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QUASSINOIDS FROM *Eurycoma longifolia*

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Abstract

By various chromatographic separations, three quassinoids, pasakbumin-C (**1**), 13*α*,21-epoxyeurycomanone (**2**), and eurylactone A (**3**) were isolated from the methanol extract of the roots of *E. longifolia.* Their structures were determined by 1D-NMR, 2D-NMR and in comparison with literature data.

Keywords. *Eurycoma longifolia;* quassinoids.

1. INTRODUCTION

Eurycoma longifolia. Jack (Simaroubaceae), an herbal known as "ba binh", "mat nhan", popularly distributed in South-East Asian countries [1]. The roots or stems of *E. longifolia* have been used in traditional and folk medicine to treat dysentery, fever, malaria, and sexual problems including male infertility [2]. The chemical components of *E. longifolia* are quassinoids [3-5], alkaloids [6], and squalenes [7, 8]. Previously, chemical investigation of this plant from Vietnam were also found the presence of quassinoids, alkaloid [9]. Specially, quassinoids were possessed antimalarial [10], antiulcer [3], cytotoxic [11], and aphrodisiac activities [12]. In this paper, we reported the isolation of three quassinoids from the roots of *E. longifolia*.

2. MATERIAL AND METHODS

2.1. Plant Material

The roots of *E. longifolia* were collected in Dak Lak Province, Vietnam, in March 2013, and identified by Dr. Bui Van Thanh, Institute of Ecology and Biological Resources, VAST. A voucher specimen was deposited at Institute of Marine Biochemistry.

2.2. General experimental procedures

All NMR spectra were recorded on a Variant 400 FT-NMR spectrometer (400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR) and chemical shifts (δ) are reported in ppm using TMS as an internal standard. Column chromatography was performed on silica gel 230-400 mesh (0.040-0.063 mm, Merck) or RP-18 resins (30-50 pm, Fujisilisa Chemical Ltd.). Thin layer chromatography was performed on DC-Alufolien $60F_{254}$ (Merck 1.05715) or RP_{18} F_{254} (Merck) plates. Compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 5 minutes.

2.3. Extraction and isolation

The dried roots of *E. longifolia* (18.0 kg) were extracted with MeOH (3×10 L, 50 ℃) under sonication for 4 h to yield 400.0 g extract. This extract was suspended in H_2O and successively partitioned with $CHCl_3$ and *n*-BuOH to obtain the $CHCl_3$ (EL1, 105.0 g), *n*-BuOH (EL2, 234.0 g), and H_2O (EL3, 60.0 g) extracts after removal of the solvents *in vacuo.* The EL1 fraction (105.0 g) was chromatographed on a silica gel column and eluting with a gradient of *n*-hexane–acetone (40:1 → 0:1, v/v) to obtain six sub-fractions, EL1A (14.2 g), EL1B (11.3 g), EL1C (17.2 g), EL1D (21.6 g), EL1E (25.2 g), and EL1F (7.3 g). The EL1D fraction was chromatographed on a silica gel column eluting with $CHCl_3$–acetone (6:1, v/v) to yield compound **1** (407.0 mg). The EL2 fraction was chromatographed on a Diaion HP-20P column eluting with H_2O containing increasing concentrations of MeOH in

water (0, 25, 50, 75, and 100 %) to obtain five sub-fraction EL2A (82.0 g), EL2B (26.3 g), EL2C (32.8 g), EL2D (12.4 g), and EL2E (72.5 g). The EL2B fraction was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (8:1, v/v) to yield compounds **2** (109.0 mg) and **3** (875.0 mg).

Pasakbumin-C (1): colorless needles, $[\alpha]_D^{25}$ +28.5 (*c* = 0.1 in MeOH), ESI-MS *m/z* 433 $[M+Na]^+$, $C_{20}H_{26}O_9$, ^{1}H- and ^{13}C-NMR, see table 1.

13*α*,21-Epoxyeurycomanone (2): colorless needles, $[\alpha]_D^{25}$ +32.1 (*c* = 0.1 in MeOH), ESI-MS *m/z* 447 $[M+Na]^+$, $C_{20}H_{24}O_{10}$, ^{1}H- and ^{13}C-NMR, see table 1.

Eurylactone A (3): colorless needles, $[\alpha]_D^{25}$ +18.0 (*c* = 0.1 in MeOH), ESI-MS *m/z* 383 $[M+H]^+$, $C_{19}H_{26}O_8$, ^{1}H- and ^{13}C-NMR, see table 1.

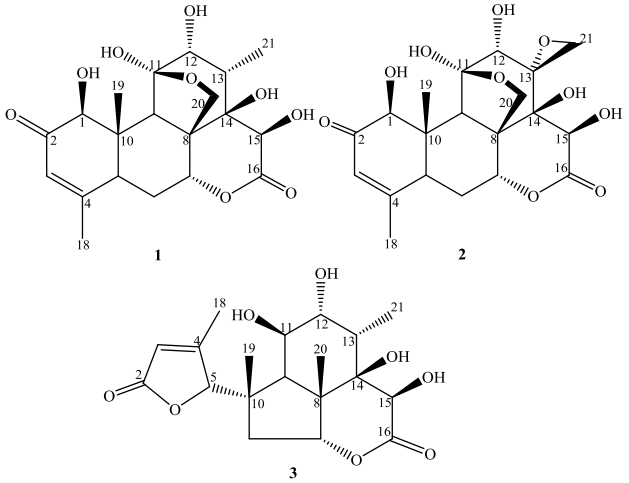

Figure 1: Chemical structures of compounds **1-3**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as colorless needles. The ^{1}H-NMR spectrum of **1** showed the signals of three methyl groups at δ_H 1.27 (d, *J* = 7.2 Hz), 1.24 (s), and 2.04 (s); two oxygenated methylene protons at δ_H 3.81 (d, *J* = 10.0 Hz) and 4.09 (d, *J* = 10.0 Hz); four hydroxy methine proton at δ_H 3.41 (d, *J* = 4.0 Hz), 4.24 (s), 4.76 (s), and 4.80 (br s); one olefinic proton at δ_H 6.07 (s). The ^{13}C-NMR and DEPT spectra of **1** revealed the signals of 20 carbons, including two carbonyl δ_C at 175.96 and 198.97; five non-protonated at δ_C 46.26, 53.39, 76.87, 110.17, and 165.34; eight methine at δ_C 42.18, 42.90, 47.30, 72.07, 76.18, 79.69, 82.57, and 125.95; two methylene at δ_C 26.19 and 67.71; three methyl carbons at 10.30, 13.23, and 22.85. The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of pasakbumin-C (table 1) [3]. The position of the carboxyl at C-16 and esterification of C-7 were verified by HMBC correlations between H-7 (δ_H 4.80)/H-15 (δ_H 4.76) and C-16 (δ_C 175.96). The HMBC correlations from H-3 (δ_H 6.07) to C-1 (δ_C 82.57)/C-2 (δ_C 198.97)/C-4 (δ_C 165.34)/C-5 (δ_C 42.90)/C-18 (δ_C 22.85) confirmed the position of carbonyl group at C-2 and double bond at C-3/C-4. In addition, the attachment of hydroxyl groups at C-12 and C-14 was determined by the HMBC correlations between H-21 (δ_H 1.27) and C-12 (δ_C 79.69)/C-13 (δ_C 42.18)/C-14 (δ_C 76.87); between H-12 (δ_H 3.41) and C-9 (δ_C 47.30)/C-11 (δ_C 110.17)/C-13 (δ_C 42.18)/C-14 (δ_C 76.87) (Figure 2). The HMBC correlations between H-20 (δ_H 3.81 and 4.09) and C-7 (δ_C 76.18)/C-9 (δ_C 47.30)/C-11 (δ_C 110.17)/C-14 (δ_C 76.87) suggested the position of the epoxy bridge at C-11/C-20. Based on the above evidence, compound **1** was determined to be pasakbumin-C.

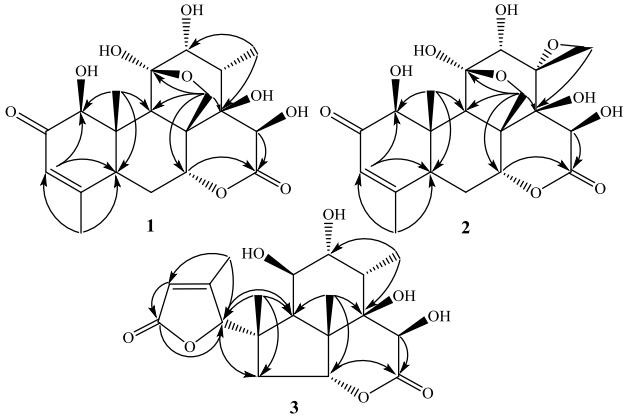

Figure 2: The key HMBC correlations of **1-3**

Compound **2** was isolated as colorless needles. The ^{1}H-NMR and ^{13}C-NMR data (table 1) were similar to those of **1** except for an epoxy group at C-13/C-21. Moreover, the NMR data of compound **2** were found to be similar to those of 13*α*,21-epoxyeurycomanone [11]. The position of epoxy group was confirmed by HMBC correlations between H-21 (δ_H 2.96) and C-12 (δ_C 79.86)/C-13 (δ_C 57.97)/C-14 (δ_C 73.69). The positions of remaining functional groups in **2** were confirmed by the analysis of HSQC and HMBC correlations. Therefore, the structure of **2** was determined to be 13*α*,21-epoxy eurycomanone.

Compound **3** was obtained as colorless needles and its molecular formula was deduced as $C_{19}H_{26}O_8$ by ESI-MS *m/z* 383 $[M+H]^+$ and ^{13}C-NMR. The ^{1}H-NMR spectrum showed signals of four methyl groups at δ_H 1.13 (s), 1.21 (d, *J* = 7.2 Hz), 1.36 (s), and 2.19 (s); four hydroxy methine protons at δ_H 4.48 (br d, *J* = 3.6 Hz), 4.33 (dd, *J* = 2.4, 2.4 Hz), 3.68 (dd, *J* = 2.4, 2.4 Hz), and 5.29 (s); one olefinic proton at δ_H 5.95 (s). The ^{13}C-NMR and DEPT spectra of **3** revealed the signals of 19 carbons, including two carbonyl δ_C at 174.00 and 176.81;

four non-protonated at δ_C 45.29, 50.97, 77.53, and 170.54; eight methine at δ_C 37.89, 48.13, 70.41, 74.25, 79.32, 88.73, 95.98, and 120.99; one methylene at δ_C 43.88; and four methyl carbons at δ_C 12.61, 16.64, 17.77, and 20.11. Analysis the NMR data of compound **3** indicated that structure of **3** was quassinoid skeleton [13]. In addition, its NMR data were similar to those of eurylactone A [13]. The HMBC correlations between H-5 (δ_H 4.82) and C-2 (δ_C 174.00)/C-3 (δ_C 120.99)/C-4 (δ_C 170.54)/C-10 (δ_C 50.97)/C-18 (δ_C 16.64); H-18 (δ_H 2.19) and C-3 (δ_C 120.99)/C-4 (δ_C 170.54)/C-5 (δ_C 95.98) suggested the *α,β*-unsaturated *γ*-lactone at C-10. The *δ*-lactone for C-7/C-18 was also confirmed by the HMBC correlations from H-7 (δ_H 4.48) to C-16 (δ_C 176.81) (figure 2). In addition, the attachment of hydroxyl groups at C-12 and C-14 was determined by the HMBC correlations from H-12 (δ_H 3.68) to C-9 (δ_C 48.13)/C-11 (δ_C 74.25)/C-13 (δ_C 37.89)/C-14 (δ_C 77.53); from H-21 (δ_H 1.21) to C-12 (δ_C 79.32)/C-13 (δ_C 37.89)/C-14 (δ_C 77.53) (figure 2). Based on the above evidence, compound **3** was determined to be eurylactone A.

Table 1: The ^{1}H- and ^{13}C-NMR data for compounds **1-3** and reference compounds

Pos.	δ_C[$]	δ_C[a,c]	δ_H[a,d] (mult., *J* = Hz)	δ_C[*]	δ_C[b,c]	δ_H[b,d] (mult., *J* = Hz)	δ_C[#]	δ_C[a,c]	δ_H[a,d] (mult., *J* = Hz)
1	84.6	82.57	4.24 (s)	84.5	82.30	4.36 (s)			
2	197.5	198.97	-	197.3	197.16	-	172.8	174.00	-
3	126.1	125.95	6.07 (s)	126.1	124.84	5.98 (s)	119.9	120.99	5.95 (s)
4	162.7	165.34	-	162.4	162.63	-	168.8	170.54	-
5	42.2	42.90	2.97 (br d, 13.2)	42.2	40.69	2.97 (br d, 13.2)	94.6	95.98	4.82 (s)
6	25.8	26.19	2.11 (m)/2.30 (br d, 13.2)	25.5	24.37	1.96 (m)/2.07 (m)	43.3	43.88	2.23 (br s)
7	75.1	76.18	4.80 (br s)	75.6	74.37	4.52 (br s)	87.4	88.73	4.48 (br d, 3.6)
8	52.7	53.39	-	53.5	51.99	-	44.6	45.29	-
9	47.0	47.30	2.83 (s)	48.4	46.62	2.89 (s)	47.9	48.13	2.52 (d, 2.0)
10	45.7	46.26	-	45.8	44.73	-	50.4	50.97	-
11	110.3	110.17	-	109.6	107.78	-	74.1	74.25	4.33 (dd, 2.4, 2.4)
12	79.7	79.69	3.41 (d, 4.0)	81.7	79.86	2.86 (s)	79.3	79.32	3.68 (dd, 2.4, 2.4)
13	42.2	42.18	2.20 (dq, 4.0, 6.8)	59.2	57.97	-	37.8	37.89	2.31 (m)
14	76.6	76.87	-	75.4	73.69	-	77.0	77.53	-
15	71.9	72.07	4.76 (s)	71.4	69.83	4.72 (s)	70.1	70.41	5.29 (s)
16	174.8	175.96	-	173.8	172.53	-	175.5	176.81	-
18	22.4	22.85	2.04 (s)	22.4	22.39	1.92 (s)	16.2	16.64	2.19 (s)
19	10.6	10.30	1.24 (s)	10.4	9.67	1.05 (s)	19.8	20.11	1.13 (s)
20	67.3	67.71	3.81 (d, 10.0)/4.09 (d, 10.0)	66.8	65.24	3.57 (d, 10.0)/4.01 (d, 10.0)	17.8	17.77	1.36 (s)
21	14.0	13.23	1.27 (d, 7.2)	46.5	44.86	2.96	13.4	12.61	1.21 (d, 7.2)

[a]Measured in CD_3OD, [b]DMSO-d_6 [c]100 MHz, [d]400 MHz, [$]$\delta_C$ of pasakbumin-C [3],
[*]δ_C of 13α,21-epoxyeurycomanone [11], [#]δ_C of eurylactone A [13].

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