Eurycomaoside: A New Quassinoid-Type Glycoside from the Roots of *Eurycoma longifolia*

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A new C_{19}-quassinoid-type glycoside has been isolated from the roots of *Eurycoma longifolia*. The structure elucidation of the compound was achieved by a combination of one- and two-dimensional NMR techniques, including ^1H–^1H-correlation spectroscopy (COSY), ^1H–^{13}C-heteronuclear correlation spectroscopy (HMQC), and ^1H–^{13}C-heteronuclear multiple-bond correlation spectroscopy (HMBC), as well as high resolution electrospray ionization Fourier transformation mass spectrometry (HR-ESI-FT-MS) data. The C(1)-glycosidation site in the quassinoid framework is encountered for the first time.

Key words *Eurycoma longifolia*; quassinoid; glycoside; Simaroubaceae

The plant *Eurycoma longifolia* JACK. is a small tree belonging to the family Simaroubaceae, and can be found in the jungle throughout Malaysia, Indochina, Borneo, and Sumatra. It is commonly known as Tongkat Ali in Malaysia and Singapore. A decoction of the roots, root bark or bark is drunk to treat diarrhea, fever, glandular swelling, bleeding, dropsy, persistent cough, and hypertension, relieve pain in the bones, as an aphrodisiac, and tonic. The pounded bark is applied to treat wounds, ulcers, syphilitic sores and headache.[1,2] In view of the fact that it has become popular throughout the world for its aphrodisiac properties, the demand for Tongkat Ali, which increased rapidly, has caused rampant encroachments in Malaysia. Thus, the plant has now been placed on the list of protected plants in the jungles of Malaysia.

Phytochemical studies carried out on *Eurycoma longifolia* showed that it possesses a series of quassinoids which are mainly responsible for its bitter taste,[3—7] triscullane-type triterpenes,[8] squalene derivatives,[9,10] biphenyl-*neo*-lignans,[11] canthin-6-one and β-carboline alkaloids.[12,13] The extracts or constituents of Tongkat Ali have been reported to have aphrodisiac,[14,15] cytotoxic,[8,13] antimalarial,[12,13] anxiolytic,[16] and antiulcer activities.[17]

The overall objective of this project is to provide pure compounds for the fingerprinting studies of Tongkat Ali. During the course of our phytochemical studies, we have isolated a new quassinoid glycoside together with four known compounds; 14,15-β-dihydroxyklaineanone,[18] 9-methoxycanthin-6-one, β-carboline-1-propionic acid, and 7-methoxy-β-carboline-1-propionic acid.[13] This paper deals with the isolation and structural elucidation of **1**.

The HR-ESI-FT-MS (high resolution electrospray ionization Fourier transformation mass spectrometry) of **1** exhibited an ion peak for $[M+Na]^+$ at *m/z* 553.2260, which is compatible with the molecular formulae $C_{25}H_{38}O_{12}$.

Inspection of the ^1H-NMR spectrum of **1** (Table 1) showed three tertiary (δ 1.72, 1.57, 1.86; Me-18, Me-19, Me-20, respectively) and a secondary methyl group (1.66 d, J=7.5 Hz, Me-21), the protons of one disubstituted double bond (δ 6.07, 5.91, each d, J=10.5 Hz), as well as one anomeric proton signal at δ 4.85 (d, J=7.5 Hz, H-1′), indicative of the presence of a β-linked sugar.

The ^{13}C-NMR spectrum of **1** displayed 25 signals, 6 of which were in good accordance with the presence of a β-glucopyranosyl moiety.[19] Acid hydrolysis of **1** followed by TLC analysis confirmed the presence of β-glucose. We favor its D absolute configuration, consistent with all other naturally occurring sugar residues in the genus *Eurycoma*. After subtraction of the 6 carbon resonances of the sugar unit, the remaining 19 resonances were attributable to a quassinoid skeleton. The quassinoid moiety signals were consistent with a $C_{19}H_{28}O_7$ framework, indicating the presence of 6 degrees of unsaturation, *i.e.* two double bonds [δ 178.9, C-15 (C=O); δ 135.7, 126.7, C-3=C-2] and four ring systems.

The covalent connectivities of the tetracyclic structure of **1** were established by analysis of the G-DQF-COSY and G-

Table 1. ^1H- and ^{13}C-Assignments of **1** (in CD_3OD+DMSO-d_6; at 500 and 125 MHz, Respectively)[a]

C/H	δ (ppm), J (in Hz)	δ (ppm)
1	4.64 br s	81.5 d
2	6.07 d (10.5)	126.7 d
3	5.91 d (10.5)	135.7 d
4	—	72.4 s
5	2.20 d (12.0)	52.4 d
6	4.62 dd (12.0, 3.5)	67.5 d
7	4.31 d (3.5)	87.5 d
8	—	45.2 s
9	2.08 br s	43.5 d
10	—	44.6 s
11	5.49 br s	72.0 d
12	3.84 br s	76.5 d
13	2.64 m	28.0 d
14	2.58 d (5.0)	56.7 d
15	—	178.9 s
18	1.72 s	24.2 q
19	1.57 s	15.2 q
20	1.86 s	21.4 q
21	1.66 d (7.5)	15.2 q
1′	4.85 d (7.5)	101.2 d
2‴	3.57[b]	75.1 d
3′	3.67 t (9.0)	78.3 d
4′	3.56[b]	71.8 d
5′	3.56[b]	77.8 d
6′	3.93 dd (4.5, 11.0), 4.14 dd (2.5, 11.0)	62.8 t

a) Assignments confirmed by DQF-COSY, HMQC and HMBC experiments. *b*) Signal pattern was unclear due to overlapping.

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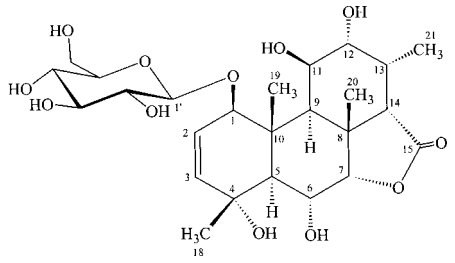

Chart 1. Structure of **1**

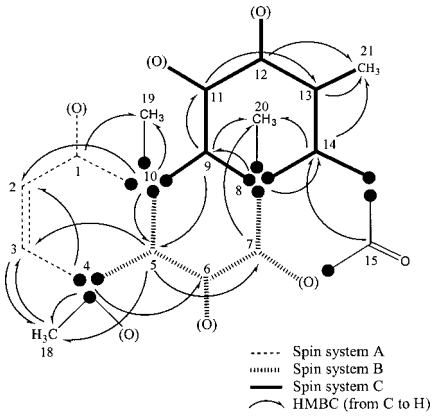

Chart 2. Partial Structures Deduced from 2D-NMR Measurements (HMQC, DQF-COSY) and Key HMBC of **1**

HMQC spectra, which revealed the presence of three isolated spin systems (Chart 1) in the quassinoid framework: "A" (H-1→H-3), "B" (H-5→H-7), "C" (H-9→H-14). The spin system A starts with the H-1 resonance (δ 4.64, br s) which showed cross peaks with an olefinic proton at δ 6.07 (d, J=10.5 Hz, H-2) in the DQF-COSY spectrum. H-2 couples with H-3 (δ 5.91, d, J=10.5 Hz), while the latter proton showed allylic coupling with H-1. The second spin system (B) could be traced from the methine proton H-5 (δ 2.20, d, J=12.0 Hz) to the oxymethine proton H-6 (δ 4.62, dd, J=12.0, 3.5 Hz) and from there to another oxymethine proton at δ 4.31 (d, J=3.5 Hz, H-7). The spin system C commences with H-9 (δ 2.08, br s) which showed correlations with the oxymethine proton at C-11 (δ 5.49, br s) which, in turn, exhibited cross peaks with the H-12 oxymethine proton (δ 3.84, br s). H-12 showed correlation with H-13 (δ 2.64, m) which, in turn, coupled with H-14 (δ 2.58, d, J=5.0 Hz) and H_3-21 (δ 1.66, d, J=7.5 Hz).

In order to establish the interfragment relationship, a gradient heteronuclear multiple-bond correlation experiment (G-HMBC) was performed (Chart 2), which not only connected the fragments but also permitted location of the glycosidic linkage and lactone. Thus, the anomeric proton of the β-D-glucopyranosyl moiety (δ 4.85, d, J=7.5 Hz, H-1′) displayed a long-range correlation to C-1 (δ 81.5, d). C-1 also showed correlations with H-2, H-3, and Me-19. The quaternary carbon signal at δ 72.4 showed HMBC connectivities to H-2, H-3, H-5, and Me-18, allowing it to be assigned unambiguously to C-4. The methine carbon at δ 43.5 was readily assigned to C-9 on the basis of long-range connectivities to H-1, H-5, H-11, Me-19, and Me-20. The position of the lactone was deduced from the long range correlation observed between C-15 and H-7. In a similar fashion, the connectivities between the partial structures (A—C) and assignment of quaternary carbon atoms, and tertiary methyl groups in the molecule were determined (Chart 2).

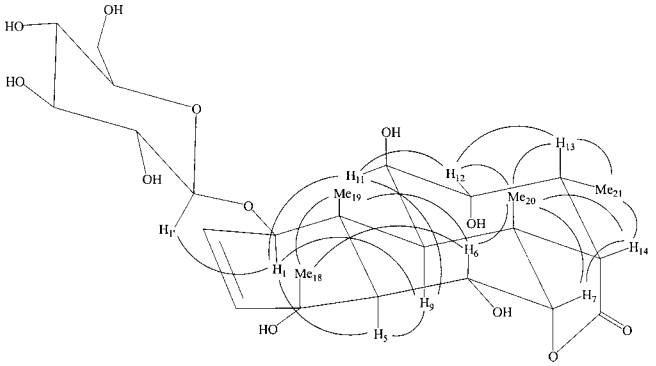

Chart 3. Selected NOESY Correlations of **1**

The relative stereochemistry of **1** was resolved by 2D-NOESY data. The cross peaks observed in the NOESY spectrum for H-9 to H-5 and H-1, H-5 to H-9 implied that these protons were cofacial (α), while observation of the key NOESY couplings from Me-18 to H-6 and Me-19, Me-19 to Me-20, and Me-20 to H-7, H-12 and H-14 revealed that these protons occupy the β face of the molecule. These results also suggested that **1** has the usual A/B *trans* and B/C *trans* ring junctions shown in Chart 3. The NOESY network from H-1 to H-1′ provided evidence for the glycosidation site. The orientation of 11β-OH was evident from the cross peaks observed from H-11 to H-9 and H-1.

From these observations, the structure of **1** was determined as shown. Compound **1**, which represents the first entry of the series of quassinoids possessing a glucosyl moiety at C-1, is named eurycomaoside.

14,15-β-Dihydroxyklaineanone, 9-methoxycanthin-6-one, β-carboline-1-propionic acid, and 7-methoxyl-β-carboline-1-propionic acid were also isolated and identified by comparison of their ^{1}H- and ^{13}C-NMR spectral data with literature values.[13,18]

Experimental

General Experimental The 1D- and 2D-NMR spectra were obtained on a Bruker® Avance DRX 500 FT spectrometer operating at 500 and 125 MHz, respectively. The chemical shift values are reported as parts per million (ppm) units relative to tetramethylsilane (TMS) for ^{1}H- and ^{13}C-; and the coupling constants are in Hz (in parentheses). For the ^{13}C-NMR spectra, multiplicities were determined by a distortionless enhancement by polarization transfer (DEPT) experiment. HR-ESI-FT-MS were obtained using a Bruker BioApex FT-MS in ESI mode.

Chromatographic Conditions TLC: precoated Si 250F plates (Baker); developing system: $CHCl_3$–MeOH–H_2O mixtures (80 : 20 : 1, 80 : 20 : 2, 70 : 30 : 3, 61 : 32 : 7); visualization: 30% H_2SO_4. Column chromatography: silica gel 230—400 mesh, RP (C-18, 40 mμ) (Merck).

Plant Material A water extract of *Eurycoma longifolia* roots was purchased from Kaden Biochemicals GMBH, Porgesring 50-22113, Hamburg.

Extraction and Isolation *Eurycoma longifolia* water extract (150 g) was suspended in 2 l of water, and then partitioned thrice with 2 l of *n*-BuOH. The *n*-BuOH portion yielded 32.2 g of a brownish extract upon concentration. The *n*-BuOH extract (30.0 g) was mounted on a column packed with Sephadex LH-20 (300 g) eluted with MeOH. Fractions were pooled into five major fractions (Frs. A—E) on the basis of their TLC profiles. An aliquot of Fr. C (1.2 g) was subjected to vacuum liquid chromatography (VLC) using reversed phase material (C-18; 250 g). Elution with increasing amounts of MeOH in H_2O (15 : 85→40 : 60) yielded compound **1** (3.0 mg).

Further studies performed on the same material have resulted in the isolation of four known compounds; 14,15-β-dihydroxyklaineanone (40.0 mg), 9-methoxycanthin-6-one (18.8 mg), β-carboline-1-propionic acid (35.0 mg), and 7-methoxy-β-carboline-1-propionic acid (7.0 mg).

Compound **1** (Eurycomaoside): $[\alpha]_D^{25}$ −10.9° (c=0.5, MeOH). IR: ν_{max} 3316, 2924, 2854, 1769, 1652, 1456, 1375, 1055, 981 cm^{-1}. ^{1}H- and ^{13}C-NMR: see Table 1. HR-ESI-FT-MS: $[M+Na]^+$ at m/z 553.2260 (Calcd for $C_{25}H_{38}O_{12}Na$, 553.2261).

Acid Hydrolysis of 1 A solution of **1** (0.5 mg) in 2 N HCl (1 ml) was refluxed for 1 h. The reaction mixture was extracted with EtOAc. After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized. The lyophilized residue was dissolved in pyridine (0.1 ml) and analyzed by TLC in EtOAc–n-BuOH–H_2O (20 : 70 : 10, v/v), together with authentic sugar samples.

Acknowledgments We thank Mr. Frank Wiggers for his assistance in obtaining the 2D-NMR spectra of **1**. This work was supported in part by the United States Department of Agriculture, ARS Specific Cooperative Research Agreement No. 58-6408-2-0009, and funded by Chromadex.

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