Phytochemistry, Vol 22, No 5, pp 1245–1247, 1983
Printed in Great Britain

0031-9422/83/051245-03$03 00/0

1,2(3)-TETRAHYDRO-3,3′-BIPLUMBAGIN: A NAPHTHALENONE AND OTHER CONSTITUENTS FROM *PLUMBAGO ZEYLANICA**

G. M. KAMAL B. GUNAHERATH, A. A. LESLIE GUNATILAKA†, M UVAIS S. SULTANBAWA‡ and S. BALASUBRAMANIAM§

Department of Chemistry, University of Peradeniya, Sri Lanka, §Department of Botany, University of Peradeniya, Sri Lanka

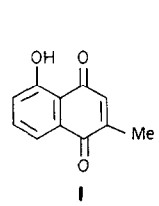
(*Received* 19 *August* 1982)

Key Word Index—*Plumbago zeylanica*, Plumbaginaceae, plumbagin; droserone, isoshinanolone; naphthalenones, 1,2(3)-tetrahydro-3,3′-biplumbagin, sitosterol, structure elucidation

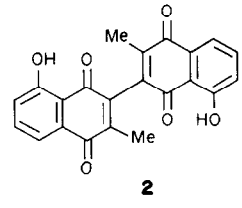
Abstract—The isolation of plumbagin, droserone, isoshinanolone and a new naphthalenone, 1,2(3)-tetrahydro-3,3′-biplumbagin is reported from the phenolic fraction of the light petrol extract of the roots of *Plumbago zeylanica*. The structure of the new naphthalenone was elucidated by means of spectroscopic data and chemical interconversions. The main constituent of the neutral fraction was shown to be sitosterol.

INTRODUCTION

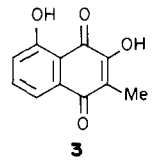
Plumbago zeylanica L., a plant with a variety of medicinal applications [1], has been previously subjected to several chemical investigations and the presence of plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone; **1**), 3,3′-biplumbagin (**2**), 3-chloroplumbagin, droserone (**3**), elliptinone, chitranone, zeylanone and isozeylanone has been reported [2–4]. In continuing our interest on the medicinal and related plants of Sri Lanka [5], we have investigated the phenolic fraction of the light petrol extract of the roots of this plant and herein we report the isolation and identification of **1**, **3**, isoshinanolone [4*R*,8-dihydroxy-3*R*-methyl-3,4-dihydro-1(2*H*)-naphthalenone; **4**] and a new naphthalenone, 1,2(3)-tetrahydro-3,3′-biplumbagin[3′,4′-dihydro-4′*R*,8,8′-trihydroxy-3,3′*R*-dimethyl-(2,2′*S*-binaphthalene)-(2′*H*)-1,1′,4-trione, **8**].



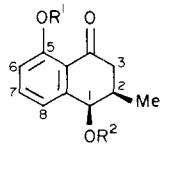
1



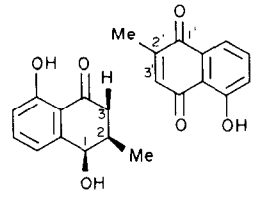
2



3



4 $R^1 = R^2 = H$
5 $R^1 = Me$, $R^2 = H$
6 $R^1 = R^2 = COMe$
7 $R^1 = R^2 = COPh$



8

RESULTS AND DISCUSSION

The hot light petrol extract of *P zeylanica* roots was separated into phenolic (sodium hydroxide-soluble) and neutral fractions. The neutral fraction constituted chiefly sitosterol The phenolic fraction on CC over acidic Si gel and elution with 0.5% and 1.0% ethyl acetate in light petrol afforded plumbagin (**1**) and droserone (**3**), respectively. Elution of the column with 3% ethyl acetate in light petrol gave isoshinanolone (**4**) as a pale yellow semi-solid, $[\alpha]_D + 24.2°$ With dimethyl sulphate–potassium carbonate it gave a monomethyl ether (**5**), mp 99–100°; with acetic anhydride–pyridine a crystalline diacetate (**6**), mp 91–93°; and with PhCOCl–pyridine it gave a non-crystalline dibenzoate (**7**), $[\alpha]_D + 135.8°$ DDQ oxidation of **4** gave plumbagin (**1**) The ^{1}H NMR spectrum of **4** was almost superimposable on that of isoshinanolone [6] The evidence for the stereochemistry at C-3 and C-4 of **4** came from its ^{1}H NMR spectrum and the CD curve of the dibenzoate (**7**) (Fig. 1) which were almost superimposable on those reported [6]. Although certain physical characteristics (mp and $[\alpha]_D$) of our sample differed from those of isoshinanolone isolated by Tezuka *et al.* [6], all the remaining physical data were in full agreement. Furthermore, our physical data, including $[\alpha]_D$, agree well with that reported for isoshinanolone obtained recently from *Aristea ecklonii* [7].

Elution of the column with 7.5% ethyl acetate in light petrol gave an orange-yellow crystalline compound,

*Part 9 in the series "Studies on Medicinal and Related Plants of Sri Lanka". For Part 8 see ref [5].

†To whom correspondence should be addressed

‡Present address Department of Chemistry, University of Maiduguri, Nigeria

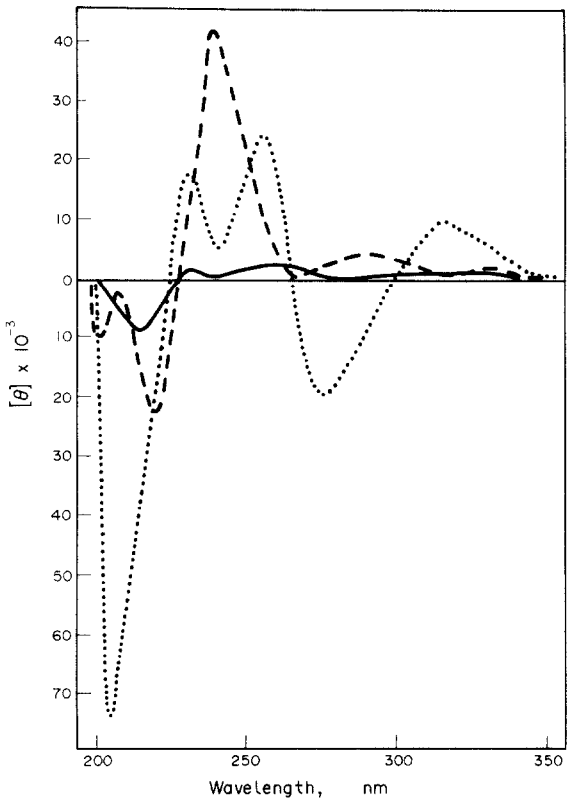


Fig 1 CD curves (in MeOH) of (—) isoshinanolone (**4**), (---) isoshinanolone dibenzoate (**7**) and ( ) 1,2(3)-tetrahydro-3,3′-biplumbagin (**8**)

$C_{22}H_{18}O_6$ mp 109–110°, $[\alpha]_D$ +69.8°, whose IR spectrum indicated the presence of hydroxyl, α,β-unsaturated carbonyl and quinone carbonyl groups (see Experimental). Comparison of the UV and ^1H NMR spectra of this quinone with plumbagin (**1**) and isoshinanolone (**4**) suggested it to be made up of these two units (see Tables 1 and 2). Absence of the vinyl proton at C-3 of the plumbagin unit indicated the possible linkage to be through the C-3 carbon atoms of the two units. This was confirmed by oxidation of the natural product with DDQ to give 3,3′-biplumbagin (**2**). Therefore, the natural quinone should be 1,2(3)-tetrahydro-3,3′-biplumbagin (**8**). It remained then to determine the stereochemical arrangement of groups at C-1–C-3. The coupling constant of H-4′ with H-3′ is 2.5 Hz suggesting that the methyl and the hydroxyl groups are *cis* (the methyl is equatorial and hydroxyl is pseudo-axial) as in **4**. Furthermore, the similarity of the CD curves of **8** and **4** (Fig 1) helped to assign the absolute configuration at C-1 as *R* [6]. The coupling constant of the H-2′ and H-3′ is 11 Hz which suggested the *trans* arrangement of groups at these two positions [8, 9].

Table 1 UV absorption maxima of compounds **1**, **3**, **4** and **8**

Compound	λ_{max}^{EtOH} nm (log ε)			
1	211 (4.39)	266 (3.99)	—	404 (3.52)
3	228 (4.62)	278 (4.52)	—	400 (3.66)
4	217 (4.52)	259 (4.09)	335 (3.76)	—
8	212 (4.70)	263 (4.32)	333 (3.77)	418 (3.63)

Elution of the column with 5% ethyl acetate in petrol afforded a new quinone, $C_{34}H_{24}O_9$, mp 246–248°, whose structure elucidation is presently underway.

Isoshinanolone (**4**) has thus far been encountered in several species of *Diospyros* (Ebenaceae) [6] and *Aristea ecklonii* [7] (Iridaceae), whereas this constitutes the first report of the occurrence of 1,2(3)-tetrahydro-3,3′-biplumbagin (**8**) in nature.

Four biosynthetic pathways have been postulated for the origin of naphthaquinones in higher plants [10]. The structural features of the naphthaquinone pigments, encountered in the genus *Plumbago*, clearly suggest that they are biosynthesized from acetate–polymalonate units and the occurrence of naphthalenones **4** and **8** provides further support for this pathway in *Plumbago* as in the genus *Diospyros*. However, the possibility of the presence of a quinone reductase enzyme in these plant species cannot be excluded, although we have failed to detect the presence of 3,3′-biplumbagin (**2**) in our extracts.

EXPERIMENTAL

General procedures. Mps are uncorr. IR: KBr discs unless otherwise stated, UV: EtOH, ^1H NMR: 60 MHz, CCl_4 and $CDCl_3$ with TMS as int. standard, TLC: Si gel (Merck) 0.10 mm, prep. TLC: 0.5 mm Si gel. Petrol refers to the fraction of bp 60–80°.

Extraction and separation of the phenolic fraction. Dried and powdered root (2.5 kg) of *P. zeylanica*, collected at Uda-

Table 2 ^1H NMR data of compounds **1**, **2**, **4** and **8** (60 MHz, CCl_4, TMS as int. standard)

Compound	Aromatic H and OH		Quinone H and Me		Reduced quinone H and Me			
	H-6–H-8	OH-5*	Me-2	H-3	H-1	H-2	H-3	Me-2
1	7.06–7.60 *m*	11.77 *br s*	2.15 *d* (J = 1.6 Hz)	6.73 *q* (J = 1.6 Hz)	—	—	—	—
2 ($CDCl_3$)	7.20–7.90 *m*	11.80 *br s*	2.06 *s*	—	—	—	—	—
4	6.66–7.37 *m*	12.17 *br s*	—	—	4.57 *d* (J = 2.5 Hz)	2.10–3.10 *m*		1.11 *d* (J = 6 Hz)
8	6.66–7.66 *m*	11.61 *br s* 11.90 *br s*	2.30 *s*	—	4.66 *d* (J = 2.5 Hz)	2.83 *m*	4.20 *d* (J = 11 Hz)	1.13 *d* (J = 7 Hz)

*Exchangeable with D_2O.

Peradeniya, was successively and exhaustively extracted with hot petrol and hot MeOH. The orange-yellow semi-solid (12.5 g), obtained after evaporation of petrol, was separated into phenolic (5 04 g) (NaOH-soluble) and neutral fractions (5 g) by the usual procedure. The phenolic fraction (5 g), which was obtained as a dark red semi-solid, was chromatographed over acid washed Si gel (120 g) made up in petrol

Plumbagin (**1**) Elution of the column with 0.5% EtOAc in petrol gave **1** (901 mg, 0.036%), mp 71–72°, lit. 73° [6], identical (mmp, IR and co-TLC) with an authentic sample [7]. For UV and ^{1}H NMR data, see Tables 1 and 2, respectively

Droserone (**3**) The fraction eluted with 1% EtOAc in petrol afforded droserone (**3**) (33 mg, 0 0013%), mp 179–180°, lit. 181° [10], UV Table 1, IR ν_{max} cm^{-1} 3308, 1643, 1625, 1580, 1435, 1395, 1360, 1320, 1310, 1293, 1202, 1160, 1095, 1053, 900, 835, 820, 750, 735, 715, ^{1}H NMR δ 11.03 (1H, *s*, exchangeable with D_2O, chelated OH), 7 70–7 10 (3H, *m*, ArH), 2 10 (3H, *s*, Ar-Me) and 1.60 (1H, *br s*, exchangeable with D_2O, OH); MS *m/z* (rel. int.). 204 0421 [M]$^+$. Calc. for $C_{11}H_8O_4$ 204.0422; 204 [M]$^+$ (100%), 176 (67), 158 (53), 148 (58), 147 (68), 130 (58), 121 (65), 102 (58), 92 (43) and 91 (43).

Isoshinanolone (**4**). Elution of the column with 3% EtOAc in petrol gave **4** (875 mg, 0.035%) as a semi-solid; $[\alpha]_D$ + 24.17°, IR ν_{max}^{film} cm^{-1}: 3500–3300, 1634, 1580, 1451, 1406, 1341, 1242, 1215, 1162, 1090, 1060, 1025, 1010, 980, 960, 940, 870, 815, 798, 745 and 715, UV and ^{1}H NMR. Tables 1 and 2, respectively; MS *m/z* 192 [M]$^+$, 177, 175, 164, 163, 159, 151, 150, 149, 146, 134, 131, 127, 122, 115, 107, 105, 94, 93, 91, 85, 83 and 77.

Isoshinanolone monomethyl ether (**5**). Isoshinanolone (70 mg) in dry Me_2CO (10 ml) was refluxed for 12 hr with Me_2SO_4 (0.3 ml) and dry K_2CO_3 (100 mg) Usual work-up and purification by prep TLC afforded **5** as a colourless crystalline solid (35 mg), mp 99–101°; IR ν_{max}^{KBr} cm^{-1} 3460, 1660, 1595, 1580, 1470, 1440, 1405, 1347, 1320, 1292, 1280, 1240, 1190, 1117, 1100, 1072, 1025, 980, 965, 940, 897, 810, 790, 750 and 710, ^{1}H NMR ($CDCl_3$). δ 7 66–6.80 (3H, *m*, ArH), 4.75 (1H, *d*, *J* = 2 5 Hz, CHOH), 3 88 (3H, *s*, OMe), 2 60 (2H, *m*, H_2-3), 2 33 (1H, *m*, H-2), 1 90 (1H, *br s*, exchangeable with D_2O, OH), 1.11 (3H, *d*, *J* = 6 Hz, CH–Me)

Isoshinanolone diacetate (**6**). Isoshinanolone (30 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (1 ml) in the usual manner to afford the diacetate **6** (28 mg) as a colourless crystalline solid, mp 91–93°, IR ν_{max}^{KBr} cm^{-1} 1765, 1730, 1680, 1605, 1460, 1450, 1430, 1410, 1370, 1340, 1325, 1310, 1260, 1240, 1190, 1160, 1140, 1090, 1050, 1015, 990, 980, 940, 920, 900, 870, 815, 790, 750, 715 and 675, ^{1}H NMR (CCl_4) δ 7.70–6 97 (3H, *m*, ArH), 6.05 (1H, *m*, CHOAc), 2 60 (3H, *m*, H-2 and H_2-3), 2 34 (3H, *s*, OCOMe), 2 07 (3H, *s*, OCOMe) and 1 14 (3H, *d*, *J* = 6 6 Hz, CHMe), MS *m/z* (rel int) 276.0994 [M]$^+$ Calc. for $C_{15}H_{16}O_5$ 276.0995; 276 [M]$^+$ (17%), 234 (80), 217 (20), 192 (88), 174 (100), 159 (38), 150 (61), 146 (74), 131 (56), 107 (27), 105 (31), 103 (31), 93 (28), 91 (38), 83 (44) and 77 (39)

Isoshinanolone dibenzoate (**7**) Isoshinanolone (50 mg) was benzoylated with PhCOCl (0 5 ml) in pyridine (2 ml) for 6 hr Usual work-up gave **7** as a colourless viscous oil (55 mg), UV λ_{max}^{MeOH} nm (log ε) 226 (4.58), 282 (3 58) and 296 (3 41), ^{1}H NMR (CCl_4) δ 8 23–7 00 (13H, *m*, ArH), 6.26 (1H, *m*, CH–OCOC$_6$H$_5$), 2 60 (3H, *m*, H-2 and H_2-3), 1.10 (3H, *d*, *J* = 6 Hz, CH–Me)

Oxidation of isoshinanolone (**4**) *to plumbagin* (**1**). Isoshinanolone (10 mg) in dioxane (2 ml) was refluxed for 12 hr with DDQ (10 mg) Evaporation of dioxane and purification by prep. TLC afforded plumbagin, mp 73–74°, identical (mmp, co-TLC and co-IR) with an authentic sample obtained above

1,2(3)-*Tetrahydro*-3,3′-*biplumbagin* (**8**). Elution of the column with 7.5% EtOAc in petrol afforded **8** as an orange coloured amorphous solid (125 mg, 0.005%), mp 109–110°, $[\alpha]_D$ + 69.8°($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3500–3200, 1660 sh, 1625, 1610, 1580, 1450, 1340, 1290, 1262, 1195, 1160, 1072, 1058, 980, 950, 915, 875, 830, 805, 740 and 700, UV and ^{1}H NMR: Tables 1 and 2, respectively; MS *m/z* (rel. int). 378 [M]$^+$ (23), 360 (100), 345 (37), 342 (19), 327 (5), 230 (12), 229 (23), 190 (16), 189 (9), 149 (21), 122 (6), 121 (35), 93 (8) and 92 (6).

Oxidation of **8** to 3,3′-*biplumbagin* (**2**). 1,2(3)-Tetrahydro-3,3′-biplumbagin (20 mg) in dioxane (5 ml) was refluxed for 48 hr with DDQ (20 mg) The major product was separated by prep TLC to afford 3,3′-biplumbagin, **2** (10 mg), mp 210–213°, lit. 214–216° [2]; IR ν_{max}^{KBr} cm^{-1}. 3480–3000 *br*, 1665, 1625, 1600, 1450, 1305, 1290, 1275, 1240, 1200, 1160, 1050, 1020 and 735; ^{1}H NMR. Table 2. This sample was shown to be identical (mmp, co-TLC, co-IR and ^{1}H NMR) with authentic 3,3′-biplumbagin [7].

Sitosterol. The neutral fraction obtained above on TLC showed the presence of essentially a single compound and this was isolated by CC on Si gel and elution with 10% EtOAc in petrol and identified as sitosterol (2.0 g, 0.08%), mp 134–136°. $[\alpha]_D$ − 35° lit. 136–137° $[\alpha]_D$ − 35° [11]. Its identity was further confirmed by mmp, co-TLC and co-IR with an authentic sample.

Acknowledgements—We thank Professor R. H. Thomson (University of Aberdeen) for valuable suggestions, MS and some CD data, Professor Carl Djerassi (University of Stanford) for CD data, Dr. V. Kumar for an authentic sample of 3,3′-biplumbagin, Professor S. Natori for the copies of IR and ^{1}H NMR spectra of isoshinanolone, and Mrs S. C. Weerasekera and Mr. P. Liyanage for technical assistance Financial support from the Special Programme of Research, Development and Research Training in Human Reproduction, W.H.O., is gratefully acknowledged

REFERENCES

1. Attygalle, J (1917) *Sinhalese Materia Medica* pp. 98–99 M. D Gunasena, Colombo.
2. Sidhu, G S. and Sankaram, A. V B (1971) *Tetrahedron Letters* 2385
3. Sankaram, A V. B., Rao, A. S. and Sidhu, G. S. (1976) *Phytochemistry* **15**, 237
4 Sankaram, A. V B., Rao, A S. and Shoolery, J N (1979) *Tetrahedron* **35**, 1777.
5 Gunatilaka, A. A. L , Silva, A. M. Y. J de, Sotheeswaran, S. and Tillekeratne, L. M. V (1982) *Phytochemistry* **21**, 2719
6. Tezuka, M , Takahashi, C , Kuroyanagi, M , Satake, M , Yoshihira, K. and Natori, S (1973) *Phytochemistry* **12**, 175.
7 Meepagala, K M , Kumar, V., Sultanbawa, M U S and Balasubramaniam, S (1981) *Sri Lanka Assoc Advmt Sci* **37**, 74
8. Pinhey, J. T and Sternhell, S. (1965) *Aust. J. Chem.* **18**, 543
9. Clark-Lewis, J. W., Jackmann, L M and Wilhams, L R (1962) *J Chem Soc* 3858.
10. Thomson, R H., (1971) *Naturally Occurring Quinones* p. 236. Academic Press, London
11. Heilbron, I. and Bunbury, H. M. (1953) *Dictionary of Organic Compounds* p. 361. Eyre & Spottiswoode, London.