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New Quassinoids from the Roots of *Eurycoma longifolia*

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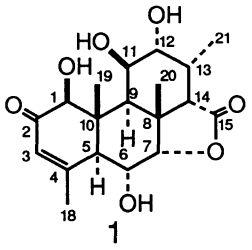
A new C_{19}-skeleton quassinoid, named longilactone and three new quassinoids, 13,21-dihydroeurycomanone, 13β,21-dihydroxyeurycomanone and 14,15β-dihydroxyklaineanone were isolated from the roots of *Eurycoma longifolia* and their structures were established by the spectral evidences.

From *Eurycoma longifolia* Jack (Simaroubaceae) that is one of famous folk medicines in the Southeast Asia, C_{20}-quassinoids; eurycomanone,[1] eurycomanol,[1] eurycomanol-2-*O*-β-D-glycopyranoside,[2] C_{19}-; eurycomalactone whose structure was revised to form a lactonic linkage at not C-7 but C-12,[3] C_{18}-; laurycolactones A and B[3] were so far isolated and some of them exhibited *in vitro* antimalarial activities.[4] Further investigation of the *n*-butanol extract resulted in isolation of new cytotoxic quassinoids, named longilactone (**1**) which is a new C_{19}-type quassinoid to form a lactonic linkage at C-7, 13,21-dihydroeurycomanone (**2**), 13β,21-dihydroxyeurycomanone (**3**) and 14,15β-dihydroxyklaineanone (**4**) along with known eurycomanone (**5**) and eurycomanol (**6**). In this communication, the structure elucidation of them and cytotoxic activities are described.

The roots of *E. longifolia* collected in Indonesia were extracted with 50% methanol, and chromatographic purification of *n*-butanol soluble fraction furnished compounds **1**-**6**.

Longilactone (**1**)[5] was suggested to be C_{19} quassinoid by ^{13}C-NMR spectrum and the molecular formula, $C_{19}H_{26}O_7$ was confirmed by high resolution MS spectrum (366.1675). The IR and UV spectra showed the presence of an α,β-unsaturated ketone (1656 cm^{-1}; 242 nm, ε=10500) and a butanolide (1769 cm^{-1}) like eurycomalactone (**7**).[3] Detailed decoupling experiments based on ^1H-^1H and ^1H-^{13}C COSY spectra in 500MHz NMR revealed us the complete assignments and coupling network as listed in Tables 1 and 2. Compared with the NMR data of **7**, the signal of H-12 (δ3.60) and also the signal of the corresponding carbon (δ76.2) showed high field shift. Furthermore, the ^1H-^1H coupling constants around C-ring were different between **1** and **7**, for example, the coupling constant (1.0Hz) between H-12 and H-14 and negligible coupling constant (ca.0Hz) between H-12 and H-13 characteristic of **7** were not observed in **1**. On the other hand, the appearence of oxygen-substituted functions at C-6 and C-7 was secured by the coupling sequence from H-5. Then, judging by the chemical shift (δ66.0 and 88.1) of these carbons and the presence of ^1H-^{13}C long rang coupling between H-7 and C-15, it turned out to substitute a hydroxyl group at C-6 and a lactonic linkage at C-7. The above all data are consistent with the proposed structure with a lactonic linkage between C-7 and C-15.

The stereostructure was corroborated by difference NOE experiments in ^{1}H-NMR. Irradiation of H-20 caused increase in the integrated intensity of H-19, H-6, H-7, H-13,and H-14, respectively. Then, NOE among H-1, H-5,and H-9 was observed as shown in Figure. Further, from the coupling constants, the structure was also explained satisfactorily.



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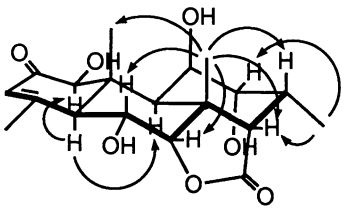
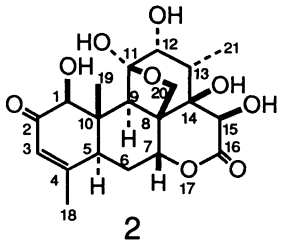


NOE enhancements

Compounds **2**[6] and **3**[7] were obtained as colorless needles, respectively and were presumed to be C_{20} quassinoids by ^{13}C-NMR spectrum. The molecular formulae of **2** and **3** were established to be $C_{20}H_{26}O_9$ (410.1582) and $C_{20}H_{26}O_{11}$ by high resolution MS and FAB MS spectra and the structures of them were considered to be closely related structures to **5** by spectral data. The IR and UV spectra indicated the presence of an α,β-unsaturated ketone (**2**; 1680 cm^{-1} and 235 nm, **3**; 1672 cm^{-1} and 240 nm) and a lactone (**2**; 1740 cm^{-1}, **3**; 1728 cm^{-1}). In ^{1}H-NMR spectrum, the signals of a doublet methyl (δ1.85) and a methine (δ2.84) coupling with the methyl were newly appeared in **2** in place of those ascribable to exomethylene in **5**. The carbon peaks (δ44.2 and 14.0) corresponding to this unit were also present. In the case of **3**, the presence of 1,2-glycol at C-13 and C-21 was implied by the carbon signals at δ77.9 and 66.8. The data described above led us to elucidate **2** and **3** as shown in Figure. The stereostructure was proved by difference NOE experiments, the integrated intensity between H-13 and H-20 in **2** and among H-21, H-12,and H-15 in **3** were increased.

Compound **4**[8] was isolated as colorless needles, mp 134-135°C, $C_{20}H_{28}O_8$ (378.1638, M^+-H_2O). The NMR data of **4** suggested that the acetal linkage between C-20 and C-11 in **2** is not present. Then, configurations of hydroxyl groups at C-11, 12, and 15 were determined by the ^{1}H-coupling constants similar to those of **1** and NOE that observed between H-20 and H-13, H-21 and H-12, and then H-9 and H-15. Absolute structures of **2**, **3** and **4** were determined to be same as **5** because of the appearence of analogous cotton curve[6-8] to **5**.

Compounds **5**[9] and **6**[10] were identical with eurycomanone and eurycomanol, respectively by comparison with various spectral data.



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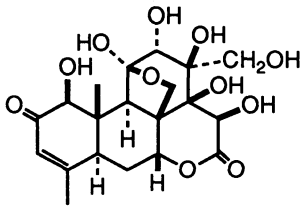

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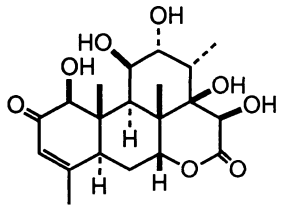

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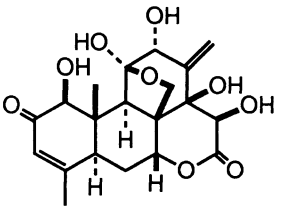

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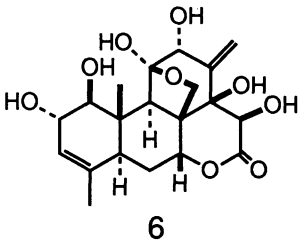

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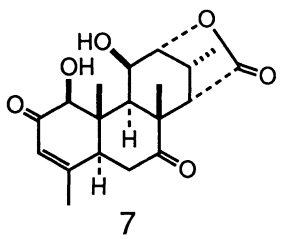

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Table 1. ^{1}H-NMR spectral data for **1-7** (500 MHz)

	1[a]	2[b]	3[b]	4[a]	5[b]	6[b]	7[c]	
H-1	3.99,s	4.39,s	4.45,s	4.08,s	4.52,s	4.04,d	4.05,brs	Coupling constants in Hz
H-2	---	---	---	---	---	4.63,m	---	
H-3	6.05,brs	6.13,brs	6.13,brs	6.04,brs	6.16,brs	5.80,brs	6.11,brs	**1**: $J_{5,6}$=11.0; $J_{6,7}$=3.9; $J_{9,11}$=2.0; $J_{11,12}$=3.0;
H-5	2.75,brd	3.17,brd	3.19,brd	2.92,brd	3.26,brd	2.82,brd	2.80,m	$J_{12,13}$=3.0; $J_{13,14}$=3.0; $J_{13,21}$=7.0
H-6e	---	2.27,dt	2.27,dt	2.22,dt	2.34,dt	2.19,dt	2.65,dd	**2**: $J_{5,6e}$=2.5; $J_{5,6a}$=14.0; $J_{6e,6a}$=14.0; $J_{6e,7}$=
H-6a	4.21,dd	2.03,td	2.07,td	2.14,td	2.03,td	1.93,td	2.75,dd	2.5; $J_{6a,7}$=2.5; $J_{12,13}$=4.1; $J_{13,21}$=7.3;
H-7	4.12,d	5.21,t	5.12,t	4.52,t	5.26,t	5.16,t	---	$J_{20,20}$=9.0
H-9	2.05,d	3.51,s	3.59,s	2.16,d	3.82,s	3.53,s	1.86,d	**3**: $J_{5,6e}$=2.5; $J_{5,6a}$=14.0; $J_{6e,6a}$=14.0; $J_{6e,7}$=
H-11	4.95,dd	---	---	5.02,t	---	---	4.78,brdd	2.5; $J_{6a,7}$=2.5; $J_{12,OH}$=4.6; $J_{21,21}$=11.6;
H-12	3.60,t	4.14,d	4.58,d	3.68,t	4.80,s	4.78,s	4.38,brd	$J_{20,20}$=8.8
H-13	2.35,m	2.84,m	---	2.36,qd	---	---	2.80,m	**4**: $J_{5,6e}$=3.0; $J_{5,6a}$=14.7; $J_{6e,6a}$=14.7; $J_{6e,7}$=
H-14	2.34,d	---	---	---	---	---	2.88,d	3.0; $J_{6a,7}$=3.0; $J_{9,11}$=3.0; $J_{11,12}$=3.0;
H-15	---	5.62,s	5.57,s	5.41,s	5.67,s	5.49,s	---	$J_{12,13}$=3.0; $J_{13,21}$=7.3
H-18	2.28,brs	1.77,brs	1.78,brs	1.99,brs	1.80,brs	1.64,brs	1.95,brs	**5**: $J_{5,6e}$=2.7; $J_{5,6a}$=14.0; $J_{6e,6a}$=14.0; $J_{6e,7}$=
H-19	1.09,s	1.62,s	1.65,s	1.21,s	1.62,s	1.74,s	1.26,s	2.7; $J_{6a,7}$=2.7; $J_{21,21}$=1.4; $J_{20,20}$=8.6
H-20	1.57,s	4.04,d	3.97,d	1.50,s	4.01,d	4.05,d	1.56,s	**6**: $J_{1,2}$=7.8; $J_{5,6e}$=2.5; $J_{5,6a}$=14.0; $J_{6e,6a}$=14.0
		4.64,d	5.20,d		4.54,d	4.55,d		$J_{6e,7}$=2.5; $J_{6a,7}$=2.5; $J_{21,21}$=1.5; $J_{20,20}$=8.5
H-21	1.38,d	1.85,d	4.61,d	1.23,d	5.66,d	5.63,d	1.16,d	**7**: $J_{5,6e}$=4.0; $J_{5,6a}$=13.5; $J_{6e,6a}$=15.5; $J_{9,11}$=
			5.01,d		6.12,d	6.09,d		3.5; $J_{11,12}$=5.0; $J_{12,13}$=ca. 0.0; $J_{12,14}$=1.0;
OH-12			7.86,d					$J_{13,21}$=7.0

a) In CD_3OD b) in pyridine-d_5 c) in $CDCl_3$, cited from Ref. 3.

Table 2. ^{13}C-NMR spectral data for **1-7** (100 MHz)

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12
1[a]	83.6	201.2	127.4	167.7	51.6	66.0	88.1	44.2	42.9	50.9	73.7	76.2
2[b]	84.6	197.5	126.1	162.7	42.2	25.8	71.9	52.7	47.0	45.7	110.3	79.7
3[b]	84.7	197.5	126.1	162.4	42.3	25.7	70.6	53.5	47.4	45.5	110.0	79.6
4[b]	84.8	199.4	124.8	165.0	43.4	26.3	71.2	49.2	44.7	44.1	74.3	80.9
5[b]	84.6	197.4	126.1	162.5	42.2	25.7	71.8	52.6	47.7	45.9	109.6	81.0
6[b]	83.8	72.7	126.9	135.0	41.6	25.6	71.9	52.7	47.9	42.3	109.7	81.1
7[c]	81.3	197.4	124.5	162.2	49.5	36.3	205.5	47.0	53.0	51.2	69.9	83.2

	C-13	C-14	C-15	C-16	C-18	C-19	C-20	C-21	
1	28.2	57.1	178.9	---	21.3	12.7	25.3	14.8	a) in CD_3OD
2	42.2	76.6	75.1	174.7	22.4	10.6	67.3	14.0	
3	77.9	78.3	74.9	173.5	22.4	11.0	67.6	66.8	b) in Pyridine-d_5
4	35.7	77.5	78.0	175.9	18.2	12.0	22.4	14.2	
5	148.0	79.4	75.9	173.8	22.4	10.4	67.7	119.3	c) in $CDCl_3$, cited from Ref. 3
6	148.5	79.4	76.5	173.8	21.2	10.9	67.8	119.3	
7	32.4	49.2	176.2	---	22.0	12.2	23.7	16.7	

Compounds **1-6** exhibited cytotoxic activities against KB and P-388 cells.[11] It seems that saturation at C-13 and C-21 such as **1**, **2**,and **4** may be structural requirement for potent activity.

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5) Longilactone (**1**): mp 130-132 °C, $[\alpha]_D$ +92.6° (c 0.19, MeOH). CD (EtOH): $[\theta]_{240}$ +22300.
6) 13,21-dihydroeurycomanone (**2**): mp 219-221 °C, $[\alpha]_D$ +46.9° (c 0.11, MeOH). CD (EtOH): $[\theta]_{240}$ -9400, $[\theta]_{322}$ +1970.
7) 13β,21-dihydroxyeurycomanone (**3**): mp 229-230 °C, $[\alpha]_D$ +17.5° (c 0.16, pyridine). CD (EtOH): $[\theta]_{245}$ -12900, $[\theta]_{320}$ +1840.
8) 14,15β-dihydroxyklaineanone (**4**): mp 134-135 °C, $[\alpha]_D$ +53.8° (c 0.29, MeOH). UV (EtOH): 240 nm (ε 8510). CD (EtOH): $[\theta]_{247}$ -9900, $[\theta]_{315}$ +2150.
9) Eurycomanone (**5**): mp 242-243 °C, $[\alpha]_D$ +34.2° (c 0.32, pyridine).
10) Eurycomanol (**6**): mp 258-260 °C, $[\alpha]_D$ +85.6° (c 0.31, pyridine).
11) Cytotoxic activities against KB and P-388 cells (IC_{50} μg/ml)

Compound	**1**	**2**	**3**	**4**	**5**	**6**
KB Cells	3.4	0.33	20	0.38	0.40	3.6
P-388 Cells	1.3	1.2	10	0.29	10	58

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