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STRUCTURE OF EBENONE, A POSSIBLE BIOGENETIC PRECURSOR OF ELLIPTINONE, FROM *DIOSPYROS EBENUM*

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Key Word Index—*Diospyros ebenum*; Ebenaceae; ebenone; β-naphthyl-1,4-naphthaquinone; elliptinone; biogenesis; ^{1}H NMR; benzene induced solvent shifts, ^{13}C NMR, mass spectra.

Abstract—The isolation and determination of the structure of ebenone, a new biogenetically significant β-naphthyl-1,4-naphthaquinone derivative, from *Diospyros ebenum* is reported.

INTRODUCTION

The formation of diospyrol (**2**) and several dimers of 7-methyljuglone (5-hydroxy-7-methyl-1,4-naphthaquinone) and plumbagin (5-hydroxy-2-methyl-1,4-naphthaquinone) present in different *Diospyros* species is presumed to involve the oxidative dimerisation of 4,5-dihydroxy-2-methyl naphthalene (**1**) [1]. Elliptinone (**4**) can be regarded as derived from diospyrol by stepwise *para* hydroxylation in either of the rings followed by oxidation (Scheme 1). We now provide circumstantial evidence for such a hypothesis by the isolation and characterisation of a β-naphthyl-1,4-naphthaquinone derivative which can be considered as an intermediate in the formation of elliptinone (**4**) from **2**.

RESULTS AND DISCUSSION

Preparative TLC of the chloroform extract of the stem bark of *D. ebenum* Koen gave five coloured bands. On elution with dichloromethane, band-3 yielded a brownish crystalline solid, mp 231–232°, which we named ebenone Its quinonoid nature was established by its reversible reduction with sodium dithionite. It did not form a quinoxaline derivative with σ-phenylenediamine and thus an σ-quinonoid structure was ruled out.

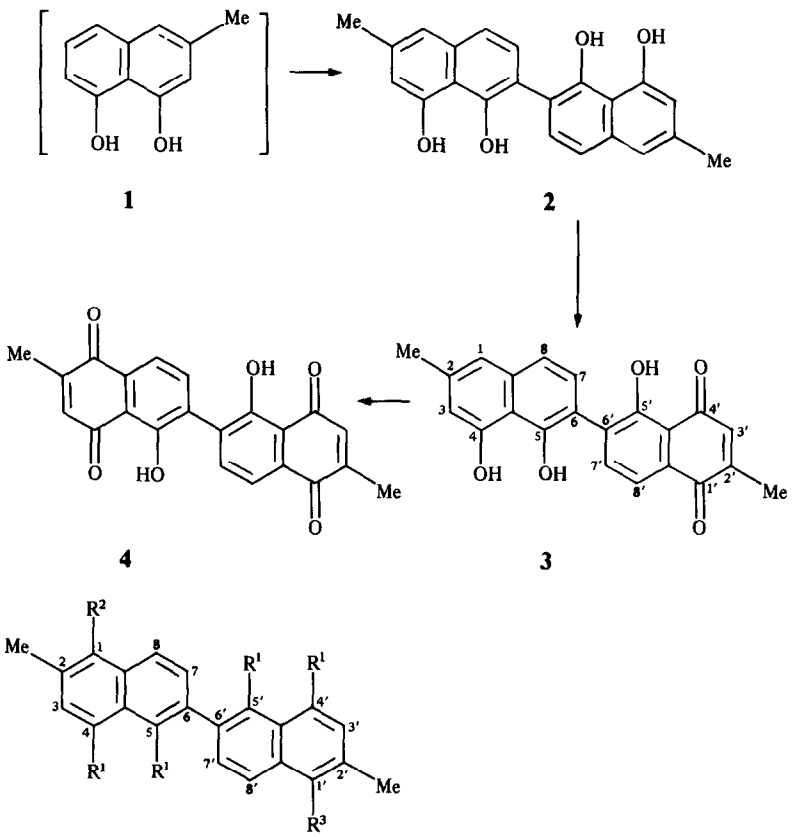
On reductive acetylation it gave a leucopentaacetate, the UV spectrum of which resembled closely that of diospyrol tetraacetate (**5**) suggesting the presence of a similar chromophore [2] (Table 1). A comparison of its molecular formula, $C_{32}H_{28}O_{10}$, with that of diospyrol tetraacetate (**5**) suggested the presence of an additional acetoxyl group which was substantiated by the successive loss of five ketene fragments from its molecular ion. Its ^{1}H NMR spectrum (Table 2) showed the presence of five acetoxyl and two aromatic methyl groups. The chemical shifts of the two acetoxyl groups at δ1.96 ($CDCl_3$) were close to the values observed for the upfield acetoxyl groups of diospyrol tetraacetate (**5**), [2] diosindigo B leucotetraacetate and diosindigo A leucotetraacetate [3], suggesting that they were *ortho* to the dimeric linkage. In contrast, the acetoxyls remote from the binaphthyl linkage were unaffected (Table 2). A pair of *meta* coupled broadened signals at δ7.12 and 7.80 which was confirmed

*The work described is taken from the Ph.D. thesis of V. V. N Reddy "Chemical Examination of some *Diospyros* Species", Osmania University, Hyderabad, 1982.

Table 1. UV and IR spectra of **3**, **5**, **6** and related compounds

	Ebenone leucopentaacetate (**6**)	Diospyrol tetraacetate (**5**)*	Ebenone (**3**)	Plumbagin	4-Hydroxy-5-methoxy 2-methylnaphthalene
λ_{max}^{MeOH} nm (log ε):	222 (4.68)	221 (4.71)	206 (4.42)	208 (4.46)	
	239 (4.80)	237 (4.98)	235 (4.76)		228 (4.49)
	290 (4 18)	290 (4.26)		264 (4.06)	
	327 (3.53)		310 (3.93)		304 (3.56)
			325 (3.91)		370 (3.55)
			340 (4.00)		332 (3.62)
			435 (3 70)	402–420 (3.57)	
λ_{infl}^{MeOH} nm (log ε):	250 (4.75)	250 (4.83)	265 (4.35)	240 (4.04)	
ν_{max}^{KBr} cm^{-1}:			1635 (>C=O)	1637 (>C=O)	3355 (O–H)
			1662	1659	
			3350 (O–H)		

*Ethanol. Data from ref. [2].



5 $R^1 = OCOMe, R^2 = R^3 = H$
6 $R^1 = R^3 = OCOMe, R^2 = H$
7 $R^1 = R^2 = R^3 = OCOMe$

Scheme 1. Probable biogenesis of ellipticinone.

by double irradiation, two pairs of *ortho* coupled protons at δ7.96 and 7.46 (J = 8.6 Hz) and δ7.86 and 7.37 (J = 8.6 Hz) established by benzene induced solvent shifts and a lone aromatic proton at δ7.20 were clearly observed (Fig. 1). Thus, structure **6** was assigned to ebenone leucopentaacetate. The pairs of peaks *a/c*, *b/d* and *f/h*, *g/i* constitute the downfield and upfield doublets of the two sets of *ortho* coupled protons respectively. Progressive addition of deuterated benzene (4, 12, 28 and 56 drops) moved peaks *b* and *d* gradually until *b* merged with *c* (12 drops) and finally shifted beyond *c*. Similarly, peaks *f* and *h* moved such that *h* closely overlapped with *g* and *f* was closer to *e*. Thus in all the spectra, the two AB quartets were clearly discernible and at the highest concentration of C_6D_6 used the most upfield limb was merged with the C_6D_5H (contaminant of C_6D_6) signal. Irradiation at δ7.96 sharpened the upfield doublet at δ7.37 and irradiation at δ7.86 sharpened the downfield doublet at

Table 2. ^{1}H NMR spectra of compounds **5–7** (89.55 MHz, TMS as int. standard)

Compound	1-H or OAc 1'-H or OAc	2-Me 2'-Me	3-H 3'-H	4-OAc 4'-OAc	5-OAc 5'-OAc	7-H 7'-H	8-H 8'-H
Ebenone leucopentaacetate ($CDCl_3$)	7.57 *s* (*br*)	2.47 *s*	7.01 *s* (*br*)	2.33 *s*	1.96 *s*	7.53 *d* (8.4)	7.71 *d* (8.4)
(**6**)	2.30 *s*	2.47 *s*	7.04 *s*	2.33 *s*	1.96 *s*	7.41 *d* (8.8)	7.73 *d* (8.8)
Ebenone leucopentaacetate [$(CD_3)_2CO$]	7.80 *s* (*br*)	2.87 *s*	7.12 *s* (*br*)	2.51 *s*	2.32 *s*	7.37 *d* (8.6)	7.86 *d* (8.6)
(**6**)	2.32 *s*	2.87 *s*	7.20 *s*	2.51 *s*	2.36 *s*	7.46 *d* (8.6)	7.96 *d* (8.6)
Diospyrol tetraacetate ($CDCl_3 + CF_3COOH$)	7.57 *d* (1.5)*	2.50 *s*	6.98 *d* (1.5)	2.34 *s*	1.96 *s*	7.33 *d* (8)	7.71 *d* (8)
(**5**) [2]							
Ellipticinone leucohexaacetate ($CDCl_3$)	2.38 *s*	2.52 *s*	7.16 *s*	2.38 *s*	1.99 *s*	7.29 *d* (9)	8.06 *d* (9)
(**7**)							

*Coupling constants (J, in Hz) are given in parentheses.

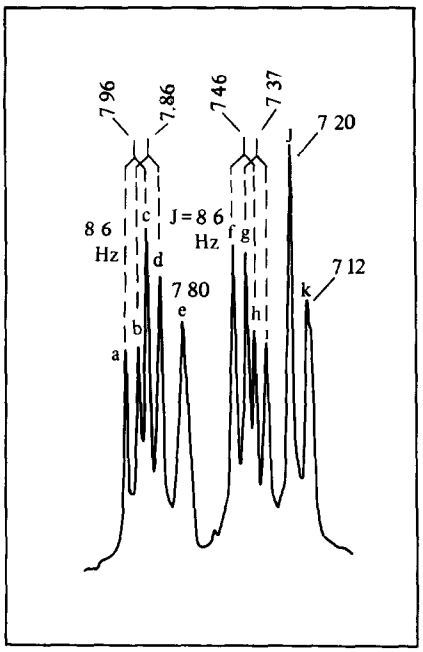


Fig. 1. ^{1}H NMR spectra (acetone-d_6) of ebenone leucopentaacetate.

δ7.46 indicating that *a*, *c*, *f*, *h* and *b*, *d*, *g*, *i* constituted the two AB quartets. These results conclusively established structure **6** for ebenone leucopentaacetate and structure **3** for ebenone.

The ^{13}C {^{1}H}NMR spectra ($CDCl_3$) of **6** showed the presence of five carbonyls at δ169.8, 169.6, 169.3, 169.0 and 168.6 and signals assignable to aromatic carbons carrying acetoxyl groups at δ145.3, 143.0 and 142.7 and two aromatic carbons carrying methyls at δ136.8 and 136.5. The region δ129.7 to 119.6 contained ten signals at δ129.7, 129.5, 129.0, 128.7, 127.9, 126.1, 123.6, 123.1, 119.7 and 119.6 accounting for the remaining 13 aromatic carbons. Both the Ar–$\underline{C}H_3$ signals were observed at δ16.6. The –OCO$\underline{C}H_3$ signals were not resolved and were seen at δ20.8, 21.2 and 21.5.

The presence of a plumbagin moiety and a 4,5-dihydroxy-2-methylnaphthalene moiety in the structure of ebenone (**3**) was indicated by the elemental composition ($C_{22}H_{16}O_5$) of its molecular ion, and its UV spectrum which resembled closely those of plumbagin and 4-hydroxy-5-methoxy-2-methylnaphthalene (Table 1). Its IR spectrum indicated the presence of hydroxyl, and chelated and unchelated carbonyl groups. In its ^{1}H NMR spectrum ($CDCl_3$), the presence of the plumbagin moiety was evident from the signals of a chelated hydroxyl (δ13.79, *s*, exchangeable with D_2O), an allylic methyl (δ2.25, *d*, J = 1.3 Hz) and a vinylic proton (δ6.88, partially resolved quartet), and the presence of the 4,5-dihydroxy-2-methylnaphthalene moiety was confirmed by the presence of the signals of an aromatic methyl (δ2.44), two phenolic hydroxyls (δ8.44, *s*, and 9.83, *s*, exchangeable with D_2O) and metacoupled aromatic protons (δ6.76, *br s* and δ7 10, *br s*). The 6,6′-linkage between these two moieties was obvious due to the presence of an *ortho* coupled AB quartet at δ7.26 and 7.41 (J = 8.9 Hz) which was assigned to H-7 and H-8 of the naphthalene moiety and a singlet at δ7.80 (2H) assigned to H-7′ and H-8′ of the plumbagin moiety (cf. elliptinone (Table 2) δ7.71, *s*, for H-7, 7′, 8.8′).

In the mass spectrum of ebenone, the $[M]^+$ ion is the base peak and an intense $[M-OH]^+$, *m/z* 343 and $[M-H_2O]^+$, *m/z* 342 ions were observed. The presence of hydroxyls at 5 and 5′ positions *ortho* to the linkage favour the elimination of a molecule of water from the $[M]^+$ ion leading to a stable furano structure.

Band 2 yielded an orange pigment which was identified as elliptinone (**4**).

EXPERIMENTAL

^{1}H and ^{13}C NMR. 89.55 MHz and 22.50 MHz respectively with TMS as internal standard; Mps: uncorr, Assigned MS fragmentations were confirmed by elemental compositions of the ions determined by high resolution mass analysis.

Plant material. The stem bark was collected at Agumbe (Karnataka), India.

Isolation of ebenone and elliptinone Shade dried finely powdered stem bark (250 g) was extracted with $CHCl_3$ (9 × 600 ml) at room temp. for 24 hr. The combined extracts were concd under red. pres. and separated by prep TLC (silica gel G (Acme) impregnated with 3% oxalic acid, $CHCl_3$) into four coloured bands: band 1 (pale yellow); band 2 (orange red); band 3 (violet); band 4 (pink); band 5 (brown) in the decreasing order of their R_f values. Compounds from bands 1, 4 and 5 have not been identified due to paucity of the material.

Ebenone (**3**). The pigment in band 3 was eluted with CH_2Cl_2 and purified by repeated recrystallization from CH_2Cl_2 and a final recrystallization from C_6H_6 to give a brown crystalline solid (50 mg), mp 231–232°. MS *m/z* (rel. int.): 362 (10), 361 (25), 360 $[M]^+$ (100), 343 (16), 342 $[M-H_2O]^+$ (34), 274 (11) High resolution MS *m/z*: 360.1000 (calc for $C_{22}H_{16}O_5$ 360.0098), 342.0900 (calc. for $C_{22}H_{14}O_4$ 342 0892), 274.1300 (calc for $C_{18}H_{10}O_3$ 274.1264).

Elliptinone (**4**). The pigment in band 2 was eluted and recrystallized from CH_2Cl_2 to give an orange crystalline solid (102 mg), mp above 300°. ^{1}H NMR (100 MHz, $CDCl_3$): δ2.22 (*d*, J = 1.5 Hz, CH_3-2, 2′), 6.83 (*q*, J = 1 5 Hz, H-3, 3′), 7.71 (H-7, 7′, 8, 8′), 12.48 (*s*, OH-5, 5′, exchangeable with D_2O). The identity was established by comparison of its TLC behaviour, mp, IR and ^{1}H NMR spectra with those of an authentic sample.

Ebenone leucopentaacetate (**6**). Ebenone (100 mg) was refluxed with Zn dust (800 mg), dry NaOAc (700 mg) and Ac_2O (10 ml) for about 2½ hr. The reaction mixture was poured onto crushed ice to give a white solid which was purified by prep. TLC (silica gel G, (Acme), $CHCl_3$–EtOAc, 4·1) and recrystallization from CH_2Cl_2–petrol (bp 40°) (1.3) to give white crystals (45 mg), mp 185° MS *m/z* (rel. int.): 572 $[M]^+$ (9), 530 $[M-CH_2CO]^+$ (16), 489 (30), 488 $[530-CH_2CO]^+$ (41), 470 $[488-H_2O]^+$ (20), 446 $[488-CH_2CO]^+$ (8), 429 (29), 428 $[446-H_2O]^+$ (100), 404 $[446-CH_2CO]^+$ (16), 388 (32), 363 (15), 362 $[404-CH_2CO]^+$ (59), 361 $[404-CH_3CO]^+$ (40), 360 $[361-H]^+$ (17), 345 (10), 344 $[362-H_2O]^+$ (33), 343 (18); High resolution MS *m/z*: 572 1665, (calc. for $C_{32}H_{28}O_{10}$ 572.1683), 530.1617 (calc. for $C_{30}H_{26}O_9$ 530.1577), 488.1458 (calc for $C_{28}H_{24}O_8$ 488 1471), 470.1372 (calc for $C_{28}H_{22}O_7$ 470.1366), 446.1314 (calc. for $C_{26}H_{22}O_7$ 446.1307), 428.1241 (calc. for $C_{26}H_{20}O_6$ 428.1260), 404.1272 (calc. for $C_{24}H_{20}O_6$ 404.1260), 362.1149 (calc. for $C_{22}H_{18}O_5$ 362.1154), 361.1066 (calc. for $C_{22}H_{17}O_5$ 361.1076), 360.0990 (calc. for $C_{22}H_{16}O_5$ 360.0998), 344.1040 (calc. for $C_{22}H_{16}O_4$ 344.1049); IR ν_{max}^{KBr} cm^{-1}: 2930, 2860, 1765 (>C=O), 1640, 1632, 1610, 1565, 1500, 1445, 1370, 1350, 1210, 1215, 1195, 1160, 1100, 1045, 1050, 935, 910, 888, 860, 820, 810, 780 and 738

Elliptinone leucohexaacetate (**7**). Elliptinone (100 mg) was

refluxed with Zn dust (800 mg), dry NaOAc (700 mg) and Ac_2O (15 ml) until the resulting soln was colourless. The reaction mixture was worked up as for **6** which was purified by CC (silica gel, Acme, less than 0.08 mm dia) and recrystallization from CH_2Cl_2–petrol (bp 40°) (1:3) to give pale yellow crystals (50 mg) mp 156–158°. MS (20 eV) *m/z* (rel. int.): 630 $[M]^+$ (3), 589 (7), 588 $[M-CH_2CO]^+$ (16), 546 $[588-CH_2CO]^+$ (43), 528 $[546-H_2O]^+$ (18), 504 $[546-CH_2CO]^+$ (11), 487 (31), 486 $[528-CH_2CO]^+$ (100), 462 $[504-CH_2CO]^+$ (12), 444 $[486-CH_2CO]^+$ (39), 443 (12), 420 $[462-CH_2CO]^+$ (7), 402 $[444-CH_2CO]^+$ (16), 378 $[420-CH_2CO]^+$ (5), 360 $[402-CH_2CO]^+$ (14); IR ν_{max}^{KBr} cm^{-1}: 1760, 1185, 1010 (aryl-acetate), ^1H NMR $[(CD_3)_2CO]$· δ2.32, 2.37, 2.57 (*s*, H-1,1′, 4, 4′, *s*, MeOCO-1, 1′, 4, 4′, 5, 5′), 2.87 (*s*, Me-2, 2′), 7.20 (*s*, H-3, 3′), 7.42 (*d*, $J = 8.8$ Hz, H-7, 7′), 7.92 (*d*, $J = 8.8$ Hz, H-8, 8′)

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