CHAPTER IV
RESULTS AND DISCUSSION

4.1 Isolated compounds from *Diospyros* spp.

A crude CH_2Cl_2 extract of dried fruits of *Diospyros ehretioides* (15.12 g) was purified by Sephadex LH-20 and HPLC, yielding four different kinds of solids: white crystals **[CPD1, CPD1-I]** (37.3 mg), light-brown solids **[CPD2, CPD2-I to CPD2-V]** (102.8 mg), orange-brown solids **[CPD3, CPD3-I]** (47.3 mg) and orange-red solids **[CPD4, CPD4-I to CPD4-III]** (79.5 mg). Due to the fact that **CPD1** and **CPD1-I** were identified by ^{1}H-NMR analyses in the same manner as other **CPDs**, therefore, only **CPD1**, **CPD2**, **CPD3** and **CPD4** could be presented. They are shown in the latter.

4.1.1 Structure elucidation of the isolated compounds from *Diospyros ehretioides* fruits

White crystals [CPD1]

A crude CH_2Cl_2 extract of dried fruits of *Diospyros ehretioides* was chromatographed on Sephadex LH-20 and re-crystallized from acetone/CH_2Cl_2 to afford white crystals **[CPD1]**. It was identified by spectroscopic techniques.

White crystals **[CPD1]** were analysed by HR-ESI-TOF mass spectrum which indicated a molecular formula as $C_{20}H_{14}0_5$ (observed *m/z* 357.0739 $(M+Na)^+$). The IR spectrum (Fig.24) showed absorption bands at 3506 and 3271 cm^{-1} (OH stretching of two hydroxyl groups), 2860-3030 cm^{-1} (CH stretching of aromatic), 1580 (C=C stretching of aromatic) and 1268-957 (C-O stretching and aromatic C-H stretching).

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The ^{1}H NMR spectrum (DMSO-d_6, Fig.18) of white crystals **[CPD1]** showed signals corresponding to three oxymethine protons at δ_H 3.51 (1H, dd, J= 4.1, 4.1 Hz), 3.61 (1H, d, J= 4.1 Hz) and 5.34 (1H, dd, J= 2.1, 5.4 Hz); nine methine protons at δ_H 6.97 (H-2), 6.97 (H-7′), 7.12 (H-5′), 7.15 (H-7), 7.25 (H-6′), 7.48 (H-5), 7.53 (H-3), 7.61 (H-4) and 7.61 (H-6); singlet signal of a hydroxyl proton at δ_H 9.91 (8′-OH); and doublet signal of a hydroxyl proton at δ_H 5.56 (1′-OH).

The ^{13}C NMR spectrum (DMSO- d_6, Fig. 19) of white crystals **[CPD1]** showed the signals for twenty carbons, including three oxymethine carbons, sixteen sp^2-hybridized carbons and a spiroketal carbon. Analyses of DEPT 135 (Fig. 19) and HMQC (Fig. 20) spectral data revealed that white crystals **[CPD1]** possessed twelve methine and eight quaternary carbons.

The ^{1}H- ^{1}H COSY spectral data (Fig. 21) demonstrated the correlations from H-3 (at δ_H 7.53) to H-2; H-4 (at δ_H 7.61) to H-3 and H-5; H-5 (at δ_H 7.48) to H-4 and H-6; H-6 (at δ_H 7.61) to H-7; H-6′ (at δ_H 7.25) to H-5′ and H-7′; H-1′ (at δ_H 5.34) to H-2′. Assignments of white crystals **[CPD1]** by analysis of ^{1}H-^{1}H COSY spectral data was difficult due to signal overlapping. Therefore, the assignment of this compound was established by HMBC correlations. The HMBC spectral data (Fig.22) showed the correlations from H-6′ (at δ_H 7.25) to C-4′a; H-5′ (at δ_H 7.12) to C-7′ and C-5′; H-2 (at δ_H 6.97) to C-1; H-6 (at δ_H 7.61) to C-5 and C-7; H-5 (at δ_H 7.48) to C-4a; H-3 (at δ_H 7.53) to C-1 and C-4.

The NOESY spectrum (Fig. 23) showed cross peaks from H-3′ to H-1′, and 1′-OH to H-7′.

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Moreover, its structure and relative configuration of white crystal **[CPD1]** was also confirmed by x-ray crystallography (Figure 3).

X-Ray crystal structure analysis of white crystal **[CPD1].** Crystal data of white crystal **[CPD1]**: monoclinic, PZ_1 , a = 9.0276(2) °A, b =8.2443(1) °A, c = 10.0741(2) °A, β = 92.92(1) °, ν = 748.80(2) °A^3, Dx = 1.483 g/cm^3, Z =2. A total of 5.320 reflections were obtained by recrystallization from acetone/CH_2Cl_2. Diffraction data were acquired on a *Bruker-Nonius kappa* CCD diffractometer with graphite-monochromated MoK_a radiation (λ= 0.71073 °A), over the θ range of 0.998-22.465°. The crystal structure was solved by direct methods using Semi-Invariants representation (SIR-97), and then all atoms except H-atoms were refined anisotropically by a full-matrix least-squares methods on F^2 using SHELXL-97 to give a final R factor of 0.0364 (R_w = 0.0982 for all data) with a data to parameter ratio of 10.81:1. Crystallographic data of white crystal **[CPD1]** have been deposited at the *Cambridge Crystallographic Data Centre* under the reference No. CCDC-261640. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

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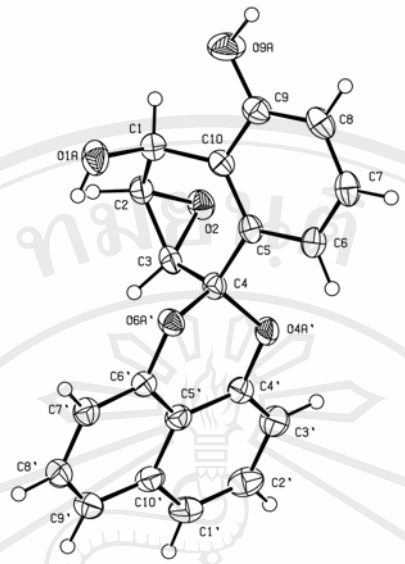
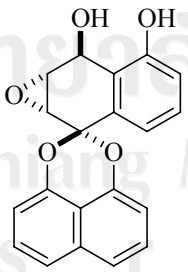


Figure 3. X-ray crystal structure of **[CPD1]** isolated from fraction B6

The ^{1}H, ^{13}C NMR data and the ^{1}H-^{1}H COSY, HMQC and HMBC spectral data (allowed the assignments of protons attached to an individual carbon) indicated that white crystals **[CPD1]** might be deoxypreussomerin JC1 **[70]**. Moreover, the ^{1}H, ^{13}C NMR together with x-ray crystallography of white crystals **[CPD1]** were compared with those reported in the literature[25], it could assure that the white crystals **[CPD1]** is palmarumycin JC1 **[70]**.



Palmarumycin JC1 **[70]**

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Orange-brown solid [CPD3]

A crude CH_2Cl_2 extract of dried fruits of *Diospyros ehretioides* was chromatographed on Sephadex LH-20 and further purified by semi-preparative HPLC, eluted with MeCN/H_2O, 65:35 to provide orange-brown solid **[CPD3]**. It was identified by spectroscopic techniques.

Orange-brown solid **[CPD3]** has been identified by direct comparison of its spectra (IR, ^1H and ^{13}C NMR and MS). It was analyzed to be $C_{20}H_{14}O_5$ from mass spectrum ($M^{+\cdot}$; *m/z* 334). The molecular formula of the compound was similar to that of palmarumycin JC1 **[70].** The IR spectrum of orange-brown solid **[CPD3]** showed characteristics of a natural keto-hydroxy deoxypreussomerin[25]. The IR spectrum (Fig. 30) showed absorption bands at 3277 cm^{-1} (OH stretching of hydroxyl group), 2922 cm^{-1} (C-H stretching of aromatic), 1644-1607 cm^{-1} (C=C stretching of aromatic), and 1269-975 (C-O stretching and aromatic C-H stretching).

The ^1H and ^{13}C NMR spectra (Table 1) indicated that three aromatic rings were present in compounds **70** and orange-brown solid **[CPD3]** were similar but the substitution pattern at C-1, C-2 and C-3 was only different. The hydroxyl group at C-1 in **70** has been oxidized to a keto group in orange-brown solid **[CPD3]**. This has also been supported by the presence of a chelated hydroxyl group (δ 12.37, 1H, brs) in the latter.

The ^1H NMR spectrum ($CDCl_3$, Fig. 25) of orange-brown solid **[CPD3]** also revealed that C-2 was not oxygenated in orange-brown solid **[CPD3]** but C-3 was oxygenated, suggesting the placement of a hydroxyl group at C-3. The ^1H NMR spectrum of orange-brown solid **[CPD3]** showed signals corresponding to methylene proton at δ_H 2.97 (1H, dd, J= 17.7, 3.9 Hz) and 3.26 (1H, dd, J= 17.8, 3.4 Hz), ten

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methine protons at δ_H 4.62 (H-3′), 6.93 (H-2), 7.11 (H-7), 7.11 (H-5′), 7.36 (H-7′), 7.46 (H-3), 7.51 (H-6), 7.57 (H-4), 7.57 (H-5), and 7.58 (H-6′). Singlet signal of a hydroxyl proton was observed at δ_H 12.37 (8′-OH).

The ^{13}C NMR spectrum ($CDCl_3$, Fig. 26) of orange-brown solid **[CPD3]** showed the signals of 20 lines. Analysis of DEPT 135 (Fig. 26) and HMQC (Fig. 27) spectral data revealed that orange-brown solid **[CPD3]** possessed ten methine, one methylene and nine quaternary carbons. The ^1H and ^{13}C NMR data readily indicated that orange-brown solid **[CPD3]** is a deoxypreussomerin derivative. The ^1H-^1H COSY (Fig. 28), HMQC (Fig. 27) and HMBC (Fig.29) spectral data allowed the assignments of protons attached to an individual carbon (Table 1).

The ^1H-^1H COSY spectral data (Fig. 28) clearly showed the correlations from H-2′ (at δ_H 2.97, 3.26) to H-3′; H-2 (at δ_H 6.93) to H-3; H-4 (at δ_H 7.57) to H-3; H-6 (at δ_H 7.51) to H-7; H-5′ (at δ_H 7.11) to H-6′ and H-6′ (at δ_H 7.58) to H-7′. The structure of orange-brown solid **[CPD3]** was also supported from the HMBC experiment. Assignment of orange-brown solid **[CPD3]** by analysis of ^1H-^1H COSY spectral data was difficult due to signal overlapping. Therefore, the assignment of this compound was established by HMBC correlations. The HMBC spectral data (Fig. 29) showed the correlations from H-2′ (at δ_H 2.97) to C-1′, C-8′a; H-2′ (at δ_H 3.26) to C-3′ and C-4′; H-3′ (at δ_H 4.62) to C-4′; H-7′ (at δ_H 7.36) to C-5′; H-6′ (at δ_H 7.58) to C-4′a and C-5′; H-5′ (at δ_H 7.11) to C-5′; H-4 (at δ_H 7.57) to C-4 and C-8a; H-2 (at δ_H 6.93) to C-2; H-5 (at δ_H 7.57) to C-5 and C-4a; H-7 (at δ_H 7.11) to C-7 and C-5.

The ^1H, ^{13}C NMR data and the ^1H-^1H COSY, HMQC and HMBC spectral data (allowed the assignments of protons attached to an individual carbon) indicated that

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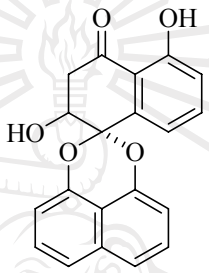
orange-brown solid **[CPD3]** might be palmarumycin JC2 **[71]**. In addition, the ^{1}H and ^{13}C NMR data of orange-brown solid **[CPD3]** were compared with those reported in the literature[25], it could assure that the orange-brown solid **[CPD3]** is palmarumycin JC2 **[71]**.



Palmarumycin JC2 **[71]**

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Table1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data (DMSO-d_6) of **Palmarumycin JC1**, and ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data ($CDCl_3$) of **Palmarumycin JC2**

	Palmarumycin JC1		**Palmarumycin JC2**	
C	δ_C, mult.[a]	δ_H, mult., *J* in Hz	δ_C, mult.[a]	δ_H, mult., *J* in Hz
1	147.2, s	-	146.3, s	-
2	108.9, d	6.97, t, 7.9	108.9, d	6.93, d, 7.6
3	127.8, d	7.53, t, 7.9	127.7, d	7.46, t, 7.7
4	120.6, d	7.61, t, 8.7	121.1, d	7.57, d, 7.6
4a	133.8, s	-	134.2, s	-
5	120.7, d	7.48, t, 7.9	121.5, d	7.57, d, 7.6
6	127.9, d	7.61, t, 8.7	127.7, d	7.51, t, 7.6
7	109.3, d	7.15, d,7.8	109.6, d	7.11, d, 7.5
8	147.3, s	-	147.1, s	-
8a	112.4, s	-	113.2, s	-
1′	59.1, d	5.34, dd, 2.1,5.4	201.0, s	-
2′	53.3, d	3.51, dd, 4.1,4.1	41.3, t	2.97, dd,17.7,3.9
				3.26, dd,17.8, 3.4
3′	49.8, d	3.61, d, 4.1	67.3, d	4.62, t, 3.6
4′	97.6, s	-	98.7, s	-
4′a	132.1, s	-	137.9, s	-
5′	117.2 , d	7.12, d, 7.4	119.9, d	7.11, d, 7.5
6′	129.1, d	7.25, t, 7.9	137.1, d	7.58, t, 7.6
7′	116.4, d	6.97, t, 7.9	118.0, d	7.36, dd, 7.6, 7.6
8′	155.7, s	-	162.2, s	-
8′a	122.6 , s	-	115.3, s	-
1′-OH	-	5.56, d, 5.6	-	-
8′-OH	-	9.91, s	-	12.37, br s

[a] Multiplicity was determined by analyses of DEPT 135 spectra

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Light-brown solid [CPD2]

A crude CH_2Cl_2 extract of dried fruits of *Diospyros ehretioides* was chromatographed on Sephadex LH-20 and further purified by semi-preparative HPLC, eluted with MeCN/H_2O, 35:65 to provide light-brown solid **[CPD2]**. It was also identified by spectroscopic techniques.

The molecular formula of light-brown solid **[CPD2],** $C_{22}H_{18}O_6$, was established by the HR-ESI-TOF-MS. The IR spectrum (Fig. 36) of light-brown solid **[CPD2]** showed conjugated C=O absorptions at 1667 and 1644 cm^{-1}. The ^1H NMR spectral data ($CDCl_3$, Fig. 31) of light-brown solid **[CPD2]** demonstrated signals of two downfield chelated OH groups at δ_H 12.52 and 12.63, four sp^2-CH groups at δ_H 6.72, 6.91, 7.04 and 7.29, an oxygenated sp^3 –CH group at δ_H 5.02, two non-equivalent CH_2, and two CH_3 groups at δ_H 1.96 and 2.05. The ^{13}C NMR spectrum (Fig. 32) of light-brown solid **[CPD2]** displayed 22 lines, while DEPT 135 and HMQC spectral data (Fig.33) revealed the presence of five CH, two CH_2, two CH_3, and 13 quaternary C-atoms. Resonances in the ^{13}C NMR spectrum at δ_C 203.8, 190.5 and 185.1, together with the IR absorptions at 1667 and 1644 cm^{-1}, revealed the presence of the C=O functionality in light-brown solid **[CPD2]**. The ^1H- ^1H COSY spectrum (Fig. 34) of light-brown solid **[CPD2]** revealed the connectivity from H-5′ to H-7′, and also showed couplings between 2-CH_3 and H-3; and H-6 and H-7. The HMBC spectrum (Fig. 35) of light-brown solid **[CPD2]** established the partial structure from C-1 to C-8: demonstrating correlations from 2-CH_3 to C-1, C-2, and C-3; H-3 to C-4; 4-OH to C-4a and C-3; and H-7 to C-5 and C-8a. The HMBC spectral data also assisted in partial structure assembling from C-1′ to C-8′ from which the following correlations were seen; 1′-OH to C-1′, C-2′ and C-8′a; 3′-CH_3 to C-3′ and C-4′; H-4′ to C-5′ and 3′-CH_3 ;

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H-5′ to C-4′a and C-8′a; H-7′ to C-8′. ^{13}C chemical shifts of quaternary C-1 at δ_C 129.8 and C-2′ at δ_C 127.4, together with the ESI-TOF-MS data, suggested the linkage between sp^2 quaternary carbons, C-1 and C-2′, leading to a structure of dimeric naphthoquinone derivative. Based on these spectral data, the structure of light-brown solid **[CPD2]** was assigned as 6′, 7′-dihydro-1′, 4, 5-trihydroxy-2,3′-dimethyl-[1,2′-binaphthalene]-5,8,8′(5H)-trione, to which the trivial name isodiospy -rol A **[72]** has been assigned. The ^1H-and ^{13}C-atoms of light-brown solid **[CPD2]** were completely assigned by analyses of 2D NMR data (Table 2). Moreover, light-brown solid **[CPD2]** possess the same basic skeleton and a reduced form of a known dimeric naphthoquinone, isodiospyrin **[23]** which was reported in the literature[10, 27]. The ^1H- and ^{13}C-NMR spectrum closely resemble that of isodiospyrin **[23]**. Compound **23** showed the 2-CH_3 and 3′-CH_3 proton at δ_H 2.03 and 2.05, respectively, the 1′-OH and 4-OH protons at δ_H 12.46 and 12.07, respectively. But the reduced form proton of light-brown solid **[CPD2]**, at H-5′ and the methylene protons, at H-6′ and H-7′ were different. Efforts to determine the absolute configuration at C-5′ of light-brown solid **[CPD2]** by the use of *Mosher ester* have met with failure, since light-brown solid **[CPD2]** was degraded to many unidentified products when treated with α-methoxy-α-[(trifluoromethyl) phenyl]acetyl chloride. Half of the light-brown solid **[CPD2]** structure is 4,8-dihydroxy-6-methyl-1-tetralone unit, known as shinanolone[26]. *(R)*-shinanolone exhibited negative optical rotation[26], while *(S)*-shinanolone showed positive optical rotation[4]. Light-brown solid **[CPD2]** exhibited negative optical rotation, $[\alpha]^{24}_D = -34.5$, therefore, the configuration of C-5′ in light-brown solid **[CPD2]** might be *(R)*, it could assure that the light-brown solid **[CPD2]** is isodiospyrol A **[72]**.

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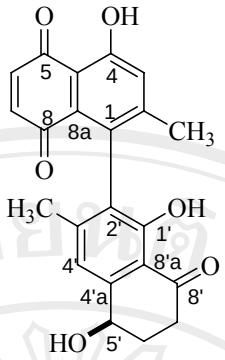

Isodiospyrol A **[72]**

Orange-red solid [CPD4]

A crude CH_2Cl_2 extract of dried fruits of *Diospyros ehretioides* was chromatographed on Sephadex LH-20 and further purified by semi-preparative HPLC, eluted with MeCN/H_2O, 45:55 to provide orange-red solid **[CPD4]**. It was identified by spectroscopic techniques.

The molecular formula of orange-red solid **[CPD4]**, $C_{22}H_{14}O_6$, was established by the HR-ESI-TOF-MS. The IR spectrum (Fig. 15) of orange-red solid **[CPD4]** showed conjugated C=O absorption at 1666 and 1641 cm^{-1}. The ^{1}H-NMR spectral data ($CDCl_3$, Fig. 10) of orange-red solid **[CPD4]** demonstrated signals of two downfield chelated OH groups at δ_H 12.07 and 12.46; six sp^2-CH groups at δ_H 6.75, 6.97, 6.98, 6.98, 7.32, and 7.63; two CH_3 groups at δ_H 2.03 and 2.05. The ^{13}C-NMR spectrum (Fig. 11) of orange-red solid **[CPD4]** displayed 22 lines, while DEPT 135 and HMQC spectral data (Fig. 12) revealed the presence of six CH, two CH_3, and 14 quaternary C-atoms. Resonances in the ^{13}C-NMR spectrum at δ_C 184.5, 185.0, 190.1, and 190.4 together with the IR absorptions at 1644 and 1666 cm^{-1} revealed the presence of the C=O functionality in orange-red solid **[CPD4]**. The ^{1}H-^{1}H COSY spectrum (Fig. 13)

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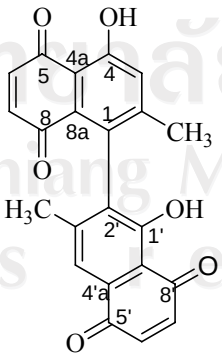
of orange-red solid **[CPD4]** showed couplings between 2-CH_3 and H-3; H-6 and H-7; and H-6′ and H-7′. The HMBC spectrum (Fig. 14) of orange-red solid **[CPD4]** established the partial structure from C-1 to C-8; demonstrating correlations from 2-CH_3 to C-1, C-2, and C-3; H-3 to C-4; 4-OH to C-4a, and C-3; H-7 to C-5 and C-8a. The HMBC spectral data also assisted in partial structure assembling from C-1′ to C-8′, from which the following correlations were seen; 1′-OH to C-1′, C-2′, and C-8′a; 3′-CH_3 to C-3′ and C-4′; H-4′ to C-5′ and 3′-CH_3; H-6′ and H-7′ to C-8′. ^{13}C NMR chemical shifts of quaternary C-1 at δ_C 135.1 and C-2′ at δ_C 130.3, together with the ESI-TOF-MS data, suggested the linkage between sp^2 quaternary C-atoms, C-1 and C-2′, leading to a structure of a dimeric naphthoquinone.

The ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HMQC and HMBC spectral data (allowed the assignments of protons attached to an individual carbon) indicated that orange-red solid **[CPD4]** might be isodiospyrin **[23].** Moreover, the ^{1}H and ^{13}C NMR data of orange-red solid **[CPD4]** compared with those reported in the literature[10, 27]. It could assure that the orange-red solid **[CPD4]** is isodiospyrin **[23].**



Isodiospyrin **[23]**

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Table 2. ^{1}H (500MHz) and ^{13}C NMR (125MHz) spectral data ($CDCl_3$) of **Isodiospyrol A** and **Isodiospyrin**

	Isodiospyrol A		**Isodiospyrin**[b]	
C	δ_C, mult.[a]	δ_H, mult., *J* in Hz	δ_C, mult.[a]	δ_H, mult., *J* in Hz
1	129.8, s	-	135.1, s	-
2	149.2, s	-	145.5, s	-
3	125.5, d	7.29, d, 0.6	121.4, d	7.63, s
4	161.7, s	-	158.6, s	-
4a	114.2, s	-	113.2, s	-
5	190.5, s	-	190.4, s	-
6	137.4, d	6.91, d, 10.1	138.8, d	6.98, dd, 10.3, 10.3
7	140.3, d	6.72, d, 10.1	140.2, d	6.98, dd, 10.3, 10.3
8	185.1, s	-	184.5, s	-
8a	128.8, s	-	128.8, s	-
2-CH_3	20.8, q	2.05, d, 0.6	20.5, q	2.03, s
4-OH	-	12.52, s	-	12.07, s
1′	159.4, s	-	162.0, s	-
2′	127.4, s	-	130.3, s	-
3′	145.8, s	-	148.2, s	-
4′	119.4, d	7.04, br s	125.8, d	7.32, s
4′a	144.3, s	-	128.6, s	-
5′	67.8, d	5.02, dd, 7.7, 3.8	185.0, d	-
6′	31.5, t	2.23, m; 2.43, m	139.6, d	6.75, d, 10.1
7′	34.7, t	2.69, m; 3.00, m	137.7, d	6.97, d, 10.2
8′	203.8, s	-	190.1, s	-
8′a	113.5, s	-	114.2, s	-
3′-CH_3	20.5, q	1.96, br s	20.7, q	2.05, s
1′-OH	-	12.63, s	-	12.46, s

[a] Multiplicity was determined by analyses of DEPT 135 spectra

[b] Isodiospyrin was compared with those reported in the literature[10, 27]

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4.1.2 Structure elucidation of the isolated compounds from *Diospyros rhodocalyx* woods

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* (5.43 g) was sequentially subjected to column chromatography on Sephadex LH-20 and silica gel to obtain: colorless solid **[CPD5]** (48 mg), opaque white solid **[CPD6]** (18.2 mg), orange-red solid **[CPD7]** (235 mg), clear white solid **[CPD8]** (15 mg) and white solid **[CPD 9]** (18 mg).

Colorless solid [CPD5]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 and silica gel**,** eluted with 4% acetone in hexane, obtained as colorless solid **[CPD5]**. It was identified by spectroscopic techniques.

Colorless solid **[CPD5]** was analysed by HR-ESI-TOF mass spectrum which indicated a molecular formula as $C_{30}H_{50}O$ (observed *m/z* 449.3861$(M+Na)^+$).

The ^1H NMR spectrum ($CDCl_3$, Fig.4) of colorless solid **[CPD5]** showed signals corresponding to ten methylene protons at δ_H 1.49; 1.24, 1.49; 1.24, 1.49; 1.24, 1.52; 1.27, 1.52; 1.27, 1.52; 1.27, 1.55; 1.3, 1.63; 1.38, 1.85; 1.6, 2.27; 2.17; five methine protons at δ_H 1.39, 1.40, 1.43, 1.62 and 2.18; an ethylene proton at δ_H 4.63 and 4.88 and seven methyl protons at δ_H 1.16, 1.16, 1.16, 1.16, 1.21, 1.21 and 1.71.

The ^{13}C NMR spectrum ($CDCl_3$, Fig. 5) of colorless solid **[CPD5]** showed the signals for thirty lines.

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By comparison of the ^{1}H and ^{13}C NMR data of colorless solid **[CPD5]** with those reported in the literature[28], it indicated that the colorless solid **[CPD5]** is lupeol **[5].**

Opaque white solid [CPD6]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 (eluted with MeOH) and silica gel**,** eluted with 5% acetone in hexane, obtained opaque white solid **[CPD6]**. It was identified by spectroscopic techniques

Opaque white solid **[CPD6]** was analysed by HR-ESI-TOF mass spectrum indicated a molecular formula as $C_{30}H_{48}O_2$ (observed *m/z* 463.3654 $(M+Na)^+$).

The ^{1}H NMR spectrum ($CDCl_3$, Fig.16) of opaque white solid **[CPD6]** showed signals corresponding to six methyl protons at δ_H 0.68, 0.75, 0.84, 0.90, 1.19 and 1.62; five methine protons at δ_H 2.80, 3.2, 4.56, 4.69 and 9.6.

The ^{13}C NMR spectrum ($CDCl_3$, Fig.17) of opaque white solid **[CPD6]** showed the signals for thirty lines.

By comparison of the ^{1}H and ^{13}C NMR data of opaque white solid **[CPD6]** with those reported in the literature[28], it indicated that the opaque white solid **[CPD6]** is betulinaldehyde **[67].**

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Orange-red solid [CPD7]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 (eluted with MeOH) to afford orange-red solid **[CPD7]**. It was identified by spectroscopic techniques

Orange-red solid **[CPD7]** was analysed by HR-ESI-TOF mass spectrum and indicated a molecular formula as $C_{22}H_{14}O_6$ (observed *m/z* 397.1216 $(M+Na)^+$).

The ^1H NMR spectrum ($CDCl_3$, Fig.8) of orange-red solid **[CPD7]** showed signals corresponding to two methyl protons at δ_H 2.24 and 2.39; six methine protons at δ_H 6.83, 6.89, 6.89, 7.06, 7.44, 7.49; two chelated hydroxyl protons at δ_H 11.81 and 12.07.

The ^{13}C NMR spectrum ($CDCl_3$, Fig.9) of orange-red solid **[CPD7]** showed the signals for twenty two lines.

By comparison of the ^1H and ^{13}C NMR data of orange-red solid **[CPD7]** with those reported in the literature[29], compound orange-red solid **[CPD7]** could be assigned as a known naphthoquinone, diospyrin **[21].**

Clear white solid [CPD8]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 (eluted with MeOH) to afford clear white solid **[CPD8]**. It was identified by spectroscopic techniques**.**

Clear white solid **[CPD8]** was analysed by HR-ESI-TOF mass spectrum and indicated a molecular formula as $C_{29}H_{50}O$ (observed *m/z* 437.7239 $(M+Na)^+$).

The ^1H NMR spectrum ($CDCl_3$, Fig.6) of clear white solid **[CPD8]** showed signals corresponding to eleven methylene protons at δ_H 1.25, 1.25, 1.29, 1.38; 1.13, 1.49; 1.24, 1.49; 1.39, 1.52; 1.27, 1.52; 1.27, 1.57; 1.32, 2.04; 1.79, 2.23; 1.98; nine methine protons at δ_H 1.40, 1.40, 1.44, 1.45, 1.46, 1.64, 1.82, 3.25 and 5.37; a hydroxyl proton at δ_H 2.0 and six methyl protons at δ_H 0.96, 1.01, 1.01, 1.06, 1.16 and 1.26.

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By comparison of the ^{1}H NMR data of clear white solid **[CPD8]** with those reported in the literature[30], it indicated that the clear white solid **[CPD8]** is β-sitosterol **[11].**

White solid [CPD9]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 (eluted with MeOH) to afford white solid **[CPD9]**. It was identified by spectroscopic techniques

White solid **[CPD9]** was analysed by HR-ESI-TOF mass spectrum which indicated a molecular formula as $C_{29}H_{48}O$ (observed *m/z* 435.7050 $(M+Na)^+$).

The ^{1}H NMR spectrum ($CDCl_3$, Fig.7) of white solid **[CPD9]** showed signals corresponding to nine methylene protons at δ_H 1.33, 1.38; 1.13, 1.49; 1.24, 1.49; 1.39, 1.52; 1.27, 1.52; 1.27, 1.57; 1.32, 2.04; 1.79, 2.23; 1.98; eleven methine protons at δ_H 1.40, 1.44, 1.44, 1.45, 1.86, 2.15, 2.33, 3.25, 5.37, 5.42 and 5.42; a hydroxyl proton at δ_H 2.0 and six methyl protons at δ_H 0.96, 1.01, 1.01, 1.16, 1.16 and 1.26.

By comparison of the ^{1}H NMR data of white solid **[CPD9]** with those reported in the literature[30], it indicated that the white solid **[CPD9]** is stigmasterol **[12].**

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4.1.3 Structure elucidation of the isolated compounds from *Diospyros glandulosa* woods

A crude CH_2Cl_2 extract of wood of *Diospyros glandulosa* (55.5 g) was sequentially subjected to column chromatography on Sephadex LH-20 and silica gel to obtain colorless solid **[CPD5-I]** (1.3 mg)**,** orange-red solid **[CPD7-I]** (159 mg), clear white solid **[CPD8-I]** (1.1 mg) and white solid **[CPD9-I]** (1.5 mg).

Colorless solid [CPD5-I]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 and silica gel**,** eluted with 4% acetone in hexane, obtained a colorless solid **[CPD5-I]**. It was elucidated by spectroscopic techniques.

Colorless solid **[CPD5-I]** was analysed by HR-ESI-TOF mass spectrum which indicated a molecular formula as $C_{30}H_{50}O$ (observed *m/z* 449.3861$(M+Na)^+$).

The ^1H NMR spectrum ($CDCl_3$, Fig.4) of colorless solid **[CPD5-I]** showed signals corresponding to ten methylene protons at δ_H 1.49; 1.24, 1.49; 1.24, 1.49; 1.24, 1.52; 1.27, 1.52; 1.27, 1.52; 1.27, 1.55; 1.3, 1.63; 1.38, 1.85; 1.6, 2.27; 2.17; five methine protons at δ_H 1.39, 1.40, 1.43, 1.62 and 2.18; an ethylene proton at δ_H 4.63 and 4.88 and seven methyl protons at δ_H 1.16, 1.16, 1.16, 1.16, 1.21, 1.21 and 1.71.

The ^{13}C NMR spectrum ($CDCl_3$, Fig. 5) of colorless solid **[CPD5-I]** showed the signals for thirty lines.

By comparison of the ^1H and ^{13}C NMR data of colorless solid **[CPD5-I]** with those reported in the literature[28], it indicated that the colorless solid **[CPD5-I]** is lupeol **[5].**

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Orange-red solid [CPD7-I]

The CH_2Cl_2 wood extract of *Diospyros glandulosa* was sequentially subjected to column chromatography on Sephadex LH-20 (eluted with MeOH) to afford orange-red solid **[CPD7-I]**. It was elucidated by spectroscopic analyses**.**

Orange-red solid **[CPD7-I]** was analysed by HR-ESI-TOF mass spectrum which indicated a molecular formula as $C_{22}H_{14}O_6$ (observed *m/z* 397.1216 $(M+Na)^+$).

The ^1H NMR spectrum ($CDCl_3$, Fig.8) of orange-red solid **[CPD7-I]** showed signals corresponding to two methyl protons at δ_H 2.24 and 2.39; six methine protons at δ_H 6.83, 6.89, 6.89, 7.06, 7.44, 7.50; two chelated hydroxyl protons at δ_H 11.82 and 12.08.

The ^{13}C NMR spectrum ($CDCl_3$, Fig.9) of orange-red solid **[CPD7-I]** showed the signals for twenty two lines.

By comparison of the ^1H and ^{13}C NMR data of orange-red solid **[CPD7-I]** with those reported in the literature[29], compound orange-red solid **[CPD7-I]** could be assigned as a known naphthoquinone, diospyrin **[21].**

Clear white solid [CPD8-I]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 (eluted with MeOH) to afford clear white solid **[CPD8-I]**. It was identified by spectroscopic techniques**.**

Clear white solid **[CPD8-I]** was analysed by HR-ESI-TOF mass spectrum which indicated a molecular formula as $C_{29}H_{50}O$ (observed *m/z* 437.7239 $(M+Na)^+$).

The ^1H NMR spectrum ($CDCl_3$, Fig.6) of clear white solid **[CPD8-I]** showed signals corresponding to eleven methylene protons at δ_H 1.25, 1.25, 1.29, 1.38; 1.13, 1.49; 1.24, 1.49; 1.39, 1.52; 1.27, 1.52; 1.27, 1.57; 1.32, 2.04; 1.79, 2.23; 1.98; nine methine protons at δ_H 1.40, 1.40, 1.44, 1.45, 1.46, 1.64, 1.82, 3.25 and 5.37; a hydroxyl proton at δ_H 2.0 and six methyl protons at δ_H 0.96, 1.01, 1.01, 1.06, 1.16 and 1.26.

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By comparison of the ^{1}H NMR data of clear white solid **[CPD8-I]** with those reported in the literature[30], it indicated that the clear white solid **[CPD8-I]** is β-sitosterol **[11].**

White solid [CPD9-I]

The CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 (eluted with MeOH) to afford white solid **[CPD9-I]**. It was identified by spectroscopic techniques

White solid **[CPD9-I]** was analysed by HR-ESI-TOF mass spectrum which indicated a molecular formula as $C_{29}H_{48}O$ (observed *m/z* 435.7050 $(M+Na)^+$).

The ^{1}H NMR spectrum ($CDCl_3$, Fig.7) of white solid **[CPD9-I]** showed signals corresponding to nine methylene protons at δ_H 1.33, 1.38; 1.13, 1.49; 1.24, 1.49; 1.39, 1.52; 1.27, 1.52; 1.27, 1.57; 1.32, 2.04; 1.79, 2.23; 1.98; eleven methine protons at δ_H 1.40, 1.44, 1.44, 1.45, 1.86, 2.15, 2.33, 3.25, 5.37, 5.42 and 5.42; a hydroxyl proton at δ_H 2.0 and six methyl protons at δ_H 0.96, 1.01, 1.01, 1.16, 1.16 and 1.26.

By comparison of the ^{1}H NMR data of white solid **[CPD9-I]** with those reported in the literature[30], it indicated that the white solid is stigmasterol **[12].**

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4.2 Biological activity of the isolated compounds

Four compounds which were isolated from *Diospyros ehretioides* were subjected to the afore-mentioned activity tests. Four compounds were isolated from *Diospyros rhodocalyx* and *Diospyros glandulosa* while betulinaldehyde **[67]** was obtained only from *Diospyros rhodocalyx*. Four compounds and five compounds, isolated from *Diospyros glandulosa* and *Diospyros rhodocalyx*, respectively, were subjected to antimalarial and antimycobacterial activity tests. Due to β-sitosterol **[11]** and stigmasterol **[12],** which were isolated from *Diospyros rhodocalyx* and *Diospyros glandulosa,* were not able to dissolve in DMSO, therefore, antimalarial and antimycobacterial activity tests were not evaluated. However, only lupeol **[5]**, diospyrin **[21]** and betulinaldehyde **[67]** were subjected to antimalarial and antimycobacterial activity tests.

4.2.1 Fruits of *Diospyros ehretioides*

Palmarumycins JC1 **[70]** and JC2 **[71]** were previously reported to possess significant antibacterial activity[25], however, they did not exhibit antibacterial activity in this study. As a part of the search for biologically-active substances from *Diospyros* spp, the author has further studied the constituents of *Diospyros ehretioides*, *Diospyros rhodocalyx* and *Diospyros glandulosa*. In this investigation, palmarumycin JC1 **[70]** did not exhibit antimalarial, antifungal, antimycobacterial and cytotoxic activities. Palmarumycin JC2 **[71]** exhibited antimalarial (IC_{50} 4.5 μg/mL), antifungal (IC_{50} 12.5 μg/mL), antimycobacterial (MIC 6.25 μg/mL) and cytotoxic (IC_{50} 11.0 μg/mL for NCI-H187 cell line) activities (Table 3). It was surprising to note that palmarumycin JC2 **[71]** possessed biological activities while its reduced form, palmarumycin JC1 **[70]**, was inactive (Table 3). The antimycobacterial activity

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of palmarumycin JC2 **[71]** was comparable to that of kanamycin sulfate and isoniazid (MIC 2.5 and 0.006 μg/mL).

In our bioassay systems, isodiospyrin **[23]** did not exhibit antimycobacterial, antifungal, antimalarial activities or cytotoxicity against Vero, KB, NCI-H187, and BC cell lines. However, it was reported to exhibit cytotoxicity against HCT-8 colon tumor and P-388 lymphocytic leukemia, termicidal and topoisomerase I inhibitory activities[31-33]. Isodiospyrol A **[72]** exhibited antimalarial (IC_{50} 2.7 μg/mL) and antimycobacterial (MIC 50 μg/mL) activities but was inactive towards *Candida albicans*. Compound **72** also exhibited cytotoxicity against BC cells (IC_{50} 12.3 μg/mL) but not towards KB and Vero cell lines (Table 3).

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Table 3. Biological activities of compounds **23, 70-72**; IC_{50} and MIC values are expressed in μg/mL (mean ± standard deviation)

Compound	Cytotoxicity (IC_{50}) (μg/mL)				Antimycobac-terial activity (MIC)[a]	Antifungal activity (IC_{50})	Antimalarial activity (IC_{50}) (μg/mL)
	NCI-H187	BC	KB	Vero	(μg/mL)	(μg/mL)	
23	>20	>20	>20	>50	>200	>20	>20
70	>20	>20	>20	>50	>200	>20	>20
71	11.0±2.5 (n=2)	>20	>20	>50	6.25	12.5±1.9 (n=2)	4.5±0.9 (n=2)
72	Not tested	12.3±1.8 (n=2)	>20	>50	50	>20	2.7±0.5 (n=2)
ellipticin	0.4±0.1	1.4±0.1	1.3±0.3	1.4±0.1			
isoniazid					0.06		
kanamycin sulfate					2.5		
amphotericin B						0.04±0.01	
dihydroartemisinin							0.004±0.001

[a] MIC values were obtained from the two-fold dilution technique, and triplicate experiments were performed, showing the same MIC value for each compound.

4.2.2 Woods of *Diospyros rhodocalyx* and *Diospyros glandulosa*

The CH_2Cl_2 wood extracts of *Diospyros rhodocalyx* and *Diospyros glandulosa* were sequentially subjected to column chromatography on Sephadex LH-20 and silica gel to obtain five compounds, lupeol **[5],** β-sitosterol **[11],** stigmasterol **[12],** diospyrin **[21]** and betulinaldehyde **[67]** which were isolated from *Diospyros rhodocalyx.* Four compounds, lupeol **[5],** β-sitosterol **[11],** stigmasterol **[12]** and diospyrin **[21]** were isolated from *Diospyros glandulosa.* Because β-sitosterol **[11]** and stigmasterol **[12],** were not able to dissolve in DMSO, therefore, antimalarial and

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antimycobacterial activity tests were not evaluated. However, only lupeol **[5],** diospyrin **[21]** and betulinaldehyde **[67]** were subjected to antimalarial and antimycobacterial activity tests. Of all the isolated compounds, diospyrin **[21]** possessed the most potent antimalarial activity against *Plasmodium falciparum* K1 with the IC_{50} value of 3.29 μg/mL and antimycobacterial activity with the MIC value at 6.25 μg/mL (Table 4).

Table 4. Biological activities of compounds **5, 11-12, 21 and 67**; IC_{50} and MIC values are expressed in μg/mL

Compound	Antimalarial activity (IC_{50}, μg/mL)	Antimycobacterial activity[a] (MIC, μg/mL)
5	>20	>20
11	Not dissolved in DMSO	Not dissolved in DMSO
12	Not dissolved in DMSO	Not dissolved in DMSO
21	3.29	6.25
67	6.25	25
isoniazid	-	0.06
kanamycin sulfate	-	2.5
dihydroartemisinine	0.004±0.001	-

[a]) MIC values were obtained from the two-fold dilution technique, and triplicate experiments were performed showing the same MIC value for each compound.

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