



6'',8'-Bisdiosquinone from *Diospyros mafiensis*

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Abstract

From the root-bark of *Diospyros mafiensis* an unusual novel tetrameric naphthoquinone, 6'',8'-bisdiosquinone, was isolated. The compound exhibited conformational isomerism and its structure has been elucidated on the basis of its spectral data. Diosindigo A and 7-methyljuglone were also identified in the stem-bark of this species. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Diospyros mafiensis*; Ebenaceae; Root-bark; 6'',8'-Bisdiosquinone; Diosindigo A; 7-Methyljuglone

1. Introduction

In continuation of our phytochemical investigations on *Diospyros mafiensis*, from the root bark of which we have isolated diosindigo A, diosquinone and 7-methyljuglone (Khan & Rwekika, 1993) and from the leaves, α -amyrin, lupeol and betulinic acid (Khan & Rwekika, 1992), we now report the isolation and identification of a new tetrameric naphthoquinone, 6'',8'-bisdiosquinone (**1**) from the root-bark of this species. This is the first tetramer containing epoxy groups and the first dimer of diosquinone. Diosindigo A and 7-methyljuglone were also identified in the stem-bark.

2. Results and discussion

The known compounds were identified by comparison with authentic samples using spectral and other physical data. From the root-bark, lupeol, diosindigo A and 7-methyljuglone were isolated. Comparative TLC also detected these two naphthoquinones in the stem-bark.

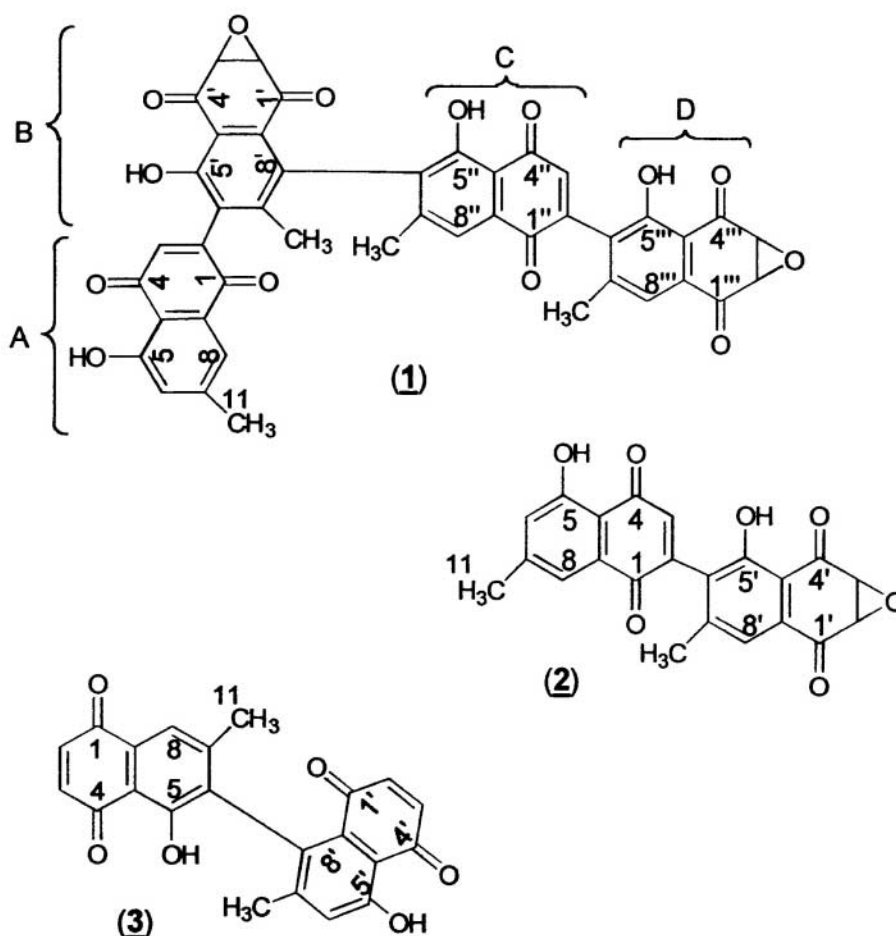
Column chromatography of the concentrated chloroform extract of the root-bark, led to the iso-

lation of 6'',8'-bisdiosquinone (**1**), a new tetrameric naphthoquinone, as a brown red crystalline compound, C₄₄H₂₆O₁₄. The tetramer was isolated from the third fraction using toluene–ethyl acetate. The structure of the compound was established by spectroscopic methods as follows.

The UV–VIS spectrum showed absorption which follows the general pattern observed for *peri*-hydroxy-naphthoquinones (Thomson, 1971, 1986, 1996). The IR spectrum showed characteristic chelated C=O absorption for *peri*-hydroxy-1,4-naphthoquinones (Thomson, 1971, 1986, 1996). The ¹H and ¹³C NMR spectra, the mass spectrum and the molecular formula indicated the compound to be an unsymmetrical tetrameric hydroxynaphthoquinone.

The ¹H NMR assignments (Table 1) for compound **1**, were made by comparison with diosquinone (**2**). All the chemical shifts are in close agreement with the values for diosquinone. The presence of four sets of resonances for epoxy, hydroxyl and methyl protons support the dimeric diosquinone structure. There is one 6-H and three 8-H type of aromatic protons. The 6-H and 8-H protons appeared as *meta*-coupled doublets ($J = 0.9$ Hz) at δ 7.13 and 7.49, respectively. The other two 8-H protons resonated as singlets at δ 7.52 and 7.50 for the remaining positions (8''-H and 8'''-H). The absence of signals for 6''-H and 8'-H indicates that the B and C moieties are linked at positions 8' and 6''. The chemical shift values for the two singlets

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for 3-H and 3''-H could be interchanged or it may be due to the different conformations of the molecule, this phenomenon being exhibited by diosquinone, which at 30° in chloroform gave two singlets each for only the 3-H and 5'-OH with a separation of 1.9 and 1.6 Hz, respectively (Thomson, 1986; Sankaram, Reddy, & Shoolery, 1987); the other resonances were unaffected. In bisdiodiosquinone at 500 MHz, similar conformational isomerism will be possible. In which case, some of the closely spaced singlets could be attributed to the presence of conformational isomers and these could include the two sets of methyls, hydroxyls and the epoxy protons, as indicated in Table 1 by the superscripts 1–7. As in the case of diosquinone, the presence of conformational isomerism is further supported by ^{13}C NMR.

^{13}C NMR assignments (Table 2) were made by comparing the chemical shifts of compound **1** with diosquinone (**2**) and isodiospyrin (**3**) (Sankaram et al., 1987; Sankaram, Reddy, & Marthandamurthi, 1986). All quaternary carbon atoms, which appeared as low intensity singlets and C–H's could easily be recognised from the DEPT spectrum. The spectrum of diosquinone exhibited much clearer signals for most of the carbons of the conformational isomers and compound

1 demonstrated comparable chemical shifts. In contrast to diosquinone, compound **1**, exhibited only one singlet for each of the carbons of C-4, C-5', C-6' and C-7'. The C-6'' to C-8' linkage is supported by the presence of a quaternary chemical shift at δ 139.4 and 120.2; these are the two singlet signals absent in the diosquinone spectrum.

The mass spectrum further support the structure with the characteristic loss of oxygen from the $[\text{M}]^+$ for epoxy compounds (Thomson, 1986), m/z 560 $[\text{M-O-ring D} + \text{H}]^+$.

The structure of the compound is thus established to be 6'',8'-bisdiodiosquinone.

3. Experimental

M.p.: uncorr. IR: KBr. UV: CDCl_3 . ^1H NMR: 500 MHz. ^{13}C NMR: 100 MHz. EIMS: probe, 30 eV. TLC: silica gel 60 F254. CC: silica gel 70–230 mesh.

Diospyros mafiensis root bark was collected from the Rufiji river delta, Tanzania, in January 1989. The plant was authenticated at the Herbarium of the Botany Department, University of Dar es Salaam, where a voucher specimen is deposited.

Table 1
Proton chemical shift assignments of 6',8''-bisdiosquinone

Moiety	Proton	6',8'-Bisdiosquinone	Diosquinone
B	2'-H	3.99 d ($J = 3.6$ Hz) ^{1 a}	3.96 d ($J = 3.8$ Hz)
D	2'''-H	3.98 d ($J = 3.6$ Hz) ^{1 a}	—
A	3-H	6.86 s ^{2 b} 6.88 s ^{2 b}	6.84 s 6.86 s
B	3'-H	4.04 d ($J = 3.6$ Hz) ^{3 c}	4.00 d ($J = 3.8$ Hz)
C	3''-H	6.86 s ^{2 b} 6.88 s ^{2 b}	—
D	3'''-H	4.03 d ($J = 3.6$ Hz) ^{3 c}	—
A	5-OH	11.87 s ⁴	11.83 s 11.48 s 11.46 s 11.48 s
B	5'-OH	11.51 s ⁵	—
C	5''-OH	11.88 s ⁴	—
D	5'''-OH	11.50 s ⁵	—
A	6-H	7.13 d ($J = 0.9$ Hz)	7.13 bs
A	8-H	7.49 d ($J = 0.9$ Hz)	7.48 s or 7.47 s
B	8'-H	—	7.48 s or 7.47 s
C	8''-H	7.52 s	—
D	8'''-H	7.50 s	—
A	7-CH ₃	2.29 s ^{6 d}	2.28 s or 2.43 s
B	7'-CH ₃	2.46 s ^{7 e}	2.43 s or 2.29 s
C	7''-CH ₃	2.45 s ^{7 e}	—
D	7'''-CH ₃	2.30 s ^{6 d}	—

a,b,c,d,e Could be interchanged. ^{1–7} Could represent pairs of conformational isomers.

Dried ground root-bark (520 g) was successively extracted $\times 3$ with petrol (40–60°) (2.5 l) and CHCl₃ (2.5 l). The petrol extract was discarded, while the CHCl₃ extract was evapd to dryness to give a solid (11.6 g, 2.2%). Part of this (6 g) afforded 3 frs on silica gel cc using toluene–EtOAc (1:1) 200 ml, (1:4) 100 ml and (1:5) 100 ml. The first fr. (220 ml) was discarded. The second fr. (80 ml) gave diosquinone, m.p. 200–200.5°, 4.5 mg (R_f 0.8, toluene), $[\alpha]^{20}_D$ –106° (CHCl₃) (Thomson, 1986). The third fr. (100 ml) on evapn and repeated recrystallization from MeOH gave 6'',8'-bis-diosquinone (**1**) as deep brown crystals, m.p. above 315°, 9.2 mg (0.004%), $[\alpha]^{25.1}_D$ –11.64° (CHCl₃; c 1.0) (R_f 0.61, toluene), having the following spectral properties: ¹H NMR: Table 1; ¹³C NMR: Table 2; IR (cm^{–1}) 1699 (m) C=O, 1644 (s) (C=O), 1614 (m) (C=O), 1566 (s) (C=C), 1369 (m), 1260 (s), 1212 (m), 869 (w), 761 (w); MS m/z (rel. int): 778 [M]⁺ (3) [found: C, 67.36%; H, 3.38% C₄₄H₂₆O₁₄ requires: C, 67.87%; H, 3.34%], 762 (2) [M–O]⁺, 560 (8) [M–O-moiety D + H]⁺, 455 (100), 439 (3), 376 (4), 281 (10), 255 (8), 188 (5) [moiety A + H]⁺ 153 (9), 137 (5) and 101 (5).

The dried powdered stem-bark (20 g) was extracted in CH₂Cl₂ for 48 h. The extract on evapn was subjected to TLC on silica gel against authentic samples. This led to the detection of diosindigo A and 7-methyl-juglone.

Table 2
Carbon-13 chemical shifts assignments of 6',8''-bisdiosquinone

Moiety	Carbon	Bisdiosquinone	Diosquinone	Isodiospyrin
A	C-1	182.4	182.4	184.4 or 184.9
B	C-1'	189.5 189.3	189.5 189.3	184.4 or 184.9
C	C-1''	182.4		
D	C-1'''	189.5 189.3		
A	C-2	145.5 145.4	145.6 145.5	139.6 or 140.2
B	C-2'	55.4	55.5	139.6 or 140.2
C	C-2''	145.5 145.4		
D	C-2'''	55.4		
A	C-3	138.9 138.7	138.8 138.8	137.7 or 138.8
B	C-3'	55.2	55.2	137.7 or 138.8
C	C-3''	138.9 138.7		
D	C-3'''	55.2		
A	C-4	188.8 —	188.9 188.8	190.1 or 190.4
B	C-4'	195.3	196.3	190.1 or 190.4
C	C-4''	195.2 188.8	196.3	
D	C-4'''	195.3 195.2		
A	C-5	161.7	161.7	158.8 or 162.1
B	C-5'	159.4 —	159.5 159.4	158.8 or 162.1
C	C-5''	161.7		
D	C-5'''	159.4		
A	C-6	124.3	124.3	135.20
B	C-6'	129.1 —	129.2 129.1	125.71
C	C-6''	139.4^a		
D	C-6'''	129.1		
A	C-7	148.7	148.7	45.5 or 148.2
B	C-7'	147.5 —	147.6 147.5	145.5 or 148.2
C	C-7''	148.7		
D	C-7'''	147.5		
A	C-8	121.3 ^b	121.2	121.4
B	C-8'	120.2^a	121.1	130.4
C	C-8''	121.3 ^b		
D	C-8'''	121.1 ^b		
A	C-9	131.5	131.6	128.7 or 129.0
B	C-9'	131.5	131.6	128.7 or 129.0
C	C-9''	131.5		
D	C-9'''	131.5		
A	C-10	112.2	112.2	113.3 or 114.3
B	C-10'	113.1	113.1	113.3 or 114.3
C	C-10''	112.2		
D	C-10'''	113.1		
A	C-11	22.3	22.2	20.4 or 20.6
B	C-11'	21.1	21.1	20.4 or 20.6
C	C-11''	22.3		
D	C-11'''	21.1		

a,b Could be interchanged.

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