A PENTACYCLIC QUINONE AND DIOSINDIGO B FROM THE HEARTWOOD OF *DIOSPYROS MELANOXYLON*

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Key Word Index—Diospyros melanoxylon; Ebenaceae; naphthaquinones; diosindigo B; rearrangement; diosindigo A; biramentacéone.

Abstract—Diosindigo A, diosindigo B and biramentacéone have been isolated from heartwood of *Diospyros* melanoxylon. A violet pigment isolated from the same source has been shown to be a new pentacyclic quinone. Sunlight causes diosindigo B to change to the pentacyclic quinone and biramentacéone. The intense peaks at m/e 389 and 388 in the mass spectrum of diosindigo B are doublets which arise by fragmentation of both M⁺ and M⁺ + 2.

The isolation and characterisation of five naphthols, four naphthaquinones and a binaphthaquinone from the heartwood of *Diosypros melanoxylon* was described in a previous report [1]. The chloroform extract of this heartwood, collected from Watangi and Medak in Andhra Pradesh, is blue in colour and was worked up with the specific aim of isolating the compound or compounds responsible for this blue colour. By preparative TLC on silica gel, four compounds were isolated from the wood collected at Medak, two of which (A and B) were blue in colour, the third (C) was violet and the fourth (D) was orange. The wood collected at Watangi contained compounds B, C and D but not compound A.

Compound A and its leucotetraacetate were found to be identical with diosindigo A (1) and its leucotetraacetate (2) respectively [2]. Compound B (molecular formula $C_{24}H_{20}O_6$) was assigned a 2,2'-binaphthyl-1,1'-quinone structure by comparison of its UV, IR and NMR spectra (Table 1) with those of diosindigo A (1) [2]. The IR spectrum of compound B showed a hydroxyl stretching absorption at 3375 cm^{-1} (*peri*-methoxynaphthol) [3], which is absent in the IR spectrum of diosindigo A (1). Compound B probably has structure 3 or 4, the former being diosindigo B [4]. The structure of diosindigo B has been established as 3 by synthesis [4].

The NMR spectrum of compound B is similar to that of diosindigo A (Table 2), suggesting the symmetrical structure 3 for the former. The difference between the NMR spectra of 1 and 3 lies in the chemical shifts of the hydroxyl protons which are relatively upfield, and the aromatic protons which are relatively downfield in the latter. The hydroxyl proton signal at δ 8.75 in 3 is close to that of *peri*methoxynaphthol derivatives [3]. The hydrogen bonding between the hydroxyl proton and the carbonyl group in diosindigo A is obviously much stronger than in compound B, where the hydroxyl group is peri to the methoxyl group only. Thus in 1 the 2H-5,5' will be more shielded by the *para* hydroxyl group than the corresponding 2H-8,8' in 3 ($\Delta \delta = 0.45$). The NMR spectrum of compound B is thus consistent with structure 3. Compound B formed a leucotetraacetate (5), which is isomeric with 2. The NMR spectra of 2 and 5 are very similar except for the position of the downfield signal (2H-5,5') which is seen at δ 8.04 in 2 and 7.40 in 5. In the latter, the 2H-8,8' are peri to the acetoxyl groups and are thus shielded by 0.64 ppm as compared to 2 where they are *peri* to the methoxyl groups. Thus structure 3 can be assigned to compound B which is therefore diosindigo B. Dr. Musgrave has kindly confirmed that our compound is diosindigo B.

Table 1. UV and	l IR	data	of	1,	3	and	6
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	Diosindigo A (1)	Diosindigo B (3)	Violet quinone (6)
λ^{CHCl_3} nm (log ε):		251 (4.36)	272.5 (4.65)
-max (8-)	294 (4.33)	293 (4.33)	309.0 (3.97)
	697 (4.45)	676 (4.42)	325.0 (3.95)
$\lambda_{i=1}^{CHCl_3}$ nm (log ε):	328 (4.10)	351 (3.94)	339.0 (3.93)
······································	,	(<i>'</i>	520.0 (3.97)
$v_{\rm max}^{\rm KBr}$ cm ⁻¹	1578 (>C=O)	3375	3350 (O-H)
		1605	1635 (>C=O)
		1580 (>C=O)	

Table 2. ¹H NMR of 1 and 3 (79.5 MHz FT NMR)

Compound	2H-3,3′	20Me-4,4′	2OH-5,5' or -8,8'	2H-6,6' or -7,7'	2Me-7,7' or -6,6'	2H-8,8' or -5,5'
Diosindigo A (1) [2,5]	8.44 (s)	4.06 (s)	13.55 (s)	6.83 (s, br.)	2.41 (s)	7.11 (s, br.)
Diosindigo B (3)	8.37 (s)	4.16 (s)	8.75 (s)	6.94 (s, br.)	2.41 (s)	7.56 (s, br.)

The MS of diosindigo B contained peaks which are formed by fragmentation of both M^+ and $M^+ + 2$ ions. The M⁺ ion (a) probably picks up two hydrogen atoms from the moisture present in the ionisation chamber to form a stable dinaphthol structure which gives rise to the $M^+ + 2$ ion (b). The abundant ions in the MS of diosindigo B, at m/e 389, 388, 374 are regarded as resulting from the M^+ (a) and M^+ + 2 (b) ions, since they are seen as doublets in the high resolution mass spectra (Scheme 1). The doublet peaks at m/e 389 analysing for C₂₄H₂₁O₅ (c) and $C_{23}H_{17}O_6$ (d) arise by the loss of -OH and -Mefrom M^+ + 2 (b) and M^+ (a) respectively. (d) loses another -Me to give the ion corresponding to $C_{22}H_{14}O_6$ (e). Similarly, the ion at m/e 388 is resolved into a doublet made up of $C_{24}H_{20}O_5$ (f) and $C_{22}H_{16}O_6$ (g); the former could arise from M^+ + 2 (b) by a loss of H₂O and the latter by a loss of -Me from h (M⁺ -1) which is incidentally the molecular ion of 6, also isolated from this wood. The ion f subsequently loses Me to give i. This fragment may also result by the loss of OMe from the M⁺ ion. The ion m/e 374 was also observed as a doublet corresponding to e and j.

Compound C had a molecular formula of $C_{23}H_{16}O_6$, and underwent reversible reduction with sodium dithionite. IR absorption bands at 3350 (OH) cm⁻¹ and 1660 and 1635 cm⁻¹ (chelated and unchelated >C=O) suggested the presence of *peri*-methoxynaphthol and *peri*hydroxynaphthaquinone systems respectively in the molecule. Its NMR spectrum showed two signals due to methyl protons (δ 2.74, $s \delta$ 2.52, s, Ar–Me), methoxyl protons (δ 4.18, s), a hydroxyl peri to the carbonyl (δ 12.13, s), two broadened singlets assignable to protons ortho to the hydroxyl groups of the peri-hydroxynaphthaquinone $(\delta 6.89)$ and *peri*-methoxy naphthol $(\delta 7.08)$ moieties of the molecule respectively, two broad singlets assignable to protons para to the hydroxyl groups of the perihydroxynaphthoquinone (δ 7.55) (H-8, δ 7.50 in 7-methyl juglone [6]) and *peri*-methoxynaphthol (δ 7.72) moieties of the molecule respectively and two singlets at δ 7.34 and δ 9.38 assignable to the lone proton and the hydroxyl proton (peri-methoxynaphthol) respectively. All the above properties were compatible with compound C having any one of the structures 6-9. Diosindigo B was converted into a violet quinone and biramentacéone by irradiation with sunlight and the former was identical with the natural product. The violet quinone should thus have structure 6. An ortho-quinonoid structure for this compound is ruled out by a negative reaction with σ -phenylene diamine. This structure was further confirmed by converting 6 into a leucotetraacetate which had the UV, IR, NMR and MS expected of 10. In the NMR spectrum of 10, H-1 is peri to the furano oxygen and absorbs relatively downfield at δ 8.03 (d, J = 2 Hz) as compared to H-8 absorbing at δ 7.67, (d, J = 2 Hz, peri to the acetoxyl group).

Compound D was identified as biramentaceone (11) by comparison of its IR and NMR spectra with those of an authentic sample. Exposure of diosindigo B (in chloroform containing ethanol or methanol) to sunlight resulted in the formation of mainly 11 and a trace of 6. It is thus difficult to

$$m/e \ 374 \ C_{22}H_{14}O_6$$

$$e$$

$$* \uparrow -Me$$

$$m/e \ 389 \ C_{23}H_{17}O_6 \qquad m/e \ 388 \ C_{22}H_{16}O_6$$

$$e$$

$$M' \ m/e \ 404 \ C_{24}H_{20}O_6 \qquad -H' \qquad m/e \ 403 \ C_{23}H_{19}O_6$$

$$M' \ m/e \ 406 \ C_{24}H_{22}O_6 \qquad -H' \qquad m/e \ 389 \ C_{24}H_{21}O_5$$

$$M' \ + 2 \ m/e \ 406 \ C_{24}H_{20}O_5 \qquad m/e \ 374 \ C_{23}H_{18}O_5$$

$$e$$

$$m/e \ 373 \ C_{23}H_{17}O_5$$

Scheme 1. Mass spectral fragmentation of diosindigo B.



say from these results whether compounds C and D are naturally-occurring or artefacts. Similar photo products are formed on irradiation of 4,4'-dimethoxy-2,2'-binaphthyl-1,1'-quinone [7].

Interestingly, this paper reports the first co-occurrence of both diosindigo A and diosindigo B in the same species and is only the second reported occurrence of diosindigo B.

EXPERIMENTAL

NMR spectra were determined at 79.5 MHz using $CDCl_3$ as solvent and TMS as int. standard. Si gel G (E. Merck 0.08 mm dia) was used for PLC. Mps are uncorr. Assigned MS fragmentations were confirmed by elemental compositions of the ions determined by high resolution mass analysis.

Plant material. The heartwoods were collected at Watangi near Kakinda and Medak (Andhra Pradesh).

Isolation of diosindigo A, diosindigo B, violet quinone and biramentacéone. 200 g of the powdered heartwood (Medak) was extracted with CHCl₃ (6×500 ml) at RT for 24 hr. The blue extracts were concd individually and separated by PLC (Si gel G/CHCl₃) into seven bands: band 1 (blue); band 2 (deep pink); band 3 (blue); band 4 (pale yellow); band 5 (pale yellow); band 6 (greenish yellow); band 7 (violet). The wood from Watangi was worked up in a similar manner: on PLC the blue band (1) corresponding to diosindigo A was absent.

Diosindigo A (1). The pigment in band 1 was eluted with CH_2Cl_2 , purified by column chromatography (Si gel) and recrystallised from CH_2Cl_2 to give blue needles (55 mg), mp above 300°, M⁺ 404.1269 (calc. for $C_{24}H_{20}O_6$ 404.1259). Reductive acetylation gave the leucotetraacetate (2): crystalline solid, mp

211–212°, M⁺ 574.1833 (calc. for $C_{32}H_{30}O_{10}$ 574.1839). The parent compound and its leucotetraacetate were identified as diosindigo A (1) and its leucoetraacetate (2) respectively by comparison with authentic samples and spectral data [2].

4,11-Dihydroxy-5-methoxy-2,9-dimethyl-dinaphtho (1,2-b: 2',3'd) furan 7,12-quinone (violet quinone) (6). The quinone was eluted from band 2 with CH₂Cl₂ and recrystallised from the same solvent to give a violet solid (30.4 mg), mp 335-338°, MS (70 eV, 180°) m/e (rel int.): 390 (6), 389 (29), 388 M^+ (100), 374 (9), 373 ($M^+ - Me$, 35), 346 (7), 345 ($M^+ - Me - CO$, 27), 317 (5), 195 (5), 194 $(M^+/2e, 15), 103(7), 97(5), 89(5), 83(5), 69(7), 58(10), 57(11), 55$ (10), 44 (16), 43 (24), m* 358.6 (388 \rightarrow 373), 319.1 (373 \rightarrow 345). The quinone (30 mg) was converted into its leucotraacetate (10), by refluxing with Ac₂O (10ml), Zn dust (270mg) and NaOAc (250 mg) until the soln was no longer coloured. The reactio mixture was poured onto crushed ice to give a solid which was purified by Si gel column chromatography and recrystallisation from CHCl₃-MeOH (3:1). Mp 334°, M⁺ 558.1516 (calc. for $C_{31}H_{26}O_{10}$, 558.1526). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 243.7 (4.38), 277.1 (4.97), 270, sh (4.82), 320.0 (4.03), 332.6 (4.02), 348.1 (4.08), 370.3 (4.08), 390.4 (4.25): IR $v_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1768, 1200, 1022 (aryl acetate); NMR: δ 2.43 (s), 2.49 (s), 2.59 (s), 2.63 (s), 2.69 (s, 2 Me, 4OAc), 4.08, (s, OMe); 7.09 (d, J = 2 Hz, H-3 and H-10), 7.67 (d, J = 2 Hz, H-8), 8.03 (d, J = 2 Hz, H-1), 7.26 (s, H-6) merged with CHCl₁ signal. MS (70 eV) m/e (rel. int.): 558 M⁺ (18), 516 $(M^+ - CH_2CO, 14), 474 (516 - CH_2CO, 20), 432 (474 - CH_2CO, 20), 432 (474$ CH₂CO, 43), 431 (20), 390 (432 - CH₂CO, 14), 389 (16), 388 (9), 375 (390 - Me, 6), 374 (5), 373 (388 - Me, 5), 346 (2), 345 (373 - CO, 4), 248 (11), 247 (10), 127 (7), 126 (5), 119 (5), 113 (10), 112 (9), 111 (8), 105 (8), 104 (5), 99 (13), 98 (10), 97 (14), 96 (7), 95 (18), 85 (39), 84 (13), 83 (19), 82 (8), 81 (8), 79 (10), 78 (56), 77 (20), 72 (7), 71 (60), 70 (21), 69 (23), 68 (7), 67 (12), 64 (5), 58 (34), 57 (94), 56 (28), 55 (29), 54(6), 53(5), 52(13), 51(12), 50(10), 45(13), 43(100), 42(23), m^* 477.1 (558 \rightarrow 516), 375.7 (474 \rightarrow 432), 352.2 (432 \rightarrow 390).

Diosindigo B. The pigment in band 3 was eluted and recrystallised from CH₂Cl₂ to give blue needles (29.2 mg), mp 232–234°, which were sparingly soluble in C_6H_6 , CHCl₃, and CH_2Cl_2 to give blue solutions. MS (70 eV, 205°) m/e (rel. int): $406 \text{ M}^+ + 2 (39), 405 (27), 404 \text{ M}^+ (86), 391 (9), 390 (27), 389$ $(M^+ - Me \text{ and } M^+ + 2 - OH, 100), 388 (80), 374 (389 - Me)$ 36), 373 (388 - Me and 404 - OMe, 83), 372 (20), 361 (9), 360 (7), 359 (388 - CHO, 22), 358 (388 - CH₂O, 17), 357 (388 - OMe, 7), 346 (11), 345 (17), 332 (5), 331 (12), 330 (16), 329 (7), 318 (7), 317 (7), 303 (7), 302 (12), 301 (7), 290 (8), 262 (5), 261 (6), 254 (5), 216 (8), $215(8), 203(9), 202.5(5), 202(M^+/2e)(19), 194(14), 194.5(12), 187$ (9), 186.5 (5), 173 (10), 172.5 (7), 165 (6), 151 (90), 135 (5), 134 (5), 106 (9), 77 (5), 58 (70), 57 (6), 55 (5), 44 (14), 43 (17), 41 (5), m* 388.5, 374.4, 373.6, 372.7, 359.6, 358.5, 357.5, 344.6. High resolution MS m/e: 406.1419 (calc. for C₂₄H₂₂O₆ 406.1416), 404.1259 (calc. for C24H20O6 404.1255), 389.1380 (calc. for C24H21O5 389.1389), 389.1089 (calc. for $C_{23}H_{17}O_6$, 389.1025), 388.1321 (calc. for C24H20O5 388.1310), 388.0982 (calc. for C23H16O6 388.0947), 374.1080 (calc. for C23H18O5 374.1154), 374.0798 (calc. for C₂₂H₁₄O₆ 374.0838).

Diosindigo B leucotetraacetate (5) Diosindigo B (43 mg) was refluxed with Zn dust (400 mg), dry NaOAc (314 mg) and Ac₂O (10 ml) until the resulting solution was colourless. The reaction

mixture was worked up as for **10** to give a white crystalline solid (22 mg), mp 232° (ethanol), M⁺ 574.1836 (Calc. for $C_{32}H_{30}O_{10}$, 574.1838). MS (70 eV, 180°) *m/e* (rel. int.): 574 M⁺ (5), 532 (M⁻ - CH₂CO, 74), 490 (532 - CH₂CO, 100), 489 (532 - MeCO, 6), 448 (490 - CH₂CO, 39), 447 (490 - MeCO, 7), 406 (448 - CH₂CO, 21), 405 (448 - MeCO, 7), 389 (406 - OH, 6), 388 (406 - H₂O, 5). 374 (389 - Me, 9), 373 (388 - Me, 8), 203 (M⁺/2e, 9), 58 (16), 43 (55), m* 451.5 (532 \rightarrow 490), 490.5 (490 \rightarrow 448), 374.8 (404 \rightarrow 389), 372.8 (406 \rightarrow 389), 358.5 (388 \rightarrow 373). UV $\lambda_{\text{max}}^{\text{EIOH}}$ nm (log ε): 226.4 (4.60), 245.3 (4.73), 292.1 (4.03), 303.5 (4.04), 316.8 inf (3.96), 332.3 (3.89). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 1752, 1205, 1010. NMR: δ 3.92 (*s*, 2 OMe-4, 4'), 6.73 (*s*, 2H-3, 3'), 2.11 (*s*, 2Ac-1, 1'), 2.38 (*s*, 2Ac-5, 5'), 2.52 (*s*, 2 Me-7, 7'), 7.11 (*d*, *J* = 1.5 Hz 2H-6, 6'), 7.43 (*br. s*, 2H-8, 8').

Biramentacéone (11). 11 was isolated from the pale yellow band running just behind diosindigo B on PLC. It was obtained as an orange crystalline solid (3 mg) by recrystallisation from CH₂Cl₂ and vacuum sublimation. Mp 247–248° (dec), M⁺ 374.0780 (calc. for C₂₂H₁₄O₆ 374.0787). IR v^{KBr}_{max} cm⁻¹ 1660, 1640. NMR: δ 2.45 (Ar-Me), 7.07 (s, 2H-3, 3'), 7.13 (d, J = 2.5 Hz, 2H-6, 6'), 7.49 (d, J = 2.5 Hz, 2H-8, 8'), 11.82 (s, 2 OH-5, 5').

Irradiation of diosindigo B. Diosindigo B (37 mg) in CHCl₃-EtOH (9:1) (95 ml) was exposed to sunlight for 2.5 hr. The reaction mixture was purified by Si gel column chromatography (eluant, CHCl₃) to give four bands. Compound 1 from a pale pink band was not identified due to paucity of the material. Compound 2 from pink band: violet solid, mp 335-338°. Identified as 6 by IR and TLC. Compound 3 from an orange band: orange solid, mp 247-248° (dec). Identified as biramentacéone by IR, TLC and NMR. Compound 4 from band 4 was not identified.

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