CHEMICAL CONSTITUENTS FROM THE CHLOROFORM EXTRACT OF THE ROOT OF *CALOTROPIS GIGANTEA* (LINN.), ASCLEPIDACEAE

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TÓM TẮT

THÀNH PHẦN HÓA HỌC CAO CHLOROFORM CỦA RỄ CÂY BỒNG BỒNG (CALOTROPIS GIGANTEA) HỌ THIÊN LÝ (ASCLEPIDACEAE)

*From the root of Calotropis gigantea, six compounds were isolated: 12-O-benzoyllineolon (**1**), 12-O-benzoyldeacetylmetaplexigenin (**2**), calotropone (**3**), 2,3-dimethoxyphenol (**4**), 2,5-dimethoxyphenol (**5**), 2-formyl-5-hydroxymethylfuran (**6**). The chemical structure of these compounds were elucidated by their NMR spectra and comparison with references.*

1. INTRODUCTION

Calotropis gigantea (Linn.) is a plant of Asclepidaceae family that wildly grows in many areas in the world such as Indonesia, China, India, Vietnam ... The leaves of *C. gigantea* were used in the treatment of paralysis, swellings and intermittent fevers. Root barks were used as the treatment of asthma, bronchitis and dyspepsia. Flowers could cure asthma, catarrh, anorexia, helmintic infection and fever [1].

The chemical constituents of *Calotropis gigantea* have been extensively investigated, leading to the isolation of many cardenolides, flavonoids, terpenes, pregnanes [2].

In this paper, we reported the isolation and structural elucidation of six compounds: 12-*O*-benzoyllineolon (**1**), 12-*O*-benzoyldeacetyl metaplexigenin (**2**), calotropone (**3**), 2,3-dimethoxyphenol (**4**), 2,5-dimethoxyphenol (**5**), 2-formyl-5-hydroxymethylfuran (**6**).

2. EXPERIMENTAL

2.1. General

The NMR spectra were measured on a Bruker Avance III 500 spectrometer, at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The HR-ESI-MS were recorded on a Brucker MicrOTOF-QII mass spectrometer. All spectra were recorded at the Central Analytical Laboratory, University of

Science, Vietnam National University, HCM city.

2.2. Plant material

Fresh roots of *Calotropis gigantea* (Linn.) were collected in Phan Thiet city, Binh Thuan province, Vietnam in May 2011. The scientific name of plant was identified by a Dr. Vo Van Chi.

2.3. Extraction and isolation

Fresh roots were washed, dried, and grounded into powder (20 kg) and then was exhaustively extracted with MeOH (30 L, reflux, 3 h x 3) to yield MeOH extract (900 g).

The MeOH extract was suspended in H_2O and successively partitioned with petroleum ether (PE), $CHCl_3$, EtOAc and *n*-butanol to yield petroleum ether extract (200 g), $CHCl_3$ extract (180 g), EtOAc extract (80 g) and *n*-butanol extract (80 g). The $CHCl_3$ extract (180 g) was re-chromatographed over silica gel eluted with $CHCl_3$-MeOH in order of increasing polarity to obtain twelve fractions (N1-N12). Fraction N3 was rechromatographed on silica gel with $CHCl_3$-MeOH (95:5) and followed by normal-phase preparative TLC with PE-$CHCl_3$ (9:1), to give **1** (6 mg) and **5** (4 mg); Fraction N4 was further separated by silica gel column chromatography, followed by normal-phase preparative TLC with $CHCl_3$-EtOAc (8:2), to give **2** (5 mg) and **6** (4 mg). Fraction N5 was re-chromatographed with $CHCl_3$/MeOH, followed by normal-phase preparative TLC with $CHCl_3$/MeOH (95:5) to yield **3** (5 mg) and **4** (6 mg).

12-O-Benzoyllineolon (1). white amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 8.10 (2H, *d*, *J* = 7.5 Hz, H-2' and H-6'), 7.54 (2H, *t*, *J* = 7.5 Hz, H-3' and H-5'), 7.60 (1H, *t*, *J* = 7.5 Hz, H-4'), 5.34 (1H, *t*, *J* = 4.0 Hz, H-6), 3.42 (1H, *m, H-3*), 4.92 (1H, *dd*, *J* = 12.0, 4.0 Hz, H-12), 3.25 (1H, *dd, J* = 10.5, 5.5 Hz, H-17), 1.33 (3H, *s,* H-18), 1.18 (3H, *s,* H-19), 2.18 (3H, *s,* H-21). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 129.2 (C-2' and C-6'), 130.1 (C-3' and C-5'), 134.0 (C-4'), 71.8 (C-3), 77.7 (C-12), 77.3 (C-8), 87.2 (C-14), 119.0 (C-6), 140.4 (C-5), 166.7 (C-7'), 216.9 (C-20), 38.0 (C-10), 54.6 (C-13), 59.0 (C-17), 12.6 (18-CH_3), 18.7 (19-CH_3), 32.6 (21-CH_3).

12-O-Benzoyldeacetylmetaplexigenin (2). white amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 7.95 (2H, *d*, *J* = 7.5 Hz, H-2' and H-6'), 7.48 (2H, *t*, *J* = 7.5 Hz, H-3' and H-5'), 7.65 (1H, *t*, *J* = 7.5 Hz, H-4'), 5.27 (1H, *t*, *J* = 3.0 Hz, H-6), 3.45 (1H, *m, H-3*), 4.83 (1H, *dd*, *J* = 11.3, 4.3 Hz, H-12), 1.67 (3H, *s,* H-18), 1.17 (3H, *s,* H-19), 2.06 (3H, *s,* H-21). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 129.3 (C-2' and C-6'), 130.3 (C-3' and C-5'), 134.0 (C-4'), 72.4 (C-3), 74.6 (C-12), 74.9 (C-8), 89.8 (C-14), 119.0 (C-6), 140.6 (C-5), 166.6 (C-7'), 216.9 (C-20), 37.9 (C-10), 58.9 (C-13), 93.0 (C-17), 10.4 (18-CH_3), 18.5 (19-CH_3), 27.7 (21-CH_3).

Calotropone (3). yellow amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 7.93 (2H, *d*, *J* = 7.5 Hz, H-2' and H-6'), 7.44 (2H, *t*, *J* = 7.5 Hz, H-3' and H-5'), 7.56 (1H, *t*, *J* = 7.5 Hz, H-4'), 5.41 (1H, *t*, *J* = 3.0 Hz, H-6), 3.51 (1H, *m, H-3*), 1,82 (1H, *m,* H-8), 4.81 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-12), 1.41 (3H, *s,* H-18), 0.99 (3H, *s,* H-19), 2.06 (3H, *s,* H-21). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 128.6 (C-2' and C-6'), 129.7 (C-3' and C-5'), 133.3 (C-4'), 71.6 (C-3), 73.3 (C-12), 37.2 (C-8), 88.4 (C-14), 121.2 (C-6), 139.8 (C-5), 165.5 (C-7'), 209.4 (C-20), 36.9 (C-10), 58.9 (C-13),

91.4 (C-17), 7.8 (18-CH_3), 19.6 (19-CH_3), 27.6 (21-CH_3).

2,3-Dimethoxyphenol (4). yellow amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 7.73 (1H, *dd*, *J*=7.8, 1.7 Hz, H-6), 7.21 (1H, *t*, *J*=8.0 Hz, H-5), 7.16 (1H, *dd*, *J*=8.0, 1.7 Hz; H-4), 4,09 (3H, *s*, 2-OCH_3), 3,91 (3H, *s*, 3-OCH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 165.0 (C-1), 148.2 (C-2), 152.1 (C-3), 117.6 (C-4), 125.0 (C-5), 124.1 (C-6), 66.2 (2-OCH_3), 56.2 (3-OCH_3).

2,5-Dimethoxyphenol (5). yellow oil, ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 7.19 (1H, *dd*, *J*=6.7, 2.6 Hz, H-4), 7.12 (1H, *d*, *J*=6.7 Hz, H-3), 7.09 (1H, *d*, *J*=2.6 Hz, H-6), 3.88 (6H, *s*, 2-OCH_3 và 5-OCH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 131.7 (C-1), 148.6 (C-2), 125.2 (C-3), 122.3 (C-4), 154.4 (C-5), 115.7 (C-6), 61.9 (2-OCH_3), 56.6 (5-OCH_3).

2-Formyl-5-hydroxymethylfuran (6). yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 6.52 (1H, *d*, *J*=3.5 Hz, H-3), 7.21 (1H, *d*, *J*=3.5 Hz, H-4), 9.61 (1H, *s*, -CHO), 4,69 (2H, *s*, -OCH_2-). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 160.4 (C-2), 109.9 (C-3), 122.3 (C-4), 152.5 (C-5), 177.6 (-CHO), 57.7 (-OCH_2-).

3. RESULTS AND DISCUSSION

Compound 1. ^{13}C-NMR spectrum of compound **1** suggested the presence of a benzoyl group δ 131.6 (C-1'), 129.2 (C-2', C-6'), 130.1 (C-3', C-5') and 134.0 (C-4'); two oxygenated sp^3 methine carbons at δ 71.8 (C-3) and 77.7 (C-12), two aliphatic sp^3 methine carbons at δ 45.3 (C-9) and 59.0 (C-17), four sp^3 quatenary carbon signals at δ 77.3 (C-8), 38.0 (C-10), 54.6 (C-13) and 87.2 (C-14); two olefinic carbon signal at δ 119.0 (C-6) and 140.4 (C-5), one ketone carbon signal at δ 216.9 (C-20); one carboxyl signal at δ 166.7 (C-7'); and three methyl group signals at δ 12.6 (C-18), 18.7 (C-19) and 32.6 (C-21). The ^{1}H-NMR spectrum showed the signal of benzoyl group [δ 8.10 (2H, *d*, *J* =7.5 Hz, H-2', H-6'), 7.54 (2H, *t*, *J* =7.5 Hz, H-3' and H-5'), 7.65 (1H, *t*, *J* =7.5 Hz, H-4')]; one olefinic proton at δ 5.27 (1H, *t*, *J* = 4.0 Hz, H-6), two oxygenated methine protons at δ 3.42 (1H, *m,* H-3) and 4.92 (1H, *dd*, *J* =12.0; 4.0 Hz, H-12) and the singlet signals of three methyl groups at δ 1.33 (*s,* H-18), 1.18 (s, H-19) and 2.18 (s, H-21). Base on these characteristics, we suggested that compound **1** was a pregnane-type sterol. The HMBC spectrum showed cross-peak of ^3J correlation between H-12 and C-7' so the benzoyl group linked to pregnane skeleton at C-12. Base on the NMR spectra and literature [3], compound **1** was identified as 12-*O*-benzoyllineolon.

Compound 2. Spectrocopic data of compound **2** showed that it was also a pregnane-type sterol because of the similarity in NMR spectra of **2** and those of **1**. However, the ^{1}H and ^{13}C-NMR spectra of **2** showed that compound lost one methine proton signal and had one more quartenary carbon**.** Moreover, NMR data of **2** showed good compatibility to the ones in literature [4] so compound **2** was proposed to be 12-*O*-benzoyldeacetylmetaplexigenin.

Compound 3. The similarity between NMR spectra of **3** and **1** indicated that **3** was also a pregnane-type sterol. Comparing the ^{13}C-NMR spectral data of **3** with those of **1** showed that **3** had also two aliphatic sp^3 methine carbons (C-8 and C-9) and two oxygenated quatenary carbons (C-14 and C-17). However the ^{13}C-NMR spectra of **3**

lost a signal of quatenary carbon at 74.6 (C-8), and appeared a signal of another quatenary carbon at 91.4 (C-17), that indicated that hydroxyl group had migrated from C-8 to C-17 in compound **3**. Through comparison of NMR data with the ones in the literature [3], compound **3** was identified as calotropone.

Compound 4. The ^{13}C-NMR spectrum of **4** showed eight signals including three aromatic quatenary carbons at δ 165.0, 148.2, and 152.1; three aromatic methine carbons signals at 117.6, 125.0, 124.1; two methoxyl carbons at δ 56.2 and 66.2. The ^{1}H-NMR of **4** showed two doublet of doublets signals at δ 7.16 (*dd*, *J*=8.0; 1.7 Hz) and 7.73 (*dd*, *J*=8.0; 1.7 Hz), one triplet signal at δ 7.21 (*t*, *J*=8.0 Hz) and two methoxyl signals at δ 4.09 and 3.91. The HSQC and HMBC experiments allowed the assignment of all proton and carbon signals of **4** as 2,3-dimethoxyphenol [5].

Compound 5. The ^{1}H-NMR spectrum of **5** showed two doublet signals at δ 7.09 (*d*, *J*=7.0 Hz), and 7.12 (*d*, *J*=2.6 Hz); one doublet of doublets signal at δ 7.19 (*dd*, *J*=7.0, 2.6 Hz) corresponding to a 1,3,4-trisubstituted phenyl group (*ABX* system) and two methoxy groups at δ 3.88 and 3.79. The ^{13}C-NMR spectrum also showed the presence of one aromatic (δ 131.7, 148.6, 154.4, 125.2, 122.3 and 115.7); and two methoxyl groups at δ 56.6 and 61.9. The HSQC and HMBC experiments allowed the assignment of all proton and carbon signals of **5** as 2,5-dimethoxyphenol [5].

Compound 6. The ^{13}C-NMR spectrum of compound **6** exhibited one aldehyde carbon at δ 177.6, two oxygenated olefinic quatenary carbons at δ 160.4 and 152.5, one oxygenated methylene carbon at δ 57.7. The ^{1}H-NMR spectrum of **6** showed two olefinic methine protons at 7.21 (1H; *d*; *J*=3.5 Hz, H-3) and 6.52 (1H; *d*; *J*=3.5 Hz, H-4) which indicated the presence of a furan ring. In addition, one aldehyde proton at δ 9.61 (1H, *s*, -CHO) and one oxygenated methylene at 4.69 (2H, *s*, -CH_2OH) were observed. The ^{1}H and ^{13}C-NMR data showed good compatibility to the ones in literature [6], so compound **6** was proposed to be hydroxymethylfurfural.

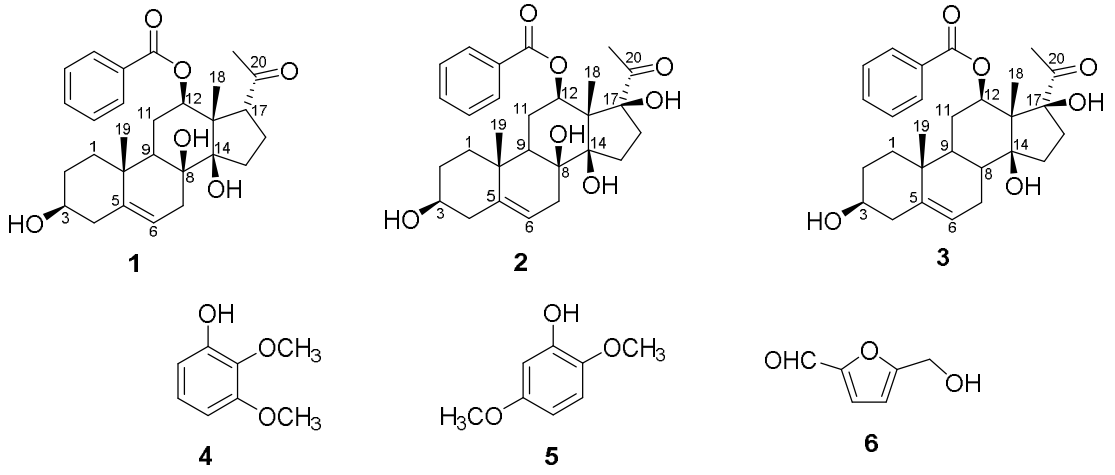

Figure 1. Chemical structure of compounds 1-6.

From the roots of *Calotropis gigantea* (Linn.), compounds **1**, **2**, **3**, **4**, **5**, **6** were isolated. Among them, **4** and **5** were first isolated from root of this plant. Further the chemical constituent and bioactivity of *C. gigantea* was carried out.

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