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# EURYCOMALIN A, A NEW DIMERIC DIHYDROBENZOFURAN FROM *EURYCOMA LONGIFOLIA*

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**Abstract —** One new dimeric 2-isopropenyl-2, 3-dihydrobenzofuran, eurycomalin A (**1**), was characterized from the methanol extract of the root of *Eurycoma longifolia* by the 1D and 2D spectral and chemical transformation studies. The reported structure of eurycomalide A (**2**) was revised through detail 2D spectral analyses.

## INTRODUCTION

*Eurycoma longifolia* (Simaroubaceae) is a tall slender shrub-tree native to Burma, Indochina, Thailand, and Southeast Asia, commonly found in the lowland forests at up to 500 meters above sealevel. *E. longifolia* is known locally as “Tongkat Ali” in Malaysia, “Pasakbumi” in Indonesia, “Cay ba binh” in Vietnam and “Ian-don” in Thailand, which has been frequently prescribed either as a single ingredient or as a mixture with other herbs. The roots of this plant are used as folk medicine for the treatment of aches, persistent fever, tertian malaria, sexual insufficiency,[1] dysentery, glandular swelling,[2-3] and as health supplements.[1] In addition, the crude extracts of this plant were reputed to increase male virility and sexual

prowers and gained notoriety as a male aphrodisiac.[4-6] From the roots, several classes of compounds have been identified and they included quassinoids,[7-12] canthin-6-one alkaloids,[7] β-carboline alkaloids,[7] tirucallane-type triterpenes,[10] squalene derivatives,[13] and biphenylneolignans.[14] Some of these constituents were shown to possess cytotoxic,[7,10,15] antimalarial,[16] antiulcer,[17] antipyretic,[18] and plant growth inhibition activities.[19] The wide range of pharmacological activities of this plant has prompted us to undertaken the chemical investigation of the methanolic extract of the root of *E. longifolia*. Herein we wish to report the isolation and characterization of one new dimeric 2-isopropenyl-2, 3-dihydrobenzo-furan **1**, and revise the reported structure of eurycomalide A (**2**).

## RESULTS AND DISCUSSION

Eurycomalin A (**1**) was isolated as optically active ($[\alpha]_D$ −61.4 $^o$) colorless syrup, with an elemental composition $C_{24}H_{22}O_6$ from its pseudomolecular ion peak in HRFABMS analysis ($[M+H]^+$, *m/z* 407.1497). The UV absorption maxima at 215, 258, and 293 nm were typical of a substituted aromatic skeleton.[20] IR absorption bands at 1609 and 1696 $cm^{-1}$ together with the carbon signal at δ 170.5 in the $^{13}C$ NMR spectrum indicated the presence of arylcarboxylic acid group in the molecule. The molecular formula and the presence of only twelve carbon signals in the $^{13}C$ NMR spectrum inferred that **1** was a symmetrical dimer. In its $^1H$ NMR spectrum, two *meta*-coupling doublets integrated for four aromatic protons were observed at δ 7.79 (*J* = 1.1 Hz, H-5) and 7.96 (*J* = 1.1 Hz, H-7) suggested the occurrence of tetrasubstituted aromatic rings. In addition, an isopropenyl dihydrofuran moiety discernable from two diastereotropic proton signals at δ 3.15 (dd, *J* = 16.0, 7.9 Hz, H-3) and 3.47 (dd, *J* = 16.0, 9.7 Hz, H-3), an oxygenated methine proton signal at δ 5.38 (dd, *J* = 9.7, 7.9 Hz, H-2), two olefinic proton singlets at δ 4.98 (H-2′) and 5.13 (H-2′), and a methyl at δ 1.78 (s, $CH_3$-3′), whose connectivity was characterized by the HMBC correlations of H-3/C-1′; H-2′/C-2, C-3; and H-3′/C-2, C-1′, C-2′. Unambiguous location of this group at C-4 and C-9 was furnished by multi-dimensional 2D spectral techniques, especially HMBC analysis, which exhibited $^2J$- and $^3J$-correlations between H-3 and C-4, C-9; and H-5 and C-3, respectively. Two strong NOEs between methylene protons of dihydrofuran and H-5 of aromatic ring and

3′-methyl protons of isopropenyl moiety also supporting the attachment of isopropenyl dihydrofuran group to C-4 and C-9. The position of carboxylic group at C-6 was also inferred by the HMBC correlations from H-5 to C-7, C-9, and C-10; and from H-7 to C-5, C-9, and C-10. A $^{3}J$-HMBC correlation between H-7 and C-8 confirmed that the two parts of this symmetric dimer were connected at C-8. Finally, the absolute configuration at C-2 of isopropenyl dihydrofuran moiety was established by comparing the sign of the Cotton effect given by the osmate ester of **1** with that of natural rutenone.[21] The positive Cotton effect at 480 nm indicated that **1** has *S* configuration at C-2. From the above spectral analyses, the structure of **1** was determined as shown in figure 1.

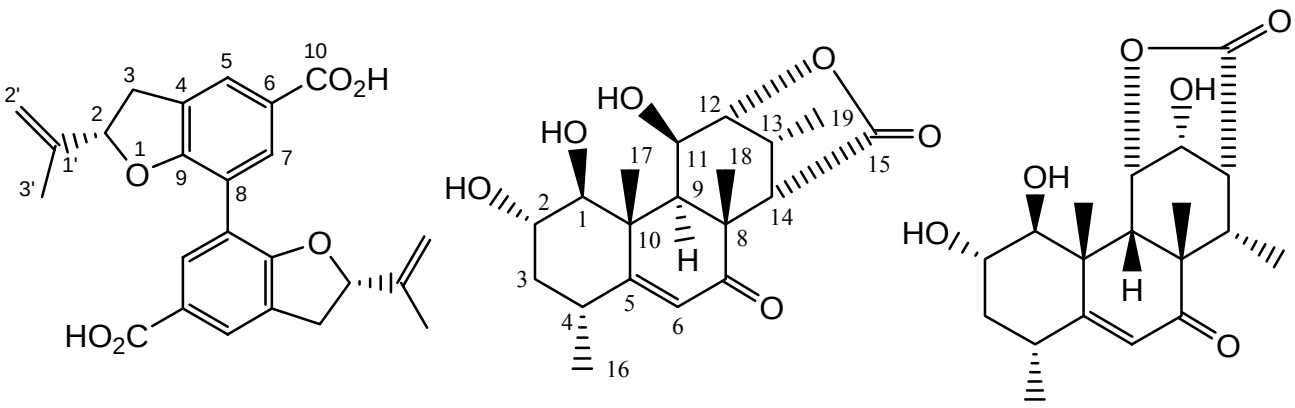


**Figure 1** Structures of Isolated Compounds

Eurycomalide A (**2**) was obtained as optically active colorless needles with mp 178 — 180 °C and $[\alpha]_D^{25}$ +31.6°. The molecular formula $C_{19}H_{26}O_6$ for **2** was established from its pseudomolecular ion peak at *m/z* 351.1806 in HRFABMS, which was reported in our previous paper as structure **3** with a rare-occurred C-ring network.[22] However, through the detailed COSY spectral analysis, it was evident that the proton signals at δ 2.46 (1H, d, *J* = 3.2 Hz, H-9), 5.78 (1H, ddd, *J* = 6.0, 4.9, 3.2 Hz, H-11), 6.91 (1H, *J* = 6.0 Hz, OH-11), and 4.49 (1H, d, *J* = 4.9 Hz, H-12) exhibited typical correlations in the C-ring of eurycoma-lactone type quassinoids.[23] The long range COSY correlation between protons at δ 3.38 (1H, br s, H-14) and 4.49 (H-12) also suggested the basic skeleton of **2** was similar with that of eurycomalactone.[23] Reassignment of chemical shifts from H-9 to H-14 and corresponding carbon signals with the aid of HMQC spectrum indicated the slight modification in the previous assignment of C-9 to C-14. In the HMBC spectrum, the $^{2}J$, $^{3}J$-HMBC correlations from a proton at δ 3.31 (H-13) to carbons at δ 47.8 (C-8)

and 177.3 (C-15), from a proton at δ 1.68 ($CH_3$-18) to carbons at δ 48.4 (C-9), 53.0 (C-14), and 199.5 (C-7), and from a proton at δ 1.92 ($CH_3$-17) to carbons at δ 46.5 (C-10), 48.4 (C-9), 85.2 (C-1), and 171.0 (C-5), provided the strong evidence for the existence of a common C-ring pattern of **2**. The NOESY correlations of $CH_3$-17 with $CH_3$-18, H-2, H-4, and OH-11, H-1 with H-3 and H-9, H-9 with H-11, H-11 with H-12, H-12 with $CH_3$-19 and H-14, and $CH_3$-18 with H-13 supported that compound **2** has same stereochemistry with that of eurycomalactone,[23] i.e., OH-2, $CH_3$-16, H-9, and $CH_3$-19 were α-oriented, however, OH-1, $CH_3$-17, $CH_3$-18, OH-11, H-12, H-13, and H-14 were β-oriented. Therefore, the complete assignments established by 1D and 2D NMR experiments confirmed that the structure of eurycomalide A should be revised as **2** shown in figure 1.

## EXPERIMENTAL

**General Experimental Procedures**. Melting points were determined on Yanaco MP-S3 micro-melting point apparatus without correction. Optical rotations were measured on a JASCO DIP-370 polarimeter. UV spectra were taken on a Hitachi UV-3210 spectrophotometer. IR spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer as KBr discs. $^1$H, $^{13}$C, COSY, HMQC, HMBC, and NOESY NMR spectra were recorded on the Bruker Avance-300 NMR spectrometer, using tetramethylsilane (TMS) as the internal standard, and all chemical shifts were reported in parts per million (ppm, δ). All the mass and high-resolution mass spectra (FAB) were obtained on a JEOL JMS-700 spectrometer. CD spectra were recorded with a JASCO J-720 spectropolarimeter.

**Plant Material**. The dried roots of *E. longifolia* Jack were collected from Malaysia in January, 2001, and authenticated by Prof. J. B. Wu, Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung. A voucher specimen (TSWu 20010005) has been deposited at the Herbarium of National Cheng Kung University, Tainan, Taiwan.

**Extraction and Isolation**. The dried roots (10.5 Kg) were cut into small pieces, extracted with methanol (20 L × 7) under reflux for 8 h, and concentrated to give dark brown syrup (500 g). The crude extract was suspended in water and partitioned with chloroform and *n*-butanol, successively, to afford four individual

portions, chloroform layer, *n*-butanol layer, water layer, and residue. The chloroform layer was concentrated *in vacuo* to leave brown syrup (50 g). The residue was chromatographed on silica gel column and eluted with gradients of chloroform and acetone to give twelve fractions. Silica gel column chromatography of fraction 9 by *n*-hexane with a step gradient of ethyl acetate gave four subfractions. Subfraction 9-2 was subjected to silica gel column chromatography with diisopropyl ether-methanol (99:1) and further recrystallization of each subfractions afforded **2** (0.9 mg). Subfraction 9-3 was purified by column chromatography on a silica gel column and eluted with mixing eluents of benzene and acetone (1:1) to yield **1** (2.5 mg).

**Preparation and CD Determination of the Osmate Ester of 1**. Stock solutions of $CH_2Cl_2$ + pyridine (3.05 mL + 0.1 mL; A) and osmic acid + $CH_2Cl_2$ (4.7 mg in 125 μL; B) were first prepared. A dry sample of **1** (1.9 mg) was dissolved in A (63 μL), and B (10 μL) was then added. After incubation for 30 min at 25°C, the mixture was diluted to 2.8 mL with $CH_2Cl_2$. The CD curve of the resulting osmate ester was immediately measured over the range 470-480 nm on a JASCO J-720 spectropolarimeter zeroed with a blank consisting of A + B. Comparison with 2'*R*-rotenone ($[\theta]_{474}$ — 5800) and shuterol ($[\theta]_{474}$ — 3100),[21] eurycomalin A ($[\theta]_{480}$+1423) was found to possess the *S* side-chain stereochemistry at C-2.

**Eurycomalin A (1)** $C_{24}H_{22}O_6$: Colorless syrup; $[\alpha]_D^{25}$–61.4 $^o$ (*c* 0.01, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) nm: 215 (4.42), 258 (4.05), 293 (3.59, sh); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3205, 2928, 2856, 1696, 1609, 1423, 1280, 1175; $^1$H NMR ($CDCl_3$, 300MHz): δ 7.96 (1H, d, *J* = 1.1 Hz, H-7), 7.79 (1H, d, *J* = 1.1 Hz, H-5), 5.38 (1H, dd, *J* = 9.7, 7.9 Hz, H-2), 5.13 (1H, s, H-2′), 4.98 (1H, s, H-2′), 3.47 (1H, dd, *J* = 16.0, 9.7 Hz, H-3), 3.15 (1H, dd, *J* = 16.0, 7.9 Hz, H-3), 1.78 (3H, s, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$, 75MHz): δ 170.5 (C-10), 160.3 (C-9), 142.4 (C-1′), 131.8 (C-7), 128.7 (C-4), 125.4 (C-5), 122.8 (C-6), 115.0 (C-8), 113.3 (C-2′), 87.8 (C-2), 34.6 (C-3), 17.0 (C-3′); FABMS *m/z* (*rel. int.* %): 407 ($[M+H]^+$, 6), 361 (9), 204 (12), 203 (16); HRFABMS *m/z* 407.1497 $[M+H]^+$ (calcd for $C_{24}H_{23}O_6$, 407.1495).

**Eurycomalide A (2)** $C_{19}H_{27}O_6$: Colorless needles (MeOH), mp 178—180 °C; $[\alpha]_D^{25}$+31.6° (*c* 0.09, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) nm: 234 (4.09), 283 (3.04); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3423, 2932, 1754, 1657, 1270; $^1$H NMR (Pyridine-$d_5$, 300MHz): δ 6.96 (1H, br s, $D_2O$ exchangeable, OH), 6.91 (1H, d, *J* =

6.0 Hz, $D_2O$ exchangeable, OH-11), 6.57 (1H, br s, $D_2O$ exchangeable, OH), 5.92 (1H, s, H-6), 5.78 (1H, ddd, *J* = 6.0, 4.9, 3.2 Hz, H-11), 4.49 (1H, d, *J* = 4.9 Hz, H-12), 4.14 (1H, m, H-2), 3.56 (1H, d, *J* = 8.7 Hz, H-1), 3.38 (1H, br s, H-14), 3.31 (1H, q, *J* = 6.9 Hz, H-13), 2.52 (1H, m, H-4), 2.46 (1H, d, *J* = 3.2 Hz, H-9), 2.13 (1H, dt, *J* = 12.3, 4.5 Hz, H-3e), 1.92 (3H, s, $CH_3$-17), 1.68 (3H, s, $CH_3$-18), 1.23 (1H, dt, *J* = 12.5, 12.3 Hz, H-3a), 1.05 (3H, d, *J* = 6.9 Hz, $CH_3$-19), 0.94 (3H, d, *J* = 6.4 Hz, $CH_3$-16); $^{13}$C NMR (Pyridine-$d_5$, 75MHz): δ 199.5 (C-7), 177.3 (C-15), 171.0 (C-5), 120.5 (C-6), 85.2 (C-1), 84.8 (C-12), 70.4 (C-2), 69.3 (C-11), 53.0 (C-14), 48.4 (C-9), 47.8 (C-8), 46.5 (C-10), 40.7 (C-3), 32.2 (C-13), 31.7 (C-4), 22.7 (C-18), 18.2 (C-16), 17.4 (C-17), 16.8 (C-19); FABMS *m/z* (*rel. int.* %): 373 ($[M+Na]^+$, 46), 351 ($[M+H]^+$, 40); HRFABMS *m/z* 351.1806 $[M+H]^+$ (calcd for $C_{19}H_{27}O_6$, 351.1808).

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