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# Synthesis and studies of antibacterial activity of pongaglabol

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**Abstract.** Pongaglabol [8-hydroxy-5-phenyl-furo[2,3-h]benzo(b)pyran-7-one] was synthesized and tested for antibacterial effects against *Shigella dysenteriae, Salmonella typhi, Streptococcus b-haemolyticus* and *Staphylococcus aureus*. The synthesized compounds were characterized using UV, IR and $^1$H NMR spectral data.

**Keywords.** Pongaglabol; *Pongamia glabra*; inhibition zone; antibacterial activity; flavone.

## 1. Introduction

*Pongamia glabra* Vent. (Leguminosae) is one of the most commonest and useful medicinal plants of India.[1] The isolation and characterization of pongaglabol has been reported earlier by Talapatra *et al*[2] from the flowers of *Pongamia glabra*. However, no proof was provided by synthesis for the structure of pongaglabol. We now report the synthesis of pongaglabol, starting from phloroacetophenone (**1**), as well as study of its antibacterial activity along with those of other related compounds.

## 2. Experimental

### 2.1 *Materials, methods and instruments*

Melting points were recorded on Gallenkamp apparatus and are uncorrected. IR spectra (KBr) were measured using a Shimadzu, DR-8001 spectrophotometer, $^1$H NMR spectra ($CDCl_3$) on a Brucker WH 200 MHz instrument with TMS as internal standard and UV spectra (MeOH) on a LKB 4053 spectrophotometer. Purity of the compounds was checked by TLC.

### 2.2 *1-(4-Allyloxy-2,6-dihydroxy-phenyl)-ethanone* (**2**)

Phloroacetophenone, **1** (10 g) in acetone (75 ml) was refluxed with allyl bromide (12·5 g) and anhyd. Potassium carbonate (60 g) for 6 h. The reaction mixture was worked up as usual by the literature procedure.[3] The reaction yielded a mixture of products, which on column chromatography gave compound **2** and was obtained as dark brown oily liquid (5·8 ml), b.p. 160–161°C (9 mm).

IR $\nu_{max}^{KBr}$: 3350, 2986, 2924, 1665, 1580, 1505, 1458, 1306, 1252, 1228, 1134, 1108, 1004, 936, 834, 728 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·58 (3H, *s*, $-COC\underline{H}_3$), 4·61 (2H, *d*, *J* = 6·9 Hz, $-OC\underline{H}_2-CH_a=CH_bH_c$), 5·89 (1H, *m*, $-OCH_2-C\underline{H}_a=CH_bH_c$), 5·22 (1H, *dd*, *J* = 7·8 and 2·65 Hz, $-OCH_2-CH_a=C\underline{H}_bH_c$), 5·24 (1H, *dd*, *J* = 13 and 2·65 Hz, $-OCH_2-CH_a=CH_b\underline{H}_c$), 6·03 (2H, *s*, $C_3-H$ and $C_5-H$), 6·48 (1H, *s*, $C_6-OH$), 12·57 (1H, *s*, $C_2-OH$, chelated).

### 2.3 *1-(3-Allyl-2,4,6-trihydroxy-phenyl)-ethanone* (**3**)

The above 1-(4-allyloxy-2,6-dihydroxy-phenyl)-ethanone (**2**, 4 g) was heated in an oil-bath cautiously and was worked up as usual by the literature procedure.[4] The pure 3-C-allylphloroacetophenone was obtained as colourless needles (1·4 g), m.p. 141–142°C.

UV $\lambda_{Max}^{MeOH}$: 221, 280 nm.

IR $\nu_{max}^{KBr}$: 3365, 3073, 2924, 1640, 1592, 1491, 1414, 1373, 1212, 1172, 1112, 1001, 976, 907, 880, 794, 754 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·55 (3H, *s*, $-COC\underline{H}_3$), 3·26 (2H, *d*, *J* = 7·2 Hz, $-C\underline{H}_2-CH_a=CH_bH_c$), 6·26 (1H, *m*, $-CH_2-C\underline{H}_a=CH_bH_c$), 4·93 (1H, *dd*, *J* = 8·1 and

*For correspondence

2·67 Hz, $-CH_2-CH_a=CH_b\underline{H}_c$), 4·97 (1H, *dd*, *J* = 13·1 and 2·67 Hz, $-CH_2-CH_a=C\underline{H}_bH_c$), 5·98 (1H, *s*, $C_3$–H), 6·45 (1H, *s*, $C_4$·OH), 6·75 (1H, *s*, $C_6$–OH), 12·38 (1H, *s*, $C_2$–OH, chelated).

### 2.4 *5-Acetyl-4,6-dihydroxy-benzofuran* (**4**)

1-(3-Allyl-2,4,6-trihydroxy-phenyl)-ethanone (**3**, 500 mg) was dissolved in ethyl acetate (150 ml), an equal volume of water and osmium tetraoxide (75 mg) was added. The mixture was stirred on a magnetic stirrer for 1·5 h during which period potassium periodate (2·5 g) was added and the mixture was stirred for two more hours. The reaction mixture was worked up as usual.[3,5] The compound **4** was crystallized from benzene:acetone (10:1) and obtained as colourless needles (250 mg), m.p. 95–96°C.

UV $l_{Max}^{MeOH}$: 241, 276, 315 nm.

IR $n_{max}^{KBr}$: 3370, 2925, 2854, 1645, 1560, 1490, 1434, 1163, 1058, 905, 800, 742 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·57 (3H, *s*, $-COC\underline{H}_3$), 6·68 (1H, *s*, $C_5$–H), 6·76 (1H, *s*, $C_6$–OH), 6·92 (1H, *d*, *J* = 2 Hz, $C_3'$–H), 7·52 (1H, *d*, *J* = 2 Hz, $C_2'$–H), 12·43 (1H, *s*, $C_2$–OH, chelated).

### 2.5 *Synthesis of (E)-1-(4,6-dihydroxybenzofuran-5-yl)-3-phenylprop-2-en-1-one* (**5**)

A mixture of 5-acetyl-4,6-dihydroxy-benzofuran (**4**, 200 mg) and benzaldehyde in ethanolic solution of KOH (5% 15 ml) was kept at room temperature for about 75 h. The reaction mixture was diluted with ice cold water, acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous $Na_2SO_4$ and evaporated to dryness. It was crystallized from benzene–petroleum spirit as yellow needles (150 mg), yield 42·85% m.p. 151–152°C, $R_f$ 0·64 (benzene:acetone; 9:1).

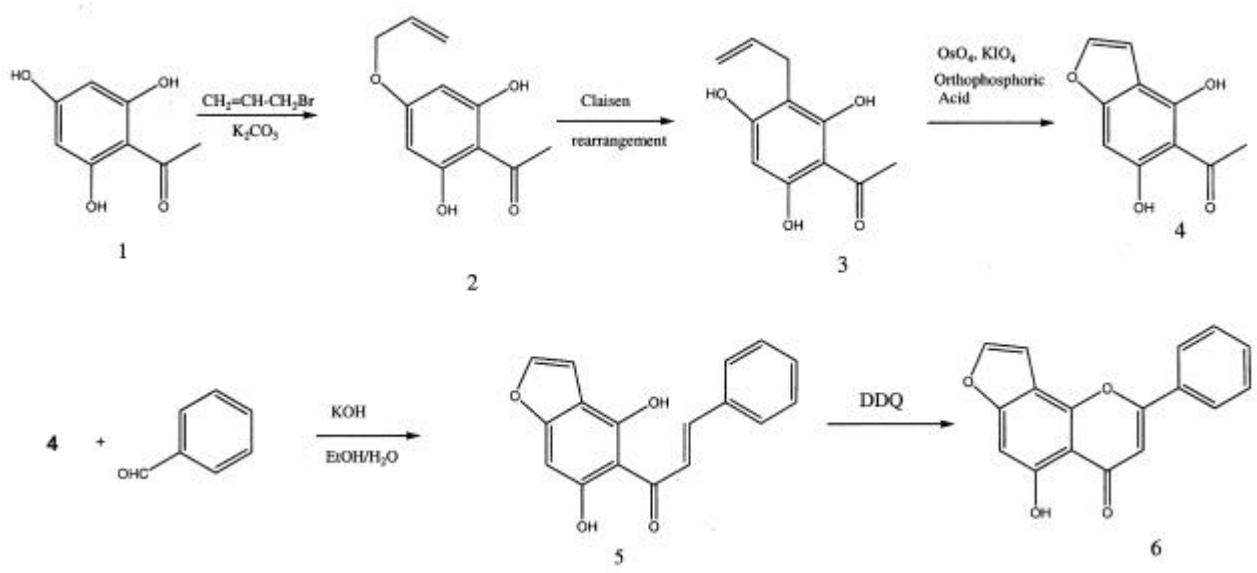
UV $l_{Max}^{MeOH}$: 245, 312 nm.

IR $n_{max}^{KBr}$: 3367, 2924, 2853, 1640, 1600, 1575, 1428, 1360, 1288, 1156, 1049, 1000, 906, 803, 756 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 6·48 (1H, *s*, $C_6'$–OH), 6·79 (1H, *d*, *J* = 2 Hz, $C_3''$–H), 6·92 (1H, *s*, $C_5'$–H), 7·16 (1H, *d*, *J* = 8·6 Hz, $C_4$–H), 7·24 (2H, *t*, *J* = 8·6 Hz, $C_3$–H and $C_5$–H), 7·31 (2H, *d*, *J* = 8·6 Hz, $C_2$–H and $C_6$–H), 7·39 (1H, *d*, *J* = 9 Hz, $C_a$–H), 7·58 (1H, *d*, *J* = 2 Hz, $C_2''$–H), 7·96 (1H, *d*, *J* = 9 Hz, $C_b$–H), 12·68 (1H, *s*, $C_2'$–OH, chelated).

### 2.6 *Synthesis of 8-hydroxy-5-phenyl-furo[2,3-h] benzo(b)pyran-7-one* (**6**)

To a solution of (E)-1-(4,6-dihydroxybenzofuran-5-yl)-3-phenylprop-2-en-1-one (**5**, 300 mg) in dry dioxane (75 ml) was added DDQ (255 mg) and the solution refluxed for 3 h. The product purified by preparative TLC over silica gel using petroleum ether–benzene (1:2) as developing solvent. It crystallized from chloroform-petroleum ether as yellow




**Scheme 1.**

needles (240 mg), yield 80%, m.p. 197–198°C (lit.[2] m.p. 198°C), $R_f$ 0·46 (benzene–ethyl acetate; 4:1). It gave green coloration with ferric chloride solution.

UV $l_{Max}^{MeOH}$: 224, 261 and 285 nm.

IR $n_{max}^{KBr}$: 2924, 2854, 1652, 1602, 1574, 1436, 1418, 1359, 1270, 1155, 1048, 801, 755 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 6·76 (1H, *s*, $C_3$–H), 6·94 (1H, *d*, $J''_{3,6}$ = 1·01 Hz, $C_6$–H), 6·99 (1H, *dd*, $J''_{3,2''}$ = 2·24 Hz, $J_{6,3''}$ = 1·01 Hz, $C''_3$–H), 7·58–7·57 (4H, *m*, $C''_2$–H, $C'_3$–H, $C'_4$–H and $C'_5$–H), 7·89–7·98 (2H, *m*, $C'_2$–H and $C'_6$–H), 12·38 (1H, *s*, $C'_2$–OH, chelated).

### 2.7 *Antibacterial screening*

The antibacterial activities of synthesized compounds **5** and **6** were studied against four bacteria, viz., *Shigella dysenteriae* ($G^-$), *Salmonella typhi* ($G^-$), *Streptococcus b-haemolyticus* ($G^+$) and *Staphylococcus aureus* ($G^+$). For the detection of antibacterial activities, the filter paper disc diffusion method[6,7] was used. Kanamycin was used as standard antibiotic for the antibacterial activities. Nutrient agar (NA) was used as basal medium for test bacteria. These agar media were inoculated with 0·5 ml of the 24 h liquid cultures containing $10^7$ microorganisms/ml. The diffusion time was 24 h at 5°C for bacteria. The incubation time was 12 h at 37°C for bacteria. Discs with only DMSO were used as control. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones.

### 2.8 *Determination of the minimum inhibitory concentration (MIC)*

To determination of the minimum inhibitory concentration (MIC) the serial dilution technique[8] were followed using nutrient broth medium. The MIC value of the compound **5** and **6** were determined against *S dysenteriae* ($G^-$) and *S b-haemolyticus* ($G^+$).

## 3. Results and discussion

### 3.1 *Synthesis of pongaglabol*

The synthesis of pongaglabol was accomplished starting from phloroacetophenone (**1**) as shown in scheme 1.

Allylation of phloroacetophenone with allyl bromide gave 1-(4-allyloxy-2,6-dihydroxy-phenyl)-ethanone (**2**), as brown oily liquid; b.p. 160–161°C. The IR spectrum of **2** showed hydroxyl signal at 3350 $cm^{-1}$ and carbonyl stretching absorption at 1665 $cm^{-1}$. The spectral data (UV, IR and $^1$H NMR, see experimental) confirmed structure **2** as 1-(4-allyloxy-2,6-dihydroxy-phenyl)-ethanone. The compound **2** on Claisen rearrangement gave the corresponding 1-(3-allyl-2,4,6-trihydroxy-phenyl)-ethanone (**3**) and was obtained as colourless needles; m.p. 141–142°C. The UV absorption maxima of **3** showed at 221 and 280 nm. The IR absorption peaks of **3** due to hydroxyl groups and carbonyl group appeared at 3365 and 1640 $cm^{-1}$ respectively. The assignment of structure **3** as 1-(3-allyl-2,4,6-trihydroxy-phenyl)-ethanone was supported by spectral data ($^1$H NMR, UV and IR, see experimental). When compound **3** was stirred with $OsO_4/KIO_4$ in presence of orthophosphoric acid yielded 5-acetyl-4,6-dihydroxy-benzofuran (**4**). It was obtained as colourless needles; m.p. 95–96°C. It gave brown colouration with $FeCl_3$ solution and its UV spectrum appeared at 241, 276 and 315 nm. The IR band at 3370 and 1645 $cm^{-1}$ indicated the presence of chelated hydroxyl and carbonyl group respectively. Spectral data (see experimental) supported compound **4** to be as 5-acetyl-4,6-dihydroxy-benzofuran.

**Table 1.** Antibacterial screening for the compounds **5** and **6**.

| Compound | Concentration | *Shigella dysenteriae* | *Salmonella typhi* | *Streptococcus b-haemolyticus* | *Staphylococcus aureus* |
|---|---|---|---|---|---|
| **5** | 100 µg/disc | 9 | – | 9 | 9 |
| | 200 µg/disc | 15* | 16* | 15* | 14 |
| **6** | 100 µg/disc | 10 | 9 | 10 | 9 |
| | 200 µg/disc | 14* | 15* | 16* | 13 |
| Kanamycin (K-30) | 30 µg/disc | 24 | 22 | 23 | 24 |

Alkaline condensation of **4** with benzaldehyde gave the corresponding chalcone **5** in good yield as yellow needles; m.p. 151–152°C. The spectral data (UV, IR and $^1$H NMR) confirmed compound **5** as (E)-1-(4,6-dihydroxybenzofuran-5-yl)-3-phenylprop-2-en-1-one. When compound **5** was refluxed with DDQ in dry dioxane for 3 h yielded 8-hydroxy-5-phenyl-furo[2,3-h]benzo(b)pyran-7-one (**6**, pongaglabol) as yellow needles; m.p. 197–198°C (lit.[2] m.p. 198°C). The spectral data of compound **6** (UV, IR and $^1$H NMR) were similar to that obtained from natural sample.

### 3.2 *Antibacterial activities*

The antibacterial activities of compounds **5** and **6** have been studied at the concentration of 100 μg/disc and 200 μg/disc against four human pathogenic bacteria. Among them, two were Gram-positive and the rest two were Gram-negative. The inhibitory effects of compounds **5** and **6** against these organisms are given in table 1.

The screening result indicate that compounds **5** and **6** showed antibacterial activities against all the bacteria tested, except that compound **5** showed no effect against *S typhi* at a concentration of 100 μg/disc.

### 3.3 *Minimum inhibitory activity*

The minimum inhibitory concentration of compounds **5** and **6** were determined against *S dysenteriae* and *S b-haemolyticus* by serial dilution method. The MIC level of compounds **5** and **6** was found to be 64 μg/ml against both *S dysenteriae* and *S b-haemolyticus* respectively.

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