Scientific Paper

SYNTHESIS AND STUDIES OF ANTIMICROBIAL ACTIVITY OF LANCEOLATIN B

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Abstract

Phytochemical studies of *Pongamia pinnata* showed an abundance of [2″, 3″: 7, 8]furanoflavone (lanceolatin B). Now, it has been synthesized from β-resacetophenone *via* chalcone precursor followed by the treatment of DMSO/I_2, diphenyl sulphide and DDQ, respectively. The antibacterial activity of [2″, 3″: 7, 8]furanoflavone and its corresponding chalcone were tested by the disc diffusion method for antibacterial effects against *Shigella dysenteriae*, *Salmonella typhi*, *Streptococcus-β-haemolyticus* and *Staphylococcus aureus*.

Key words: *Pongamia pinnata*, lanceolatin B, antibacterial activity

Introduction

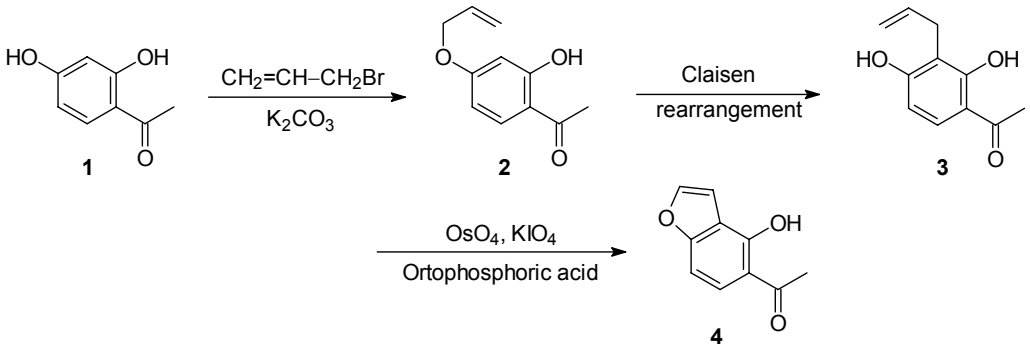
Pongamia pinnata (Leguminosae) is one of the most useful medicinal plants of India[1] as well as Bangladesh.[2] All its parts have been extensively studied and are known to contain [2″, 3″: 7, 8]furanoflavone named as lanceolatin B.[3-7] All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, painful rheumatic joints wounds, ulcers, etc.[8] Pirrung, M. C. *et al*.[9] reported the synthesis of lanceolatin B through dipolar cycloaddition of diazocyclohexene-1, 3-diones, leading to benzofuran derivatives. This paper now reports herein a synthesis of [2″, 3″: 7, 8]furanoflavone (lanceolatin B) and study the antibacterial activity of this furanoflavone along with its chalcone precursor. The synthetic method of [2″, 3″:7, 8]furanoflavone has been accomplished in five steps starting from β-resacetophenone (**1**).

Results and discussion

The synthesis of [2″, 3″:7, 8]furanoflavone (lanceolatin B) was accomplished starting from β-resacetophenone (**1**) as shown in Scheme 1 and Scheme 2.

β-Resacetophenone (**1**) when refluxed with allyl bromide in presence of K_2CO_3 and acetone yielded 4-O-allyresacetophenone[10] (**2**), which on Claisen-rearrangement

gave 3-C-allylresacetophenone[11] (**3**). The compound **3** was subjected to OsO_4/KIO_4 oxidative double bond cleavage followed by orthophosphoric acid cyclization to 2-hydroxyfurano(2′, 3′ : 4, 3)acetophenone[12] (**4**).

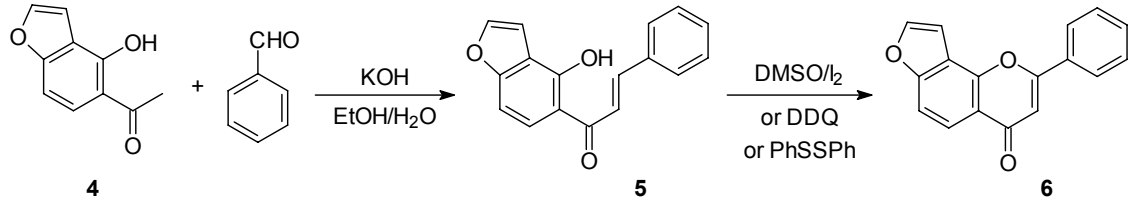


Scheme 1

Claisen condensation of compound **4** with benzaldehyde yielded the corresponding chalcone **5** in good yield (53.70%) and it was obtained as yellow needles, mp 112-114 °C. The UV spectrum of compound **5** showed characteristics absorption band at 254, 276 and 328 nm. The IR spectrum of compound **5** showed absorption frequencies at 3452 and 1645 cm^{-1} indicating the presence of a hydroxyl, a conjugated carbonyl group and the absorption peaks at 1600 and 1592 cm^{-1} indicated the presence of unsymmetric ethylenic double bond and aromatic rings, respectively. In its ^1H NMR spectrum, a set of *trans*-olefinic proton at δ 7.58 and 8.04 (each d, J = 16.0 Hz) and a chelated hydroxyl group at δ 12.68 assigned to C_2′-OH proton. The ^1H NMR also exhibited the presence of two olefinic protons of a fused furan ring at δ 7.12 and 7.79 (d, J = 2.1 Hz). In the aromatic region, a set of two *ortho*-coupled protons of the A-ring at δ 7.05 and 7.91 (J = 8.9 Hz) and five protons of the B-ring at δ 7.21-7.30 (m) were observed.

The compound **5**, which on separately treatment with DDQ, $DMSO/I_2$ and diphenyl disulphide gave the desired compound **6** (lanceolatin B) in excellent yield. The spectral data (UV, IR and ^1H NMR) and melting point of compound **6** were very much similar with the natural sample of [2″, 3″:7, 8]furanoflavone (lanceolatin B).

Antibacterial activities. The antibacterial activities of compounds **5** and **6** have been assayed using a filter paper disc diffusion method[13,14] 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.



Scheme 2. Synthesis of lancheolatin B.

Table 1. Antibacterial screening for the compound **5** and **6**.[a]

Compound	Concentration	S. dysenteriae	S. typhi	S-β-haemolyticus	S. aureus
5	100 μg/disc	9	-	9	10
	200 μg/disc	15	16	15	14
6	100 μg/disc	10	9	10	9
	200 μg/disc	14	15	16	13
K-30 [b]	30 μg/disc	24	22	23	24

[a] Inhibitory activity is expressed as the diameter (in mm) of the observed inhibition zone.
[b] Kanamycin-30.

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

Minimum Inhibitory Activity. The minimum inhibitory concentration of the compounds 5 and 6 were determined against *S. dysenteriae* and *S-β-haemolyticus* by serial dilution method.[15] The MIC level of both the compound **5** and **6** was found 64 μg/mL against *S. dysenteriae* and *S-β-haemolyticus*, respectively.

Experimental

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 ($CDCl_3$) were recorded on a Bruker WH 200 MHz instrument with TMS as an internal standard and UV spectra (MeOH) on a LKB 4053 spectrophotometer. Purity of the compounds was checked by tlc. **CAUTION:** benzene is flammable, may cause cancer, and should be handled with care.

Synthesis of [2″, 3″ : 4′, 3′] furanochalcone (5). A mixture of 2-hydroxyfurano(2′, 3′ : 4, 3)acetophenone (**4**, 10 mmol, 1.76 g) and benzaldehyde (1.1 eqiv., 1.16 g) in ethanolic solution of KOH (5%, 15 mL) was kept at room temperature for about 75 hr. The reaction mixture was diluted with ice-cold water, acidified with cold diluted HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous

Na_2SO_4 and evaporated to dryness. It was purified by preparative tlc (petroleum ether : acetone; 10:1) and crystallized from benzene-petroleum ether as yellow needles (yield 1.55 g, 53.70%), mp. 112-114 °C, R_f 0.64 (benzene: acetone; 9:1). Anal. Calcd for $C_{17}H_{12}O_3$: C 77.26, H 4.58. Found: C 77.41, H 4.42. UV λ_{max}^{MeOH}: 254, 276, 328 nm. IR ν 3452, 1645, 1600, 1592, 1470, 1420, 1375, 1325 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.05 (d, 1H, *J* 8.9 Hz, C_5'-H), 7.12 (d, 1H, *J* 2.1 Hz, C_4''-H), 7.21-7.30 (m, 5H, C_2-H, C_3-H, C_4-H, C_5-H and C_6-H), 7.58 (d, 1H, *J* 16.0 Hz, C_α-H), 7.79 (d, 1H, *J* 2.1 Hz, C_5''-H), 7.91 (d, 1H, *J* 8.9 Hz, C_6'-H), 8.04 (d, 1H, *J* 16.0 Hz, C_β-H), 12.68 (s, 1H, C_2'-OH, chelated).

Synthesis of [2″, 3″ : 7, 8] furanoflavone (6) using DDQ. The chalcone (**5**, 0.75 mmol, 200 mg) in dry dioxane (50 mL) was added DDQ (0.68 mmol, 155 mg) and the solution refluxed for 3 hr. The product was purified by preparative tlc over silica gel using petroleum ether - benzene (1:2) as developing solvent. It crystallized from chloroform-petroleum ether as colorless needles (yield 120 mg, 60%), mp 137-138 °C (Lit.[6] mp 137 °C), R_f 0.46 (benzene - ethyl acetate; 4:1). It gave positive Mg/HCl (yellow colouration) and Labat tests and a blue fluorescence in UV light. Anal. Calcd for $C_{17}H_{10}O_3$: C 77.85, H 3.84. Found: C 77.66, H 3.68. UV λ_{max}^{EtOH}: 250, 330 nm. IR ν 1644, 1580, 1405, 1360, 1345 cm^{-1}. ^1H NMR ($CDCl_3$) δ 6.86 (s, 1H, C_3-H), 7.22 (br d, 1H, *J* 2.1 Hz, C_4''-H), 7.55-7.61 (m, 4H, C_3'-H, C_4'-H, C_5'-H, and C_6-H), 7.78 (d, 1H, *J* 2.1 Hz, C_5''-H), 7.95 (m, 2H, C_2'-H and C_6'-H), 8.15 (d, 1H, *J* 9.9 Hz, C_5-H).

Synthesis of [2″, 3″ : 7, 8] furanoflavone (6) using DMSO/I_2. The chalcone (**5**, 0.75 mmol, 200 mg) was suspended in dimethyl sulfoxide (DMSO, 6 mL) and a crystal of iodine[16] was added to it. The mixture was refluxed for 15 min in an oil bath and diluted with water. The solid obtained was filtered off, washed with 20% aq. sodium thiosulfate. It was purified by preparative tlc over silica gel GF_{254} using hexane-acetone (10:1) as eluting solvent and crystallized from ether as pale yellow needles (yield 135 mg, 67.50%), mp 137-138 °C, R_f 0.46 (benzene - ethyl acetate; 4:1). It gave positive Mg/HCl and Labat tests and a blue fluorescence in UV light. Spectral data of this flavone (**6**) was identical to that prepared by DDQ method.

Synthesis of [2″, 3″ : 7, 8] furanoflavone (6) using Ph-S-S-Ph. The chalcone (**5**, 0.75 mmol, 200 mg) was pasted with diphenyl sulphide[17] (0.68 mmol, 125 mg) in a mortar and the mixture was transferred to a 100 mL three necked round bottom flask equipped with nitrogen inlet and outlet tubes. The central neck was closed by a glass stopper. The

flask was then dipped into a silicon oil bath and heated at 265 °C under nitrogen atmosphere until the distillation of the thiols formed through the other outlet tube ceased (2.5 hr). The reaction mixture was then cooled at room temperature and 20 mL chloroform was added. The organic layer was washed with water several times. It was dried over anhydrous sodium sulfate and the solvent was removed by distillation. The product crystallized from ether as pale yellow needles (yield 130 mg, 65%), mp 137-138 °C, R_f 0.46 (benzene - ethyl acetate; 4: 1). It gave positive Mg/HCl and Labat tests and a blue fluorescence in UV light. Spectral data for this flavone (**6**) were also identical to that prepared by DDQ and DMSO/I_2 method.

Antibacterial screening. The antibacterial activities of synthesized compounds **5** and **6** were studied against four human pathogenic bacteria, viz., *S. dysenteriae* (G^-), *S. typhi* (G^-), *S-β-haemolyticus* (G^+) and *S. aureus* (G^+). For the detection of antibacterial activities the filter paper disc diffusion method[13,14] was performed. Each sample was dissolved in dimethyl sulfoxide (DMSO), an aliquot (10 μL) of the solution was dropped on to a paper disk (5mm diameter, Whatman No. 2 filterpaper). Kanamycin was used as standard antibiotics 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 hr liquid cultures containing 10^7 microorganisms/mL. The diffusion time was 24 hr at 5 °C for bacteria. The sample containing air-dried paper disk was then placed on the agar. The incubation time was 12 hr at 37 °C for bacteria. Disks with only DMSO were used as control. Inhibitory activity was measured with a transparent ruler (in mm) as the diameter of the observed inhibition zones.

Determination of the Minimum Inhibitory Concentration (MIC). Minimal inhibitory concentration is defined as the lowest concentration that inhibits bacterial growth. For determination of the minimum inhibitory concentration (MIC) the serial dilution technique[15] were followed using nutrient broth medium. The MIC value of the compound **5** and **6** were determined against *S. dysenteriae* (G^-) and *S-β-haemolyticus* (G^+).

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Povzetek

Fitokemične študije *Pongamia pinnata* so pokazale prisotnost [2″, 3″: 7, 8]furanoflavona (lanceolatin B). Tega smo pripravili iz 2,4-dihidroksiacetofenona preko halkonskega prekurzorja, s sledečo obdelavo z DMSO/I_2, difenil sulfidom in nato DDQ. Antibakterijsko aktivnost pripravljenih spojin smo testirali na *Shigella dysenteriae*, *Salmonella typhi*, *Streptococcus-β-haemolyticus* in *Staphylococcus aureus*.