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Chemical constituents of *Plumbago indica* roots

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Isolation of a new naphthoquinone 3-*O*-3′-bidroserone, along with naphthoquinone derivatives 2,3-epoxyplumbagin and plumbagic acid is reported from the roots of *Plumbago indica*. The structures have been elucidated on the basis of chemical and spectral evidence. The configuration of plumbagic acid has been assigned.

A good number of α-naphthoquinones and flavonoids have been reported[1-9] from the well reputed medicinal plant, *Plumbago indica* Linn. (syn. *P. rosea* L)[4]. Our further study on its chemical constituents has resulted in the isolation of one more naphthoquinone and two naphthoquinone derivatives from the ethyl acetate extract of the roots. This paper describes the isolation and characterization of these compounds.

PI-31 designated as 3-*O*-3′-bidroserone, $C_{22}H_{14}O_7$, [Ml$^+$ 390, mp 188°C, was obtained as orange needles. It exhibited UV-Vis absorption bands in MeOH at 226 (log ε, 4.60), 282 (4.34) and 408 (3.88) nm characteristic of dihydroxy-1,4-naphtho quinones[5,6]. The IR spectrum in KBr showed the presence of hydroxyl (3325 cm^{-1}), aryl ether (2850, 1201 cm^{-1}), and unchelated and chelated carbonyl (1665 sh and 1625 cm^{-1}) functions. The ^{1}H NMR spectrum displaying signals for two vinylic methyl groups (δ, 2.08, 6H, s, Me-2,2′), six aromatic protons (δ 7.14-7.28, 2H, q, H-6,6′; δ 7.52-7.76, 4H, m, H-7,7′, 8,8′) and two phenolic hydrogen bonded hydroxyl protons (δ 11.12, 2H, s, exchangeable with D_2O, HO-5,5′), was very similar to that of droserone **1** and 3-hydroxyjuglone **2**[5,6]. These data can be satisfied by considering structure **3** for the compound PI-31, which was also supported by the EI mass spectrum[7] showing significant mass peaks at m/z 390 [M]$^+$ (20%), 375 [M–Me]$^+$ (25), 374 [M–O]$^+$ (100), 373 [M–OH]$^+$ (4), 372 [M–H_2O]$^+$ (9), 362 [M–CO]$^+$ (12), 357 [M–Me–H_2O]$^+$ (27), 334 [M–2CO]$^+$ (9), 319 [M–2CO–Me]$^+$ (8), 203 [one monomeric unit]$^+$ (12), 188 [another monomeric unit+H]$^+$ (18), 187 (6), 121 (24), 120 (16) and 92 (24). The 3-*O*-3′ ether linkage between the two quinone units in structure **3** was also suggested by its ^{13}C NMR spectrum, which exhibited carbon signals at δ 8.73 (C-11, 11′), 119.83 (C-8,8′), 121.86 (C-10,10′), 123.13 (C-6,6′), 132.80 (C-9,9′), 135. (C-2,2′), 137.47 (C-7,7′), 152.79 (C-3,3′), 161.23 (C-5,5′), 183.04 (C-1,1′) and 184.75 (C-4,4′). The carbon signals at δ 8.73, 152.79 and 184.75 ppm were in good agreement with the reported data (δ 7.9, 154.0 and 181.5 ppm) for *ortho*-methyl hydroxy grouping in aristolindiquinone **4**[8]. The compound on methylation with MeI in the presence of Ag_2O afforded a dimethyl ether **5** as orange crystals, $C_{24}H_{18}O_7$, [M]$^+$ 418, mp 160°C. This dimethyl ether showed ^{1}H NMR spectrum similar to that of the parent compound except for the signal of two phenolic hydroxyl protons, which disappeared and two methoxyl appeared at δ 3.98 (6H, s). All these observations collectively led to the assignment of 3-*O*-3′-bidroserone structure **3** for the compound PI-31. It may be noted that the occurrence of droserone **1** in *P. indica* has been reported earlier[3].

PI-32 designated as 2,3-epoxyplumbagin, $C_{11}H_8O_4$, [M]$^+$ 204, mp 155°C was obtained in pale yellow needles. The structure of this compound was assigned as **6** from the study of its spectral data (UV, IR, ^{1}H NMR and MS) as well as through the preparation of its methyl ether **7**, $C_{12}H_{10}O_4$, [M]$^+$ 218, mp 115°C and direct comparison (mixed m.p., co-TLC and superimposable IR spectra) with the product of plumbagin **8** obtained from the oxidation with alkaline hydrogen peroxide[9]. It may be known synthetically but no spectral data were reported elsewhere to the best of our knowledge. However, this is the first report of its natural occurrence. The compound may be an artefact formed during extraction procedure.

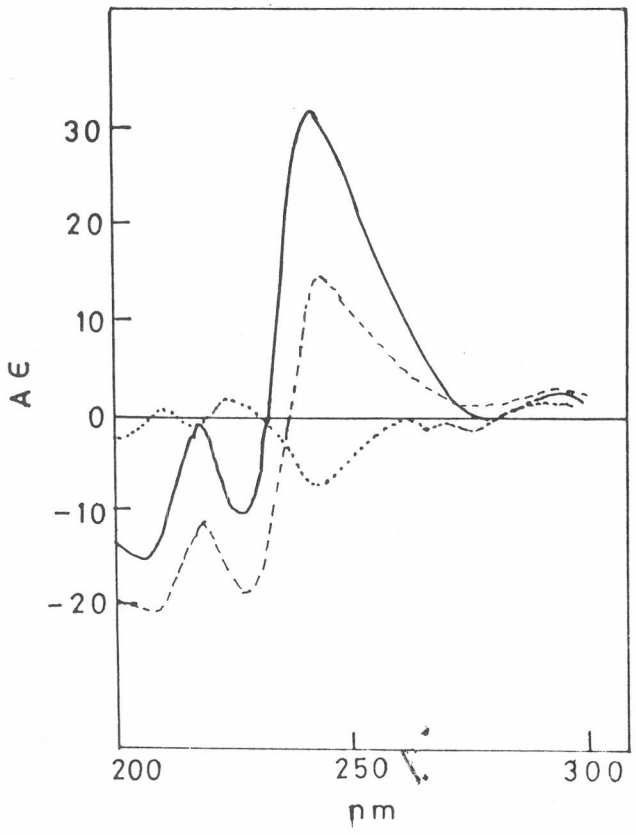


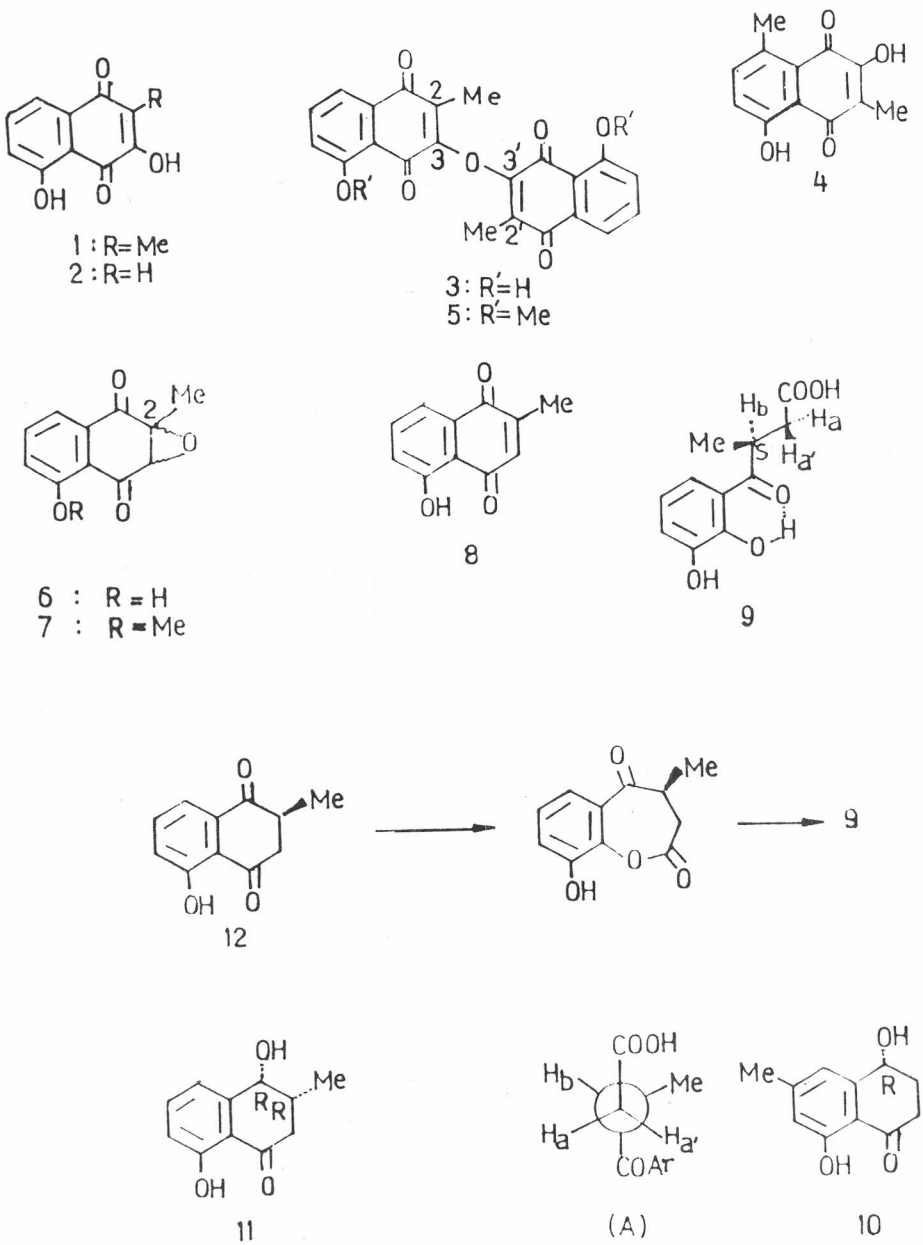
Figure 1—CD curves of plumbagic acid (— · —), and dibenzoates of shinanolone (···) and isoshinanolone (—) in methanol.

PI-33, $C_{11}H_{12}O_5$, $[M]^+$ 224, $[\alpha]_D$+4.2° ($CHCl_3$), mp 112°C, showed UV spectrum in MeOH at 220 (log ε, 4.17), 268 (4.02) and 348 (3.45) nm and IR absorption bands in KBr at 3460 (OH), 1708 (COOH) and 1640 (chelated >C=O) cm^{-1}, similar to those reported for plumbagic acid **9**, mp 110°C, isolated from *Plumbago zeylanica*[10]. However, our observation on its ^1H NMR spectrum in $CDCl_3$ was different from the reported value of some proton signals. The ^1H NMR spectrum displayed signals at δ 1.30 (3H, d, *J*=8 Hz, Me-3), 2.50 (1H, dd, *J*=16, 4 Hz, Ha′-2), 3.02 (1H, dd, *J*=16,10 Hz, Ha-2), 3.90 (1H, br sextet, *J*=8 Hz, H-3), 5.76 (2H, brs, HO-3′ and HOOC-1), 6.84 (1H, t, *J*=8 Hz, H-5′), 7.16 (1H, dd, *J*=8, 2 Hz, H-4′), 7.38 (1H, dd, *J*=8, 2Hz, H-6′) and 12.40 (1H, s, exchangeable with D_2O, HO-2′). The C-2 methylene protons appeared as double doublets with different *J* values instead of reported same value (*J*=16 Hz) because the geminal pair H_a and $H_{a'}$ are diastereotopic. They are coupled to each other with the largest coupling constant (16 Hz) and also each of them are coupled to H_6 with different coupling constants, one large (10 Hz) and the other small (4 Hz). This difference in coupling constants can be accounted by the Newman formula (**A**) of the compound. We have also confirmed the carbon skeletal structure of **9** by ^{13}C NMR studies with DEPT experiments. The aroyl carbonyl carbon appeared much downfield than the benzoyl carbonyl carbon (δ 195 ppm) because of intramolecular H-bonding with C-2′–OH group and attachment with a secondary alkyl group[11]. The skeletal structure **9** was also supported by its EI-mass spectrum which showed significant mass peaks at m/z 224 $[M]^+$ (40%), 206 $[M–H_2O]^+$ (40), 179 $[M–COOH]^+$ (35), 178 (8), 164$[M–CH_3COOH]^+$ (15), 138(31), 137(100), 136(13), 109(12) and 81(24). The configuration at C-3 of the compound was not assigned earlier. We have assigned it as *S* by correlation of its CD curve with those of dibenzoates of shinanolone **10** and isoshinanolone **11** (Figure 1). It may be noted that the absolute configuration at C-4 of shinanolone **10** was assigned earlier as *R* and that of isoshinanolone **11** as *R* and at C-3 as *R* [12]. However, further study is necessary for its confirmation as aromatic chirality method is not safely applicable to this compound because plumbagic acid is an aryl open chain ketonic acid and both shinanolone **10** and isoshinanolone **11** are tetralones. Thus, the structure of plumbagic acid was assigned as 3*S*-methyl-4(2′,3′-dihydroxyphenyl)-4-oxobutanoic acid **9**. This compound is possibly derived in plant by oxidation of dihydroplumbagin **12** to a lactone followed by hydrolytic cleavage of lactone ring (Scheme I). This is the first report of the occurrence of plumbagic acid from *P. indica*. Although the compound has been reported to cure cough and to relieve phlegm by Qian[10], studies for evaluation of other pharmacological activities are in process.

Experimental Section

General. Melting points are uncorrected. IR spectra were recorded in KBr and ^1H NMR spectra in $CDCl_3$ on a 100 MHz Jeol Fx-100 instrument and ^{13}C NMR spectra (including DEPT) in $CDCl_3$ on a 400 MHz Bruker WM-400 instrument. Petrol refers to the fractions of bp 60-80°C. Silica gel (Merck, 60-120 mesh) was used for column chromatography (CC) and silicagel G (Merck) for thin layer chromatography.

Extraction and isolation of compounds. The dried and powdered roots (1 kg) of *P. indica*, collected at Hoogly, West Bengal and Jampai,



Scheme I

Tripura, was successively extracted with hot petrol and hot EtOAc in a Soxhlet extractor. The EtOAc extract after removal of the solvent under reduced pressure gave a dark brown semisolid (12.2 g), which was separated into phenolic and neutral fractions by treatment with 5% NaOH and subsequent acidification of the aqueous layer with 2*M* HCl and extraction with diethyl ether. The phenolic fraction (6.35 g) was column chromatographed using solvents of less polarity to high polarity.

The fraction eluted with 30% petrol in benzene yielded a residue of two compounds which on repeated column chromatography afforded plumbagin **8** in orange needles (0.3 g, 3×10^{-2}%) mp 78°C (lit.[13], m.p. 78°C); 2,3-epoxyplumbagin **6** in pale yellow needles (0.035 g, 3.5×10^{-3}%), mp 155°C. The fraction eluted with 50% petrol in benzene afforded 3-*O*-3′-bidroserone **3** in orange needles (0.045 g, 4.5×10^{-3}%), mp 188°C.

The fraction eluted with 25% $CHCl_3$ in EtOAc afforded a residue which on crystallization from C_6H_6–$CHCl_3$ mixture yielded PI-33 as pale brown prisms, mp 112°C (0.2 g, 2×10^{-2}%).

PI-32 (2,3-Epoxyplumbagin, 6): UV(MeOH): 230 (log ε 4.38), 258 (3.96) and 338 (3.68) nm; IR(KBr): 3470-3430 br (OH), 2860 and 1265 (–O–), 1685 and 1635 (unchelated and chelated >C=O) cm^{-1}; ^1H NMR: δ 1.54 (3H, s, Me-2), 3.96 (1H, s, H-3), 6.60-7.66 (3H, m, H-6,7,8) and 12.00 (1H, s, exchangeable with D_2O, HO-5); MS(EI): 204 $[M]^+$ (100%), 176 $[M–CO]^+$ (24), 148 $[M–2CO]^+$ (19), 121 (26), 120 (13) and 92(11).

PI-33 (Plumbagic acid, 9): ^{13}C NMR: δ 18.33 (Me-3), 38.72 (C-3), 38.87 (C-2), 117.33 (C-1′), 119.89 (C-4′), 120.46 (C-6′), 120.59 (C-5′), 145.74 (C-3′), 150.24 (C-2′), 177.88 (C-1), 208.83 (C-4).

Methylation of 3-*O*-3-bidroserone 3. A solution of **3** (15 mg) in CH_2Cl_2 (3 mL) was stirred with CH_3I (1.5 mL) in the presence of Ag_2O (50 mg) for 15 hr at room temperature. The reaction mixture was filtered and the filtrate concentrated and purified through CC when a dimethyl ether **5** of **3** was obtained in orange needles, mp 160°C (8 mg, 54%); UV(MeOH): 224 nm (log ε, 4.56), 276 (4.26) and 376 (3.72); IR: 2850 (aromatic ether), 1660 (unchelated >C=O), 1620 cm^{-1} (chelated >C=O); ^1H NMR: δ 2.10 (6H, s, Me-2,2′), 3.98 (6H, s, MeO-5,5′), 7.20-7.30 (2H, H-6,6′), 7.60-7.85 (4H, m, H-7.7′, 8,8′).

Methylation of 2,3-epoxyplumbagin 6. A solution of **6** (10 mg) in $CHCl_3$ (3 mL) was stirred with CH_3I (1 mL) and Ag_2O (50 mg) for 10 hr at room temperature. The reaction mixture was on usual work-up and column purification afforded colourless needles of the methyl ether **7** of **6**, mp 115°C (7 mg, 70%); UV(MeOH): 226 (log ε 4.32), 250 (3.92) and 330 (3.58); IR: 2848 and 1251 (–O–), 1690 cm^{-1} (>C=O); ^1H NMR: δ 1.72 (3H, s, Me-2), 3.84 (1H, s, H-3), 3.98 (3H, s, MeO–5), 7.30 (1H, dd, *J*=8, 2 Hz, H-6), 7.58 (1H, dd, *J*=8, 2 Hz, H-8), 7.72 (1H, t, *J*=8 Hz, H-7).

Oxidation of plumbagin 8 with alkaline H_2O_2. A solution of **8** (20 mg) in EtOH (4 mL) was stirred with 30% H_2O_2 (3 mL) and K_2CO_3 (50 mg) for 2 hr at room temperature. The reaction mixture was evaporated to a residue and extracted with petrol. The petrol extract on column purification afforded 2,3-epoxyplumbagin **6** (12 mg, 60%), mp 154°C.

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