

## Studies on Medicinal and Related Plants of Sri Lanka. Part 18.<sup>1</sup> Structure of a New Naphthoquinone from *Plumbago zeylanica*.

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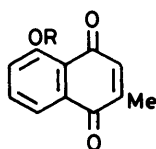
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The isolation of chitranone (3), zeylanone (4), maritnone (5), 2-methylnaphthazarin (6), plumbazeylanone, and a new naphthoquinone, methylene-3,3'-diplumbagin (7) from the phenolic fraction of the light petroleum extract of the roots of *Plumbago zeylanica* (Plumbaginaceae) is reported. The dimethyl ether of the new quinone has been synthesized from plumbagin methyl ether.

*Plumbago zeylanica* (Plumbaginaceae) is a perennial herb with long succulent roots, growing in the low country, especially in the warm dry districts of Sri Lanka. The roots of this plant have been well known in India for a long time for their medicinal properties.<sup>2</sup> Previous studies on Indian *P. zeylanica* led to the isolation of naphthoquinones, plumbagin (1), 3,3'-biplumbagin, 3-chloroplumbagin, droserone, elliptnone, chitranone, zeylanone and isozeylanone.<sup>3-5</sup> In an investigation of *P. zeylanica* collected in Sri Lanka we have now found additional plumbagin-related quinones (see also refs. 6 and 7).

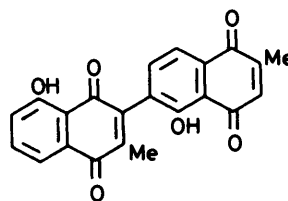
physical data and by comparison with authentic samples. The fraction eluted with 7.5% ethyl acetate in light petroleum yielded, on further purification by p.t.l.c. four quinones. The least polar, C<sub>23</sub>H<sub>16</sub>O<sub>6</sub>, m.p. 208–210 °C, was found to be a new quinone and its structure was elucidated as methylene-3,3'-diplumbagin (7) from the following evidence.

The i.r. and u.v. spectra of this quinone are similar to those of plumbagin. The <sup>1</sup>H n.m.r. of the new quinone showed a D<sub>2</sub>O exchangeable singlet at δ 11.93, a multiplet at δ 7.63–7.13, a broad singlet at δ 3.96 and a sharp singlet at δ 2.30 in the ratio of

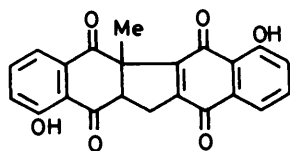


(1) R = H

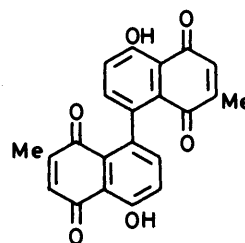
(2) R = Me



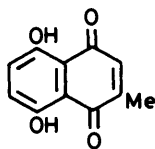
(3)



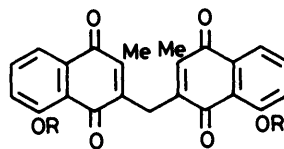
(4)



(5)



(6)



(7) R = H

(8) R = Me

The phenolic fraction of the light petroleum extract of the roots of *P. zeylanica* was chromatographed on a column of silica gel and eluted with light petroleum containing increasing amounts of ethyl acetate. The fraction eluted with 1% ethyl acetate in light petroleum yielded two compounds, identified as plumbagin (1) and 2-methylnaphthazarin (6) from their

1:3:1:3. The sharpness of the methyl singlet coupled with the absence of vinylic protons indicated the presence of a C-3 substituent in the plumbagin entity. The <sup>1</sup>H n.m.r. data together with the molecular formula suggested that the pigment contains two plumbagin units connected by a methylene bridge (δ 3.96, br s) suggesting structure (7). This was confirmed by

comparison of its dimethyl ether (8) with a synthetic sample prepared from plumbagin (1) as described below.

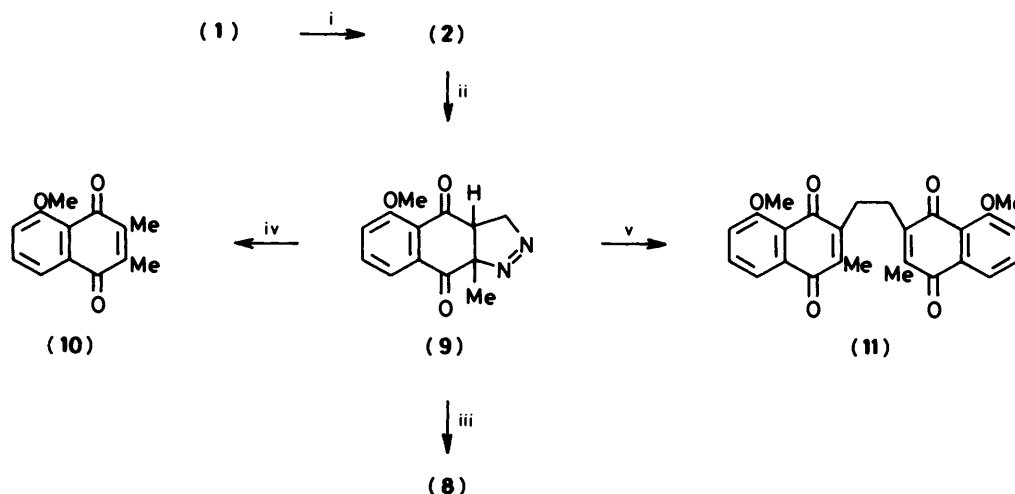
Plumbagin was converted into its methyl ether (2) by treatment with iodomethane in chloroform in the presence of silver oxide.<sup>8</sup> This was treated with diazomethane at low temperature under anhydrous conditions in ether<sup>9</sup> to yield the benzindazole dione (9). The mass spectrum of compound (9) showed a peak at highest  $m/z$  value 216 ( $C_{13}H_{12}O_3$ ) corresponding to  $M^+ - N_2$  and characteristic of this type of benzindazole dione. Similarly,<sup>9</sup> heating (9) in toluene for several minutes yielded exclusively 2,3-dimethyljuglone methyl ether (10). The mass spectrum of compound (10) was identical with that of the benzindazole dione (9). Furthermore, in basic medium, compound (9) behaved like the benzindazole dione derived from 2-methyl-1,4-naphthoquinone<sup>9</sup> giving the ethylene-bridged biquinone (11) (Scheme). However when treated with a

possibility plumbagin dissolved in chloroform was applied on a silica gel plate, allowed to stand for 30 min and eluted with chloroform. The only mobile spot was found to be that due to plumbagin. Some (polymeric) material remained at the base but no spots were detected corresponding to other quinones in this study.

### Experimental

**General Procedures.**—M.p.s are uncorrected. I.r. spectra were recorded for KBr discs, u.v. spectra for ethanol solutions and  $^1H$  n.m.r. spectra for solutions in  $CDCl_3$  unless otherwise stated.

**Extraction of *P. zeylanica* Roots.**—The dried and powdered root (2.3 kg) of *P. zeylanica* collected at Palatupana, Hambantota district, Sri Lanka was successively and ex-



**Scheme.** Reagents and conditions: i,  $MeI-Ag_2O-CHCl_3$ , 27 °C; ii,  $CH_2N_2$ -ether, 0 °C, 3 h; iii, slight excess of (2)- $CHCl_3-MeOH-NaOH$ , 20 min; iv, toluene-reflux 20 min; v,  $CHCl_3-MeOH-NaOH$ , 20 min

slight excess of plumbagin methyl ether (2) in the presence of aqueous sodium hydroxide in methanol and chloroform, the benzindazole dione (9) yielded the methylene-bridged dimer (8) identical with the dimethyl ether of the natural product (see Experimental section). This constitutes the first report of the occurrence of methylene-3,3'-diplumbagin (7) in nature. However, methylene-3,3'-diplumbagin has been obtained together with several other dimers of plumbagin during a biomimetic-type reaction of plumbagin with its hydroquinone in buffered methanolic solution.<sup>10</sup> Possible participation of methanol (as formaldehyde) in the formation of compound (7) in this reaction has been suggested and it is possible that the biosynthesis of methylene-3,3'-diplumbagin in *P. zeylanica* would involve an analogous reaction.

The three subsequent compounds isolated from the above fraction were found to be naphthoquinones with dimeric plumbagin structures and were identified as chitrone (3), zeylanone (4) and maritnone (5) (see Experimental section for details). Maritnone has been reported previously in *Diospyros* spp.<sup>11</sup> (Ebenaceae) but not in Plumbaginaceae. The fraction eluted with 10% ethyl acetate in light petroleum yielded, on further purification by p.t.l.c., isoshinanolone<sup>6</sup> and plumbazeylanone.<sup>7</sup> The detailed structure elucidation of plumbazeylanone will be presented elsewhere.

It is possible that some of the quinones encountered are artefacts arising due to oxidation and/or changes during chromatography of plumbagin (1). In order to rule out this

haustively extracted with hot light petroleum (b.p. 60–80 °C) and methanol. The dark brown semi-solid (33.47 g, 1.45%) obtained after removal of light petroleum under reduced pressure, was separated into phenolic (22.8 g,  $9.91 \times 10^{-1}\%$ ) and neutral (6.41 g,  $2.7 \times 10^{-1}\%$ ) fractions by treatment with 2M sodium hydroxide and subsequent acidification of the aqueous layer and extraction with ether. The phenolic fraction (22.8 g) which was obtained as a dark red semi-solid was chromatographed on a column of acid washed silica gel (450 g) made up in light petroleum and eluted with light petroleum containing increasing amounts of ethyl acetate.

**Isolation of 2-Methylnaphthazarin (6).**—The fraction eluted with 1% ethyl acetate in light petroleum yielded, on further purification by p.l.c. (benzene:chloroform, 1:1), 2-methylnaphthazarin (6) (0.033 g,  $1.4 \times 10^{-3}\%$ ), m.p. 173–174 °C (lit.,<sup>12</sup> 174–175 °C) as a dark reddish-violet crystalline solid, identical with an authentic sample (m.p., mixed m.p. and c.t.l.c.);  $\lambda_{max}$ . (log  $\epsilon$ ) 214 (4.50), 280 (3.92), 478 (3.69), 508 (3.72), and 544 nm (3.50);  $m/z$  (%) 204 ( $M^+$ , 100), 189 (13), 176 (50), 147 (13), 108 (11), and 91 (5) (Found:  $M^+$ , 204.0425.  $C_{11}H_8O_4$  requires  $M$ , 204.0422).

**Isolation of Methylene-3,3'-diplumbagin (7).**—The fraction obtained from the column on elution with 7.5% ethyl acetate in light petroleum yielded, after further purification by p.l.c. (benzene, two developments), compound (7) as orange yellow

needles (0.030 g,  $1.3 \times 10^{-3}\%$ ), m.p. 208–210 °C (from chloroform–light petroleum);  $\lambda_{\max}$ . (log  $\epsilon$ ) 215 (3.68), 247 (3.80), 275 (3.75), and 410 nm (3.44);  $\nu_{\max}$ . 3 660–3 160br, 2 910, 1 655, 1 632, 1 597, 1 455, 1 380, 1 360, 1 305, 1 290, 1 265, 1 195, 1 150, 1 065, 1 092, 1 020, 960, 850, 840, 792, 755, 745, 715, and 700  $\text{cm}^{-1}$ ;  $\delta$  (60 MHz) 11.93 (2 H, s,  $2 \times \text{OH}$ ; exchangeable with  $\text{D}_2\text{O}$ ), 7.63–7.13 (6 H, m, ArH), 3.96 (2 H, br s,  $\text{CH}_2$ ), and 2.30 (6 H, s,  $2 \times \text{Me}$ );  $m/z$  (%) 388 ( $M^+$ , 100), 373 (56), 370 (12), 345 (13), 213 (30), 121 (23), 130 (9), 92 (16) and 74 (21) (Found:  $M^+$ , 388.0952.  $\text{C}_{23}\text{H}_{16}\text{O}_6$  requires  $M$ , 388.0947).

**Methylation of Compound (7).**<sup>8</sup>—Silver(i) oxide (100 mg) and iodomethane (1 ml) was added to a well stirred solution of compound (7) (10 mg) in chloroform (0.5 ml) and stirring was continued overnight at room temperature. The reaction mixture was filtered and the filtrate evaporated under reduced pressure to give crude 5,5'-dimethoxy-2,2'-dimethyl-3,3'-methylenedi-1,4-naphthoquinone (8) which, on recrystallisation from chloroform–methanol, yielded yellow needles, m.p. 243–244 °C (9 mg, 84%) (lit.,<sup>10</sup> 241–242 °C);  $\nu_{\max}$ . 2 930, 1 654, 1 620, 1 576, 1 476, 1 456, 1 436, 1 335, 1 270, 1 245, 1 190, 1 160, 1 110, 1 056, 990, 971, 954, 835, 792, 776, 746, and 700  $\text{cm}^{-1}$ ;  $\delta$  (220 MHz) 7.76–7.13 (6 H, m, ArH), 3.97 (6 H, s,  $2 \times \text{OMe}$ ), 3.94 (2 H, s,  $-\text{CH}_2-$ ), and 2.30 (6 H, s,  $2 \times \text{Me}$ );  $m/z$  (%) 416 ( $M^+$ , 100), 401 (65), 373 (18), 227 (76), 135 (17), and 76 (28) (Found:  $M^+$ , 416.1272.  $\text{C}_{25}\text{H}_{20}\text{O}_6$  requires  $M$ , 416.1260).

**Synthesis of 5,5'-Dimethoxy-2,2'-dimethyl-3,3'-methylenedi-1,4-naphthoquinone (8).**—(a) **Methylation of Plumbagin (1).**<sup>8</sup> Plumbagin (750 mg) was dissolved in chloroform (10 ml), silver(i) oxide (3 g) and iodomethane (10 ml) were added and the solution was stirred overnight at room temperature (27 °C). Work-up as above, and recrystallisation from chloroform–light petroleum gave plumbagin methyl ether (2) as yellow needles (740 mg, 92%), m.p. 94–95 °C (lit.,<sup>13</sup> 94 °C).

(b) **Conversion of plumbagin methyl ether (2) into benzindazole dione (9).**<sup>9</sup> An ethereal solution of diazomethane was prepared from Diazald (6.54 g) in the usual manner. This was dried by decantation onto potassium hydroxide pellets, every 0.5 h for 2 h, followed by slow redistillation. Plumbagin methyl ether (2) (500 mg) was dissolved in anhydrous ether (6 ml; sodium dried and redistilled) and added to an excess of well stirred ethereal diazomethane solution at 0 °C. After 3 h, the flocculent precipitate formed was collected and washed with light petroleum (b.p. 40–60 °C). The mother liquor was concentrated under reduced pressure at low temperature, and triturated with light petroleum (b.p. 40–60 °C) to yield another crop of the product. This was collected and washed with light petroleum. The combined product was dissolved in the minimum volume of chloroform and light petroleum (b.p. 40–60 °C) was added until precipitation was incipient, and the solution kept at  $-5$  °C overnight. The solvent was removed by filtration and the resulting solid was dried *in vacuo* at room temperature to obtain off-white crystals of compound (9) (466 mg, 77%), m.p. 110–112 °C (decomp.),  $\nu_{\max}$ . 2 990, 2 935, 2 850, 1 685, 1 660sh, 1 472, 1 449, 1 432, 1 372, 1 300, 1 288, 1 274, 1 245, 1 226, 1 183, 1 140, 1 107, 1 078, 1 066, 1 042, 1 026, 997, 947, 930, 910, 880, 855, 826, 766, 720 and 690  $\text{cm}^{-1}$ ;  $\delta$  (60 MHz) 7.80–7.00 (3 H, m, ArH), 5.07 (2 H, d,  $J$  8 Hz,  $\text{CH}_2\text{N}=\text{N}$ ), 3.97 (3 H, s, OMe), 3.00 (1 H, t,  $J$  8 Hz, CH), and 1.73 (3 H, s, Me);  $m/z$  (%) 216 ( $M^+ - \text{N}_2$ , 100), 201 (20), 187 (23), 173 (22), 160 (7), 158 (10), 145 (12), 135 (15), 115 (17), 104 (8), 76 (25), 58 (31), and 43 (60) (Found  $M^+ - \text{N}_2$ , 216.0784.  $\text{C}_{13}\text{H}_{12}\text{O}_3$  requires  $M$ , 216.0785).

(c) **Preparation of 5,5'-Dimethoxy-2,2'-dimethyl-3,3'-methylenedi-1,4-naphthoquinone (8).**—The foregoing benzindazole dione (9) (50 mg) and plumbagin methyl ether (2) (51 mg) were dissolved in chloroform (0.6 ml) and methanol (8.6 ml) was

added, followed by 1M sodium hydroxide (0.05 ml). Nitrogen was evolved and the solution immediately became deep red. A few minutes later the solution changed to green and a solid started to separate. Stirring was continued for 2 minutes, the yellow crystals were collected, washed several times with methanol, and recrystallised from methanol–chloroform giving compound (8) as yellow needles (26 mg, 41%), m.p. 244–245 °C, identical (mixed m.p., i.r., m.s. and  $^1\text{H}$  n.m.r.) with the above methylated natural sample.

**Reactions of Benzindazole Dione (9).**—(a) **Thermal decomposition.** The benzindazole dione (9) (100 mg) was suspended in toluene (8 ml) and refluxed for 20 min during which time the reaction mixture turned bright yellow. The major compound was isolated by p.l.c. and crystallised from chloroform–light petroleum to give 5-methoxy-2,3-dimethyl-1,4-naphthoquinone (10) as yellow needles (60 mg, 68%), m.p. 144–145 °C;  $\nu_{\max}$ . 1 655, 1 630, 1 580, 1 470, 1 442, 1 370, 1 330, 1 280, 1 270, 1 215, 1 183, 1 152, 1 100, 1 060, 1 010, 962, 920, 826, 770, 730, and 690  $\text{cm}^{-1}$ ;  $\delta$  (220 MHz) 7.90–7.10 (3 H, m, ArH), 4.04 (3 H, s, OMe), 2.18 (3 H, s, Me), and 2.16 (3 H, s, Me);  $m/z$  (%) 216 ( $M^+$ , 100), 201 (20), 187 (19), 173 (18), 145 (12), 128 (8), 115 (14), 104 (14), 76 (30), and 58 (61) (Found:  $M^+$ , 216.0784.  $\text{C}_{13}\text{H}_{12}\text{O}_3$  requires  $M$ , 216.0785).

(b) **Base-catalysed reaction.**—The benzindazole dione (9) (25 mg) was dissolved in chloroform (0.2 ml) and methanol (6 ml) was added, followed by 1M sodium hydroxide (0.02 ml). The deep red colour formed immediately after addition of sodium hydroxide faded to a yellow. After 20 min the yellow crystals were collected, washed several times with methanol, and dried *in vacuo* to give 5,5'-dimethoxy-2,2'-dimethyl-3,3'-ethylenedi-1,4-naphthoquinone (11) (10 mg, 45%), m.p. 289–290 °C;  $\nu_{\max}$ . 2 940, 1 652, 1 630, 1 582, 1 472, 1 443, 1 432, 1 370, 1 342, 1 330, 1 278, 1 250, 1 220, 1 187, 1 143, 1 092, 1 071, 1 033, 970, 927, 860, 832, 790, 740, and 705  $\text{cm}^{-1}$ ;  $\delta$  (60 MHz) 7.90–7.10 (6 H, m, ArH), 4.00 (6 H, s,  $2 \times \text{OMe}$ ), 2.80 (4 H, s,  $\text{CH}_2\text{CH}_2$ ), and 2.30 (6 H, s,  $2 \times \text{Me}$ );  $m/z$  (%) 430 ( $M^+$ , 100), 415 (15), 397 (9), 267 (6), 241 (19), 240 (15), 215 (30), 201 (16), 187 (9), 144 (6), 128 (12), 115 (13), 76 (20), 58 (11), and 43 (29) (Found:  $M^+$ , 430.1416.  $\text{C}_{26}\text{H}_{22}\text{O}_6$  requires  $M$ , 430.1416).

**Isolation of Chitranone (3).**—Further purification by p.l.c. in benzene (two developments) of the fraction obtained on elution of the column with 7.5% ethyl acetate in light petroleum yielded chitranone (0.016 g,  $6.9 \times 10^{-4}\%$ ) as red crystals, m.p. 116–118 °C (lit.,<sup>4</sup> 118–120 °C). Its identity was confirmed by spectral characteristics and direct comparison with the i.r. spectrum of an authentic sample,  $\lambda_{\max}$ . (log  $\epsilon$ ) 250 (4.20) and 426 nm (4.00);  $\nu_{\max}$ . 2 920, 1 660, 1 640, 1 610, 1 450, 1 425, 1 360, 1 285, 1 255, 1 230, 1 200, 1 155, 1 130, 1 050, 1 030, 985, 900, 850, 835, 750, 740, 735, and 710  $\text{cm}^{-1}$ ;  $m/z$  (%) 374 ( $M^+$ , 100), 359 (16), 346 (9), 331 (5), 329 (6), 317 (5), 303 (5), 187 (6), 121 (12), 120 (9), 92 (15), and 57 (13) (Found:  $M^+$ , 374.0770.  $\text{C}_{22}\text{H}_{14}\text{O}_6$  requires  $M$ , 374.0790).

**Isolation of Zeylanone (4).**—P.l.c. separation in benzene (two developments) of the fraction obtained from the column by elution with 7.5% ethyl acetate in light petroleum afforded zeylanone (0.025 g,  $1.0 \times 10^{-3}\%$ ) as bright orange crystals, m.p. 214–216 °C (lit.,<sup>5</sup> 212–214 °C). Its identity was confirmed by comparison of spectral data ( $^1\text{H}$  n.m.r., i.r. and m.s.) with those reported for zeylanone;<sup>5</sup>  $\delta$  (220 MHz) 12.29 and 12.07 (each 1 H, s, exchangeable with  $\text{D}_2\text{O}$ , OH), 7.76–7.20 (6 H, m, ArH), 3.58–3.36 (2 H, m,  $\text{CH}_2$ ), 3.32–2.98 (1 H, m,  $>\text{CH}$ ), and 1.86 (3 H, s, Me), the  $^1\text{H}$  n.m.r. spectrum is superimposable with that of authentic zeylanone;<sup>5</sup>  $m/z$  (%) 374 ( $M^+$ , 100), 359 (62), 227 (7), 212 (14), 149 (19), 121 (22), 120 (54), and 92 (39) (Found:  $M^+$ , 374.0781.  $\text{C}_{22}\text{H}_{14}\text{O}_6$  requires  $M$ , 374.0790).

*Isolation of Maritinone (5).*—This was isolated from the same column fraction by p.l.c. in benzene (two developments) and purified by crystallisation from methanol, m.p. 190–192 °C (lit.,<sup>11</sup> 193–195 °C), (0.014 g,  $6.0 \times 10^{-4}\%$ ). Its identity was confirmed by comparison of spectra (i.r. and <sup>1</sup>H n.m.r.) with those of authentic maritinone;  $\nu_{\max}$ . 1 656, 1 642, 1 612, 1 485, 1 455, 1 415, 1 382, 1 360, 1 320, 1 218, 1 025, 1 010, 992, 900, 837, and 780  $\text{cm}^{-1}$ ;  $m/z$  (%) 374 ( $M^+$ , 100), 359 (5), 331 (26), 303 (52), 278 (44), 250 (32), 188 (14), 161 (12), 139 (23), 120 (12), 95 (14), 77 (24), and 58 (50) (Found:  $M^+$ , 374.0782.  $\text{C}_{22}\text{H}_{14}\text{O}_6$  requires  $M$ , 374.0790).

*Isolation of Plumbazeylanone and Isoshinanolone.*—The column fraction eluted with 10% ethyl acetate in light petroleum on further separation by p.l.c. (eluant: dichloromethane) afforded plumbazeylanone<sup>7</sup> (0.189 g,  $8.2 \times 10^{-3}\%$ ) as a bright orange crystalline solid, m.p. 246–248 °C, and isoshinanolone<sup>6</sup> (0.90 g,  $3.9 \times 10^{-2}\%$ ) as a pale yellow semi-solid.

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