

## Chemical Constituents from the Flowers of *Homalium tomentosum*

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### Abstract

The phytochemical investigation of the flowers of *Homalium tomentosum* led to the isolation of three known compounds: 3-phenylisocoumarin (**1**), 2-( $\beta$ -glucopyranosyloxy)-7-[(2-oxo-2-phenyl)ethyl]benzoyl-5-hydroxybenzyl alcohol (**2**) and cochinolide- $\beta$ -glucopyranoside (**3**). Their structures were determined by analysis of spectroscopic data as well as comparison of NMR data with those previously reported.

**Keywords:** *Homalium tomentosum*, *Homalium* spices, isocoumarin, phenolic glycoside

### 1. Introduction

*Homalium tomentosum* is a plant of Flacaurtiaceae family. It is medium size plant which normally found in area of Thailand and the root of this plant are used as an astringent (Wiar, C. 2006). Previous chemical investigations of *Homalium* species were found various types of compounds such as xanthenes from *H. paniculiflorum* (Wu, S.Y.; *et al.*, 2015), alkaloids from *H. pomyense* (Pais, M.; *et al.*, 1973), benzofuranones from *H. brachybotry* (Mosaddik, A., Forster, P. I., Waterman, P. G., 2007), phenolic glycosides from *H. longifolium* (Shaari, K. and Waterman, P. G., 1994 and 1995b), *H. cochochinesis* (Ishikawa, T.; *et al.*, 2004) and *H. ceylanicum* (Liu, L., *et al.*, 2013 and Ekabo, O. A., *et al.*, 1993a, b). In addition, cochinolide, tremulacin and cochinchiside B which were isolated from *H. cochochinesis* showed weak activity against herpes simplex virus and tremulacin displayed weak anti-HIV activity (Ishikawa, T.; *et al.*, 2004) along with xanthenes derivatives, homapanicones A and B, showed cytotoxicities against various human cancer cell lines (Wu, S.Y.; *et al.*, 2015). From the literature review, the chemical investigation of the flowers of this plant has not been reported and various types of compounds of this genus displayed interesting biological activities. In this work, we describe the isolation and structural elucidation of three known compounds isolated from the flowers of this plant.

### 2. Materials and Methods

Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and reported without correction. Infrared spectra (IR) were obtained on a Perkin Elmer Spectrum GX FT-IR system and recorded on wave number ( $\text{cm}^{-1}$ ).  $^1\text{H}$  and  $^{13}\text{C}$ -Nuclear magnetic resonance spectra ( $^1\text{H}$  and  $^{13}\text{C}$  NMR) were recorded on a FTNMR, Bruker Avance 300 MHz or 500 MHz spectrometers using tetramethylsilane (TMS) as an internal standard. Spectra were recorded

as chemical shift parameter ( $\delta$ ) value in ppm down field from TMS ( $\delta$  0.00). Ultraviolet spectra (UV) were measured with a UV-160A spectrophotometer (SHIMADSU). Principle bands ( $\lambda_{\max}$ ) were recorded as wavelengths (nm) and log  $\epsilon$  in methanol solution, respectively. Optical rotations were measured in methanol solution with sodium D line (590 nm) on a JASCO P-120 automatic polarimeter. Thin-layer chromatography (TLC) and precoated thin-layer chromatography were performed on silica gel 60 GF<sub>254</sub> (Merck). Column chromatography was carried on silica gel (Merck) type 100 (70-230 mesh ASTM), Sephadex LH-20 or reverse phase C<sub>18</sub> silica gel.

Extraction and isolation: The flowers of *H. tomentosum* were extracted with acetone and methanol to give the crude acetone (4.88 g) and methanol (11.15 g) extracts. The crude acetone extract was separated by column chromatography over silica gel. Elution was performed with 100% dichloromethane followed by increasing amount of methanol and finally with 100% methanol to afford thirteen fractions (Fr1-13). Fraction 4 was separated by column chromatography over Sephadex LH-20 with 50% methanol in dichloromethane to give compound **1** (17.8 mg). Fraction 10 was purified by column chromatography over Sephadex LH-20 with 50% methanol in dichloromethane to yield three subfractions (Fr101-3). Subfraction 10-2 was separated by column chromatography over Sephadex LH-20 with 50% methanol in dichloromethane to obtain compound **2** (19.0 mg). The crude methanol extract was separated by column chromatography over Sephadex LH-20 with 100% methanol to give four fractions (M1-4). Fraction M3 was separated by column chromatography over Sephadex LH-20 with 100% methanol to yield five subfractions (M31-35). Subfraction M32 was purified by column chromatography over reverse phase silica gel with 70% methanol in water followed by increasing amount of methanol and finally with 100 % methanol to afford compound **3** (3.3 mg).

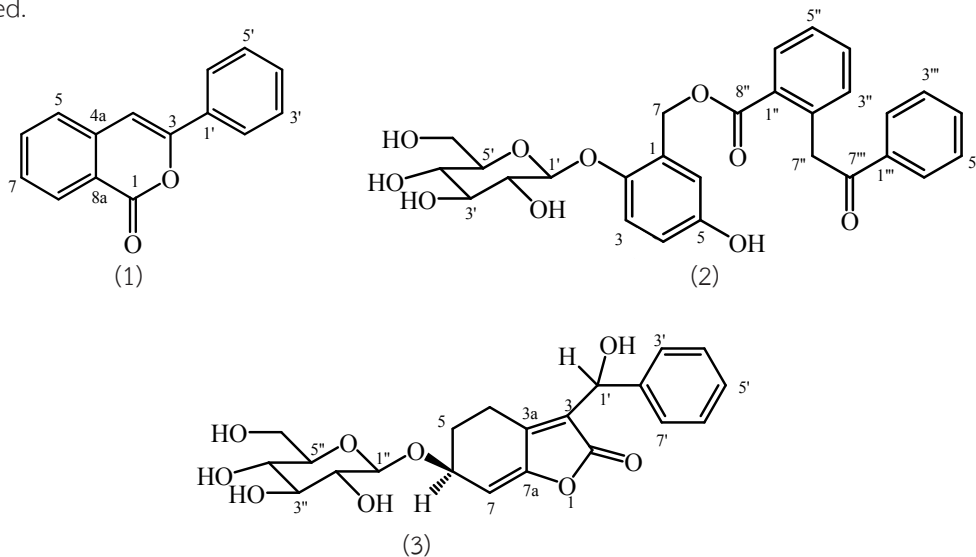
3-Phenylisocoumarin (**1**): brown solid; mp = 80-82 °C; IR (film) 1730 cm<sup>-1</sup>; UV (MeOH) 296, 310 and 340 nm; <sup>1</sup>H NMR (300 MHz in CDCl<sub>3</sub>):  $\delta_{\text{H}}$  6.97 (s, 1H, H-4), 7.47 (m, 2H, H-5, H-7), 7.48 (m, 3H, H-3', H-4', H-5'), 7.73 (dt, 2.0, 8.0, 1H, H-6), 7.89 (dd, 2.0, 8.4, 2H, H-2', H-6') and 8.32 (d, 8.0, 1H, H-8); <sup>13</sup>C NMR (75 MHz in CDCl<sub>3</sub>):  $\delta_{\text{C}}$  101.83 (C-4), 120.65 (C-8a), 125.29 (C-3', C-5'), 125.97 (C-5), 128.18 (C-7), 128.86 (C-4'), 129.72 (C-8), 129.99 (C-2', C-6'), 132.00 (C-1'), 134.88 (C-6), 138.00 (C-4a), 153.74 (C-3) and 162.00 (C=O)

2-( $\beta$ -glucopyranosyloxy)-7-{2-[(2-oxo-2-phenyl) ethyl]bezoyl}-5-hydroxybenzyl alcohol (**2**): white solid; mp= 118-120 °C; IR (film) 1653 and 3429 cm<sup>-1</sup>; UV (MeOH) 231 and 285 nm;  $[\alpha]_{\text{D}}^{24}$  -19.2 (c 0.3, MeOH); <sup>1</sup>H NMR (300 MHz in Acetone-d<sub>6</sub>):  $\delta_{\text{H}}$  3.44 (m, 4H, H-2'-H-5'), 3.68 (dd, 4.5, 11.1, 1H, H-6a'), 3.85 (dd, 4.5, 11.1, 1H, H-6b'), 4.76 (d, 7.2, 1H, H-1'), 4.83 (s, 2H, H-7''), 5.26 (d, 13.2, 1H, H-7a), 5.30 (d, 13.2, 1H, H-7b), 6.70 (dd, 3.0, 8.7, 1H, H-4), 6.85 (d, 3.0, 1H, H-6), 7.05 (d, 8.7, 1H, H-3), 7.40 (d, 7.8, 1H, H-3''), 7.45 (dt, 1.2, 7.8, 1H, H-5''), 7.53 (m, 1H, H-4''), 7.58 (dd, 1.5, 7.2, 1H, H-4'''), 7.63 (m, 2H, H-3''' and H-5'''), 8.07 (dd, 1.5, 7.2, 2H, H-2''' and H-6'''), 8.11 (dd, 1.2, 7.8, 1H, H-6''); <sup>13</sup>C NMR (75 MHz in Acetone-d<sub>6</sub>):  $\delta_{\text{C}}$  44.55 (C-7''), 61.63 (C-7), 61.81 (C-6'), 70.44 (C-4'), 76.84 (C-2'), 77.01 (C-3'), 77.84 (C-5'), 102.86 (C-1'), 115.17 (C-6), 115.53 (C-4), 117.72 (C-3), 127.02 (C-1 and C-5''), 128.05 (C-2''' and C-6'''), 128.57 (C-4''), 130.12 (C-1''), 132.22 (C-4'''), 130.68 (C-6''), 132.82 (C-3'', C-3''' and C-5'''), 137.31 (C-1'''), 137.69 (C-2'') 148.67 (C-2), 152.62 (C-5), 166.61 (C-8'') and 196.66 (C-7''')

Cochinolide- $\beta$ -glucopyranoside (**3**); brown gum; IR (film) 1,640 and 3,418  $\text{cm}^{-1}$ ; UV (MeOH) 278 nm;  $[\alpha]_{\text{D}}^{24} +54.17$  (c 0.03, MeOH);  $^1\text{H}$  NMR (300 MHz in Acetone- $d_6$ ):  $\delta_{\text{H}}$  1.94 (*m*, 2H, H-5), 2.67 (*ddd*, 4.8, 8.0, 18, 1H, H-4a), 3.11 (*ddd*, 4.8, 8.0, 18, 1H, H-4b), 3.18 (*dt*, 3.9, 7.8, 1H, H-2''), 3.34 (*m*, 1H, H-5''), 3.40 (*dt*, 3.9, 7.8, 2H, H-3'' and H-4''), 3.72 (*dd*, 5.4, 11.0, 1H, H-6a''), 3.84 (*ddd*, 2.4, 5.4, 11.0, 1H, H-6b''), 4.49 (*d*, 7.8, 1H, H-1''), 4.63 (*dd*, 4.5, 10, 1H, H-6), 5.71 (*d*, 4.2, 1H, H-1'), 5.98 (*d*, 4.5, 1H, H-7), 7.27 (*t*, 7.5, 1H, H-5'), 7.34 (*t*, 7.5, 2H, H-4' and H-6'), 7.46 (*d*, 7.5, 2H, H-3' and H-7');  $^{13}\text{C}$  NMR (75 MHz in Acetone- $d_6$ ):  $\delta_{\text{C}}$  19.67 (C-4), 28.19 (C-5), 62.08 (C-6''), 68.17 (C-1'), 70.81 (C-5''), 71.76 (C-6), 73.15 (C-3''), 73.97 (C-2''), 76.77 (C-4''), 102.59 (C-1''), 111.12 (C-7), 126.07 (C-2'), 126.69 (C-3' and C-7'), 127.42 (C-4' and C-6'), 128.26 (C-5''), 142.39 (C-3), 148.68 (C-3a), 149.91 (C-7a) and 168.50 (C-2)

### 3. Results and Discussion

Three known compounds were isolated from the flowers of *H. tomentosum*. Their structures were identified by analysis of spectroscopic data and comparison of NMR data with those previously reported.



Compound **1**, the  $^1\text{H}$  NMR spectrum displayed signals of the 1,2-disubstituted benzene [ $\delta_{\text{H}}$  7.47 (*m*, 2H), 7.73 (*dt*, 2.0, 8.0, 1H) and 8.32 (*d*, 8.0, 1H)], monosubstituted benzene [ $\delta_{\text{H}}$  7.48 (*m*, 3H) and 7.89 (*dd*, 2.0, 8.4, 2H)] and trisubstituted double bond [ $\delta_{\text{H}}$  6.97 (*s*, 1H)] units which was confirmed by HMBC and  $^1\text{H}$ - $^1\text{H}$  COSY data. The  $^{13}\text{C}$  NMR and DEPT 135° spectra displayed one carbonyl ( $\delta_{\text{C}}$  162.00), four quaternary ( $\delta_{\text{C}}$  120.65, 132.00, 138.00, and 153.74) and ten methine ( $\delta_{\text{C}}$  101.83, 125.29 x 2, 125.97, 128.18, 128.86, 129.72, 129.99 x 2 and 134.88) carbons. From HMBC data, the olefinic proton of the trisubstituted double bond (H-4,  $\delta_{\text{H}}$  6.97) showed a cross peak with C-5 ( $\delta_{\text{C}}$  125.97) of the 1,2-disubstituted benzene and the aromatic proton, H-8 ( $\delta_{\text{H}}$  8.32), of the 1,2-disubstituted benzene displayed a cross peak with ester carbonyl carbon (C-1,  $\delta_{\text{C}}$  162.0) which were confirmed the trisubstituted double bond and ester carbonyl carbon connected at C-4a ( $\delta_{\text{C}}$  138.00) and C-8a ( $\delta_{\text{C}}$  120.65), respectively and the chemical shift value of C-3 ( $\delta_{\text{C}}$  153.74) of the trisubstituted double bond,

established an isochromenone unit. The aromatic proton, H-2' ( $\delta_{\text{H}}$  7.89) of the monosubstituted benzene showed a cross peak with C-3 of the isochromenone unit which was confirmed the monosubstituted benzene connected at C-3 of the isochromenone unit. Compound 1 was 3-phenylisocoumarin (Shaari, K. and Waterman, P. G., 1995a).

Compound 2, the  $^1\text{H}$  NMR spectrum showed signals of the monosubstituted benzene [ $\delta_{\text{H}}$  7.58 (*dd*, 1.5, 7.2, 1H), 7.63 (*m*, 2H) and 8.07 (*dd*, 1.5, 7.2, 2H)], 1,2,4-trisubstituted benzene [ $\delta_{\text{H}}$  6.70 (*dd*, 3.0, 8.7, 1H), 6.85 (*d*, 3.0, 1H) and 7.05 (*d*, 8.7, 1H)], 1,2-disubstituted benzene [ $\delta_{\text{H}}$  7.40 (*d*, 7.8, 1H), 7.45 (*dt*, 1.2, 7.8, 1H), 7.53 (*m*, 1H) and 8.11 (*dd*, 1.2, 7.8, 1H)], oxymethylene protons [ $\delta_{\text{H}}$  5.26 (*d*, 13.2, 1H) and 5.30 (*d*, 13.2, 1H)], methylene proton [ $\delta_{\text{H}}$  4.83 (*s*, 2H)] and glucose unit: one anomeric proton [ $\delta_{\text{H}}$  4.76 (*d*, 7.2, 1H)], two nonequivalent oxymethylene protons [ $\delta_{\text{H}}$  3.68 (*dd*, 4.5, 11.1, 1H) and 3.85 (*dd*, 4.5, 11.1, 1H)] and four methine protons [ $\delta_{\text{H}}$  3.44 (*m*, 4H)] units. The coupling constant value of the anomeric proton ( $J = 7.2$  Hz) indicated that the glucose unit would be a  $\beta$ -glucopyranose. The  $^{13}\text{C}$  NMR and DEPT 135° spectra presented two carbonyl ( $\delta_{\text{C}}$  166.61 and 196.66), six quaternary ( $\delta_{\text{C}}$  127.02, 130.12, 137.31, 137.69, 148.67 and 152.62), fifteen methine ( $\delta_{\text{C}}$  70.44, 76.84, 77.01, 77.84, 102.86, 115.17, 115.53, 117.72, 127.02, 128.05, 128.57, 130.68, 132.22, and 132.82 x 2) and three methylene ( $\delta_{\text{C}}$  44.55, 61.63 and 61.81) carbons. The HMBC data, the oxymethylene protons (H-7a and H-7b,  $\delta_{\text{H}}$  5.26 and 5.30) displayed cross peaks with C-2 ( $\delta_{\text{C}}$  148.67) and C-6 ( $\delta_{\text{C}}$  115.17) of the 1,2,4-trisubstituted benzene, suggested that the oxymethylene carbon (C-7,  $\delta_{\text{C}}$  61.63) was connected at C-1 ( $\delta_{\text{C}}$  127.02) of the 1,2,4-trisubstituted benzene. The hydroxyl groups were located at C-2 and C-5 ( $\delta_{\text{C}}$  152.62) according to the chemical shift values to form a 2,5-dihydroxybenzyl alcohol unit. The aromatic proton, H-2''' ( $\delta_{\text{H}}$  8.07), of the monosubstituted benzene showed a cross peak with ketone carbonyl carbon (C-7'',  $\delta_{\text{C}}$  196.66). This data established a benzoyl moiety. The methylene proton (H-7'',  $\delta_{\text{H}}$  4.83) gave cross peaks with C-1''' ( $\delta_{\text{C}}$  137.31) of the benzoyl moiety and C-1'' ( $\delta_{\text{C}}$  130.12) of the 1,2-disubstituted benzene, indicated that the methylene carbon (C-7'',  $\delta_{\text{C}}$  44.55) was attached at C-1''' of the benzoyl moiety though a ketone linkage and C-2'' ( $\delta_{\text{C}}$  137.69) of the 1,2-disubstituted benzene. The aromatic proton, H-6'' ( $\delta_{\text{H}}$  8.11) of the 1,2-disubstituted benzene showed a cross peak with ester carbonyl carbon (C-8'',  $\delta_{\text{C}}$  166.61) and the oxymethylene protons (H-7a and H-7b) of the 2,5-dihydroxybenzyl alcohol unit presented a correlation with ester carbonyl carbon (C-8''), indicated that the 2,5-dihydroxybenzyl alcohol unit was connected at C-1'' of the 1,2-disubstituted benzene though an ester linkage. In addition, the anomeric proton (H-1',  $\delta_{\text{H}}$  4.76) of the  $\beta$ -glucopyranose presented a cross peak with C-2 of the 2,5-dihydroxybenzyl alcohol unit, indicated that the  $\beta$ -glucopyranose was attached at C-2 of the 2,5-dihydroxybenzyl alcohol unit though an ether linkage. Compound 2 was a 2-( $\beta$ -glucopyranosyloxy)-7-[2-[(2-oxo-2-phenyl)ethyl]benzoyl]-5-hydroxybenzyl alcohol (Shaari, K. and Waterman, P. G., 1995b).

Compound 3, the  $^1\text{H}$  NMR data showed signals of the monosubstituted benzene [ $\delta_{\text{H}}$  7.46 (*d*, 7.5, 2H), 7.34 (*t*, 7.5, 2H) and 7.27 (*t*, 7.5, 1H)], trisubstituted double bond [ $\delta_{\text{H}}$  5.98 (*d*, 4.5, 1H)], two methylene groups [ $\delta_{\text{H}}$  2.67 (*ddd*, 4.8, 8.0, 18.0, 1H), 3.11 (*ddd*, 4.8, 8.0, 18.0, 1H) and 1.94 (*m*, 2H)], two oxymethine protons [ $\delta_{\text{H}}$  5.71 (*d*, 4.2, 1H) and 4.63 (*dd*, 4.5, 10.0, 1H)] and one glucose unit: one

anomeric proton [ $\delta_{\text{H}}$  4.49 (*d*, 7.8, 1H)], two nonequivalent oxymethylene protons [ $\delta_{\text{H}}$  3.72 (*dd*, 5.4, 11.0, 1H) and 3.84 (*ddd*, 2.4, 5.4, 11.0, 1H)] and four methine protons [ $\delta_{\text{H}}$  3.18 (*dt*, 3.9, 7.8, 1H), 3.34 (*m*, 1H) and 3.40 (*dt*, 3.9, 7.8, 2H)] units. The glucose unit would be a  $\beta$ -glucopyranose according to the coupling constant value of anomeric proton ( $J = 7.8$  Hz) (Ishikawa, T., *et al.*, 1998). The  $^{13}\text{C}$  NMR and DEPT  $135^\circ$  spectra showed one carbonyl ( $\delta_{\text{C}}$  168.50), four quaternary ( $\delta_{\text{C}}$  126.07, 142.39, 148.68 and 149.91) and eleven methine ( $\delta_{\text{C}}$  68.17, 70.81, 71.76, 73.15, 73.97, 76.77, 102.59, 111.12, 126.69, 127.42 and 128.26) and three methylene ( $\delta_{\text{C}}$  19.67, 28.19 and 62.08) carbons. From  $^1\text{H}$ - $^1\text{H}$  COSY data, the methylene protons ( $\text{H}_2$ -5,  $\delta_{\text{H}}$  1.94) gave cross peaks with  $\text{H}_2$ -4 ( $\delta_{\text{H}}$  2.67 and 3.11) and H-6 ( $\delta_{\text{H}}$  4.63) along with the oxymethine proton (H-6) showed cross peaks with  $\text{H}_2$ -5 and the olefinic proton, H-7 ( $\delta_{\text{H}}$  5.98) of the trisubstituted double bond. These data established a  $-\text{CH}_2\text{CH}_2\text{CH}(\text{OH})\text{CH}=\text{C}-$  moiety. The HMBC data, the methylene protons,  $\text{H}_2$ -5 and the oxymethine proton H-6 presented cross peaks with C-3a ( $\delta_{\text{C}}$  148.68) and C-7a ( $\delta_{\text{C}}$  149.91), respectively. This result established a cyclohexene. The methylene proton,  $\text{H}_2$ -4 showed HMBC correlations with C-2 ( $\delta_{\text{C}}$  168.50) and C-3 ( $\delta_{\text{C}}$  142.39) as well as the chemical shift value of C-7a, established a dihydrobenzofuranone unit. The oxymethine proton (H-1',  $\delta_{\text{H}}$  5.71) gave cross peak with C-3a of the dihydrobenzofuranone unit and C-3' ( $\delta_{\text{C}}$  126.69) of the monosubstituted benzene, suggesting that the oxymethine group was connected at C-3 of the dihydrobenzofuranone unit and C-2' ( $\delta_{\text{C}}$  126.07) of the monosubstituted benzene. In addition, the anomeric proton (H-1'',  $\delta_{\text{H}}$  4.49) of the  $\beta$ -glucopyranose presented a cross peak with C-6 ( $\delta_{\text{C}}$  71.76) of the dihydrobenzofuranone unit. This data confirmed that the  $\beta$ -glucopyranose was connected at C-6 of the dihydrobenzofuranone unit through ether linkage. Compound **3** was a cochinolide- $\beta$ -glucopyranoside (Ishikawa, T., *et al.*, 1998).

#### 4. Conclusion

Three known compounds: 3-phenylisocoumarin (**1**), 2-( $\beta$ -glucopyranosyloxy)-7-[2-[(2-oxo-2-phenyl)ethyl]benzoyl]-5-hydroxybenzyl alcohol (**2**) and cochinolide- $\beta$ -glucopyranoside (**3**) were isolated from the flowers of *H. tomentosum*. Compound **2** is phenolic glycosides of 2,5-dihydroxybenzyl alcohol which is the most compound found in *Homalium* spp. and this is the first report on the isolation of compound **2** from this plant.

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