

## Synthesis and antimicrobial activity of 4',5,7-trihydroxy-3'-prenylflavanone

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**Abstract.** 4',5,7-Trihydroxy-3'-prenylflavanone was synthesized and tested for antibacterial effects against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The synthesized compounds were characterized using UV, IR, MS and <sup>1</sup>H and <sup>13</sup>C NMR data. The antibacterial screening of the synthesized compounds were performed *in vitro* by the filter paper disc diffusion method.

**Keywords.** 4',5,7-Trihydroxy-3'-prenylflavanone; antibacterial activity; flavanone.

### 1. Introduction

Flavonoids are a group of common and naturally occurring polyphenolic compounds that are widely found in the plant kingdom.<sup>1</sup> They occur naturally as plant pigments in a broad range of fruits and vegetables as well as beverages such as tea, red wine, coffee and beer.<sup>2</sup> Flavonoids have been reported to exert multiple biological effects including antimicrobial,<sup>3</sup> cytotoxicity,<sup>4</sup> anti-inflammatory<sup>5</sup> as well as anti-tumor activities.<sup>6</sup> In this regard, several flavonoids-bearing hydroxyl groups on the A or B ring have been reported to be potential antioxidant agents. It is now well established that such potency is mainly due to the ability of hydroxyl groups to donate hydrogen which enable the flavonoids to undergo a redox reaction that helps them to scavenge free radicals.<sup>7</sup> In addition, the presence of hydroxyl groups in the skeleton also contribute to high affinity for proteins and therefore acts as inhibitors of microbial enzymes<sup>8</sup> and inhibition of NADH dehydrogenase of mitochondrial inner membranes.<sup>9</sup>

Prenylated flavanones are a unique class of naturally occurring flavonoids characterized by the presence of a prenylated side chain in the flavonoid skeleton. It was reported that one phenolic group

and certain degree of lipophilicity are required for the activity of the flavonoids.<sup>10</sup> Substitution of the flavonoid ring system with prenyl groups would increase their lipophilicity and consequently enhance their interaction with cellular membranes.<sup>11</sup> 4',5,7-Trihydroxy-3'-prenylflavanone (**1**) has been isolated for the first time in 1989 from the chloroform extract of the stem bark of *Erythrina eriotriocha*.<sup>12</sup> This paper now reports the short and facile synthesis of 4',5,7-trihydroxy-3'-prenylflavanone (**1**) or 3'-prenylnaringenin. 4',5,7-Trihydroxy-3'-prenylflavanone (**1**) and its corresponding chalcone were screened *in vitro* for their antibacterial activity against four human pathogenic bacteria, *Bacillus subtilis* (G<sup>+</sup>), *Escherichia coli* (G<sup>-</sup>), *Staphylococcus aureus* (G<sup>+</sup>) and *Pseudomonas aeruginosa* (G<sup>-</sup>).

### 2. Experimental

#### 2.1 Materials, method and instruments

Melting points were recorded on a Leica Galen III Kofler micro melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Shimadzu 8000 or Perkin–Elmer series 1600 spectrometers as thin film (NaCl windows) for liquid samples or KBr pellet for solid samples. Mass spectral data were obtained from Kent Mass Spectrometry

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Service, UK.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (300 and 75 MHz respectively) were recorded on a Bruker Avance 300 Spectrometer using  $\text{CDCl}_3$  and DMSO as solvent. Reactions were monitored by thin-layer chromatography (tlc) carried out on 0.2 mm Merck pre-coated silica gel plates (60  $F_{254}$ ).

## 2.2 4-Hydroxy-3-prenylbenzaldehyde (3)

4-Hydroxybenzaldehyde (**2**) (1.1 g, 9.02 mmol) was dissolved in 10% aqueous KOH solution (12 mL) and 1-bromo-2-methyl-but-2-ene (2.5 g, 16.78 mmol) was added drop-wise within 30 min at room temperature. The reaction mixture was stirred at rt for 48 h and then acidified with HCl (10%) and extracted with EtOAc (3  $\times$  25 mL). The organic layer was washed with water and brine, followed by drying over anhydrous  $\text{MgSO}_4$  and the solvent was evaporated under reduced pressure. The resulting syrup was chromatographed on a silica gel column (PE : EtOAc, 4 : 1) to give 4-hydroxy-3-prenylbenzaldehyde (**3**) as colourless crystals with m.p. 53–54°C (lit.<sup>13</sup> 57–60°C) in 12.8% yield.

$R_f$  = 0.18 (PE : EtOAc, 9 : 1); IR  $\nu_{\text{max}}$  (KBr)  $\text{cm}^{-1}$ : 3215 (OH), 1658 (C=O), 1597 and 1446 (aromatic C=C), 1376 (C–O).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ) ppm: 1.75 (6H, s, H-4' and H-5'), 3.43 (2H, d,  $J$  = 7.5 Hz, H-1'), 5.35 (1H, m, H-2'), 6.24 (1H, br, –OH), 6.94 (1H, d,  $J$  = 7.5 Hz, H-5), 7.66–7.72 (2H, with unresolved couplings due to overlapping, H-2 and H-6) and 9.86 (1H, s, CHO).

## 2.3 4-Methoxymethoxy-3-prenylbenzaldehyde (4)

To a solution of 4-hydroxy-3-prenylbenzaldehyde (**3**) (150 mg, 0.79 mmol) in  $\text{CH}_2\text{Cl}_2$  (8 mL) at 0°C, *N,N*-diisopropylethylamine (5 mL) was added, this was followed by the addition of DMAP (40.0 mg, 0.33 mmol) and the reaction mixture was stirred for 15 min. MOMCl (70 mg, 0.89 mmol) was then added drop-wise at 0°C, and the mixture was stirred for 15 min, after which time the temperature was increased to room temperature with stirring overnight. The reaction mixture was then poured into water and extracted with  $\text{CHCl}_3$  (2  $\times$  20 mL). The  $\text{CHCl}_3$  extracts were washed with water and brine, followed by drying over anhydrous  $\text{MgSO}_4$ . The solvent was evaporated under reduced pressure, and the resulting syrup was chromatographed on a silica gel column (PE : EtOAc, 1 : 4) to give 4-methoxymethoxy-3-prenylbenzaldehyde (**4**) (132 mg, 71.0%) as a colorless liquid;  $R_f$  0.46 (PE : EtOAc, 1 : 4).

IR  $\nu_{\text{max}}$  (neat)  $\text{cm}^{-1}$ : 2823 and 2733 (C–H aldehyde), 1686 (C=O), 1600 and 1451 (C=C aromatic), 1253 (C–O).

NMR  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) ppm: 1.74 (3H, s, C-4'), 1.77 (3H, s, C-5'), 3.39 (2H, d,  $J$  = 7.5 Hz, C-1'), 3.50 (3H, s, –OCH<sub>3</sub>), 5.27–5.35 (3H, m, –OCH<sub>2</sub> and C-2'), 7.18 (1H, d,  $J$  = 7.5 Hz, H-5), 7.69–7.72 (2H, m, H-2 and H-6), 9.89 (1H, s, –CHO).

## 2.4 2'-Hydroxy-4,4',6'-tris(methoxymethoxy)-3-prenylchalcone (6)

A solution of 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone<sup>14</sup> (**5**) (100 mg, 0.39 mmol) and 4-methoxymethoxy-3-prenylbenzaldehyde (**4**) (100 mg, 0.43 mmol) in ethanol (10 mL), and 50% aq. solution of KOH (0.8 mL) was added. The mixture was then stirred at rt for 48 h. The mixture was poured into iced water, acidified with HCl (10%), and extracted with dichloromethane, and then washed with water and brine. The organic layer was dried over anhydrous  $\text{MgSO}_4$ , and evaporated under reduced pressure. The residual orange syrup was chromatographed on a silica gel column (PE : EtOAc, 9 : 1) to afford 2'-hydroxy-4,4',6'-tris(methoxymethoxy)-3-prenylchalcone (**6**) (139 mg, 76.1%) as a yellow crystalline with m.p. 43–45°C and  $R_f$  0.31 (PE : EtOAc, 4 : 1).

IR  $\nu_{\text{max}}$  (KBr)  $\text{cm}^{-1}$ : 3439 (OH), 1622 (C=O), 1590 and 1415 (C=C aromatic), 1149 (C–O).

NMR  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) ppm: 1.75 (3H, s, H-5''), 1.78 (3H, s, H-4''), 3.37 (2H, d,  $J$  = 7.2 Hz, H-1''), 3.51 (9H, m, 3x –OCH<sub>3</sub>), 5.21–5.36 (7H, m, 3x –OCH<sub>2</sub> and H-2''), 6.27 (1H, d,  $J$  = 2.4 Hz, H-3'), 6.33 (1H, d,  $J$  = 2.4 Hz, H-5'), 7.10 (1H, d,  $J$  = 9.0 Hz, H-5), 7.43–7.45 (2H, m, with unresolved couplings due to overlapping, H-2 and H-6), 7.78 (1H, d,  $J$  = 15.6 Hz, H- $\alpha$ ), 7.85 (1H, d,  $J$  = 15.6 Hz, H- $\beta$ ), 13.93 (1H, s, –OH).

NMR  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) ppm: 17.5 (C-5'', CH<sub>3</sub>), 25.5 (C-4'', CH<sub>3</sub>), 28.5 (C-1'', –CH<sub>2</sub>), 55.5–56.5 (3x OCH<sub>3</sub>, CH<sub>3</sub>), 94.0 (–OCH<sub>2</sub>, CH<sub>2</sub>), 94.5 (C-1', C-4°), 95.0 (C-5', C-H), 95.5 (–OCH<sub>2</sub>, CH<sub>2</sub>), 98.0 (C-3', C-H), 114.0 (C-5, C-H), 122.0 (C-2'', C-H), 125.5 (C- $\alpha$ , C-H), 127.5 (C-6, C-H), 129.5 (C-3, C-4°), 130.0 (C-2, C-H), 131.5 (C-1, C-4°), 133.0 (C-3'', C-4°), 143.0 (C- $\beta$ , C-H), 157.0 (C-2', C-4°), 160.0 (C-4, C-4°), 163.5 (C-6', C-4°), 167.5 (C-4', C-4°) and 193.0 (C=O, C-4°).

MS:  $m/z$  472 [ $\text{M}^+$ ,  $\text{C}_{26}\text{H}_{32}\text{O}_8$ , (13)], 473 (8), 427 (18), 395 (16), 367 (20), 263 (36), 259 (100), 241 (64), 231 (27), 227 (22) and 197 (17).

### 2.5 4',5,7-Trihydroxy-3'-prenylflavanone (1)

A solution of 2'-hydroxy-4,4',6'-tris(methoxymethoxy)-3-prenylchalcone (6) (0.050 mg, 0.11 mmol) in MeOH (6 mL) was added 10% HCl (1 mL) and refluxed for 1 h. Then NaOAc was added and the resulting mixture was refluxed for the next 3 h. The mixture was cooled and water (20 mL) was added and extracted with EtOAc (20 mL  $\times$  2), dried over anhydrous MgSO<sub>4</sub>, filtered, and evaporated to dryness to give 4',5,7-trihydroxy-3'-prenylflavanone (1) (32.16 mg, 89.33%) as a brown solid with m.p. 129–131°C (lit.<sup>15</sup> 134–135°C) and *R<sub>f</sub>* 0.21 (PE : EtOAc, 1 : 5).

IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3406 (OH), 1664 (C=O), 1611 and 1451 (C=C aromatic), 1166 (C–O).

UV  $\lambda_{\max}$  (EtOH) nm: 336 (shoulder), 289; NaOH; 325, 248; AlCl<sub>3</sub>; 381, 306; AlCl<sub>3</sub>/HCl; 372, 307; NaOAc; 330, 286; NaOAc/H<sub>3</sub>BO<sub>3</sub>; 329 (sh), 286.

NMR  $\delta_{\text{H}}$  (acetone) ppm: 1.70 (6H, s, H-4'' and H-5''), 2.73 (1H, dd, *J* = 17.4 and 3.3 Hz, H-3a), 3.20 (1H, dd, *J* = 17.4 and 12.9 Hz, H-3b), 3.36 (1H, d, *J* = 7.5 Hz, H-1''), 5.35 (1H, m, H-2''), 5.44 (1H, dd, *J* = 12.9 and 3.0 Hz, H-2), 5.97 (2H, s, H-6 and H-8), 6.90 (1H, d, *J* = 8.1 Hz, H-5'), 7.23 (1H, dd, *J* = 8.1 and 2.1 Hz, H-6'), 7.29 (1H, d, *J* = 2.1 Hz, H-2'), 8.45 (1H, s, –OH), 9.61 (1H, s, –OH), 12.18 (1H, s, –OH).

NMR  $\delta_{\text{C}}$  (acetone) ppm: 17.0 (C-5'', CH<sub>3</sub>), 25.0 (C-4'', CH<sub>3</sub>), 28.5 (C-1'', CH<sub>2</sub>), 42.5 (C-3, CH<sub>2</sub>), 79.0 (C-2, CH), 95.0 (C-8, CH), 96.0 (C-6, CH), 102.5 (C-4a, C-4°), 115.0 (C-5', CH), 122.5 (C-2'', CH), 125.0 (C-6', CH), 128.0 (C-2', CH), 128.2 (C-1', C-4°), 130.0 (C-3'', C-4°), 132.0 (C-3', C-4°), 154.9 (C-4', C-4°), 155.5 (C-8a, C-4°), 164.5 (C-5, C-4°), 166.5 (C-7, C-4°) and 196.0 (C=O, C-4°).

CIMS: *m/z* 340 [M<sup>+</sup>, C<sub>20</sub>H<sub>20</sub>O<sub>5</sub>, (65)], 341 (100), 339 (32), 285 (13), 286 (15), 188 (20), 175 (87), 153 (17), 141 (14) and 107 (10).

### 2.6 Antibacterial screening

The antibacterial activities of the synthesized compounds (1) and (6) were studied against four bacteria, viz. *Bacillus subtilis* (G<sup>+</sup>), *Escherichia coli* (G<sup>-</sup>), *Staphylococcus aureus* (G<sup>+</sup>) and *Pseudomonas aeruginosa* (G<sup>-</sup>). For the detection of antibacterial activities, the filter paper discs diffusion method was used.<sup>16</sup> Streptomycin sulphate was used as positive control. Nutrient agar (NA) was used as basal medium for test bacteria. The discs were prepared by impregnating them in methanol solution of each

sample (1 mg/1 mL). Each culture was prepared to a turbidity equivalent to McFarland and spread on the test tube. The paper disc containing the compound were placed on the agar surface previously inoculated with suspension of each microbes to be tested. All determinations were made in duplicate. Inhibition diameter were determined after incubation at 37°C  $\pm$  1 for 24 h. The antimicrobial activity was indicated by the presence of the clear inhibition zones around each disc.

### 2.7 Minimum inhibition concentration

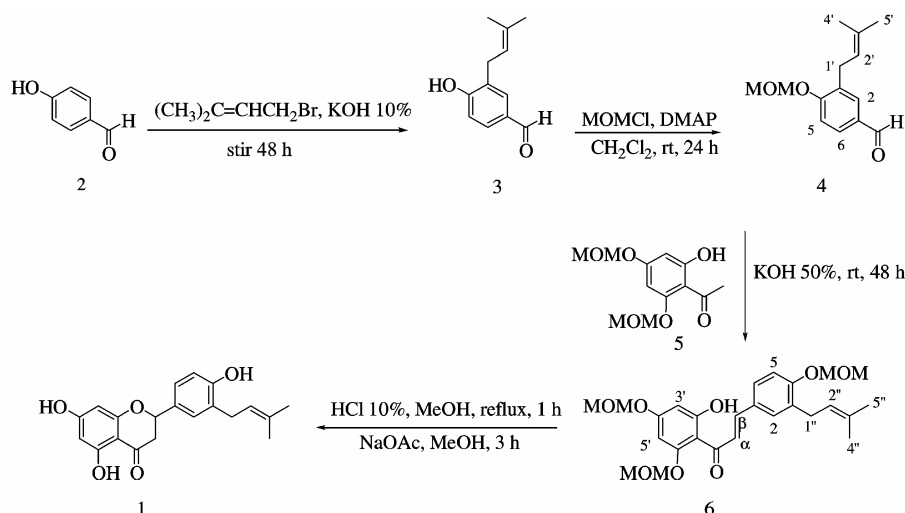
The determination of the minimum inhibitory concentration (MIC), the serial dilution technique were followed using nutrient broth medium. The MIC was defined as the lowest concentration of samples that had restricted the growth of microbial.<sup>17</sup> The MIC value of compound (1) were determined against *Escherichia coli* (G<sup>-</sup>).

## 3 Results and discussion

### 3.1 Synthesis of 4',5,7-trihydroxy-3'-prenylflavanone (1)

The synthesis of 4',5,7-trihydroxy-3'-prenylflavanone (1) was accomplished using the route shown in scheme 1. Lack of a general, efficient and regioselective method for the introduction of a prenylated side chain was a major challenge for the synthesis of this particular compound. Nevertheless, the initial step which was the introduction of a prenyl group to 4-hydroxybenzaldehyde (2) was carried out. The synthesis of 4-hydroxy-3-prenylbenzaldehyde (4) was achieved by treatment of 1-bromo-3-methylbut-2-ene or prenyl bromide with 4-hydroxybenzaldehyde (2) in KOH 10% solution for 48 h at room temperature.<sup>18</sup> The prenylation of (2) gave the desired product (3) in only 12.8% along with 4-*O*-prenylbenzaldehyde as a by-product.

The hydroxyl group of 4-hydroxy-3-prenylbenzaldehyde (3) was then protected as –OMOM derivative (4) using MOMCl, DMAP, NEt(*i*-Pr)<sub>2</sub> in CH<sub>2</sub>Cl<sub>2</sub> to furnish 4-methoxymethoxy-3-prenylbenzaldehyde (4) as a colourless liquid in 71% yield. The Claisen condensation of compound (4) with 2-hydroxy-4,6-bis(methoxymethoxy)-acetophenone (5) yielded the corresponding chalcone (6) in 76.1% yield with m.p. 43–45°C as yellow crystalline. The IR spectrum of



Scheme 1.

chalcone (**6**) showed an absorption frequency at  $1622\text{ cm}^{-1}$  indicating the presence of a conjugated carbonyl group and a broad peak at  $3439\text{ cm}^{-1}$  due to the hydroxyl groups. The  $^1\text{H}$  NMR spectrum exhibited the characteristic signals due to *trans*-olefinic protons at  $\delta$  7.78 (1H, *d*,  $J = 15.6$  Hz, H- $\alpha$ ) and 7.85 (1H, *d*,  $J = 15.6$  Hz, H- $\beta$ ) whereas the prenyl moiety resonated at  $\delta$  1.75 (3H, *s*,  $-\text{CH}_3$ ), 1.78 (3H, *s*,  $-\text{CH}_3$ ), 3.37 (2H, *d*,  $J = 7.2$  Hz,  $-\text{CH}_2$ ) and  $\delta$  5.21–5.36 (7H, *m*, 3x  $-\text{OCH}_2$  and H-allylic). The phenolic proton appeared at  $\delta$  13.93. The aromatic protons of ring A appeared at  $\delta$  6.27 (*d*,  $J = 2.4$ , H-3') and 6.33 (*d*,  $J = 2.4$ , H-5'). The other aromatic protons of ring B appeared at  $\delta$  7.10 (*d*,  $J = 9.0$ , H-5) and both H-2 and H-6 appeared at  $\delta$  7.43–7.45 (*m*) integrating for two protons.

The final step in the synthetic route to flavanone (**1**) was to cyclise the corresponding chalcone (**6**). Prior to this, chalcone (**6**) which was subjected to deprotection *via* acid hydrolysis (HCl 10%) and subsequent treatment with excessive NaOAc in methanol has provided flavanone (**1**) as a brown solid with m.p. 129–131°C (lit.<sup>15</sup> 134–135°C). Interestingly, this reaction gave the desired flavanone (**1**) as a single product in excellent yield (89.3%).

The presence of a broad peak at  $3406\text{ cm}^{-1}$  due to the hydroxyl groups in the IR spectrum suggested that the protecting groups have been removed. The UV spectrum of flavanone (**1**) showed an intense band II at 289 nm with only a shoulder at 336 nm representing band I, which was typical for flavanone.<sup>19</sup> The  $^1\text{H}$  NMR data of flavanone (**1**) showed the signals of the prenyl group were still intact at  $\delta$  1.70 (6H, *s*, 2x  $-\text{CH}_3$ ), 3.36 (1H, *d*,  $J = 7.5$  Hz,  $-\text{CH}_2$ ) and 5.35 (1H, *m*, H-allylic). The formation of

flavanone (**1**) was deduced from the characteristic signals at  $\delta$  2.73 (1H, *dd*,  $J = 17.4$  and 3.3 Hz, H-3a), 3.20 (1H, *dd*,  $J = 17.4$  and 12.9 Hz, H-3b) and 5.44 (1H, *dd*,  $J = 12.9$  and 3.0 Hz, H-2). In addition, the signal of phenolic protons were observed at  $\delta$  8.45 (1H, *s*, H-4'), 9.61 (1H, *s*, H-7) and 12.18 (1H, *s*, H-5). The aromatic protons of ring B gave an AMX coupling system at  $\delta$  6.90 (1H, *d*,  $J = 8.1$  Hz, H-5'), 7.23 (1H, *dd*,  $J = 8.1$  and 2.1 Hz, H-6'), 7.29 (1H, *d*,  $J = 2.1$  Hz, H-2') whereas the protons of ring A were seen at  $\delta$  5.44 (1H, *dd*,  $J = 12.9$  and 3.0 Hz, H-2) and 5.97 (2H, *s*, H-6 and H-8). The spectral data of compound (**1**) (UV, IR, MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR) were similar to that obtained from natural sample.<sup>12</sup>

### 3.2 Antibacterial screening

The antibacterial activities of compounds (**1**) and (**6**) has been assayed at the concentration 1000  $\mu\text{g}/\text{mL}$  against four human pathogenic bacteria. Among them, two were gram-positive and the other two were gram-negative. The inhibitory effect of compounds (**1**) and (**6**) against these organisms are given in table 1. The screening results indicate that only compound (**1**) was active against a gram-negative bacteria, *Escherichia coli* with a mean zone of inhibition  $12.5 \pm 0.3$  mm (table 1).

### 3.3 Determination of the minimum inhibitory concentration (MIC)

The active sample in the disc diffusion method was then tested for its activity by the serial dilution method

**Table 1.** Antibacterial screening for the compounds (1) and (6).

Organism	Diameter of the zone of inhibition (mm)		
	Chalcone (6)	Prenylflavanone (1)	Streptomycin sulphate
<i>Bacillus subtilis</i>	–	–	22.0 ± 0.3
<i>Staphylococcus aureus</i>	–	–	22.5 ± 0.7
<i>Escherichia coli</i>	–	12.5 ± 0.3	22.0 ± 0.0
<i>Pseudomonas aeruginosa</i>	–	–	22.0 ± 0.0

Data represent mean ± SD of three independent experiments performed in duplicate

to determine the minimum inhibition concentration (MIC-value). The MIC value obtained for 4',5,7-trihydroxy-3'-prenylflavanone (1) was 1000 µg/mL against *Escherichia coli*.

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