Indian Journal of Chemistry
Vol. 62, February 2023, pp. 126-130
DOI: 10.56042/ijc.v62i2.67779

Synthesis and evaluation of mosquito larvicidal activity of pongamol and lanceolatin B

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Received 21 October 2022; accepted (revised) 12 January 2023

Pongamol and lanceolatin B are two well known active components present in different parts of karanj (*Pongamia glabra*) tree. However, the level of their content is very low and structural similarity with karanjin (major furanoflavonoid) makes their isolation in pure form difficult. This has compelled chemist to synthesize pongamol and lanceolatin B from simpler raw materials. The present research work is one such attempt to synthesize pongamol and lanceolatin B from 4-hydroxy-2-methoxybenzaldehyde using an alternate and efficient pathway. Structural confirmation of synthesized pongamol and lanceolatin B are based on NMR (^{1}H and ^{13}C) and mass and purity by HPLC. X-ray crystallographic study of synthesized lanceolatin B is also carried for the first time to confirm its structure. Both these compounds are evaluated for mosquito larvicidal activity against early 4th instar larvae of *Culex quinquefasciatus* strain for 24 h. Both these compounds exhibited very good mosquito larvicidal activity. Activity of pongamol (LC_{50} = 327.4 ± 108 ppm) is found to be better than lanceolatin B (LC_{50} = 897.6 ± 140 ppm).

Keywords: Synthesis, Pongamol, Lanceolatin B, Larvicidal activity, X-ray crystallography

Phytochemical investigation of *Pongamia glabra*, an Indian medicinal plant revealed the presence of several bioactive constituents[1]. Karanjin, pongamol and lanceolatin B are three major bioactive constituents present in different parts of tree. Karanjin and lanceolatin belong to the class of furanoflavonoid whereas pongamol is a furanohydroxychalcone. Difficulty in isolating pongamol and lanceolatin in pure condition prompted chemists to synthesize them after confirming their structure[2]. Back in 1955, Mukerjee and Seshadri[3a] reported first synthesis of pongamol starting from 4-methoxybenzofuran-5-carboxylic acid. Since then many new methodologies, alternate pathways and varying starting materials were reported by several researchers. Different starting materials such as 7-hydroxy flavones[3b], resacetophenone[3c], 3-(bromomethyl)-4hydroxy-2-methoxybenzoic acid[3d], 4-methoxy benzofuran-5-carboxylic acid[3e], 2-diazocyclohexane-1, 3-dione[3f], *etc*. are reported in the literature for the synthesis of pongamol and lanceolatin.

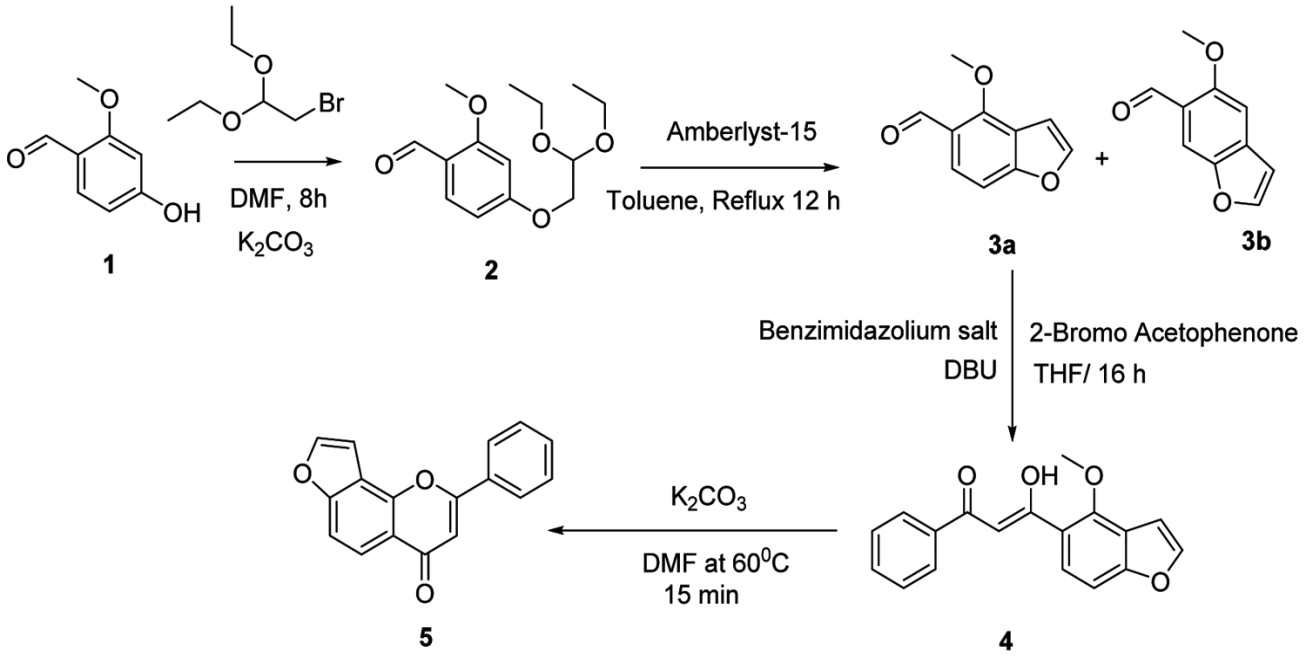
Many synthetic strategies for flavones are applied to furanoflavones. The most popular methodology adopted for the synthesis of pongamol and lanceolatin is Baker-Venkataraman (B-V) rearrangement for the synthesis of flavones[4]. However, over a period of time several alternate cleaner, much milder and efficient pathways revealed in the literature for the synthesis of pongamol and lanceolatin. Starting from resacetophenone, several researchers carried out synthesis of lanceolatin following initially a regioselective O-alkylation with bromoacetaldehyde diethylacetal, followed by O-acylation with benzoyl chloride, rearrangement with potassium-t-butoxide and finally acid catalyzed cyclization[5]. Syed Alam[3c] carried out base catalyzed O-alkylation of β-resacetophenone with allyl bromide, which then undergoes Claisen rearrangement to give 3-C-allylresacetophenone, followed by OsO_4/KIO_4 catalyzed oxidative double bond cleavage, acid induced cyclization and Claisen condensation with benzaldehyde to give pongamol. Final ring closer to get lanceolatin was carried by treatment either with DDQ or DMSO/I_2 or diphenyl disulphide. Lee and Morehead[3e] reported a new and separate route for the

synthesis of pongamol and lanceolatin *via* methyl ester of karanjic acid starting from diazacyclohexane-1,3-dione. Lee and Kang[6] though adopted B-V approach, but harsh acid catalyzed cyclization was replaced with $InCl_3$-catalyzed intramolecular dehydrative cyclization of furanochalcone to furanoflavone. Many such cyclization approaches reported in the literature is discussed by Sharma *et al.*[7] Authors also reported a much simpler and efficient K_2CO_3-mediated tandem O-arylation and C-O bond cleavage for the conversion of pongamol to lanceolatin[7]. Synthetic strategy adopted in the present manuscript is an amalgamation of some of these reported approaches. However, an alternate starting material, 4-hydroxy-2- methoxybenzaldehyde is proposed in the present work. Synthesis of pongamol and lanceolatin is achieved in 3 and 4 steps respectively in good yield. Structural confirmation of synthesized pongamol and lanceolatin B are based on NMR (^1H and ^{13}C) and mass and purity by HPLC. X-ray crystallographic study of synthesized lanceolatin B is also carried for the first time to confirm its structure. Synthesized compounds are evaluated for mosquito larvicidal activity against early 4th instar larvae of *Culex quinquefasciatus* strain.

Results and Discussion

Synthesis

Synthesis was initiated by reacting 4-hydroxy-2-methoxybenzaldehyde with bromoacetaldehyde diethyl acetal in presence of anhydrous K_2CO_3 in dry DMF to give 4-(2,2-diethoxyethoxy)-2-methoxybenza ldehyde in 75% of isolated yield[8]. The cyclization of formed methoxy benzaldehyde was carried out by refluxing in toluene in presence of Amberlyst-15 giving a mixture of two benzofurans, 4-methoxybenzofuran-5-carbaldehyde (3a) as major and 5-methoxybenzofuran-6-carbaldehyde (3b) as minor. Column chromatographic purification of desired benzofuran (3a) afforded 77% of isolated yield[9]. Singh *et al*. reported a new N-heterocyclic carbene promoted intermolecular acylation of α-haloketone with aromatic aldehyde for synthesis of 1,3-diketone[10]. The same umpoulong strategy was applied in the present synthesis of furanochalcone, pongamol. Intermolecular acylation of 2-bromoacetophenone with 4-methoxybenzofuran-5-carbaldehyde (3a) was conducted in presence of N,N-dibenzyl benzimidazolium chloride and DBU to get pongamol in 80% of isolated yield after column purification (Scheme 1). Conversion of pongamol to lanceolatin was achieved within 15 min at 27°C by a K_2CO_3 catalyzed intramolecular tandem O-arylation *via* C-O bond cleavage[7] (Scheme 1). Synthesized pongamol and lanceolatin B were characterized by NMR (^1H and ^{13}C) and mass after ascertaining their purity by HPLC. HPLC analysis indicated that the synthesized pongamol is 99.6% pure whereas lanceolatin B is 99.8%.



Scheme 1 — Synthesis of Pongamol (4) and Lanceolatin B (5) from 4-hydroxy-2-methoxy benzaldehyde

Mosquito Larvicidal Assay

Karanjin, the principal furanoflavonoid compound present in different parts of karanja tree is found to possess excellent mosquito larvicidal activity against fourth instar larvae of *Culex quinquefasciatus* mosquito strain[11]. In our earlier work, it was observed that a crude extract from deoiled seed cake containing 19-20% of karanjin along with karanja fatty acid methyl esters (48-52%), lanceolatin (6-7%) and other furanoflavonoids (22-26%) exhibited significantly higher larvicidal activity (LC_{50} = 2.29 ± 3.45 ppm) compared to pure karanjin (LC_{50} = 209.4 ± 48.9 ppm)[11,12]. Though synergism of the crude extract may be the reason for showing exceptional larvicidal activity, but it is envisaged that both pongamol and lanceolatin B may show similar larvicidal activity like karanjin due to their structural similarity with karanjin, especially lanceolatin B. Standard bioassay procedure was adopted for ascertaining mosquito larvicidal activity of pongamol and lanceolatin B against early 4th instar larvae of *Culex quinquefasciatus* strain over a period of 24 h. Both these compounds exhibited very good mosquito larvicidal activity (Table 1). Though the activity of pongamol (LC_{50} = 327.4 ± 108 ppm) was found to be at par with karanjin (LC_{50} = 209.4 ± 48.9 ppm) but lanceolatin B exhibited poor activity compared to karanjin (LC_{50} = 897.6 ± 140 ppm) despite its structural resemblance with karanjin.

X-ray Crystallography

X-ray crystallographic study of natural pongamol is already reported in the literature confirming its enol form rather than β-diketo form[13]. Thorough literature survey indicated no such study was carried out on lanceolatin B. In order to reconfirm its structure, X-raystudy of lanceolatin B was carried out as per the standard protocol (Fig. 1).

Experimental Details

All the chemicals used in this study were obtained from different commercial sources and were used without any further purification. Reactions were monitored on micro TLC with UV detection. Final purifications were carried out using silica gel (Rankem) 60-120 mesh. All ^{1}H and ^{13}C NMR spectra were recorded on ADVANCE-300 and 400 (300 and 400 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR). Chemical shifts are reported in δ (ppm) with reference to TMS as internal standard. Molecular weights of unknown compounds were identified by ES-MS and HR-MS (Electron Spray Ionization Technique). HPLC analysis was performed on HPLC equipment (Agilent 1260) equipped with Diode Array Detector. Analytical separation was carried out on reversed phase column, RP-C18 (250 × 4.6 mm i.d. × 3 µm) (Merck) using 90:10 (v/v) of Methanol:water in isocratic elution mode. Data and chromatograms were collected using Open lab Chemstation chromatopac software system.

Synthesis of 4-(2,2-diethoxyethoxy)-2-methoxy benzaldehyde, 2

To a stirred solution of 4-hydroxy-2-methoxybenzaldehyde (5.0 g, 32.86 mmol) in DMF (50 mL) was added K_2CO_3 (9.0 g, 65.72 mmol) followed by bromoacetaldehyde diethyl acetal (5.93 mL, 39.43 mmol). Then reaction mixture was warmed to 50°C and stirred for 3 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT, diluted with water and extracted with EtOAc. The organic layer was dried over anhyd. Na_2SO_4, filtered and concentrated. The residue was purified by column

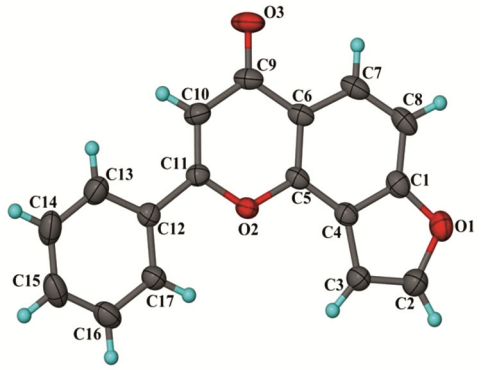


Fig. 1 — ORTEP diagram of synthesized lanceolatin B with the atom-numbering. Displacement ellipsoids are drawn at the 35% probability level and H atoms are shown as small spheres of arbitrary radius

Table 1 — Larvicidal activity (in ppm) for 24 h of pongamol and lanceolatin B against 4th instarlarvae of *Culex quinquefasciatus* mosquitoes

Compd	Mosquito Larvicidal Activity(Mean ± SE)		Chi square	Regression coefficient
	LC_{50} (ppm)	LC_{90} (ppm)		
Pongamol	327.4 ± 108	953.2 ± 822.4	0.48	2.76
Lanceolatin	897.6 ± 1404	3113.5 ± 9186	0.54	2.37

Negative control (acetone) – no activity; *level of significance: 0.05

chromatography on silica gel by eluting with ethyl acetate: hexane (5:95 v/v) to yield 4-(2,2-diethoxyethoxy)-2-methoxybenzaldehyde as light yellow colour liquid (6.6 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.2 (s, 1H, Ar-CHO), 7.9 (s, 1H, Ar-H), 7.1 (d, 2H, Ar-H), 4.9 (m, 1H, O-CH-O), 4.2 (m, 6H, 3 × O-CH_2), 3.9 (m, 3H, O-CH_3), 1.2 (m, 6H, 2 × -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.2, 165.0, 163.4, 130.5, 119.2, 106.3, 101.3, 100.1, 98.4, 68.5, 62.7, 62.3, 55.3, 31.7, 15.2; HR-MS (ESI): *m/z* [M+H+] Calcd for $C_{14}H_{21}O_5$: 269.13835. Found: 269.13835.

Synthesis of 4-methoxybenzofuran-5-carb-aldehyde, 3a

To a stirred solution of 4-(2,2- diethoxyethoxy)-2-methoxybenzaldehyde (6.0 g, 22.39 mmol) in toluene (50 mL) was added amberlyst-15 (1.0 g). Then reaction mixture was stirred at 110°C for 16 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT, diluted with water and extracted with EtOAc. The organic layer was dried over anhyd. Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel by eluting with ethyl acetate: hexane (10:90 v/v) to yield **3a** as light white colour solid, m.p.93-95°C (3.0 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.5 (s, 1H, Ar-CHO), 8.2 (d, 1H, Ar-H), 7.7 (d, 1H, Ar-H), 7.1 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 4.0 (s, 3H, O-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.6, 159.4, 159.58, 144.81, 120.9, 120.8, 119.8, 106.01, 93.7, 54.6, 28.5; HR-MS (ESI): *m/z* [M+H+] Calcd for $C_{10}H_9O_3$: 177.05462. Found: 177.05462.

Synthesis of (Z)-3-hydroxy-3-(4-methoxybenzo-furan-5-yl)-1-phenylprop-2-en-1-one, 4

In a solution of benzimidazolium salt (1.36 g, 4.54 mmol) in THF was added **3a** (2.0 g, 11.36 mmol) under nitrogen atmosphere at 27°C and stirred for 10 min at the same temperature. After that DBU (0.690 g, 4.5 mmol) was added to the stirred reaction mixture with syringe followed by the addition of 2-bromo acetophenone (2.26 g, 11.36 mmol) in THF and stirred for 4 h at 27°C. After completion of the reaction, as judged by TLC the solvent was evaporated using rotary evaporator, water was added and the product was extracted with ethyl acetate and dried over anhyd. Na_2SO_4. The crude product was purified by using column chromatography by eluting with ethyl acetate: hexane (30:70 v/v) which afforded the title compound as brownish colour solid, m.p.135-137°C (2.35 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0 (d, 2H, , Ar-H), 7.9 (d, 1H, Ar-H), 7.7(d, 1H, Ar-H), 7.65-7.60 (m, 1H, Ar-H), 7.5-7.45 (m, 1H, Ar-H), 7.35 (s, 1H, COCH=CH), 7.2 (d, 1H, Ar-H), 7.0 (d, 1H, Ar-H), 4.2 (s, 3H, O-Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 186.13, 184.30, 158.73, 153.70, 144.80, 135.61, 132.22, 128.70, 128.60, 128.30, 127.10, 126.50, 122.20, 119.50, 107.0, 105.30, 97.90, 61.16; HR-MS (ESI): *m/z* [M+H+] Calcd for $C_{18}H_{15}O_4$: 295.09640. Found: 295.09649.

Synthesis of 2-phenyl-4*H*-furo(2,3-h)-chromen-4-one, 5

To a stirred solution of pongamol (0.5 g, 1.7006 mmol) in DMF was added K_2CO_3 (0.470 g, 3.401 mmol) and reaction mixture was stirred at 60°C for 12 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT, diluted with water and extracted with DCM. The organic layer was dried over anhyd. Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel by eluting with ethyl acetate: hexane (30:70 v/v) to yield Lanceolatin as white solid, m.p.125-127°C (0.360 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.2 (d, 1H, Ar-H), 7.9 (d, 2H, Ar-H), 7.7(d, 1H, Ar-H), 7.63-7.60 (m, 4H, Ar-H), 7.35-7.45 (d, 1H, Ar-H), 6.8 (s, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.24, 162.63, 158.34, 150.80, 145.82, 131.72, 131.56, 129.17, 126.16, 121.74, 119.34, 117.14, 110.77, 107.98, 104.18; HR-MS (ESI): *m/z* [M+H+] Calcd for $C_{17}H_{11}O_3$: 263.07012. Found: 263.07027.

Mosquito Larvicidal Assay

The larvicidal activity of the synthesized pongamol and lanceolatin B was determined by standard bioassay procedure. A laboratory colony of early 4th instar larvae of *Culex quinquefasciatus* was used for the larvicidal activity, which was maintained at 26±2ºC,70%±10% RH, under 14 L: 10 D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio. Ten of early 4th stage larvae of *Culex quinquefasciatus* were kept in a 100 mL of distilled water. Each treatment was replicated 6 times. Stock solutions of known concentration of synthesized pongamol and lanceolatin B were prepared in acetone. Different concentrations of 100, 200 and 300 ppm were prepared and efficacy was studied against larvae of *Culex quinquefasciatus* mosquito larvae. A negative (Acetone) control was set up along with the bio-efficacy studies. Larval mortality (in percentage) was recorded after 24 h treatment and probit analysis was performed for calculating the LC_{50} and LC_{90} values representing the concentration in ppm that killed 50% and 90% of larvae, respectively[14].

Crystal Data of Lanceolatin B

$C_{17}H_{10}O_3$, M = 262.25, Monoclinic, space group P21/n (No.14), a = 5.743(2)Å, b = 17.265(6)Å, c = 12.777(5)Å, α = 90°, β = 95.043(7)°, γ = 90°, V = 1262.0(8)Å3, Z = 4, Dc = 1.380 g/cm^3, F000 = 544, Bruker D8 QUEST PHOTON-100, Mo-Kα radiation, λ = 0.71073 Å, T = 293(2)K, 2θmax = 55°, μ= 0.095 mm^{-1}, 15685 reflectionscollected, 2890 unique (Rint = 0.0356), 181 parameters, R1 = 0.0478, wR2 = 0.1050, R indices based on 1905 reflections with I > 2σ(I) (refinement on F2), Final GooF = 1.046, largest difference hole and peak = –0.114 and 0.119 e.Å^{-3}.

Data collection and structure solution details

Single crystal X-ray data were collected at RT on a Bruker D8 QUEST equipped with a four-circle kappa diffractometer and Photon 100 detector. An Iμs microfocus Mo source (λ=0.71073Å) supplied the multi-mirror monochromated incident beam. A combination of Phi and Omega scans were used to collect the necessary data. Integration and scaling of intensity data were accomplished using SAINT program[15]. The structures were solved by Direct Methods using SHELXS97 (Ref. 16) and refinement was carried out by full-matrix least-squares technique using SHELXL-2014/7 (Ref. 16,17) Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were positioned geometrically and treated as riding on their parent C atoms, with C-H distances of 0.93–0.97 Å, and with U_{iso}(H) = 1.2U_{eq} (C) or 1.5U_{eq} for methyl atoms. The CCDC No 2202114 deposition number contains the supplementary crystallographic data for this paper which can be obtained free of charge at https://www.ccdc.cam.ac.uk/structures/.

Conclusion

The present work describes synthesis of pongamol and lanceolatin in an alternate and efficient pathway starting from 4-hydroxy-2-methoxybenzaldehyde. Both these compounds were characterized using NMR (^{1}H and ^{13}C) and mass and purity by HPLC. The structure of synthesized lanceolatin B is also confirmed by X-ray crystallography, which has not been reported earlier. Mosquito larvicidal activity of both the synthesized compounds were evaluated against early 4th instar larvae of *Culex quinquefasciatus* strain for 24 h. Pongamol and lanceolatin exhibited very good mosquito larvicidal activity against the studied strain. However the activity of pongamol is found to be better than lanceolatin B but at par with karanjin.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

Acknowledgement

The authors thank Director, CSIR-IICT for support in carrying out the research work (IICT/Pubs./2022/277) and Dr Jagadeesh Babu Nanubolu for carrying out the X-ray crystallographic study.

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