Facile Synthesis and Antibacterial Activity of Naturally Occurring 5-Methoxyfuroflavone

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A convenient synthesis of 5-methoxyfuroflavone (6, pongaglabol methyl ether), a constituent of some Pongamia or Millettia genus, was achieved by starting from 2,4-dihydroxy-6-methoxyacetophenone *via* a chalcone precursor, followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). This five-step reaction (total yield: 21.6%) is more facile with that of previously utilized procedures using each different starting material. Antibacterial activities of the above compound and its precursor chalcones, which also belongs to the class of furoflavonoids, were tested by the disc diffusion method against *Shigella dysenteriae*, *Salmonella typhi*, *Streptococcus-β-haemolyticus*, and *Staphylococcus aureus*. 5-Methoxyfuroflavone showed moderate bactericidal activity against all tested bacterial strains, whereas its corresponding chalcone compound revealed a selective activity.

Key words 5-methoxyfuroflavone; furoflavonid; facile synthesis; antibacterial activity

Furoflavones are an abundant subclass of the flavonoid major class of natural product compounds and are widely distributed in the plant kingdom and in dietary foods.[1—3] During the last few decades, nearly 300 chemically exclusive furoflavones have been isolated from plants.[2,4] Isolation and characterization of 5-methoxyfuroflavone (**6**, 5-methoxy-2-phenyl-4*H*-furo[2,3-*h*]chromen-4-one), which is also known as pongaglabol methyl ether, have been reported from *Pongamia glabra*, *P. pinnata*, *Millettia pachycarpa*, *M. sanagana*, and *Ochna squarrosa*.[2—5] In the structure of furoflavones, the furan moiety is annellated at ring A of the 4*H*-chromen-4-one skeleton in a linear or angular position, *i.e.* linked to either C-6/C-7 or C-7/C-8, respectively. The furan ring in 5-methoxyfuroflavone is located at the C-7/C-8 (angular) positions in ring A.

In 1956, Pavanaram and Row[6] first reported on the synthesis of 5-methoxyfuroflavone from the *O*-benzoyl derivative of 5-acetyl-4-hydroxy-6-methoxycoumarone, followed by formation of diketone and cyclization of diketone using an acetic acid and hydrochloric acid mixture in twelve steps, with a 20.29% overall yield. Thereafter, Aneja *et al.*[7] improved its synthesis with a 26.33% yield in seven steps by modification of the above method for comparison and establishment of the structure of 6-methoxyfuroflavone isolated from *P. glabra*, starting from chrysin, a naturally occurring flavone. However, chrysin (5,7-dihydroxyflavone) has to be prepared from 2,4,6-trihydroxyacetophenone as a starting material for total synthesis of 5-methoxyfuroflavone in two steps. According to the aforesaid method, the furan ring was built up in the final step, followed by Claisen migration, ozonolysis, and polyphosphoric cyclization of 7-*O*-allyl-5-*O*-methylchrysin. However, we were able to improve the synthetic method by reducing steps (total five steps) as well overall yield (21.6%) by building of the furan ring prior to formation of the flavone skeleton, which is more facile compared to that of previous methods. In the present study, we describe an alternative convenient method for synthesis of 5-methoxyfuroflavone (**6**), starting from commercially available 2,4-dihydroxy-6-methoxyacetophenone (**1**) in five steps.

We also examined the ability of synthetic natural furoflavone **6** to inhibit the growth of *Shigella dysenteriae* (G^-), *Salmonella typhi* (G^-), *Streptococcus-β-haemolyticus* (G^+), and *Staphylococcus aureus* (G^+) together with its chalcone precursor **5**. To date, the bactericidal action of compounds **5** and **6** has not been reported.

Synthesis of 5-Methoxyfuroflavone (6, Pongaglabol Methyl Ether) The crucial starting material **4** for preparation of 5-methoxyfuroflavone (**6**) was readily synthesized in

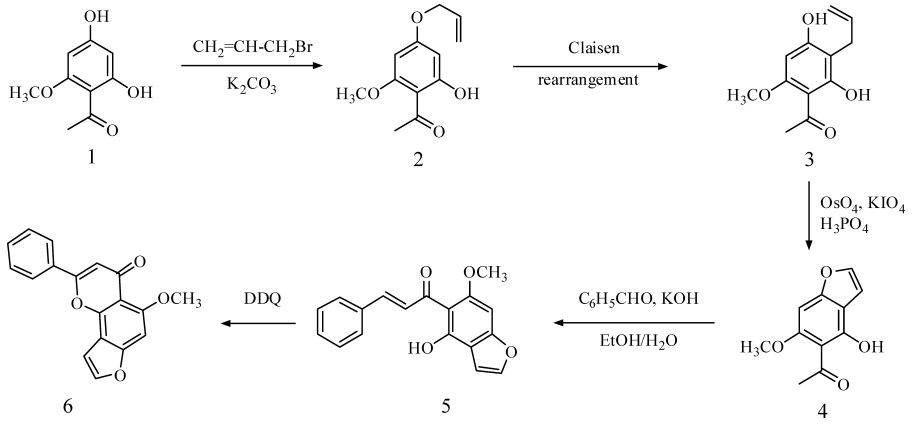

Chart 1. Procedure for Synthesis of 5-Methoxyfuroflavone (**6**)

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Table 1. Antibacterial Activities of Compounds **5** and **6** and Standard Reference Compounds

Compd.	Concn (μg/disc)	Growth inhibition area (mm)[a]			
		Shigella dysenteriae	*Salmonella typhi*	*Streptococcus-β-haemolyticus*	*Staphylococcus aureus*
5	100	0±0	0±0	6.7±1.2	6.0±1.7
	200	0±0	0±0	8.0±1.0	8.67±2.1
6	100	4.5±1.0	5.0±1.5	8.0±1.8	7.5±1.0
	200	7.0±1.5	7.0±1.0	11.0±0.8	10.5±0.5
Ciprofloxacin	30	29.0±1.0	31.0±1.5	27.0±0.8	28.0±1.0
Kanamycin	30	24.0±1.2	22.0±1.0	23.0±0.6	24.0±0.5

a) Inhibitory activity is expressed as the diameter (in mm) of the observed inhibition zone. Data are means±S.D. for at least three experiments.

three steps from 2,4-dihydroxy-6-methoxyacetophenone (**1**), as shown in Chart 1. Chemoselective *O*-allylation[8] of **1** using allyl bromide with potassium carbonate in acetone gave 1-(4-allyloxy-2-hydroxyl-6-methoxyphenyl)ethanone (**2**) as an oily liquid in a 51.0% yield. The chemical shift of *O*-allyl group could be assigned in usual way,[9] *i.e.* two *O*-methylene protons at δ 4.98 as a doublet (J=6.9 Hz), one olefinic proton at δ 6.01 as a multiplet and two olefinic protons at δ 5.61 (J=7.9, 2.66 Hz) and 5.68 (J=13.1, 2.66 Hz) as two double-doublets. The presence of a singlet for chelated hydroxyl group at δ 12.69 assigned to C_2–OH proton, indicating the chemoselective 4-*O*-allylation of compound **1**. Next, the regioselective Claisen rearrangement[10] of compound **2** under pyrolytic conditions yielded the corresponding 1-(3-allyl-2,4-dihydroxy-6-methoxyphenyl)ethanone (**3**) as colorless needles. In its ^{1}H-NMR spectrum, the *C*-allyl group was appeared in usual way[9] *i.e.* two *C*-methylene protons at δ 3.29 as a doublet (J=7.2 Hz), one olefinic proton at δ 6.55 as a multiplet and two olefinic protons at δ 5.21 (J=8.1, 2.67 Hz) and 5.30 (J=13.1, 2.67 Hz) as two double-doublets. Two singlets for a free and chelated hydroxyl group at δ 6.48 and 12.58 assigned to C_4– and C_2–OH protons, respectively, which suggested the stereoselective *C*-allylation of compound **2**. The site-selective [3,3] Claisen rearrangement preferably occurred at C_3-position than C_5-position of compound **2**, which also supported by previous reports.[7,11] Careful analysis of the reaction mixture after Claisen rearrangement also revealed formation of small amounts (5%) of the isomer of **3** as 1-(5-allyl-2,4-dihydroxy-6-methoxyphenyl)-ethanone, which was identified by spectral analysis (data not shown). When compound **3** was subjected to OsO_4/KIO_4 oxidation in the presence of orthophosphoric acid, 5-acetyl-4-methoxy-6-hydroxybenzofuran (**4**) was produced as colorless needles. The one-pot furan ring formation of **3** was carried out according to the known procedures.[8,11] Two *cis*-olefinic protons of fused-furan ring of compound **4** were appeared at δ 6.99 and 7.61 as two doublets (J=2.0 Hz). A singlet for chelated hydroxyl group at δ 12.89 confirms the existence of a OH group at C_2-position of compound **4**. The other ^{1}H-NMR peaks of compound **4** were observed in appropriate positions. Aldol condensation of **4** with benzaldehyde using potassium hydroxide in ethanol gave the corresponding (*E*)-1-(4-hydroxy-6-methoxybenzofuran-5-yl)-3-phenylprop-2-en-1-one (**5**) in a 77% yield. Its ^{1}H-NMR spectrum revealed the presence of a set of *trans*-olefinic protons at δ 7.28 and 7.68 (each d, J=16.0 Hz) in addition to two olefinic protons of a fused furan ring at δ 6.99 and 7.61 (d, J=2.0 Hz) in ring A. Two singlets at δ 13.01 and 3.83 showed the presence of a chelated hydroxyl (C_2'-OH) group and a methoxy group, respectively, in the A ring. The five and one aromatic proton of rings B and A appeared as a multiplet at δ 7.52—7.65 (m, 5H) and a singlet at δ 7.22, respectively. When compound **5** was treated with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) in dry dioxane, 5-methoxyfuroflavone (**6**; 5-methoxy-2-phenyl-4*H*-furo[2,3-*h*]chromen-4-one) was produced as yellow needles in good yield (83%). Spectral data and the physical properties of **6** were consistent with those of natural 5-methoxyfuroflavone reported in the literature.[5]

Table 2. MIC Levels of Compounds **5** and **6** and Standard Reference Compounds

Compd.	Test organism, MIC (μg/ml)[a]	
	Shigella dysenteriae	*Streptococcus-β-haemolyticus*
5	—	128
6	256	128
Ciprofloxacin	4	8
Kanamycin	8	8

a) MIC values were obtained in triplicate.

Antibacterial Evaluation Compounds **5** and **6** were evaluated for their antibacterial activities against four strains, including *Shigella dysenteriae* (G^-), *Salmonella typhi* (G^-), *Streptococcus-β-haemolyticus* (G^+), and *Staphylococcus aureus* (G^+), and their activities were compared with standard antibiotics. Antibacterial activity of 5-methoxyfuroflavone (**6**) was higher than that of the chalcone precursor **5**; further, **6** showed bactericidal activity against all tested strains, whereas **5** showed selective activity (Table 1). The effect of bacterial growth inhibition of both compounds was weaker than that of the antibiotics ciprofloxacin or kanamycin. The minimum inhibitory concentrations (MIC) of both compounds were calculated against *S. dysenteriae* and *S.-β-haemolyticus* by serial dilution method. As presented in Table 2, compound **6** showed MIC levels of 256 and 128 μg/ml, respectively.

In conclusion, we have described a facile methodology for the synthesis of 5-methoxyfuroflavone which displays a moderate antibacterial activity against both Gram-positive and Gram-negative bacterial strains. The present methodology would be helpful to synthesize more derivatives of natural as well as synthetic furoflavonoids with wide range of biological activity.

Experimental

General Melting points were determined on a Yanagimoto MP 500D and were uncorrected. ^{1}H-NMR spectra were recorded on a Brucker WH 300 MHz spectrometer and chemical shifts were expressed as δ values (ppm) using tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out on a Merck Si gel 60 (0.2—0.5 mM). Purity of the compounds was checked by TLC. Compound **1** was supplied from BBP (China) and other reagents were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Preparation of 1-(4-Allyloxy-2-hydroxyl-6-methoxyphenyl)ethanone (2) 2,4-Dihydroxy-6-methoxyacetophenone (**1**) (6 g, 0.033 mol) in acetone (60 ml) was refluxed with allyl bromide (4.5 g, 0.037 mol) and anhyd. K_2CO_3 (20 g, 0.15 mol) for 6 h. After completion of the reaction, it was acidified with dil. HCl and the mixture was partitioned between water and ether. Ether was evaporated, and the residue was subjected to silica gel column chromatography (*n*-hexane/dichloromethane=20 : 1), affording compound **2** as an oily liquid; yield 51.0% (0.017 mol, 3.74 g); ^{1}H-NMR ($CDCl_3$) δ: 2.58 (3H, s, $COCH_3$), 3.91 (3H, s, OCH_3), 4.98 (2H, d, J=6.9 Hz, OCH_2), 6.01 (1H, m, C<u>H</u>=CH_2), 5.61 (1H, dd, J=7.9, 2.66 Hz, CH=C<u>H</u>$_a$<u>H</u>$_b$), 5.68 (1H, dd, J=13.1, 2.66 Hz, CH=C<u>H</u>$_a$<u>H</u>$_b$), 6.93 (1H, d, J=2.4 Hz, H-3), 7.01 (1H, d, J=2.4 Hz, H-5), 12.69 (1H, s, OH). *Anal.* Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.94; H, 6.41.

Preparation of 1-(3-Allyl-2,4-dihydroxy-6-methoxyphenyl)ethanone (3) Compound **2** (3 g, 0.014 mol) was heated cautiously in an oil-bath at 150—160 °C and worked up as usual to give **3** as colorless needles; yield 85% (0.012 mol, 2.64 g), mp 89—91 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.62 (3H, s, $COCH_3$), 3.29 (2H, d, J=7.2 Hz, C<u>H</u>$_2$), 3.91 (3H, s, OCH_3), 6.55 (1H, m, C<u>H</u>=CH_2), 5.21 (1H, dd, J=8.1, 2.67 Hz, CH=C<u>H</u>$_a$<u>H</u>$_b$), 5.30 (1H, dd, J=13.1, 2.67 Hz, CH=C<u>H</u>$_a$<u>H</u>$_b$), 7.03 (1H, s, H-3), 6.48 (1H, s, C_4–OH), 12.58 (1H, s, C_2–OH). *Anal.* Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.92; H, 6.29.

Preparation of 5-Acetyl-4-methoxy-6-hydroxybenzofuran (4) Compound **3** (500 mg, 2.3 mmol) was dissolved in orthophosphoric acid (150 ml) and an equal volume of water and osmium tetraoxide (75 mg, 0.034 mmol) was added. The mixture was stirred on a magnetic stirrer for 1.5 h during which period potassium periodate (2.5 g, 11 mmol) was added and the mixture was stirred for two more hours. The reaction mixture was partitioned between water and ether; the ether layer was then dried with anhydrous sodium sulfate overnight. The solvent was evaporated, and the residue was subjected to silica gel column chromatography (*n*-hexane/ethyl acetate=20 : 1) to furnish **4** as colorless needles; yield 78% (1.8 mmol, 0.37 g), mp 72—73 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.59 (3H, s, $COCH_3$), 3.93 (3H, s, OCH_3), 7.12 (1H, s, H-5), 6.92 (1H, d, J=2.0 Hz, OCH=C<u>H</u>), 7.56 (1H, d, J=2.0 Hz, OC<u>H</u>=CH), 12.89 (1H, s, OH). *Anal.* Calcd for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 64.21; H, 4.95.

Synthesis of (*E*)-1-(4-Hydroxy-6-methoxybenzofuran-5-yl)-3-phenyl-prop-2-en-1-one (5) A mixture of compound **4** (310 mg, 1.5 mmol) and benzaldehyde (320 mg, 3 mmol) in ethanolic solution of KOH (5%, 15 ml) was kept at room temperature for about 72 h. The reaction mixture was diluted with ice cold water, acidified with cold dil. HCl, and the mixture was partitioned between water and ether. After the ether layer was dried with anhydrous sodium sulfate overnight, the solvent was evaporated. The remaining residue was subjected to silica gel column chromatography (*n*-hexane/dichloromethane (DCM)=15 : 1) to yield compound **5** as pale yellow needles; yield 77.0% (1.16 mmol, 0.342 g); mp 112—116 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 3H, OCH_3), 6.99 (d, 1H, J=2.0 Hz, OCH=C<u>H</u>), 7.22 (s, 1H, H-5′), 7.52—7.65 (m, 5H, H-2, 3, 4, 5, 6), 7.28 (d, 1H, J=16.0 Hz, C_α-H), 7.61 (d, 1H, J=2.0 Hz, OC<u>H</u>=CH), 7.68 (d, 1H, J=16.0 Hz, C_β-H), 13.01 (s, 1H, OH); *Anal.* Calcd for $C_{18}H_{14}O_4$: C, 73.46; H, 4.79. Found: C, 73.61; H, 4.67.

Synthesis of 5-Methoxy-2-phenyl-4*H*-furo[2,3-*h*]chromen-4-one (6) Compound **5** (441 mg, 1.5 mmol) in dry dioxane (75 ml) was added with DDQ (340 mg, 1.5 mmol) and the solution was refluxed for 3 h. Following removal of dioxane, the residue was subjected to silica gel column chromatography (*n*-hexane/ethyl acetate=9 : 1), providing the desired compound **6** as colorless needles; yield 83% (1.25 mmol, 0.364 g); mp 178—180 °C (lit.[5] mp 176 °C); ^{1}H-NMR ($CDCl_3$) δ: 3.91 (s, 3H, OCH_3), 6.89 (s, 1H, H-3), 7.02 (s, 1H, H-6), 7.12 (d, 1H, J=2.0 Hz, OCH=C<u>H</u>), 7.59—7.67 (m, 5H, H-2′, 3′, 4′, 5′, 6′), 7.79 (d, 1H, J=2.0 Hz, OC<u>H</u>=CH). *Anal.* Calcd for $C_{18}H_{12}O_4$: C, 73.97; H, 4.14. Found: C, 73.88; H, 4.26.

Antibacterial Screening The antibacterial effect was determined by the filter paper disc diffusion method.[12] Discs with only dimethyl sulfoxide (DMSO) were used as control, and ciprofloxacin and kanamycin were used as positive controls. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones in triplicate. The minimum inhibitory concentration (MIC, in μg/ml) was determined against *S. dysenteriae* (G^-) and *S.-β-haemolyticus* (G^+) using nutrient broth medium (DIFCO) by serial dilution technique.[12] MIC was considered to be the lowest concentration of the tested compound (in DMSO) that inhibits the growth of bacteria.

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