

# Coumarins from Myanmar edible fruit tree (*Casimiroa edulis*)

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## Coumarins from Myanmar edible fruit tree (*Casimiroa edulis*)

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This research expresses the phytochemical study from the Myanmar edible fruit tree, *Casimiroa edulis* (Rutaceae). The result revealed that the isolation and identification of two furanocoumarins (bergapten **1** and isopimpinellin **2**) from the stem bark of this plant. Their molecular structures were elucidated and identified by using NMR spectroscopy in combination with IR, UV and HRMS spectra data, respectively. Furthermore, these two compounds were investigated for their anti-diabetic activity. According to the result, bergapten **1** and isopimpinellin **2** are not essentially good for anti-diabetic activity. This is the first report of two furanocoumarins from the Myanmar edible fruit tree.

Keywords: *Casimiroa edulis*, furanocoumarins, NMR spectroscopy, anti-diabetic activity.

### Introduction

*Casimiroa edulis* La Llave (Rutaceae) is a genus of comprising only 10 species which is distributed in tropical and subtropical regions, including Myanmar. Among them, *Casimiroa edulis* is the well-known species<sup>1,2</sup>. The traditional used and the pharmacological studies of the leaves and seeds of *Casimiroa edulis* displayed various biological activities. In Myanmar, this plant is named Thar-kyar-thee.

Phytochemical studied from genus *Casimiroa* assist to the separation of various chemical constituents such as 18 flavonoids (isolated from leaves, fruits, and seeds), 24 alkaloids (isolated from leaves, fruits, bark, and seeds), and 16 coumarins (isolated from leaves, fruits, and seeds). Pharmacological investigations of isolated flavonoids are antioxidant, anti-mutagenic, solid tumor selective cytotoxicity, vasodilation and radical-scavenging activities, the investigations of isolated alkaloids are anti-mutagenic, solid tumor selective cytotoxicity, anti-hypertensive, cardiovascular activities, and the investigations of isolated coumarins are anti-coagulant, vasodilation and radical-scavenging, adipogenesis, solid tumor selective cytotoxicity activities, respectively<sup>3-14</sup>. This

paper deals with the structure elucidation of two furanocoumarins from the stem bark of *Casimiroa edulis*.

### Experimental

#### General:

The infrared spectra (IR) were obtained on FTIR-8400S (Shimadzu) using KBr. UV spectra were examined in MeOH by using UV-Vis Shimadzu spectrometer. Column chromatography (CC) was achieved on silica gel (BW-820H). Analytical thin-layer-chromatography (TLC) was completed at room temperature on pre-coated Kieselgel silica gel 60 F<sub>254</sub> (Merck) aluminium plates. Melting points were determined by fisher john melting point apparatus. All NMR spectra were checked in CDCl<sub>3</sub> by using a Bruker Avance 3 (<sup>1</sup>H: 600 MHz and <sup>13</sup>C: 151 MHz) with TMS as an internal standard. Chemical shifts are described in part per million ( $\delta$ , ppm) based on their solvent signal (chloroform-*d*:  $\delta_H$  7.26,  $\delta_C$  77.16) coupling constants *J* value in Hertz (Hz). HR-FAB-MS was attained with a JEOL JMS HX-110 mass spectrometer.

#### Plant material:

*Casimiroa edulis* La Llave was collected from Namp-see

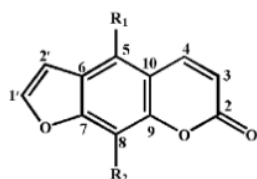
Village, Taunggyi (Shan State), Myanmar, in August 2016, and was identified by the Professor of Botany Department, University of Taunggyi.

#### Extraction and isolation:

The dry sample of *Casimiroa edulis* (1 kg) was exhaustively extracted with MeOH (3 L) at room temperature for four weeks. The methanol extract was concentrated under pressure using rotator evaporator for dryness. Then, the methanolic extract was partition between *n*-hexane and MeOH (300 ml×3, v/v). The concentrated MeOH extract (50 g) was subjected to vacuum liquid chromatography (VLC) and eluted with *n*-hexane-EtOAc in a stepwise gradient solvent system (10:0, 6:4, 0:10, v/v) to afford several fractions. Column eluate was collected in 150 ml glass bottles to yield 28 fractions and examined under UV. Based on analytical TLC, the fractions were grouped and evaporated on a rotary evaporator to afford six fractions (J-1 to J-6). Fractions J-5 (2 g) were additionally purified by silica gel column chromatography with a gradient solvent system of *n*-hexane in EtOAc (10:0, 0:10) to give colourless needles of compound **1**, and pale-yellow crystalline solids of compound **2**.

#### Results and discussion

Compound **1** was obtained as colorless needle crystals. Its melting point was 186–206°C. Based on the molecular ion peak at  $m/z$  217.0501  $[M + H]^+$  (Calcd. 216.0423) in the HR-FAB-MS, the molecular formula was determined as  $C_{12}H_8O_4$ . IR spectrum informed the characteristic of sharp bands at 3143, 3115, 3088, 3010, 2924, 2852, 1737, 1720, 1625 and 1581  $cm^{-1}$  which could be assigned to  $-OCH_3$ ,  $sp^2-CH$ ,  $sp^3-CH$ , lactone  $C=O$ ,  $C=C$  ring skeletal stretching vibration, respectively. The UV spectrum showed the bands



Comps.	R <sub>1</sub>	R <sub>2</sub>
<b>1</b>	OCH <sub>3</sub>	H
<b>2</b>	OCH <sub>3</sub>	OCH <sub>3</sub>

Fig. 1. Chemical structure of compound **1** and **2**.

at  $\lambda_{max}$  221, 249, 259, 268, and 311 nm, which were typical of a furanocoumarin skeleton. The  $^1H$  NMR spectrum was exhibited as an easily distinguishable pair of doublets at  $\delta_H$  6.28 (d, 1H, 9.8 Hz, H-3) and  $\delta_H$  8.16 (d, 1H, 9.8 Hz, H-4). It also displayed two furan doublets at  $\delta_H$  7.60 and 7.02 (each, d, 1H, 2.4 Hz, H-2' and H-3'), and an aromatic singlet at  $\delta_H$  7.14 (s, 1H, H-8). Combining analysis of  $^{13}C$  NMR and DEPT spectra data, compound **1** displayed 12 carbons resonance, including five methine, one oxygenated and six quaternary carbons, respectively. In the  $^1H$ - $^1H$  COSY experiment, the cross-peak correlations of H-3 with H-4, and the cross-peak correlations of H-2' through H-3' were observed. The long-range HMBC attachment from H-3 to C-2 and C-10, H-4 to C-2 and C-9, H-8 to C-6, C-7, C-9 and C-10, H-2'' to C-3'', C-6 and C-7, H-3'' to C-2', C-6 and C-7 were also observed. The remaining one singlet proton at  $\delta$  4.27 (integrating for three protons) showed the presence of methoxy group on the aromatic ring. This confirmation was deduced by HMBC spectrum, which confirms that methoxy proton showed the long-range correlation with the C-5 carbon at  $\delta_C$  149.6. Analysis of UV, IR, 1D, 2D NMR and HRMS spectra as well as on comparison with Literature, were allowed to determine the structure of compound **1** as bergapten<sup>15–17</sup>.

Compound **2** was obtained as pale-yellow crystalline solids with a melting point at 178–180°C. Its molecular formula was assigned as  $C_{13}H_{10}O_5$  based on HR-FAB-MS ( $m/z$  247.0606  $[M + H]^+$ , Calcd. 246.0528). The UV spectrum ex-

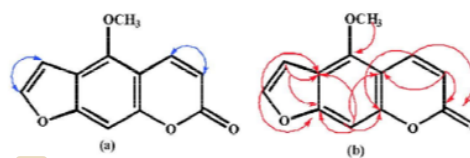


Fig. 2.  $^1H$ - $^1H$  COSY correlations (a) and major  $^1H$ - $^{13}C$  long-range HMBC (b) correlation in compound **1**.

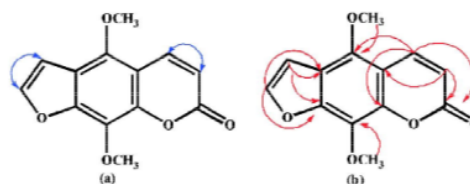


Fig. 3.  $^1H$ - $^1H$  COSY correlations (a) and major  $^1H$ - $^{13}C$  long-range HMBC (b) correlation in compound **2**.

hibited the absorption maxima at 241, 248, 268 and 311 nm, which is consistent with the presence of a furocoumarin. The IR spectrum revealed the presence of an  $\alpha,\beta$ -unsaturated lactone ( $\nu_{\max}$  1751 and 1718  $\text{cm}^{-1}$ ), methoxy group (3132 and 3159  $\text{cm}^{-1}$ ), C-H stretching vibration of  $\text{sp}^2$  hydrocarbon (3084 and 3005  $\text{cm}^{-1}$ ), asymmetrical and symmetrical C-H stretching vibration of  $\text{sp}^3$  hydrocarbon (2843 and 2953  $\text{cm}^{-1}$ ), and C=C ring skeleton stretching vibration ( $\nu_{\max}$  1604 and 1593  $\text{cm}^{-1}$ ) groups.  $^{13}\text{C}$  NMR of compound **2** included 13 carbon signals, comprising 2 methoxy carbon signals, 4 methine carbon signals, and 7 quaternary carbon signals, which were classified by DEPT and HSQC experiment. Its  $^1\text{H}$  NMR spectrum was exhibited a distinct pair of doublets at  $\delta_{\text{H}}$  6.27 and 8.11 (each, 1H, 9.8 Hz) assignable to C-3, C-4 protons, a furan doublet at  $\delta_{\text{H}}$  7.62 and 6.99 (each, 1H, 2.2 Hz) assignable to C-2', C-3' protons. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum showed a correlation between H-3 and H-4 protons while H-2' proton had a correlation to the H-4' proton. In HMBC spectrum, the following correlations were observed; the proton signal at  $\delta_{\text{H}}$  6.27 was connected with C-2 and C-10, the proton signal at  $\delta_{\text{H}}$  8.11 was connected with C-2 and C-9, the proton signal at  $\delta_{\text{H}}$  7.62 was correlated with C-2', C-6 and C-7, the proton signal at  $\delta_{\text{H}}$  6.99 was correlated with C-3', C-6 and C-7. The remaining methoxy protons at  $\delta$  4.16 (integrating for 6 protons) were placed at C-5 and C-8 on the

**Table 1.**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data of compound **1** in  $\text{CDCl}_3$  and bergapten  $^1\text{H}$  in  $\text{CDCl}_3$

Position	Compd. <b>1</b>		Lit. [Ref. 16]	
	$^1\text{H}$ NMR <sup>a</sup>	$^{13}\text{C}$ NMR <sup>b</sup>	$^1\text{H}$ NMR <sup>c</sup>	$^{13}\text{C}$ NMR <sup>d</sup>
2	—	161.2	—	161.4
3	6.28 (d, 9.8 Hz)	112.6	6.27 (d, 9.8 Hz)	112.6
4	8.16 (d, 9.8 Hz)	139.2	8.15 (d, 9.8 Hz)	139.4
5	—	149.6	—	149.7
6	—	112.7	—	112.9
7	—	158.4	—	158.5
8	7.14 (s)	93.9	7.13 (s)	94.0
9	—	152.7	—	151.9
10	—	106.5	—	106.6
2'	7.60 (d, 2.4 Hz)	144.8	7.59 (d, 2.4 Hz)	144.9
3'	7.02 (d, 2.4 Hz)	105.0	7.02 (d, 2.4 Hz)	105.2
5-OMe	4.27 (s)	60.1	4.27 (s)	60.3
8-OMe	—	—	—	—

<sup>a</sup>Spectra recorded in 600 MHz, <sup>b</sup>spectra recorded in 151 MHz, <sup>c</sup>spectra recorded in 400 MHz and <sup>d</sup>spectra recorded in 100 MHz.

**Table 2.**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data of compound **2** in  $\text{CDCl}_3$  and isopimpinellin in  $\text{CDCl}_3$

Position	Compd. <b>2</b>		Lit. [Ref. 16]	
	$^1\text{H}$ NMR <sup>a</sup>	$^{13}\text{C}$ NMR <sup>b</sup>	$^1\text{H}$ NMR <sup>c</sup>	$^{13}\text{C}$ NMR <sup>d</sup>
2	—	160.4	—	160.4
3	6.27 (d, 9.8 Hz)	112.8	6.26 (d, 9.8 Hz)	112.8
4	8.11 (d, 9.8 Hz)	139.4	8.10 (d, 9.8 Hz)	139.3
5	—	144.3	—	144.3
6	—	114.8	—	114.8
7	—	150.0	—	150.0
8	—	128.2	—	128.2
9	—	143.7	—	143.7
10	—	107.6	—	107.6
2'	7.62 (d, 2.2 Hz)	145.1	7.61 (d, 2.3 Hz)	145.1
3'	6.99 (d, 2.2 Hz)	105.1	6.99 (d, 2.3 Hz)	105.1
5-OMe	4.16 (s)	61.7	4.16 (s)	61.7
8-OMe	4.16 (s)	60.8	4.14 (s)	60.8

<sup>a</sup>Spectra recorded in 600 MHz, <sup>b</sup>spectra recorded in 151 MHz, <sup>c</sup>spectra recorded in 400 MHz and <sup>d</sup>spectra recorded in 100 MHz.

basic of long range HMBC correlations. With the aid of above data and previous report, compound **2** was identified as isopimpinellin<sup>16–18</sup>.

## Conclusions

In this study, two known compounds were obtained from the stem bark of *Casimiroa edulis*. The structure of isolated compounds identified as bergapten **1** and isopimpinellin **2** based on their spectroscopic data, such as UV-Vis, FTIR, 1D NMR, 2D NMR, and HRMS. The isolated compounds are not important for their anti-diabetic activity. A lot of researchers were reported these two compounds from many sources of natural product. This is the first report on the occurrence of bergapten and isopimpinellin from Myanmar edible fruit tree *Casimiroa edulis*.

## Acknowledgements

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## Supporting Information

1D and 2D NMR spectrum are described.

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