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Quassinoids from *Eurycoma longifolia*

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Abstract

Three quassinoids, eurycolactone D (**1**), eurycolactone E (**2**) and eurycolactone F (**3**) were isolated from the roots of *Eurycoma longifolia* Jack. The structures of **1**–**3** were elucidated by spectroscopic methods, and that of **3** was further confirmed by X-ray crystallography. The known quassinoids, laurycolactone B (**4**) and eurycomalactone (**5**) were also identified. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Eurycoma longifolia* Jack; Simaroubaceace; Bitter principles; Spectroscopy; X-ray crystallography; Quassinoids; Eurycolactone D; Eurycolactone E; Eurycolactone F

1. Introduction

Eurycoma longifolia Jack, an indigenous plant to Southeast Asia, is a tall slender shrub tree commonly found as an understory in the lowland forests at up to 500 m above sea level (Goh et al., 1995). Over the years, phytochemical studies carried out on the roots of this plant showed that among others, it possesses bitter constituents collectively known as quassinoids (Thoi and Suong, 1970; Suong et al., 1982; Darise et al., 1982, 1983; Bates et al., 1984; Chan et al., 1986, 1989, 1991, 1992; Morita et al., 1990; Kardono et al., 1991; Tada et al., 1991; Itokawa et al., 1992, 1993; Ang et al., 1995a,b). The wide spectrum of pharmacological activities viz. antimalarial (Chan et al., 1986, 1989; Kardono et al., 1991; Ang et al., 1995a, b), antiulcer (Tada et al., 1991), antipyretic (Chan et al., 1995) and cytotoxic to cancer cells (Morita et al., 1990; Kardono et al., 1991; Itokawa et al., 1992) associated with these quassinoids has further prompted us to continue on with our investigation for new quassinoids from this plant (Ang et al., 2000).

Hence, we wish to report on the isolation and characterisation of the C_{18}-quassinoid, eurycolactone D (**1**) and the two C_{19}-quassinoids, eurycolactone E (**2**) and eurycolactone F (**3**) along with the two known quassinoids, laurycolactone B (**4**) (Suong et al., 1982) and eurycomalactone (**5**) (Suong et al., 1982). The structures of these quassinoids were elucidated based on analysis of spectral evidence.

2. Results and discussion

Eurycoma longifolia Jack roots were procured from Penang Island, Malaysia and were extracted with MeOH. The MeOH extract was partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$-soluble portion was subjected to CC over silica gel and subsequently Diaion HP-20 resin. Repeated purifications using prep. HPLC with a RP column employing various mobile phases led to the isolation of the C_{18}-quassinoid, eurycolactone D (**1**) and the two C_{19}-quassinoids, eurycolactone E (**2**) and eurycolactone F (**3**) along with two known quassinoids, laurycolactone B (**4**) and eurycomalactone (**5**).

Eurycolactone D (**1**) was obtained as colourless prisms. Its molecular formula, $C_{18}H_{22}O_5$, was determined by HRFABMS and was two hydrogen atoms more than that of **4** which possessed a molecular formula of $C_{18}H_{20}O_5$. Comparison of the NMR spectra

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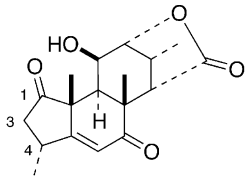
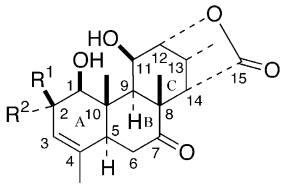
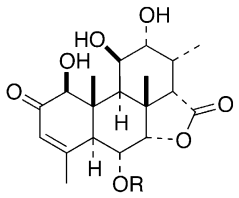


1: Eurycolactone D
4: Laurycolactone B ($\Delta^{3,4}$)



2: Eurycolactone E (R^1 = H, R^2 = OH)
5: Eurycomalactone (R^1, R^2 = O)



3: Eurycolactone F (R = Ac)
6: Longilactone (R = H)

(Tables 1 and 2) with those of **4** revealed that **1** had the same B and C rings as in **4** and that, accordingly, the structural differences between these two quassinoids resided only in the A ring. The most notable differences observed in the ^{13}C NMR spectrum were that C-3 and C-4 resonances of **1** were observed as a methylene (δ 45.1) and a methine (δ 32.6) carbon, respectively. Thus, **1** appeared to be a 3,4-dihydro form of **4**. The stereochemistry of 4-Me was determined to be in an α-configuration by observing a NOESY correlation between 10-Me and 4-H (Fig. 1). The NOESY correlations between 8-Me and 13-H, 11-OH and 13-H, and among 8-Me, 10-Me and 11-OH showed that these groups and hydrogen were all in β-configurations. From these findings, structure **1** was deduced for eurycolactone D.

Eurycolactone E (**2**) was obtained as colourless needles. The molecular formula was established as $C_{19}H_{26}O_6$ by HRFABMS. The similarity of the NMR spectra between **2** and **5** suggested that these compounds possessed the same B and C ring structures. Observation of the C-2 resonance at δ 74.2 as a methine carbon, the 2-H resonance correlated to 1-H, 3-H, 5-H and 4-Me in the ^1H–^1H COSY spectrum, and the absence of an UV absorption of a conjugated ketone suggested that **2** is a C-2 hydroxyl analogue of **5**.

The stereochemistry of this compound was confirmed by NOESY experiments (Fig. 2). NOESY correlations were observed among 1-H, 5-H and 9-H, among 6-H_β, 8-Me and 10-Me, among 8-Me, 10-Me and 11-OH, among 8-Me, 11-OH and 13-H, and between 1-OH and 10-Me. These data showed that the A/B and B/C ring junctures were both in a *trans* relationship, and that the configuration of the 1-OH and 11-OH groups were both in β-configurations. By contrast, the stereochemistry of the 2-OH group was assigned to an α-configuration by observing the NOESY correlation between 2-H and 10-

Table 1
^1H NMR chemical shifts assignments for eurycolactones D–F (**1**–**3**) in C_5D_5N[a]

Position	1	2	3
1		4.04 (*m*)	4.25 (*d*, 2.5)
2		4.65 (*br m*)	
3 (α)	2.81 (*dd*, 18.8, 9.7)	5.74 (*m*)	6.20 (*m*)
(β)	1.82 (*dd*, 18.8, 10.5)		
4	3.07 (*m*)		
5		2.48 (*br d*, 13.9)	3.23 (*d*, 12.0)
6 (α)	5.98 (*d*, 2.1)	2.61 (*dd*, 15.4, 3.9)	5.74 (*dd*, 12.0,3.8)
(β)		2.91 (*t*, 15.4)	
7			4.55 (*d*, 3.8)
9	2.48 (*d*, 3.4)	2.10 (*d*, 3.4)	2.79 (*br s*)
11	5.59 (*m*)	5.54 (*m*)	5.79 (*m*)
12	4.47 (*dd*, 4.9, 1.0)	4.49 (*dd*, 4.8, 1.0)	4.14 (*m*)
13	3.33 (*q*, 7.0)	3.14 (*q*, 6.9)	2.77 (*m*)
14	3.35 (*d*, 1.0)	3.28 (*d*, 1.0)	2.49 (*d*, 5.5)
4-Me	0.98 (*d*, 6.5)	1.54 (*s*)	1.97 (*s*)
8-Me	1.71 (*s*)	1.73 (*s*)	1.87 (*s*)
10-Me	1.81 (*s*)	1.66 (*s*)	1.49 (*s*)
13-Me	1.05 (*d*, 7.0)	1.03 (*d*, 6.9)	1.75 (*d*, 7.4)
1-OH		7.35 (*d*, 4.0)	6.18 (*d*, 2.5)
2-OH		6.40 (*d*, 6.4)	
11-OH	7.70 (*d*, 5.9)	6.00 (*d*, 5.5)	6.56 (*d*, 5.3)
12-OH			6.42 (*d*, 3.6)
6-OAc			2.15 (*s*)

[a] The spectra were obtained at 500 MHz. Chemical shifts are reported in ppm relative to C_5D_4HN resonance at 7.21 ppm. *J*-Values are given in parentheses in Hz.

Me. These observations along with the molecular formula demonstrated by HRFABMS have led to the conclusion that eurycolactone E has structure **2**.

Eurycolactone F (**3**) was obtained as colourless prisms. Its molecular formula, $C_{21}H_{28}O_8$, was established by HRFABMS. Its NMR spectra were similar to those of longilactone **6** (Morita et al., 1990) except that **3** possesses an acetyl group (δ_H 2.15 and δ_C 21.2, and δ_C 170.3). The presence of an acetyl group was also suggested by IR absorption at 1739 cm^{-1} (acetate) and its molecular formula which was more than that of **6** by a C_2H_2O unit. The position of the acetyl group was assigned to the C-6 oxygen atom by the observation of an HMBC correlation between 6-H (δ 5.74) and the acetyl-carbonyl carbon (δ 170.3). The stereochemistry of **3** was confirmed by the analysis of its NOESY spectrum (Fig. 3). Correlations were observed among 1-H, 5-H and 9-H, among 6-H, 8-Me and 10-Me, among 8-Me, 11-OH and 13-H, between 7-H and 8-Me, 11-OH and 12-H, and 12-H and 13-H. These data showed that the A/B and the B/C ring junctures were both in a *trans* relationship, and the configuration of 6-OAc, 12-OH and 13-Me were in α-configurations, while the configuration of 1-OH, 7-H, 8-Me and 10-Me were in β-configurations. From these findings, the structure of eurycolactone F was proposed to be structure **3**. This structure was confirmed by X-ray crystallography (Fig. 4).

Table 2
^{13}C NMR chemical shifts assignments for eurycolactones D–F (**1**–**3**) in C_5D_5N[a]

Position	1	2	3
1	214.2	82.4	83.1
2		74.2	199.4
3	45.1	126.8	127.5
4	32.6	133.7	162.1
5	173.2	49.2	47.2
6	118.8	37.0	68.5
7	198.3	207.5	82.4
8	47.5	51.8	43.5
9	41.7	49.9	41.7
10	52.7	44.2	50.2
11	67.2	70.2	73.2
12	84.7	84.2	75.7
13	32.3	32.8	27.6
14	52.9	53.8	56.0
15	177.1	176.9	176.3
4-Me	15.3	20.4	23.4
8-Me	22.4	24.1	21.1
10-Me	19.0	11.6	12.5
13-Me	16.9	16.8	15.2
6-OAc			170.3
			21.2

[a] The spectra were obtained at 125 MHz. Chemical shifts are reported in ppm relative to the solvent resonance at 135.5 ppm.

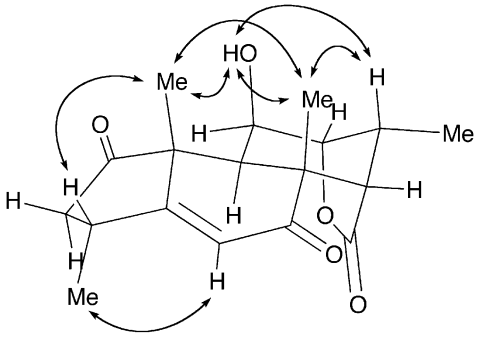


Fig. 1. Selected NOESY correlations for eurycolactone D (**1**).

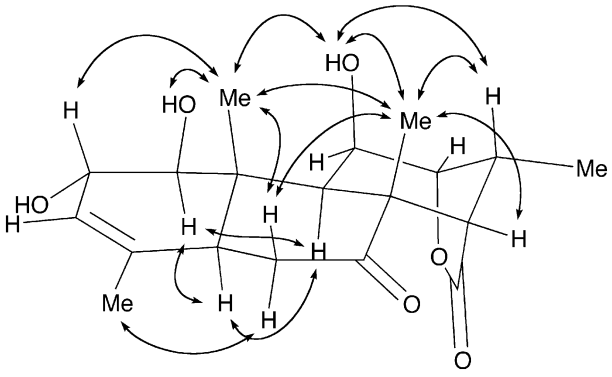


Fig. 2. Selected NOESY correlations for eurycolactone E (**2**).

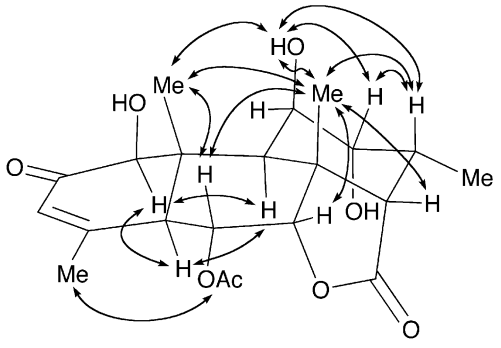


Fig. 3. Selected NOESY correlations for eurycolactone F (**3**).

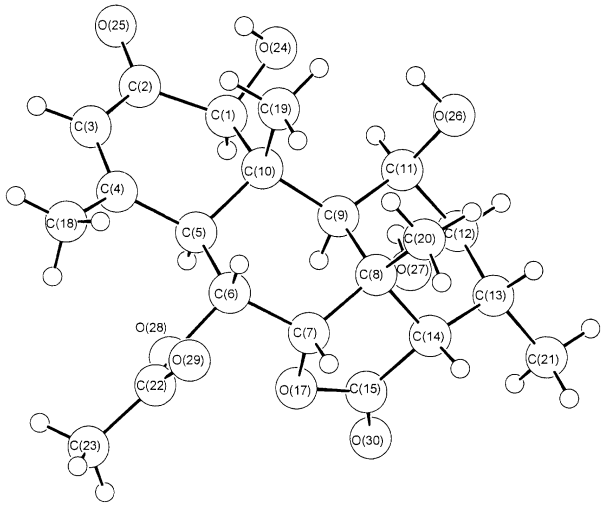


Fig. 4. Crystal structure of **3**.

3. Experimental

3.1. General

Optical rotations were measured on a Jasco DIP-360 digital polarimeter, whereas UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. IR spectra were measured on a Jasco FT/IR 410 spectrophotometer, whereas NMR spectra were obtained using a Bruker DRX-500 spectrometer. Mass spectra were acquired with a VG AutoSpec E spectrometer, with X-ray diffraction data being measured on a MacScience MXC18 diffractometer. Prep. HPLC was performed on a Tosoh CCPP-D system equipped with a UV detector and a reversed-phase column, TSK-Gel ODS-80 TM (20×300 mm), using mixed solvent systems of MeOH/H_2O (35:65–70:30) or H_2O/MeCN (70:30–80:20) at a flow rate of 10 ml/min.

3.2. Plant material

Eurycoma longifolia Jack roots were procured from Penang Island, Malaysia. It was authenticated by comparison with a voucher specimen previously deposited at the School of Pharmaceutical Sciences, University Science Malaysia, Minden, Penang, Malaysia.

3.3. Extraction and isolation

Air dried roots (50 kg) of *E. longifolia* were milled and the fatty substances were removed by petrol before being extracted with MeOH. The extract was then conc. under red. pres. to give a dark brown viscous residue (1.5 kg) and was subsequently partitioned between $CHCl_3$ and H_2O.

The $CHCl_3$-soluble portion (50 g) was subjected to CC [silica gel; $CHCl_3$/MeOH (1:0–2:1) gradient solvent system] followed by elution with MeOH and was separated into nine frs. The second fr. (17 g dry wt) was further subjected to CC [Diaion HP-20; (MeOH and Me_2CO) successively] to produce three sub. frs. Upon standing, the first sub. fr. (10.4 g) produced 0.87 g of crude crystals and was further subjected to prep. HPLC employing either H_2O/MeCN or H_2O/MeOH solvent systems to give two known quassinoids, **4** (6.5 mg) and **5** (24.2 mg). Their structures were confirmed by comparing their spectral data with those previously reported (Suong et al., 1982). The mother liquor was separated by using prep. HPLC with either H_2O/MeOH or H_2O/MeCN solvent systems to afford compounds **1** (7.7 mg), **2** (33.8 mg), **3** (10.6 mg) along with additional **4** (139.0 mg) and **5** (44.7 mg).

3.4. Eurycolactone D (*1*)

Colourless prisms, mp 236–238 °C (from EtOAc); $[\alpha]_D^{28}$: −61° (c 0.08, MeOH); UV (MeOH) λ_{max} nm (log ε): 227 (3.92); IR ν_{max} (film) cm^{-1}: 3483 (hydroxyl), 1775 (γ-lactone), 1745 (ketone), 1670 (α, β-unsaturated ketone); ^1H and ^{13}C NMR: refer to Tables 1 and 2. HRFABMS m/z 319.1524 $[M+H]^+$ $C_{18}H_{23}O_5$, calc. 319.1545.

3.5. Eurycolactone E (*2*)

Colourless needles, mp 208–212 °C (from MeOH); $[\alpha]_D^{28}$: +72° (*c* 0.34, MeOH); IR ν_{max} (film) cm^{-1}: 3468 (hydroxyl), 1775 (γ-lactone), 1709 (ketone); ^1H and ^{13}C NMR: refer to Tables 1 and 2. HRFABMS m/z 351.1818 $[M+H]^+$ $C_{19}H_{27}O_6$, calc. 351.1808.

3.6. Eurycolactone F (*3*)

Colourless prisms, mp 243–244 °C (from MeOH–H_2O); $[\alpha]_D^{28}$: +215° (c 0.11, MeOH); UV (MeOH) λ_{max} nm (log ε): 240 (4.04); IR ν_{max} (film) cm^{-1}: 3516 (hydroxyl), 1776 (γ-lactone), 1739 (acetate), 1664 (α, β-unsaturated ketone); ^1H and ^{13}C NMR: refer to Tables 1 and 2. HRFABMS m/z 409.1872 $[M+H]^+$ $C_{21}H_{29}O_8$, calc. 409.1862.

3.7. Crystal data for compound *3*

$C_{21}H_{28}O_8$, $M=408.40$, 0.35×0.3×0.3 mm, orthorhombic, $P2_12_12_1$, $a=12.000(3)$, $b=15.908(3)$, $c=10.161(2)$ Å, $V=1939.7(7)$ Å^3, $T=298$ K, $Z=4$, λ(Cu-K_α) = 1.541178 Å, 2192 reflections measured, 2099 unique reflections, $R=0.069$, $Rw=0.097$. The structure was solved by direct methods and expanded using Fourier techniques; X-ray data have been submitted to the Cambridge Crystallographic Centre.

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