CHEMICAL INVESTIGATION OF *DIOSPYROS MOLLIS*, GRIFF [EBENACEAE]; CHEMICAL CONSTITUENTS OF THE BLACK HEARTWOOD. THE FINAL CHAPTER.

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ABSTRACT

Investigation of the black heartwood of Diospyros mollis, *Griff led to the isolation of naphthoquinone dimer* ***3****, naphthalene* ***8****, and naphthaldehydes* ***9*** *and* ***10****. While* ***10*** *has earlier been isolated from other* Diospyros *species compounds* ***3****,* ***8****, and* ***9*** *are novel.*

INTRODUCTION

Diospyros mollis (Ebenaceae) is a big tree distributed throughout Thailand. Its fresh berries have long been used very effectively against intestinal hook-worms,[1] and the active principle has been identified as **1**,[2] the diglucoside of diospyrol **2**.[3] While the results concerning the study of *Diospyros mollis* berries have been well documented,[4] the study of its heartwood has never been reported. A cross-section of the tree trunk shows it to compose of two layers, the outer greenish brown layer which is easily attacked by pests and the inner core which is composed of very heavy, extremely hard and durable, lustrous jet black wood (Fig. 1). It is not surprising, therefore, that Asian people value the *Diospyros mollis* wood for use in high quality furniture and as a consequence the tree has become scarce and risk extinction.[5] The remaining *Diospyros mollis* plants in Thailand have now been classified as a protected species and the Forestry Department has embarked on a plantation programme to introduce new young cultures to the wild. Chemical investigation of the black heartwood of this plant has led to isolation of the naphthoquinone dimer **3**, naphthalene derivative **8**, and an abundance of fluorescent naphthaldehydes **9** and **10**.

Fig. 1 A cross-section of the *Diospyros mollis* tree trunk;
A – Blackcore, B – Part missing, destroyed by pest

RESULTS AND DISCUSSION

The grounded black core of *Diospyros mollis* tree trunk (1 Kg) was soaked in methylene chloride (5 L) at room temperature for seven days after which it was filtered off and the soaking process repeated twice. The combined fluorescent dark filtrate was evaporated to dryness to give a black gum (67.8 g). Silica gel column chromatography of this material using gradient elution, starting with 50% methylene chloride in hexane and finishing with pure methylene chloride, effected separation into four fractions. Further purification of these fractions by silica gel quick columns using 40% methylene chloride in hexane as eluent followed by crystallization finally yielded respectively compounds **8** (0.32 g, 0.032%), **3** (0.075 g, 0.0075%), **9** (0.43 g, 0.043%) and **10** (8.05 g, 0.805%).

The molecular formula of naphthoquinone dimer **3**, $C_{23}H_{16}O_6$, was deduced from its mass spectral (*m/e* 388, M^+; HRMS found: 388.0927) and elemental (found: C, 70.79; H, 3.82%) analyses. NMR (in $CDCl_3$-CF_3COOH) absorptions of **3** revealed much of its structural details in which two methyl groups respectively attached to the *p*-quinone and aromatic moieties appeared at δ 1.96 and 2.51 as two singlets while that of the methoxy group resonated at δ 4.03. The presence of a 1,2,3-trisubstituted aromatic nucleus (ring D) was clearly evident from the absorptions of three adjacent aromatic protons at δ 6.76 (d, J=7.6 Hz), 7.16 (d, J=8.8 Hz) and 7.62 (dd, J=7.6 and 8.8 Hz). Two *meta* protons on ring A appeared as two doublets at δ 7.27 and 7.61 (J=1.4 Hz) while the singlet at δ 7.04 could be assigned as the lone *p*-quinone proton on ring B.

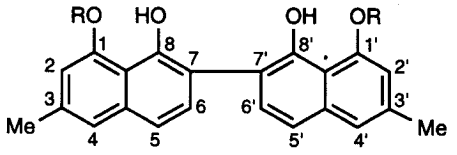
The presence of one phenolic group in the dimer **3** was confirmed by chemical reactions. Methylation of the dimer **3** using silver oxide-methyl iodide in chloroform provided the corresponding methyl ether **4** as orange prisms (mp. 249-250°C, decomposed, from chloroform-hexane) and the acetate **5** (orange prisms, mp. 280-281°C, decomposed, from chloroform-hexane) was obtained by standard treatment of **3** with acetic anhydride in pyridine.

The NMR spectrum of the methyl ether **4** displayed two methyl singlets at δ 4.01 and 4.03 which accounted for the original and newly introduced methoxy groups, while that of the acetate group in **5** resonated at δ 2.48. The low field shift (0.3 ppm) of the (*peri*-position) proton originally resonating at δ 7.61 (d, J=1.4 Hz) in the spectrum of starting material **3** to δ 7.91 in that of its acetate **5** strongly suggested that the free hydroxy group in **3** was located on ring A and therefore the methoxy group would have to be accommodated by ring D.

The above evidence revealed the unsymmetrical structural feature of the quinone dimer **3**. Although many possible structures were considered only **3** (7-2' linkage) and **6** (6-2' linkage) were consistent with the observed NMR data. Despite the fact that it was not a straightforward process to distinguish between structures **3** and **6** by spectroscopic means, structure **6** was rejected on biosynthetic ground. Both diospyrol and its diglucoside, respectively **2** and **1**, were apparently biosynthesized from an oxidative coupling of two naphthol monomers **7** and, indeed, compound **7** was later isolated from the young berries of *Diospyros mollis*.[6] While couplings of two monomers **7** between 7-7' and 7-2' positions, giving skeletons **2** and **3** respectively, are considered as favourable processes the combination of two monomers at positions 6 and 2' to finally give compound **6** was believed to be very unlikely.[7] Structure **3** was therefore proposed to be the quinone dimer isolated from the heartwood of *Diospyros mollis*.

Compound **8** was isolated as colorless prisms (mp. 106-107°C from methylene chloride-hexane) whose mass spectral (*m/e* 216, M^+) and elemental (found C, 72.33; H, 5.60%) analyses indicated the molecular formula, $C_{13}H_{12}O_3$. NMR spectrum of **8** exhibited three singlets at δ 2.40 (3H), 3.97 (3H) and 6.12 (2H) respectively of the aromatic methyl, methoxy and methylene dioxy groups. Four aromatic protons of the naphthalene nucleus in **8** appeared as two sets of protons having the *meta*- (δ 6.55 and 7.10, J=1.5 Hz, H-2 and H-4) and *ortho*- (δ 7.06 and 7.24, J=9 Hz, H-6 and H-5) relationships. The described data confirmed the structural integrity of the naphthalene derivative **8**. In fact, another structural possibility in which the methylene dioxy moiety is situated at positions 5, 6 on the naphthalene nucleus (see structure **8**) was also considered but later rejected on the basis of data from the nmr NOE experiments of compound **8**.

Structure elucidation of the highly fluorescent naphthaldehydes **9** (pale yellow needles, mp. 185-186°C from methylene chloride-hexane) and **10** (yellow needles, mp.110-111°C from ethanol) presented



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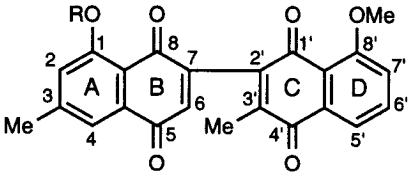
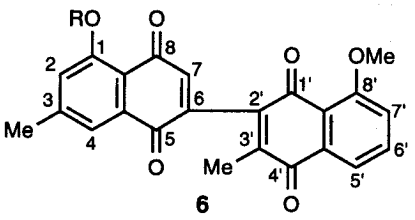
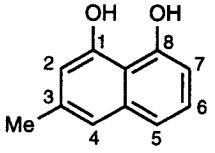
2, R = H



3, R = H
4, R = Me
5, R = COMe



6



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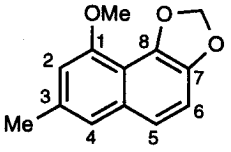

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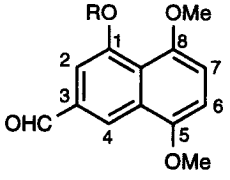
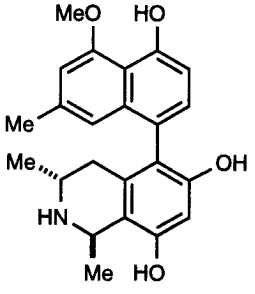


9, R = H
10, R = Me
11, R = COMe



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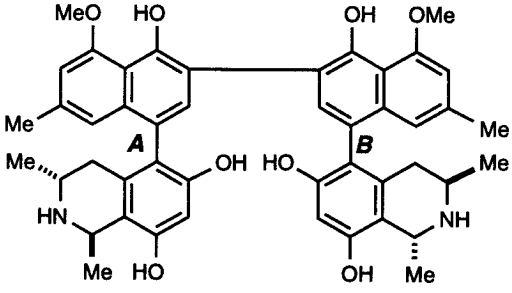
	Stereogenic Axis Configuration	
	A	***B***
12a, Michellamine A	*S*	*S*
12b, Michellamine B	*R*	*S*
12c, Michellamine C	*R*	*R*



no problem. The NMR spectrum of **9** ($C_{13}H_{12}O_4$; *m/e* 232, M^+; found: C, 67.56; H, 5.20%; λ_{max} in EtOH, 222 (log ε 4.42), 257 (log ε 4.47), 320 (log ε 3.67) and 396 (log ε 3.92) nm; ν_{max} in $CHCl_3$; 3400, 1690 cm^{-1}) showed two low field single proton singlets at δ 9.57 (disappeared with D_2O) and 10.10 assignable to the phenolic and aldehyde moieties respectively. The two methoxy groups in **9** resonated as two singlets at δ 4.00 and 4.07 while the remaining four hydrogens in the molecule were divided into two sets of *meta*- (δ 7.33 and 8.25, J=2 Hz) and *ortho*- (δ 6.73 and 6.91, J=9 Hz) aromatic protons at positions 2, 4 and 6, 7 of the naphthol nucleus.

Methylation of **9** (Me_2SO_4/K_2CO_3/acetone/reflux) provided the corresponding methyl ether whose physical and spectroscopic properties were found to be identical in all respects to the trimethoxynaphthaldehyde **10** which was isolated in large amount from the same plant specimen. Compound **10** was previously reported to be a constituent of the heartwood of *Diospyros quiloensis*,[8] and the fresh roots of *Diospyros mollis*.[9] The acetate **11** (yellow prisms, mp. 117-118°C from methylene chloride-hexane) was prepared in a straightforward manner from **9** and its spectroscopic data were also in agreement with the proposed structure.

It is highly interesting to note that michellamine B **12b**, a highly promising naturally occurring anti-HIV agent from *Ancistrocladus korupensis*,[10-12] is a naphthylisoquinoline alkaloid dimer whose core structure resembles that of diospyrol **2**. Undoubtedly, michellamines **12** are biosynthetically formed from oxidative coupling of the corresponding monomer, korupensamine A **13**. Compound **13**, an antimalarial displaying reasonably potent *in vitro* activity against *P. faciparum* and *P. berghei*, was found to co-exist with michellamines in plant,[13] and it has been synthesized[14-16] and oxidatively coupled to yield the dimer **12**.[17]

EXPERIMENTAL

Column chromatography was carried out on silica gel 60, 63-200 μm(Merck). ^1H NMR spectra were recorded on Varian EM-360, Bruker AM 400 or Bruker 300-DPX instrument in $CDCl_3$ or $CDCl_3$+CF_3COOH with TMS as internal standard. IR spectra were taken on a Jasco model A-302 spectrometer or Perkin Elmer 2000N FT Raman. Low-resolution mass spectra (70 eV) were run on Finnigan MAT INCOS 50 or JMS-DX 300 JEOL mass spectrometer. Elemental analyses were carried out on a Perkin Elmer Elemental Analyser 2400 CHN. UV spectra were measured with a Milton Roy Spectronic 3000 Array Spectrophotometer using EtOH as the solvent. Melting points were determined on Electrothermal melting point apparatus and were uncorrected.

COMPOUND 3

Orange needles, mp. 292-293°C (decomposed) (from chloroform-carbon tetrachloride); λ_{max}(EtOH), 246 (log ε 4.50), 430 (log ε 4.13) nm; ν_{max}(Nujol), 3560, 1665, 1660, 1645, 1270, 1125 cm^{-1}; *m/e* (relative intensity), 388 (M^+, 32), 375 (33), 360 (83), 343 (34), 215 (41), 202 (66), 189 (44), 115 (36), 106 (100); HRMS; Calcd. for $C_{23}H_{16}O_6$ [M^+]: 388.3820. Found: 388.0927; ^1H NMR (δ in $CDCl_3$-CF_3COOH), 1.96 (s, 3H, -Me), 2.51 (s, 3H, -Me), 4.03 (s, 3H, -OMe), 6.76 (d, J=7.6 Hz, 1H, H-7'), 7.04 (s, 1H, H-6), 7.16 (d, J=8.8 Hz, 1H, H-5'), 7.27 (d, J=1.4 Hz, 1H, H-2), 7.61 (d, J=1.4 Hz, 1H, H-4), 7.62 (dd, J=7.6, 8.8 Hz, 1H, H-6'). Analysis; Calcd. for $C_{23}H_{16}O_6$: C, 71.13; H, 4.15. Found: C, 70.79; H, 3.82%.

Methylation (MeI/Ag_2O/chloroform) of **3** provided **4** as orange prisms, mp. 249-250°C (decomposed) (from chloroform-hexane); ν_{max}(Nujol), 1653, 1598, 1575, 1262, 1069 cm^{-1}; *m/e* (relative intensity), 402 (M^+, 83), 389 (44), 374 (70), 357 (100), 345 (51), 215 (24), 202 (28); HRMS; Calcd. for $C_{24}H_{18}O_6$ [M^+]: 402.4092. Found: 402.1114; ^1H NMR (δ in $CDCl_3$-CF_3COOH), 1.92 (s, 3H, -Me), 2.54 (s, 3H, -Me), 4.01 (s, 3H, -OMe), 4.03 (s, 3H, -OMe), 6.72 (d, J=7.6 Hz, 1H, H-7'), 6.89 (s, 1H, H-6), 7.09 (d, J=8.7 Hz, 1H, H-5'), 7.23 (broad s, 1H, H-2), 7.53 (dd, J=7.6, 8.7 Hz, 1H, H-6'), 7.63 (broad s, 1H, H-4).

Acetylation (Ac_2O/pyridine) of **3** provided **5** as orange prisms, mp. 280-281°C (decomposed) (from chloroform-hexane); ν_{max}(Nujol), 1767, 1656, 1605, 1581, 1269, 1123 cm^{-1}; *m/e* (relative intensity), 388 (M^+-42, 72), 360 (100), 343 (17), 215 (11), 202 (14), 189 (10), 106 (10); HRMS; Calcd. for $C_{23}H_{16}O_6$ [M^+-42]: 388.3820. Found: 388.0995; ^1H NMR (δ in $CDCl_3$-CF_3COOH), 1.90 (s, 3H, -Me), 2.48 (s, 3H, -Me),

2.53 (s, 3H, -Me), 4.00 (s, 3H, -OMe), 6.69 (d, J=7.6 Hz, 1H, H-7'), 6.81 (s, 1H, H-6), 7.07 (d, J=8.7 Hz, 1H, H-5'), 7.30 (d, J=0.7 Hz, 1H, H-2), 7.49 (dd, J=7.6, 8.7 Hz, 1H, H-6'), 7.91 (d, J=0.7 Hz, 1H, H-4).

COMPOUND 8

Colorless prisms, mp. 106-107°C (from methylene chloride-hexane); λ_{max}(EtOH), 234 (log ε 4.14), 309 (log ε 3.94), 356 (log ε 3.75); ν_{max}($CHCl_3$), 1615, 1530, 1270, 1060 cm^{-1}; *m/e* (relative intensity), 216 (M^+, 100), 201 (89), 173 (12), 143 (8), 128 (9), 115 (32), 108 (11); ^1H NMR (δ in $CDCl_3$), 2.40 (s, 3H, -Me), 3.97 (s, 3H, -OMe), 6.12 (s, 2H, $-OCH_2O-$), 6.55 (d, J=1.5 Hz, 1H, H-2), 7.06 and 7.24 (AB system, J=9 Hz, 2H, H-5 and H-6), 7.10 (d, J=1.5 Hz, 1H, H-4). Analysis; Calcd. for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.33; H, 5.60%.

COMPOUND 9

Pale yellow needles, mp. 185-186°C (from methylene chloride-hexane); λ_{max}(EtOH), 222 (log ε 4.42), 257 (log ε 4.47), 320 (log ε 3.67), 396 (log ε 3.92) nm; ν_{max}($CHCl_3$), 3400, 1690, 1615, 1520, 1260, 1060 cm^{-1}; *m/e* (relative intensity), 232 (M^+, 71), 217 (100), 202 (10), 201 (15), 189 (13), 173 (12), 116 (8), 115 (12); ^1H NMR (δ in $CDCl_3$), 4.00 (s, 3H, -OMe), 4.07 (s, 3H, -OMe), 6.73 and 6.91 (AB system, J=9 Hz, 2H, H-6 and H-7), 7.33 (d, J=2 Hz, 1H, H-2), 8.25 (d, J=2 Hz, 1H, H-4), 9.57 (s, 1H, disappeared with D_2O, -OH), 10.10 (s, 1H, -CHO). Analysis; Calcd. for $C_{13}H_{12}O_4$: C, 67.24; H, 5.21. Found: C, 67.56; H, 5.20%.

Acetylation (Ac_2O/pyridine) of **9** provided the corresponding acetate **11** as yellow prisms, mp. 117-118°C (from methylene chloride-hexane); ν_{max}($CHCl_3$), 1765, 1690, 1610, 1515, 1270, 1050 cm^{-1}; *m/e* (relative intensity), 274 (M^+, 33), 232 (88), 217 (100), 115 (11); ^1H NMR (δ in $CDCl_3$), 2.35 (s, 3H, -CO-Me), 3.87 (s, 3H, -OMe), 3.97 (s, 3H, -OMe), 6.78 and 6.96 (AB system, J=9 Hz, 2H, H-6 and H-7), 7.55 (d, J=2 Hz, 1H, H-2), 8.67 (d, J=2 Hz, 1H, H-4), 10.15 (s, 1H, -CHO). Analysis; Calcd. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.14. Found: C, 65.57; H, 4.84%.

COMPOUND 10

Yellow needles, mp. 110-111°C (from ethanol) (lit.,[8] mp. 111-112.5°C from methanol, lit.,[9] mp. 110-111°C from ethanol); λ_{max}(EtOH), 220 (log ε 4.47), 259 (log ε 4.50), 315 (log ε 3.67), 393 (log ε 3.95) nm; ν_{max}($CHCl_3$), 1690, 1600, 1520, 1270, 1087, 1080 cm^{-1}; *m/e* (relative intensity), 246 (M^+, 100), 231 (88), 203 (18), 160 (12), 123 (10), 115 (20); ^1H NMR (δ in $CDCl_3$), 3.93 (s, 3H, -OMe), 4.00 (s, 3H, -OMe), 4.05 (s, 3H, -OMe), 6.83 and 7.02 (AB system, J=9 Hz, 2H, H-6 and H-7), 7.32 (d, J=2 Hz, 1H, H-2), 8.38 (d, J=2 Hz, 1H, H-4), 10.13 (s, 1H, -CHO). Analysis; Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.61; H, 5.81%.

Compound **10** was also obtained from the methylation of **9** under the standard conditions (Me_2SO_4/K_2CO_3/acetone/reflux).

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