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A QUASSINOID GLYCOSIDE FROM THE ROOTS OF *EURYCOMA LONGIFOLIA*

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Key Word Index—*Eurycoma longifolia*, Simaroubaceae, eurycomanol-2-*O*-β-D-glucopyranoside, eurycomanol, quassinoid, antimalarial

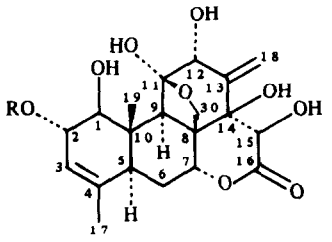
Abstract—A new quassinoid glycoside, eurycomanol-2-*O*-β-D-glycopyranoside, and eurycomanol have been isolated as antimalarial components of *Eurycoma longifolia*.

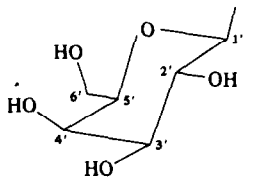
INTRODUCTION

As part of our search for antimalarial constituents among local medicinal herbs, we have studied *Eurycoma longifolia* Jack., known locally as 'Tongkat Ali'. This plant is used as a traditional treatment for persistent fevers and tertian malaria [1]. In previous study [2], we reported on the isolation and antimalarial activities of several quassinoids, the bitter principles of this plant. This communication describes the structural determination of eurycomanol-2-*O*-β-D-glycopyranoside (**1**), a new quassinoid glycoside, and the antimalarial activity of **1** and that of eurycomanol (**2**), also isolated from the same source.

RESULTS AND DISCUSSION

The *n*-butanol extract of *E longifolia* roots, on silica gel column chromatography, gave several fractions with antimalarial activity. Further purification of these fractions afforded the glycoside (**1**) and eurycomanol (**2**). The latter (**2**) was identified from spectroscopic data and by direct comparison with an authentic specimen [2, 3]. The glycoside **1** gave peaks at *m/z* 573 $[M+H]^+$, 595 $[M+Na]^+$ and 665 $[M+glycerol+H]^+$ in positive SIMS, from which the molecular formula, $C_{26}H_{36}O_{14}$, was deduced and confirmed by an elemental analysis as $C_{26}H_{36}O_{14}\cdot 2H_2O$. It was a hexoside from a peak at *m/z* 411 corresponding to $[M-C_6H_{10}O_5+H]^+$. Acid hydrolysis of **1** with 0.1 M HCl at 45° gave several degraded



1 R = 

2 R = H

products and little aglycone. The sugar moiety was however identified as D-glucose by TLC and by GC (after trimethylsilylation). Enzymatic hydrolysis with β-glucosidase gave the intact aglycone which was confirmed as **2** and indicated a β-D-glucosidic linkage in **1**. The overlapped high field ^{1}H NMR signals of **1** were fully assigned by ^{1}H–^{1}H COSY spectra and were almost identical to those of **2** except for the presence of additional sugar proton signals, which were consistent with a glucosidic structure [4]. Comparative ^{13}C NMR spectral studies of **1** and **2** (Table 1) confirmed that six additional carbons were present in **1** corresponding to those of D-glucose and a glycosylation shift of $\Delta\delta$9.7 (C_5D_5N) and $\Delta\delta$8.7 (DMSO-d_6) observed at C-2 pointed to the structure as eurycomanol-2-*O*-β-D-glucopyranoside.

Plasmodium falciparum, Gombak A strain, which was moderately chloroquine resistant (IC_{50}, 0.144 ± 0.042 μg/ml) and sensitive to quinine (IC_{50}, 0.069 ± 0.001 μg/ml), was used as the antimalarial test parasite. Further purifications were performed on the active fractions with IC_{50} of less than 5 μg/ml. Pure glycoside **1** gave an IC_{50} of 1.590 ± 0.169 μg/ml which was comparable to that for the aglycone (**2**) (IC_{50}, 1.544 ± 0.137), suggesting that eurycomanol is possibly the active component. Both **1** and **2** were however less potent than chloroquine and quinine against *P. falciparum*, Gombak A strain.

Table 1. ^{13}C NMR chemical shifts of compounds **1** and **2**

C	1*	1†	2‡	2§
1	84.0	83.1	83.8	82.2
2	82.5	79.9	72.8	71.2
3	—	124.0	126.9	125.6
4	—	134.8	135.1	133.9
5	41.3	—	41.6	39.9
6	25.5	24.4	25.7	24.6
7	71.7	69.9	71.9	70.3
8	52.8	51.2	52.7	51.2
9	47.8	46.1	47.9	46.4
10	42.5	–	42.4	41.2
11	109.6	107.8	109.8	107.8
12	81.0	79.1	81.1	79.3
13	147.9	146.0	—	146.0
14	79.4	77.7	79.4	77.7
15	76.5	75.0	76.5	75.1
16	173.8	172.2	173.8	172.4
17	21.3	20.9	21.3	21.0
18	120.1	118.9	119.3	119.0
19	10.8	9.8	10.8	9.8
30	68.0	66.9	67.9	66.3
1′	106.2	105.3		
2′	76.1	74.0		
3′	78.6	76.6		
4′	71.9	70.2		
5′	78.5	76.2		
6′	62.7	60.9		

*Measured at 25 MHz in C_5D_5N
‡Measured at 22.5 MHz in C_5D_5N
†Measured at 22.5 MHz in DMSO-d_6
§Measured at 25 MHz in DMSO-d_6 [4]
—Overlapped with solvent peaks

EXPERIMENTAL

Plant material. The roots of *E. longifolia* were collected from the Penang Botanical Garden Forest Reserve, Malaysia. A voucher specimen has been deposited at the School of Pharmaceutical Sciences, Science University of Malaysia.

Extraction, isolation and antimalarial evaluation. The dried MeOH extract (60 g) of the defatted powdered root (2 kg) was partitioned between $CHCl_3$ and H_2O. The aq. layer was subsequently extracted with *n*-BuOH, satd. with H_2O. The *n*-BuOH residue (9 g) was further subjected to CC [silica gel, $CHCl_3$–MeOH–H_2O (7:3:1)] to give eight pooled fractions. Each fraction was monitored for 50% inhibition (IC_{50}) against *Plasmodium falciparum* (Gombak A strain) cultured continuously *in vitro* with human erythrocytes [5]. Parasitaemia was determined for each serial dilution of the fractions and their IC_{50} values were calculated from linear regression line analysis. Fractions with IC_{50} of less than 5 μg/ml, were further purified by CC, prep. TLC and semiprep. HPLC [Lobar Lichroprep RP-8 (Merck) column (10 mm × 240 mm), MeOH–H_2O (2:3), refractive index, flow rate, 1.5 ml/min] to yield **1** (160 mg) and **2** (195 mg).

Eurycomanol-2-O-β-D-glucopyranoside (**1**). Colourless needles from MeOH, mp 230–233°. $[\alpha]_D^{25}$ +78° (pyridine, *c* 1.00) (Found: C, 51.29; H, 6.42. $C_{26}H_{36}O_{14}$ $2H_2O$ requires: C, 51.31; H, 6.62%). IR ν_{max}^{KBr} cm^{-1}: 3430–3360, 2900, 1745. ^{1}H NMR (400 MHz, C_5D_5N) δ: 1.49 (3H, *br s*, Me-10), 1.64 (3H, *s*, Me-4), 1.85 (1H, *ddd*, *J* = 15.0, 13.0, 2.0 Hz, H-6a), 2.13 (1H, *ddd*, *J* = 15.0, 2.0, 2.0 Hz, H-6e), 2.76 (1H, *dd*, *J* = 13.0, 2.0, H-5), 3.47 (1H, *s*, H-9), 3.98 (1H, *dd*, *J* = 8.5, 5.3, 2.3 Hz, H-5′), 4.01 (1H, *d*, *J* = 8.0 Hz, H-30), 4.09 (1H, *dd*, *J* = 8.6, 7.7 Hz, H-2′), 4.10 (1H, *d*, *J* = 8.0 Hz, H-1), 4.21 (1H, *dd*, *J* = 8.6, 8.9 Hz, H-3′), 4.25 (1H, *dd*, *J* = 8.9, 8.5 Hz, H-4′), 4.39 (1H, *dd*, *J* = 11.0, 5.3 Hz, H-6′), 4.52 (1H, *d*, *J* = 8.0 Hz, H-30), 4.56 (1H, *dd*, *J* = 11.0, 2.3 Hz, H-6′), 4.59 (1H, *m*, H-2), 4.78 (1H, *s*, H-12), 5.14 (1H, *dd*, *J* = 2.0, 2.0 Hz, H-7), 5.23 (1H, *d*, *J* = 7.7 Hz, H-1′), 5.49 (1H, *s*, H-15), 5.61 (1H, *d*, *J* = 2.0 Hz, H-18), 5.80 (1H, *br s*, H-3), 6.08 (1H, *d*, *J* = 2.0 Hz, H-18). ^{13}C NMR: Table 1. SIMS *m/z*: 687 [M + glycerol + Na]$^+$, 669 [M − H_2O + glycerol + Na]$^+$, 665 [M + glycerol + H]$^+$, 595 [M + Na]$^+$, 573 [M + H]$^+$, 411 [M − $C_6H_{10}O_5$ + H]$^+$.

Eurycomanol (**2**). Colourless needles from EtOH, mp 272–274° (Found: C, 57.01; H, 6.48. $C_{20}H_{28}O_8$, $\frac{1}{2}H_2O$ requires: C, 57.27; H, 6.49%). ^{1}H NMR (400 MHz, C_5D_5N) δ: 1.63 (3H, *br s*, Me-10), 1.78 (3H, *s*, Me-4), 1.93 (1H, *ddd*, *J* = 15.0, 13.0, 2.5 Hz, H-6a), 2.19 (1H, *ddd*, *J* = 15.0, 2.5, 2.5 Hz, H-6e), 2.85 (1H, *dd*, *J* = 13.0, 2.5 Hz, H-5), 3.61 (1H, *s*, H-9), 4.09 (1H, *d*, *J* = 8.0 Hz, H-1), 4.09 (1H, *d*, *J* = 8.0 Hz, H-30), 4.61 (1H, *d*, *J* = 8.0 Hz, H-30), 4.68 (1H, *m*, H-2), 4.86 (1H, *s*, H-12), 5.17 (1H, *dd*, *J* = 2.5, 2.5 Hz, H-7), 5.57 (1H, *s*, H-15), 5.68 (1H, *d*, *J* = 2.0 Hz, H-18), 5.84 (1H, *br s*, H-3), 6.16 (1H, *d*, *J* = 2.0 Hz, H-18). ^{13}C NMR: Table 1.

Acid hydrolysis of **1**. An aq. soln (10 ml) of **1** (30 mg) in 0.1 M HCl was warmed at 45° for 4 days. The reaction mixture was neutralized with concn. $Ba(OH)_2$ $8H_2O$ and then centrifuged. The supernatant liquid was evapd. *in vacuo* and the residue was subjected to prep. TLC ($CHCl_3$–MeOH–H_2O, 6:4:1) to isolate the sugar portion (5 mg). This was identified as D-glucose from direct comparison against sugar standards on TLC (NaH_2PO_4 $2H_2O$–silica gel, *iso*-PrOH–Me_2CO–0.1 M lactic acid, (2:2:1) and by GC of its TMS ethers [HMDS (100 μl) + TMCS (50 μl) + sugar (2–3 mg) in 0.5 ml dry pyridine] under the following conditions: 5% SE-30 Gas Chrom Q, 100–120 mesh column (1.75 mm × 2 m), column temp. 180° (isothermal), injection temp. 250°, FID temp. 250°, flow rate, 30 cm^3/min.

Enzymatic hydrolysis of **1**. An aq. soln (5 ml) of **1** (10 mg) was treated with Naringinase (Sigma No. N-8631, 250 mg; β-glucosidase activity 5 units/g) at 37° for 3 days. The reaction mixture

was filtered and then freeze-dried. The residue was treated with EtOH to yield **2** (6 5 mg) as colourless needles (undepressed mixed melting and superimposable IR spectra with authentic **2**. The sugar was reconfirmed as D-glucose by the procedure as described for acid hydrolysis

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A STEROID FROM *CALOTROPIS PROCERA*

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Key Word Index—*Calotropis procera*, Asclepiadaceae; procesterol; hydroxyketone, (24*S*)-24-ethyl stigmast-4-en-6α-ol-3-one

Abstract—Procesterol, a new steroidal hydroxy ketone, has been isolated from the fresh and undried flowers of *Calotropis procera*. The chemical and spectral studies identified it as a C-6, C-24 diepimer of stigmast-4-en-6β-ol-3-one.

INTRODUCTION

Calotropis procera R. Br. (Asclepiadaceae) grows widely in tropical regions of Asia and Africa. The milky juice of this plant is used by the natives of India as a purgative, while the flowers are considered digestive, stomachic, tonic and useful in cough, asthma, catarrh and loss of appetite. The root bark is said to promote secretions and to be useful in treating skin diseases, enlargement of the abdominal viscera, intestinal worms, ascites and anasarca [1]. We have previously reported a new terpenoid from this plant [2] Following further studies on the fresh and undried flowers a new keto-sterol has been isolated and named as procesterol Its stereostructure has been elucidated as (24*S*)-24-ethyl-stigmast-4-en-6α-ol-3-one, through chemical and spectral studies

RESULTS AND DISCUSSION

Procesterol (**1**) crystallized from acetone, mp,. 167°, $[\alpha]_D+21.5°$; its HRMS gave a molecular ion peak at *m/z* 428.6654 corresponding to the molecular formula $C_{29}H_{48}O_2$ (calcd 428.7010) The molecular ion peak was also confirmed by FD mass spectrometry [3]. The strong absorption band at 1675 and 1640 cm^{-1} in its IR spectrum revealed the presence of an α,β-unsaturated keto function and another band at 3575 cm^{-1} was due to hydroxyl absorption. The secondary nature of alcoholic group in **1** was shown by its oxidation with Jones reagent to a diketone **1a**. The ^1H NMR spectrum showed the presence of 6-methyl groups out of which one was primary [δ0.847, 3H, *t*, $J=7.3$ Hz], three secondary [δ0.905, 0.827, 0.847, 3×3H, *d*, $J=6.3$, 6 7 Hz] and two tertiary [δ1.397, 0 760, 2×3H, *s*]. Further signals were observed for vinylic proton [δ5.780, 1H, *d*, $J=1.8$ Hz] and the proton geminal to hydroxyl group [δ4.450, 1H, *oct.*, $J=12$ 1, 4.7, 1.8 Hz]. The ^{13}C NMR spectrum revealed 29 carbon atoms in the molecule. The multiplicity assignments were made by DEPT experiments [4] which showed the presence of six methyl, 10 methylene and nine methine carbon atoms. The quaternary carbon atoms were determined by subtracting DEPT spectra from broad band ^{13}C NMR spectrum.

Procesterol showed in its mass spectrum prominent [M − side chain]$^+$ (*m/z* 287), [M − side chain − 42]$^+$ (*m/z*

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