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Prenylated isoflavonoids isolated from the leave of *Cudrania tricuspidata* Carr. Bur.

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***Abstract*— By various chromatography methods, three known isoflavones were isolated from the leave of *Cudrania tricuspidata*. Their structures were identified to be lupalbigenin (1), isolupalbigenin (2) and wighteone (3) by 1D and 2D-NMR analysis and in comparison with previous reported data.**

Keywords—Cudrania tricuspidata; isoflavonoid; lupalbigenin; isolupalbigenin, wighteone.

I. INTRODUCTION

Cudrania tricuspidata belong to Moraceae family, widely grow in Asia, China, Korea and Vietnam, treatment of inflammation, cancer, influenza, hepatitis, and neuritis [1-5]. In China, root and leave used to produce herb tea and functional drinking thousands year ago [6]. Recent decades, *C. tricuspidata* is one of the most important medicinal plants treating cancer in Korea [7]. This plant has been reported rich of prenylated flavonoids and xanthones which possessed anti-cancer, anti-inflammation, anti-obesity, and neuroprotective effects [2, 8-10]**.** There is few research about chemical components and bioactivities of this plant in Vietnam. In our present working, structure of three isoflavonoids, lupalbigenin (**1**), isolupalbigenin (**2**) and wighteone (**3**) were elucidated.

II. MATERIALS AND METHODS

A. General experiment procedures

The nuclear magnetic resonance (NMR) spectra were measured using a BRUKER avance 500 MHz spectrometer (Bruker, Karlsruhe, Germany) with TMS as the internal standard. The electrospray ionization (ESI) mass spectra were performed on an AGILENT 1100 LC-MSD trap spectrometer. Silica gel (70-230, 230-400 mesh, Merck, Whitehouse Station, NJ, USA), reversed phase C-18 resins (75 µm, YMC Co. Ltd., Kyoto, Japan), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used as absorbents in the column chromatography (CC). Thin layer chromatography (TLC) plates (silica gel 60 F_{254} and reversed phase C-18 F_{254}, 0.25 µm) were purchased from Merck KGaA (Merck, Darmstadt, Germany). Spots were detected under UV radiation (254 and 365 nm) and by spraying the plates with 10% H_2SO_4 followed by heating with a heat gun.

B. Plant materials

The leave of *C.tricuspidata* were collected at Vu Thu, Thai Binh province, Vietnam in April 2015. Its scientific name was identified by MSc Do Thanh Tuan, Thaibinh Medical University, Thai Binh province. A voucher specimen (TB15.2015) is deposited at the Herbarium of Thaibinh Medical University.

C. Extraction and Isolation

The dried and powdered of leave of C. tricuspidata (1.0 kg) were sonically extracted with methanol at 50 ^{0}C for three times (4.0 L each). After removal of the solvents under vacuo obtained 200 g crude extract. This crude extract was suspended with distilled water (1.5 L) and successively partitioned with chloroform, ethyl acetate (three times, 1.5 L each) to give respective soluble extracts, chloroform (MQC, 80.0 g), ethyl acetate (MQE, 30.0 g) and water layer. The MQC fraction was roughly separated on a normal silica gel column, eluting with gradient of solvent systems of *n*-hexane in acetone (0-100% acetone, 1.0 L stepwise) to yield eight fractions, MQC1 - MQC8. Fraction MQC5 (6.0 g) continuously separated on a normal silica gel column with eluted solvent system *n*-hexane/acetone (4/1, v/v) to obtain three sub-fractions, MQC5A - MQC5C. The sub-fraction MQC5A (1.4 g) was purified firstly on a reversed-phase C-18 column eluting with methanol/water (1/1, v/v), and further purified on a normal silica gel column eluting with dichloromethane/methanol (20/1, v/v) to obtain two compounds, **1** (30 mg) and **2** (23 mg). The MQC7 fraction was chromatographed on a reversed-phase C-18 column eluting with acetone/water (2.5/1, v/v) to yield four sub-fractions, MQC7A - MQC7D. Compound 3 (21 mg) was obtained from sub-fraction MQC7D (0.8 g) on a normal silica gel column, eluted with *n*-hexane/acetone (4/1, v/v).

- **Lupalbigenin (1):** yellow amorphous powder, $C_{25}H_{26}O_5$ (M 406). ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1.
- **Isolupalbigenin (2):** yellow amorphous powder, $C_{25}H_{26}O_5$ (M 406). ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1.
- **Wighteone (3):** yellow amorphous powder, $C_{20}H_{18}O_5$ (M 338). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 1.

TABLE I. NMR DATA FOR COMPOUNDS **1** - **3**

	1			**2**		**3**		
C	$^{\#}\delta_C$	$\delta_C{}^{a,c}$	$\delta_H{}^{a,d}$ (mult., *J* = Hz)	$\delta_C{}^{a,c}$	$\delta_H{}^{a,d}$ (mult., *J* = Hz)	$^{@}\delta_C$	$\delta_C{}^{b,c}$	$\delta_H{}^{b,d}$ (mult., *J* = Hz)
2	152.8	152.3	7.74 (s)	152.7	7.89 (s)	153.8	154.5	8.02 (t. 3.5)
3	123.7	123.7	-	123.4	-	122.3	124.6	-
4	181.2	180.9	-	181.3	-	180.4	182.3	-
5	159.8	159.3	-	159.7	-	159.0	160.5	-
6	110.1	111.6	-	98.9	6.29 (s)	111.2	113.1	-
7	161.6	161.7	-	161.8	-	162.1	163.6	-
8	94.0	93.2	6.34 (s)	106.7	-	93.0	93.9	6.39 (d. 3.5)
9	156.3	156.0	-	155.4	-	155.5	158.7	-
10	106.0	105.4	-	105.4	-	105.4	106.1	-
1'	123.2	122.4	-	122.4	-	121.5	123.5	-
2'	130.7	130.2	7.17 (d. 3.0)	130.2	7.20 (d. 2.0)	130.3	131.4	7.38 (d. 8.5)
3'	127.2	127.9	-	127.9	-	115.2	116.3	6.87 (d. 8.5)
4'	154.9	154.7	-	154.8	-	157.5	157.6	-
5'	116.0	115.5	6.83 (d. 8.0)	115.3	6.85 (d. 8.0)	115.2	116.3	6.87 (d. 8.5)
6'	128.3	127.8	7.19 (dd. 8.5. 2.0)	127.8	7.23 (dd. 8.0. 2.0)	130.3	131.4	7.38 (d. 8.5)
1"	29.7	28.7	3.37 (t. 8.0)	28.8	3.38 (d. 7.0)	21.2	22.3	3.37 (br s)
2"	121.6	122.0	5.35 (t. 7.0)	122.0	5.35 (t. 7.0)	122.3	123.4	5.25 (t. 7.0)
3"	135.2	133.3	-	133.5	-	130.8	132.1	-
4"	25.7	25.6	1.74 (s)	25.7	1.75 (s)	25.6	25.9	1.68 (s)
5"	17.9	17.7	1.75 (s)	17.7	1.74 (s)	17.8	17.9	1.79 (s)
1'''	21.4	21.4	3.37 (t. 8.0)	21.4	3.43 (d. 6.5)			
2'''	121.2	121.9	5.28 (t. 7.0)	122.0	5.22 (tm. 7.0)			
3'''	136.1	132.6	-	132.2	-			
4'''	25.7	25.6	1.69 (s)	25.6	1.69 (s)			
5'''	17.8	17.7	1.80 (s)	17.8	1.80 (s)			

*) Overlapped signals.

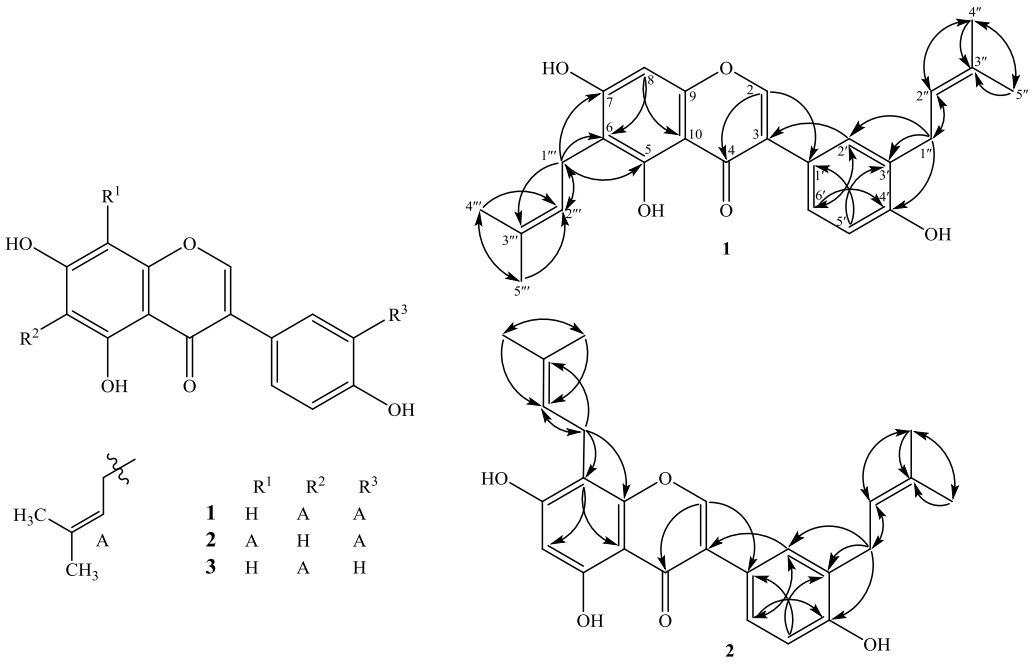

Fig1. Chemical structures of compounds **1**-**3** and key HMBC correlations of **1 and 2**

III. RESULTS AND DISCUSSION

Compound **1** was obtained as yellow amorphous powder. The proton ^{1}H-NMR of **1** indicated two γ,γ-dimethylallyl groups and an 1,3,4-trisubstituted at 7.18 (1H, dd, *J* = 8.5, 2.0 Hz), 7,17 (1H, d, *J* = 3.0 Hz) and 6.83 (1H, d, *J* = 8.0 Hz). The first group was obtained at δ_H 1.69 and 1.80 (3H each, s), 3.37 (2H, t, *J* = 8.0 Hz), and 5.28 (1H, t, *J* = 7.0 Hz). The remaining group was obtained at δ_H 1.74 and 1.75 (3H x 2, s), 3.37

(2H, d, J = 8.0 Hz) and 5.35 (1H, t, J = 7.0 Hz). The carbon ^{13}C-NMR and DEPT spectra of **1** revealed signals of 25 carbons which were classified to be 12 non-protonated carbons, 7 methine carbons, 2 methylene carbons and 4 methyl groups. Basically, the NMR spectra of **1** were closed to those of lupalbigenin [11] (Table 1). The position of two γ,γ-dimethylallyl groups were determined with the aid of HMBC analysis. The HMBC correlations between two methyl groups δ_H 1.69 (H-4‴) and 1.80 (H-5‴) and carbon δ_C 121.9 (C-2‴); between methylene signal δ_H 3.37 (H-2‴) and carbons δ_C 159.3 (C-5)/ 111.6 (C-6)/ 161.7 (C-7)/ 121.9 (C-2‴) indicated the location of the first γ,γ-dimethylallyl group at carbon C-6. The location of second γ,γ-dimethylallyl group at carbon C-3′ was assigned with the aid of HMBC correlations between methylene signal δ_H 3.37 (H-2″) and carbons δ_C 130.2 (C-2′)/ 127.9 (C-3′)/ 154.7 (C-4′)/ 122.0 (C-2″). Consequently, compound **1** was elucidated to be lupalbigenin.

Compound **2** was also obtained as yellow amorphous powder. Analysis of ^{1}H-, ^{13}C-NMR of **2** also indicated an isoflavone with two γ,γ-dimethylallyl groups as **1**. The difference between **1** and **2** is the replacement of carbon methine at δ_C 93.2 (C-8) in **1** by a carbon methine at δ_C 98.9 (C-6) in **2** (Table 1) suggested the new location of the γ,γ-dimethylallyl group at C-8. The HMBC correlations between methylene signal δ_H 3,43 (H-1‴) and carbons δ_C 161,8 (C-7)/ 106,7 (C-8)/ 155,4 (C-9) confirmed that. Based above NMR evidence, compound **2** was determined to be isolupalbigenin [12].

Compound **3** was also obtained as yellow amorphous powder. The proton ^{1}H-NMR of **1** showed two doublet signals of a 1,4-disubtituted aromatic ring at δ_H 7.38 (2H, d, J = 8.5 Hz), 6.87 (2H, d, J = 8.5 Hz); and a γ,γ-dimethylallyl group at δ_H 1.68 và 1.79 (3H each, s), 3.37 (2H, brs), and 5.25 (1H, t, J = 7.0 Hz). The carbon ^{13}C-NMR and DEPT spectra of **2** showed 20 carbons which were classified to be 11 non-protonated carbons, 6 methine carbons, 1 methylene carbon and 2 methyl groups. Comparison with NMR data of **1** and **2**, the position of γ,γ-dimethylallyl group was assigned at C-6 (Table 1). Consequently, compound **3** was elucidated to be wighteone [13].

All compounds showed strong activity toward human leukemia HL-60 cells with IC_{50} ranging from 5.1 ± 0.1 to 18.0 ± 1.7 μM. Mitoxantrone, an anticancer agent treating acute myeloid leukemia, was used as positive control. Results of NO measurement indicated that compound **3** was good NO inhibitor with IC_{50} value 18.0 μM [14]. Thus, species C. tricuspidata could be used as anti-cancer and anti-inflammatory agent.

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