_{30}O_{11}$ $[M]^+$, 494.1788).

Compound 3: colorless, amorphous solid; $[\alpha]^{22}_D$ +124 (*c* 0.05, MeOH); ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 412 $[M]^+$, 394 $[M - H_2O]^+$; HREIMS *m/z* 412.1748 (calcd for $C_{20}H_{28}O_9$ $[M]^+$, 412.1733).

Compound 4: colorless, amorphous solid; $[\alpha]^{22}_D$ +90 (*c* 0.2, MeOH); ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 396 $[M]^+$, 378 $[M - H_2O]^+$; HREIMS *m/z* 396.1414 (calcd for $C_{19}H_{24}O_9$ $[M]^+$, 396.1420).

Compound 5: colorless, amorphous solid; $[\alpha]^{21}_D$ +39 (*c* 0.1, MeOH); ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; FABMS *m/z* 365 $[M + H]^+$; HRFABMS *m/z* 387.1440 (calcd for $C_{19}H_{24}O_7Na$ $[M + Na]^+$, 387.1420).

Compound 6: colorless, amorphous solid; $[\alpha]^{22}_D$ +7 (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1775, 1715, 1680, 1600; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 362 $[M]^+$; HREIMS *m/z* 362.1353 (calcd for $C_{19}H_{22}O_7$ $[M]^+$, 362.1366).

Compound 7: colorless, amorphous solid; $[\alpha]^{22}_D$ +8 (*c* 0.08, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3450, 1770, 1715, 1670; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 364 $[M]^+$, 346 $[M - H_2O]^+$; HREIMS *m/z* 364.1533 (calcd for $C_{19}H_{24}O_7$ $[M]^+$, 364.1522).

Compound 8: colorless, amorphous solid; $[\alpha]^{22}_{D}$ +120 (*c* 0.04, MeOH); ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 366 $[M]^+$, 348 $[M - H_2O]^+$; HREIMS *m/z* 366.1647 (calcd for $C_{19}H_{26}O_7$ $[M]^+$, 366.1679).

Compound 9: colorless, amorphous solid; $[\alpha]^{22}_{D}$ +31 (*c* 0.08, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3350, 1773, 1709, 1589; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m/z* 350 $[M]^+$, 332 $[M - H_2O]^+$; HREIMS *m/z* 350.1724 (calcd for $C_{19}H_{26}O_6$ $[M]^+$, 350.1729).

Compound 10: colorless, amorphous solid; $[\alpha]^{22}_{D}$ +27 (*c* 0.06, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1776, 1710, 1597; ^{1}H NMR, Table 1; EIMS *m/z* 366 $[M]^+$, 348 $[M - H_2O]^+$; HREIMS *m/z* 366.1653 (calcd for $C_{19}H_{26}O_7$ $[M]^+$, 366.1679).

Cell Line. Highly metastatic HT-1080 human fibrosarcoma cells (ATCC#CCL-121) were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in MEMα that was supplemented with 10% FBS, 0.1% $NaHCO_3$, and 1% antibiotic antimycotic solution.

Cytotoxic Assay. Cell viability in the presence or absence of test compounds was determined using the standard MTT assay[20] as described previously.[21] In brief, exponentially growing cells were harvested and plated in 96-well plates (2×10^3 cells/well). After 24 h incubation at 37 °C under a humidified 5% CO_2 atmosphere to allow cell attachment, the cells were treated with varying concentrations of test compounds in their respective medium (100 μL). After 72 h incubation, 100 μL of MTT solution (0.5 mg/mL) was added to the wells. After 2 h incubation, the formazan formed was extracted with DMSO, and its amount was measured spectrophotometrically at 550 nm with a Perkin-Elmer HTS-7000 bioassay reader (Norwalk, CT). Cell viability and IC_{50} values were calculated from the mean values of data from three wells. Cellular viability (%) = $[\{Abs_{(test\ sample)} - Abs_{(blank)}\}/\{Abs_{(control)} - Abs_{(blank)}\}] \times 100$.

Acknowledgment. We are thankful to Dr. Quan Le Tran, National University-Hochiminh City, Hochiminh City, Vietnam, for providing the stems of *E. longifolia*. This work was supported in part by a Grant-in-Aid for International Scientific Research (No. 16406002) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR (except for **2** and **10**) spectra for **1**−**10**, COSY spectrum for **2** and **10**, and the structures for **11**−**24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP900486F