Polyphenols from the roots of *Plumbago rosea*

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Two flavonoids and two carboxylic acids have been isolated from the ethyl acetate extract of the roots. The structures of these compounds have been established as myricetin-3,3',5',7-tetra methyl ether 1, amelopsin-3',4',5',7-tetramethyl ether 2, plumbagic acid 3 and roseanoic acid 4 on the basis of UV, IR, 1H and 13C NMR and mass spectrum studies. The two carboxylic acids 3 and 4 are reported here for the first time. Compounds 1 and 2 are the first report from the genus *plumbago*.

**Keywords:** *Plumbago rosea* L., plumbagic acid, roseanoic acid, myricetin-3,3',5',7-tetra methyl ether, amelopsin - 3',4',5',7-tetramethyl ether

The drug kodiveli described in Siddha system of medicine is known as chitraka in Ayurveda1. In Siddha system2, three varieties (blue, red and white) of kodiveli are described. They are botanically identified as *Plumbago auriculata, Plumbago indica* Linn. syn. *P. rosea* Linn. and *Plumbago zeylanica* Linn. (Family: Plumbaginaceae) respectively. *P. rosea* Linn.3 is a pretty ornamental plant, frequently grown in gardens for its showy bright red flowers. It is widely distributed in the tropics. *P. rosea* contains a higher percent of plumbagin4,5 the most abundant and characteristic phytochemical of these species, which is associated with a variety of biological activities6,7. *P. zeylanica* had been studied in greater detail8,11 and a number of other benzoquinones, including dimers of plumbagin, have been reported12. Earlier workers have reported the characterization of several compounds namely plumbagin13, 6-hydroxy plumbagin, sitosterol, stigmasterol, campesterol, plumbagic acid lactone, two flavonyl methyl ethers-azaleatin, cyarin and two aliphatics — palmitic acid and myricyl palmitate from *P. rosea*.14,15 The present paper deals with the isolation of two novel carboxylic acids and flavonoids from the ethyl acetate extract of roots of *P. rosea* and their structural determination based upon the chemical and spectroscopic data. Plumbagin, α-amyрин, α-amyrin acetate, β-sitosterol, *n*-octacosanol and β-sitosterol-3β-D-glucoside were also characterized.

**Results and Discussion**

Compound 1 was crystallized from benzene, m.p. 205°C. It responded to the characteristic colour test for flavones with Mg/HCl and for phenol with neutral FeCl3. It gave yellow colour with alkali. The molecular formula of compound 1 was established as C19H18O8 m/z (M+ 374). The 1H NMR spectrum displayed signals for the presence of four methoxy groups at δ 3.89, 3.92, 3.95, 3.97. There were two doublets at δ 6.38 and 6.48 with J = 2.2 Hz which were assigned to H-6 and H-8. A two proton singlet at 7.46 corresponded to H-2' and H-6'. Broad singlets at 6.69 and 11.55 were assigned to 4' and 5 hydroxyl groups respectively. In the 13C NMR there were eleven singlets, four doublets and four quartets which confirmed the structure of the compound as myricetin-tetra methyl ether 1. Compound 2 m.p. 180°C, was readily soluble in alkali giving a yellow colour. With neutral FeCl3, it gave a greenish blue colour. It responded to Shinoda’s test for flavonoid. The molecular formula of 2 was established as C19H20O8 m/z (M+ 376). The 1H NMR spectrum showed two meta coupled protons of ring A viz. H-6 and H-8 at δ 6.06 and 6.11 (J = 2.0 Hz). The two equivalent protons H-2' and H-6' appeared as two proton doublet at δ 6.76. H-2 appeared as one proton doublet. The two methoxyl groups was shown as singlets at δ 3.81 and 3.87. Two methoxyl groups were observed at δ 3.92. The broad one proton signal at δ 11.18 was assigned to 5-hydroxyl. All the above data suggested the structure of the compound to be amelopsin-3',4',5',7-tetramethyl ether 2. The 13C NMR spectrum, as assigned, also confirmed the structure. Compound 3 was crystallized from benzene. It gave green colour.
with FeCl₃ and red colour with Conc. H₂SO₄. m.p. 109°C. C₁₁H₁₂O₅ m/z (M⁺ 224). It was a phenolic carboxylic acid as it dissolved in NaHCO₃ and gave green colour (catechol) with FeCl₃. The UV-Vis (217, 266, 347 nm) and IR spectral data (3300 (hydroxyl), 2850 (carboxylic acid), 1730 (acid carbonyl) and 1700 (keto carbonyl) was characteristic of a ortho-dihydroxy acetophenone which was further corroborated. The ¹H NMR spectrum showed the presence of three aromatic protons at δ 6.81 (t), 7.12 (dd, J=8 and 1.4 Hz) and 7.35 (dd, J=8 and 1.4 Hz). These chemical shifts and coupling constants were characteristic of a 1,2,3-trisubstituted benzene ring. In the mass spectrum of the compound there were fragments corresponding to m/z 137. The methylene protons were observed at δ 2.5 (dd, 1H, J=17.3 and 5.4 Hz) and at 3.0 (dd, 1H, J=17.3 and 8.8 Hz). The chemical shift of these geminal protons were conclusive proof enough that this carbon atom is attached to an asymmetric center and this centre should be a –CH (CH₃) COOH which constitutes the rest of the carbons and protons. As could be rationalized there was a single proton at δ 3.91 (ddq, J = 8.7, 5.4 and 7.2 Hz) and a doublet of three protons at 1.27 (d, 3H, J=7.2 Hz). It was, therefore, evident that the side chain consisted of the following moiety –COCH₂-CH (CH₃) COOH which should be attached next to the vicinal hydroxyls. Structure 3 explains and rationalizes all these features. In the ¹³C NMR spectrum of the compound, there were five singlets, four doublets, one triplet and one quartet and their multiplicities were in agreement with structure 3.

Compound 4 was crystallized from CHCl₃ and ethyl acetate. m.p.215°C. It showed UV-Vis absorption at 229, 284, and 371 nm characteristic of a benzopyrone. The functionalities were an α,β-unsaturated carbonyl, IR 1688 cm⁻¹, δ C 180.6 (s), a carboxylic acid, IR 1700 cm⁻¹, δ C 168.1 (s), a vinylic methyl, δ 2.44 (d, 3H, J = 0.6 Hz) and δ C 20.0 (q). Signal for a proton appeared at δ 6.2 as a broad singlet (J = 0.6 Hz). The chemical shift of the methyl and its coupling constant were in agreement with the methyl group at position 2 and the proton at position 3. Therefore, the carboxylic acid should be placed in the ring. The presence of three aromatic protons in a row was deduced from the ¹H NMR spectrum. From the ¹H NMR of aromatic protons, the alternate possibility of carboxylic acid group at position 5 was ruled out. The structure 4 with carboxylic acid attached to position 8 was favourable, as this structure alone could account for proton at δ 8.47 due to the deshielding by pyrone carbonyl group. In ¹³C NMR all the carbon atoms were accounted with their correct multiplicities.

**Experimental Section**

All melting points are uncorrected and determined on a heating block apparatus. UV-Vis spectra were recorded on a Shimadzu UV-Vis spectrophotometer model UV-1601 using spectroscopic grade methanol. FT-IR spectra were recorded on a Perkin-Elmer grating spectrophotometer in KBr disc. The ¹H and ¹³C NMR spectra were recorded on a Bruker instrument at 400 and 100 MHz respectively. The solvents used were CDCl₃, DMSO-d₆ and CD₃OD
with TMS as the internal standard. EI-MS mass spectra were recorded on a Varian instrument at 70 eV by the direct inlet method. All chemicals and solvents used were of analytical grade.

Plant material

Roots of *P. rosea* were collected from Erode, Tamil Nadu and were identified by botanists at CSMDRIAS (CCRAS), Arumbakkam, Chennai. A voucher specimen (00518) has been deposited in the herbarium of this institute.

Extraction and isolation

Coarsely powdered roots of *P. rosea* (500 g) were extracted with ethyl acetate in an aspirator bottle for 72 hr. The solvents were removed by distillation on a water bath, the last traces being removed under vacuum to yield a residue (16 g). Ethyl acetate extract (14 g) was chromatographed over a column of silica gel (acme, 100-200 mesh, 1:22) using n-hexane, benzene, chloroform, ethyl acetate and their mixtures successively. n-hexane eluates removed almost all the plumbagin present. Fractions collected with 2% ethyl acetate in benzene, on removal of the solvent yielded a solid 3 which was crystallized from benzene (0.95 g), m.p. 109°C. Elution of the column with 20% ethyl acetate in benzene and removal of the solvent yielded the compound 4 which was recrystallised from chloroform and ethyl acetate.

**Myricetin-3', 4', 5', 7-tetra methyl ether, 1**

Yellow amorphous powder; m.p. 205°C; 1H NMR (400 MHz, CDCl3): δ 3.89, 3.92, 3.95, 3.97 (3H, each s, 4 × OCH3), 6.38 (1H, d, J = 2.2 Hz, H-6), 6.48 (1H, d, J = 2.2 Hz, H-8), 5.97 (6H, 2 × OCH3). 3.96 (6H, s, 2 × OCH3), 4.56 (1H, d, J = 6 Hz), 4.98 (1H, d, J = 6 Hz, H-3), 6.06 (1H, d, J = 2 Hz, H-6), 6.11 (1H, d, J = 2 Hz, H-8), 6.76 (2H, s, H-2' and H-6'), 11.18 (s, -OH); 13C NMR (100 MHz, CDCl3): δ 195.6 (s, C-4), 168.8 (s, C-7), 163.6 (s, C-9), 162.7 (s, C-5), 153.4 (s, C-3' and C-5'), 131.4 (s, C-4' or C-1'), 120.4 (s, C-4' or C-1'), 104.4 (d, C-2' and C-6'), 95.6 (d, C-6), 94.7 (d, C-8); 83.7(d, C-3), 72.3 (d, C-2), 60.8 (q, OCH3), 56.1 (q, 2 × OCH3), 55.8 (q, OCH3); EIMS (70 eV): m/z 376 [M+]2, 348, 347, 319, 210, 195, 167.

**Plumbagic Acid, 3**

Yellow amorphous powder; m.p. 103°C; UV-Vis (EtOH): 217 (2.3), 266 (1.5), 347.6 (0.3) nm; IR (KBr): 3300 (hydroxyl), 2850 (carboxylic acid) and 1688 (β-unsaturated) cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 31.27 (3H, d, J = 7.2 Hz, -CH (CH3) COOH), 3.5 (1H, d, J = 17.3 and 8.8 Hz, CH2CH (CH3) COOH), 3.91 (1H, dd, J = 8.7, 5.4 Hz, CH2CH (CH3) COOH), 6.81 (1H, t, H-5), 7.12 (1H, dd, J = 8 and 1.4 Hz, H-4), 7.35 (1H, dd, J = 8 and 1.4 Hz, H-6), 11.16 (s, -OH); 13CNMR (100 MHz, CDCl3): δ 131.4 (s, C-1), 150.2 (s, C-2), 145.6 (s, C-3), 119.1 (d, C-4), 120.5 (d, C-5), 120.6 (d, C-6); 208.6 (s, > CO), 36.7 (t, -CH2-), 36.8 (d, -CH(CH3)COOH), 174.2 (s, -COOH), 18.4 (q, CH3); EIMS (70 eV): m/z 224 [M+]2, 206 [ M' - 18], 178 (M'-18-28), 137, 136, 109, 108, 91, 81, 63, 53.

**Roseanoic acid, 4**

Brown amorphous powder; m.p. 215°C; UV-Vis (EtOH): 229, 284, 371 nm; IR (KBr): 1700 (carboxylic acid) and 1688 (β-unsaturated carbonyl) cm⁻¹; 1H NMR (400 MHz, CDCl3): 2.44 (3H, d, J = 0.6 Hz, CH3), 6.36 (1H, bs, H-3), 7.67 (1H, dd, J = 8.4 and 1.7 Hz, H-7), 7.77 (1H, dd, J = 8.4 and 7.5 Hz, H-6), 8.47 (1H, dd, J = 7.5 and 1.7 Hz, H-5); 13CNMR (100 MHz, CDCl3): 180.6 (s, C-4), 168.1 (s, -COOH), 165.3 (s, C-9), 157.6 (s, C-2), 133.9 (d, C-5), 132.9 (d, C-7), 132.0 (s, C-10), 123.1 (d, C-3), 120.4 (s, C-8), 110.6 (d, C-6) and 20 (q, CH3); EIMS (70 eV): m/z 204 [M']2.

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