

Note

A novel alkaloid, four alkaloid precursors and a coumarin from *Feronia limonia*

P Pitchai^{a,b,c}, R Ulagi^a, P S Mohan^{*a} & R M Gengan^c

^aDepartment of Chemistry, School of Chemical Sciences,
Bharathiar University, Coimbatore 641 046, India

^bP G and Research Centre, Department of Chemistry, Government
Arts College (Autonomous) Kumbakonam 612 001, India

^cDepartment of Chemistry, Durban University of Technology,
Steve Biko Campus, Durban 4000, South Africa

E-mail: ps_mohan_in@yahoo.com

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A new quinoline alkaloid has been isolated from the flowers of *Feronia limonia* along with four alkaloid precursors, one coumarin, a known steroid stigmaterol and *p*-coumaric acid. The structures of compounds have been elucidated on the basis of spectroscopic data and comparison with the literature.

Keywords: Quinoline alkaloid, coumarin, *Feronia limonia*

Feronia limonia (Linn.) Swingle syn. *F. elephantum* (Linn.) Correa, belonging to the *Rutaceae* family, is a commonly occurring tropical plant species endemic to the Indian subcontinent. Different parts of this plant have been used as crude drugs by traditional healers to treat a variety of ailments; its extracts possess various medicinal properties *viz.*, vasodilator, anti-microbial, laxative, purgative, astringent, anti-hypertensive^{1,2} and is used for the treatment of gastrointestinal disorders^{3,4}.

The *Rutaceae* family is known for its abundance of anthranilic acid derived alkaloids, coumarins, limonoids and flavonoids⁵. Constituents from the root bark of *Feronia limonia* are coumarins like bergapten, steroids, alkaloids, fatty acids, aromatic hydrocarbons and glycosides⁶⁻⁸. An acidic polysaccharide from the fruit shows good activity against tumor cells⁷. Constituents from the root and stem bark are stigmaterol, friedelin, β -sitosterol-3-*O*- β -D-glucopyranoside, bergapten, xanthotoxin, scopoletin, isoimperatorin, osthol and 6,7-dimethoxycoumarin⁸. Surprisingly, neither phytochemical nor pharmacological research is documented on the flowers of *Feronia limonia* and hence the current research thrust^{9,10} is aimed at this plant species.

This paper reports the isolation and structural elucidation of eight compounds including a novel quinoline alkaloid **6** from the acetone extract of dried flowers of this plant. To the best of the knowledge, compounds **2** and **7** are isolated for the first time from this particular plant source whilst compounds **3**, **4** and **5** are reported for the first time in plants.

Results and Discussion

Air-dried flowers of *Feronia limonia* were de-waxed with *n*-hexane and exhaustively extracted with acetone in a Soxhlet apparatus until the extract became colourless. On cooling, a precipitate was observed. After further allowing to stand for 12 hr, the precipitate was filtered and kept aside for analysis. The supernatant liquid was concentrated *in vacuo*, the mixture separated using column chromatography and the products were purified by recrystallization. Eight compounds were characterized in all. **Figure 1** presents a flow chart indicating the solvent system used for eluting compounds **1-8**.

Characterization of Compound 1

The fraction which eluted with 2% of ethyl acetate (EA) in petroleum ether (PE) was subjected to repeated recrystallization from ethanol to produce a white amorphous solid; it melted at 169°C, and showed $[\alpha]_D^{25} -51^\circ$ (CHCl₃). It was characterised as stigmaterol **1**.

Characterization of Compound 2

Elution with 3% EA in PE gave compound **2**. It fluoresces bright blue under UV light. It was purified by recrystallization from chloroform and it melted at 190°C and was identified as bergapten **2**.

Characterization of Compounds 3-5

Fractions eluted with the solvent system in the range 4-9% EA in PE (**Figure 1**) was monitored by thin layer chromatography (TLC); these fractions showed three spots. Hence each fraction was then re-chromatographed to afford three crystalline compounds **3**, **4** and **5**.

The IR spectra of **3**, **4** and **5** showed the N-H stretching at 3292 cm⁻¹, amide C=O stretching at

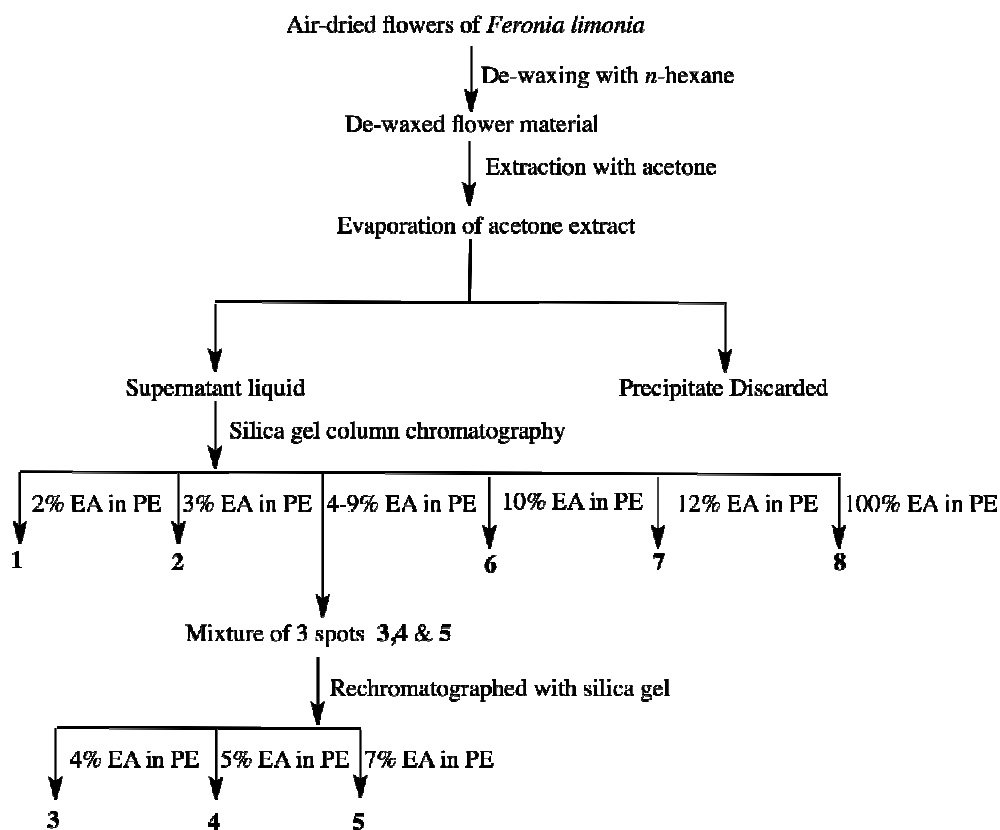
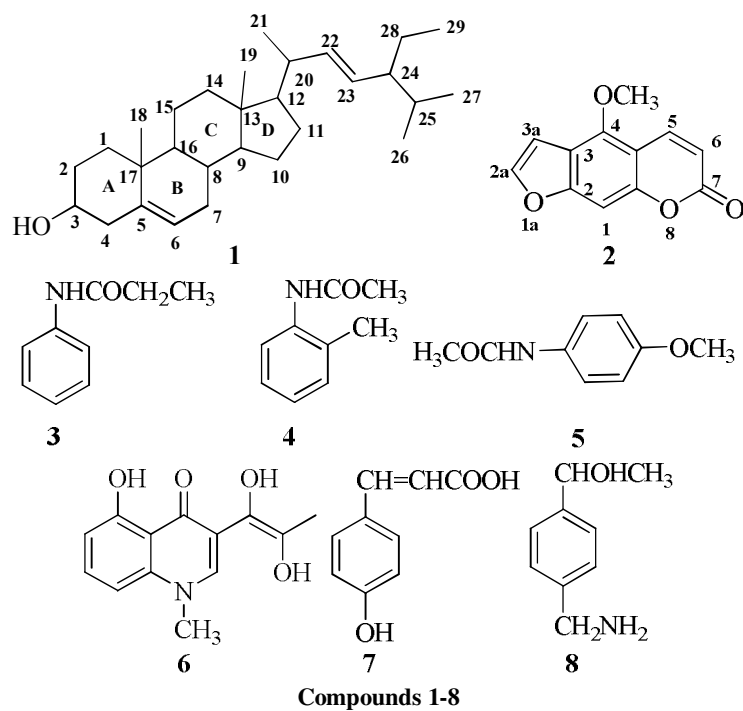


Figure 1 — Flow chart showing the methodology and eluting solvents used for purification of compounds 1-8 by column chromatography



1650 cm^{-1} and aromatic C=C stretching at 1610 and 1520 cm^{-1} . ^1H NMR spectrum of all three compounds showed one broad proton singlet at δ 7.2 which was exchangeable with D_2O , confirming the presence of NH group. The spectral evidence indicated that **3**, **4** and **5** possessed a common structural skeleton and were identified as *N*-phenylpropanamide **3**, *N*-(2-methylphenyl) acetamide **4** and *N*-(4-methoxyphenyl) acetamide **5**. These three amides were obtained for the first time from a plant source, also in large quantities, and believed to be the precursors for the biochemical synthesis of complex plant alkaloids.

Characterization of Compound 6

Elution with 10% EA in PE yielded a yellow powder in very small quantity. The IR spectra showed a characteristic aromatic ketonic C=O stretching at 1650 cm^{-1} and a vinylidene C-H stretching and bending at 3090 and 901 cm^{-1} . The chelated hydroxyl group was observed at 3434 cm^{-1} . The ^1H NMR spectrum showed a D_2O exchangeable singlet at δ 16.5 and it was assigned to C₅. The pair of doublets at δ 8.09 ($J = 8.0$ Hz) and δ 7.7 ($J = 7.2$ Hz) were assigned to C₈-H and C₆-H, respectively. An aromatic triplet at δ 7.5 ($J = 8$ Hz) was assigned to C₇-H and one proton singlets at δ 9.4 and δ 9.5 were assigned to the two hydroxyl groups present in the alkenyl carbon. Three proton singlets at δ 1.7 and 2.7 corresponded to the methyl groups present in the side chain and *N*-atom, respectively. The mass spectrum indicated the molecular ion peak m/z 247; the peak at m/z 219 was due to the loss of a carbonyl group (C=O m/z 28). The peak at m/z 202 was due to loss of a hydroxyl group from m/z 219 and this formed the base peak. The ^{13}C NMR confirmed the presence of a carbonyl group at δ 189.0, *N*-Me group at δ 47.0 and =C-Me group at δ 17.0. Based on the above spectral data, **6** was identified as 5-hydroxy-3-(1,2-dihydroxyprop-1-enyl)-1-methyl-quinolin-4-one. There was no literature data available, with which to make comparison. The occurrence and identity of this compound is reported for the first time from a plant as a natural source. The number of naturally occurring *N*-methyl-4-quinolones with hydroxylated vinylidene substitution at C₃ is rare and this compound presumably is a precursor of other furanoquinoline alkaloids.

Characterization of Compound 7

Elution with 12% EA in PE yielded a gummy residue which on repeated recrystallization with chloroform afforded **7** as colourless needles; it melted

at 218°C. The mass spectrum showed a molecular ion peak at m/z 164 and elemental analysis corresponded to the molecular formula $\text{C}_9\text{H}_8\text{O}_3$ and was identified as *p*-coumaric acid.

Characterization of Compound 8

Elution with 100% EA yielded **8** as a white solid. It melted at 190°C and elemental analysis indicated the molecular formula $\text{C}_9\text{H}_{13}\text{ON}$. The ^1H NMR spectrum showed doublets at δ 7.10 ($J = 8.5$ Hz) and 6.68 ($J = 8.5$ Hz) each integrating for two protons thereby suggesting the presence of a 1,4-disubstituted aromatic ring. A doublet for three protons at δ 2.27 ($J = 8.5\text{Hz}$) and a quartet for one proton at δ 4.49 ($J = 4.5\text{Hz}$) were accounted for the presence of a -CH-CH₃ group. A multiplet at δ 2.50 was attributed to -CH₂- bonded to a *N*-atom. A one proton broad singlet at δ 5.0 was assigned to the hydroxyl proton. Based on the above data, along with δ 70.8 and 59.8 for the carbons which bonded with oxygen and nitrogen respectively in the ^{13}C NMR, **8** was identified as 1-[4-aminomethyl](phenyl) ethanol. The chiral centre of **8** could not be established since only a merge quantity was isolable. To the best of the knowledge, **8** is reported for the first time from a natural plant source.

Experimental Section

Melting points were determined on an electrically heated melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FTIR spectrophotometer as KBr discs unless otherwise indicated. ^1H NMR spectra were obtained on a Bruker (500 MHz) instrument in CDCl_3 , D_2O or $\text{DMSO}-d_6$ solutions using tetramethylsilane as an internal standard. J values are given in Hz. Thin layer chromatography were run on glass plates coated with silica gel G and visualized using iodine vapour. All the basic chemicals and solvents were purchased from Rankem (India).

Collection and identification of plant material

The plant materials were collected during April 2005 from Perundurai, Erode District, Tamil Nadu, India and identified by Dr. Geetha, Department of Botany, Govt. College, Tiruppur, TN, India.

De-Waxing of flower

The fresh flowers were dried under shade at RT for 15 days. The dried flowers (2.5 kg) were chopped, extracted with *n*-hexane and left overnight. The

Table I — ^{13}C NMR chemical shifts of stigmaterol

Carbon	^{13}C NMR	Carbon	^{13}C NMR	Carbon	^{13}C NMR
1	37.2	11	21.1	21	19.0
2	28.2	12	39.7	22	138.3
3	71.8	13	42.2	23	129.2
4	40.5	14	56.8	24	50.1
5	140.7	15	24.4	25	29.1
6	121.7	16	26.0	26	21.2
7	31.6	17	56.7	27	21.1
8	31.9	18	12.0	28	23.0
9	51.2	19	19.4	29	12.3
10	36.5	20	36.1		

solvent was filtered, collected and concentrated in a rotary evaporator. The waxy material was discarded and not used further.

Extraction of the leaves

Wet flowers were air-dried for 3 hr to remove excess hexane and then extracted with acetone in a Soxhlet apparatus for 5 days. The acetone extract was allowed to stand for slow evaporation at RT. A mixed yellow solid and a white crystalline compound, which gradually separated out, were filtered and kept aside. The supernatant liquid of acetone extract was concentrated under reduced pressure and the gummy material (300 g), thus obtained was subjected to silica gel column chromatography using a gradient of petroleum ether : ethyl acetate as mobile phase as described in **Figure 1**.

Compound 1

It was purified by recrystallization from ethanol. Yield 100 mg; m.p. 169°C; IR (KBr): 3434, 2934, 1462, 1382, 1061, 969 cm^{-1} ; ^1H NMR (CDCl_3): δ 0.68 (s, 3H, $\text{C}_{18}\text{-H}$), 0.98 (s, 3H, $\text{C}_{19}\text{-H}$), 1.00 (d, 3H, $\text{C}_{21}\text{-H}$, $J = 6.5$ Hz), 0.82 (d, 3H, $\text{C}_{26}\text{-H}$, $J = 6.0$ Hz), 0.77 (d, 3H, $\text{C}_{27}\text{-H}$, $J = 6.0$ Hz), 0.78 (t, 3H, $\text{C}_{29}\text{-H}$, $J = 7.5$ Hz), 4.98 (dd, 1H, $\text{C}_{23}\text{-H}$, $J = 15.5, 8.5$ Hz), 5.11 (dd, 1H, $\text{C}_{22}\text{-H}$, $J = 15.5, 8.5$ Hz), 5.32 (bs, 1H, $\text{C}_6\text{-H}$); ^{13}C NMR (CDCl_3): δ **Table I**; EIMS: m/z (rel.int.%) 412 (32), 394 (98), 382 (22), 271 (60), 255 (58), 213 (30), 159 (41), 145 (53), 107 (52), 95 (60), 83 (88), 69 (72), 55 (100), 43 (63).

Compound 2

Compound **2** was eluted with 3% ethyl acetate in petroleum ether and purified by recrystallization from

chloroform. Yield 60 mg; m.p. 190°C; UV-Vis (MeOH), nm: 224, 249, 266 and 310; IR (KBr): 1720, 1615, 1466, 1356, 1213 cm^{-1} ; ^1H NMR (CDCl_3): δ 4.30 (s, 3H, $-\text{OCH}_3$), 6.30 (d, 1H, $\text{C}_6\text{-H}$, $J = 9.6$ Hz), 7.05 (d, 1H, $\text{C}_{3a}\text{-H}$, $J = 2.4$ Hz), 8.19 (d, 1H, $\text{C}_5\text{-H}$, $J = 9.6$ Hz), 7.62 (d, 1H, $\text{C}_{2a}\text{-H}$, $J = 2.8$ Hz), 7.17 (s, 1H, $\text{C}_1\text{-H}$); ^{13}C NMR (CDCl_3): δ 161.3 (C_7), 112.6 (C_6), 144.8 (C_5), 152.7 (C_4), 112.5 (C_3), 158.4 (C_2), 93.8 (C_1), 106.3 (C_{3a}), 149.5 (C_{2a}), 60.1 ($-\text{OCH}_3$); EIMS: m/z (rel.int.%) 216 [M^+] (100), 201 (30), 173 (46), 145 (22), 89 (10).

Compound 3

The petroleum ether-ethyl acetate (96:4) eluate afforded colourless crystals. Yield 50 mg; m.p. 104°C; IR (KBr): 3280, 1667, 1603, 1548, 1498 cm^{-1} ; ^1H NMR (CDCl_3): δ 7.51 (d, 2H, C_2 & $\text{C}_6\text{-H}$, $J = 8.0$ Hz), 7.31 (t, 2H, C_3 and $\text{C}_5\text{-H}$, $J = 7.6$ Hz), 7.10 (t, 1H, $J = 7.4$ Hz, $\text{C}_4\text{-H}$), 2.39 (q, 2H, $\text{CH}_2\text{-H}$, $J = 7.6$ Hz), 1.25 (t, 3H, $\text{CH}_3\text{-H}$, $J = 7.6$ Hz), 7.2 (bs, 1H, NH); ^{13}C NMR (CDCl_3): δ 137 (C_1), 119 (C_2 and C_6), 129 (C_3 and C_5), 124 (C_4), 30.8 (CH_2), 9.7 (CH_3), 172 ($\text{C}=\text{O}$); EIMS: m/z (rel.int.%) 149 (38), 93 (100), 77 (10), 65 (14), 57 (28), 51 (8).

Compound 4

Elution with petrol ether-ethyl acetate (95:5) gave compound **4** as colourless crystals. Yield 30 mg; m.p. 106°C; IR (KBr): 3291, 1651, 1610, 1530, 1485 cm^{-1} ; ^1H NMR (CDCl_3): δ 7.18 (d, 1H, $\text{C}_3\text{-H}$, $J = 10.8$ Hz), 7.00 (t, 2H, C_4 and $\text{C}_5\text{-H}$, $J = 7.4$ Hz), 7.73 (d, 1H, $\text{C}_6\text{-H}$, $J = 7.6$ Hz), 2.20 (s, 3H, $\text{COCH}_3\text{-H}$), 2.10 (s, 3H, $\text{CH}_3\text{-H}$), 7.20 (bs, 1H, NH); ^{13}C NMR (CDCl_3): δ 135 (C_1), 130 (C_2), 129 (C_3), 125 (C_4), 127 (C_5), 123 (C_6), 24 (COCH_3), 168 ($\text{C}=\text{O}$), 17 (CH_3); EIMS: m/z (rel.int.%) 149 (30), 107 (100), 65 (6), 77 (18), 51 (9), 43 (36).

Compound 5

Further elution with 7% ethyl acetate in petroleum ether gave compound **5** as colourless crystals (35 mg); m.p. 125°C; IR (KBr): 3243, 1649, 1605, 1560, 1512 cm^{-1} ; ^1H NMR (CDCl_3): δ 6.85 (d, 2H, C_2 and $\text{C}_6\text{-H}$, $J = 9.2$ Hz), 7.38 (d, 2H, C_3 and $\text{C}_5\text{-H}$, $J = 9.2$ Hz), 2.2 (s, 3H, $\text{COCH}_3\text{-H}$), 3.8 (s, 3H, $\text{OCH}_3\text{-H}$), 7.2 (bs, 1H, NH); ^{13}C NMR (CDCl_3): δ 130 (C_1), 121 (C_2 & C_6), 114 (C_3 & C_5), 156 (C_4), 168 ($\text{C}=\text{O}$), 55.5 (OCH_3), 24 (COCH_3); EIMS: m/z (rel.int.%) 165 (74), 123 (66), 108 (100), 80 (10), 43 (14).

Compound 6

Elution with 10% ethyl acetate in petroleum ether gave compound **6** as a yellow powder. Yield 10 mg; m.p. 210°C (d); IR (KBr): 3434, 3090, 1650, 1620, 1508, 901 cm⁻¹; ¹H NMR (CDCl₃): δ 9.20 (s, 1H, H-2), 8.09(d, 1H, C₈-H, *J* = 8.0 Hz), 7.70 (d, 1H, C₆-H, *J* = 7.2 Hz), 7.50 (t, 1H, C₇-H, *J* = 8 Hz), 2.70 (s, 3H, *N*-CH₃-H), 1.70(s, 3H, C₂-CH₃-H), 16.50 (bs, 1H, C₅-OH-H), 9.40 (s, 1H, C₂-OH-H), 9.50 (s, 1H, C₁-OH-H); EIMS: *m/z* (rel.int.%) 247 (20), 219 (70), 202 (100), 173 (22), 43 (16).

Compound 7

From the petroleum ether-ethyl acetate (88:12) eluate, *p*-coumaric acid was isolated; it was and further purified by washing with petroleum ether to produce colourless crystals from chloroform. Yield 40 mg; m.p. 217°C; UV-Vis (MeOH), nm: 312, 220, 210; IR (KBr): 3383, 1676, 1596, 831 cm⁻¹; ¹H NMR (CDCl₃): δ 7.60 (d, 1H, C₂-H, *J* = 15.9 Hz) 6.31 (d, 1H, C₃-H, *J* = 15.9 Hz), 7.53 (d, 2H, C₂ and C₆-H, *J* = 8.6 Hz), 6.89 (d, 2H, C₃ and C₅-H, *J* = 8.6 Hz), 3.20 (bs, 1H, C₄-OH); ¹³C NMR (CDCl₃): δ 125.6 (C₁), 129.4 (C₂ and C₆), 115 (C₃ and C₅), 159.1 (C₄), 166.8 (C₁), 144.1 (C₂) and 114.4 (C₃); EIMS: *m/z* (rel.int.%) 164 [M⁺] (100), 147 [M-OH]⁺ (42), 119 [M-OH, CO] (28) and 91 [M-C₂HO₃]⁺ (24).

Compound 8

Colourless crystals, Yield 35 mg; m.p. 165°C; IR (KBr): 3288, 1605, 1504 and 1265 cm⁻¹; ¹H NMR (CDCl₃): δ 7.10 (d, 2H, C₂ and C₆-H, *J* = 8.5 Hz), 6.68 (d, 2H, C₃ and C₅-H, *J* = 8.5 Hz), 4.49 (q, 1H, O-CH-H, *J* = 4.2 Hz), 2.27 (d, 3H, CH₃-H, *J* = 8.5 Hz),

2.50 (m, 2H, *N*-CH₂-H); ¹³C NMR (CDCl₃): δ 156.1 (C₁), 134.8 (C₄), 129.3 (C₂), 126.9 (C₆), 114.9 (C₅), 114.6 (C₃), 70.8 (O-CH-C), 59.8 (CH₂-N), 35.9 (CH₃).

Conclusion

Eight compounds were isolated from the flowers of *Feronia Limonia* including a novel quinoline alkaloid, one coumarin, a known steroid stigmasterol and *p*-coumaric acid.

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