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3′-METHOXYDIOSPYRIN, A 7-METHYLJUGLONE DIMER FROM *DIOSPYROS MANNII*

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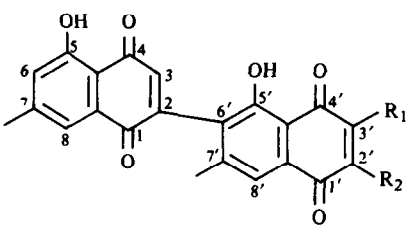
Key Word Index—*Diospyros mannii*; Ebenaceae; 7-methyljuglone dimers; diospyrin; 3′-methoxydiospyrin; triterpenes.

Abstract—Examination of two samples of stem bark of *Diospyros mannii* yielded three naphthoquinones and three triterpenes. Five of the compounds, 7-methyljuglone, diospyrin, lupeol, betulin and betulinic acid, were previously known from other *Diospyros* species and the sixth was characterized as the new naphthoquinone dimer, 3′-methoxydiospyrin.

Diospyros mannii Hiern is a small to medium-sized tree found throughout the rain forest zone of west tropical Africa [1]. In this paper, we report the results of phytochemical analyses of samples of stem bark of this previously uninvestigated species collected in both Cameroon and Ghana and the characterization of a new juglone derivative, 3′-methoxydiospyrin (**3**).

Extraction of the stem bark of material from Cameroon with petrol (bp 40–60°), followed by prep. TLC, gave a wax, the C-2 to C-6′ linked 7-methyljuglone dimer diospyrin (**1**) and the common triterpenes lupeol and betulinic acid. Diospyrin was identified by comparison of physical and spectroscopic data (notably ^{1}H NMR) with that published [2, 3] and the two triterpenes by direct comparison with authentic material. The wax was analysed by GC and EIMS which revealed it to be C_{20}–C_{28} saturated fatty acids (mainly C_{22}, C_{24}, C_{26} and C_{28}) esterified to C_{26} and C_{28} saturated alcohols.

Subsequent extraction with methanol followed by prep. TLC gave further **1** and a yellow-orange compound with mp 220–225°. Accurate mass measurement suggested $C_{23}H_{16}O_7$ and both UV and IR spectra were typical of a juglone derivative. The ^{1}H NMR spectrum showed signals for 7- and 7′-methyl groups, H-6, H-8, H-8′ and H-3, that were in close agreement with those observed for **1**. Of the two hydrogen-bonded hydroxyl protons one, at δ 11.88, agreed closely with that for the 5-hydroxyl group of **1** but the other occurred at δ 12.00 (cf. δ 12.18 for the 5′-hydroxyl in **1**). The two remaining signals, singlets at δ 6.17 (1H) and 3.92 (3H), differed from **1** and must be assigned to a proton and a methoxy substituent which requires this compound to be either 2′-methoxy (**2**) or 3′-methoxydiospyrin (**3**). The EIMS fragmentation confirms that the compound is a benzenoid-quinonoid-linked dimer [4] and agrees with the proposed structures **2** and **3**.



1 $R_1 = R_2 = H$

2 $R_1 = H$, $R_2 = OMe$

3 $R_1 = OMe$, $R_2 = H$

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The methoxy substituent was assigned to C-3′ on the basis of its effect on the resonance position of the 5′-hydroxyl signal. Moore and Scheuer [5] have shown that the chemical shift of the 5-hydroxyl proton of a juglone derivative is deshielded by *ca* δ 0.30 by C-2 oxygenation and shielded by *ca* 0.20 by C-3 oxygenation. Thus, the shielded position of the 5′-hydroxyl signal (δ 12.00) in the methoxydiospyrin compound can only be explained by placement of the methoxy group at C-3′ and allows the assignment of structure **3**.

A similar examination of a second sample of stem bark, collected in Ghana, gave **1**, lupeol and betulinic acid and, in addition, 7-methyljuglone and the triterpene betulin. No trace of **3** could be found in this sample.

3′-Methoxydiospyrin (**3**) does not appear to have been isolated previously. In view of its occurrence only in the methanol extract it seemed possible that it was an artefact of the extraction procedure, derived from **1** or from the corresponding 2′,3′-epoxide, diosquinone, a compound known from other *Diospyros* species [4, 6]. However, no trace of diosquinone could be detected in any *D. mannii* extract and prolonged refluxing of **1** in methanol under both acidic and basic conditions failed to show the formation of any methoxy derivatives.

EXPERIMENTAL

Plant material. Stem bark of *D. mannii* was collected in the Douala-Edea Forest Reserve, Cameroon (voucher: McKey and Gartlan, 143, deposited at the Herbarium of the Royal Botanic Gardens, Kew) and in the Kade Forest Reserve, Ghana (voucher: Hall and Nabooh, GC46657, deposited at the Herbarium of the University of Ghana).

Isolation of compounds from Cameroon material. Ground stem bark (322 g) was extracted with petrol (bp 40–60°) and then MeOH. On concn the petrol extract gave a wax (460 mg) followed by **1** (98 mg). The supernatant was subjected to CC over Si gel, eluting with $CHCl_3$ followed by $CHCl_3$–EtOAc (95:1) to give further **1** (50 mg) and a mixture of **1** and a number of triterpenes. Prep. TLC of the mixture on Si gel ($CHCl_3$) gave further **1** (70 mg), lupeol (10 mg) and betulinic acid (25 mg).

Concn of the MeOH extract gave a residue (3.4 g) which was dissolved in $CHCl_3$, filtered and subjected to prep. TLC on Si gel ($CHCl_3$–EtOAc, 9:1) to give further **1** (30 mg) and **3** (40 mg).

Isolation of compounds from Ghanaian material. Concn of the petrol (bp 40–60°) extract of ground stem bark (105 g) followed by prep. TLC on Si gel ($CHCl_3$) gave 7-methyljuglone (1 mg), **1** (40 mg), lupeol (15 mg), betulin (5 mg) and betulinic acid (8 mg).

Diospyrin (**1**). Yellow-orange needles from petrol (bp 40–60°)–$CHCl_3$, mp 257–258° (decomp.) (lit. [4] 257°). Found: $[M]^+$ 374.0802; $C_{22}H_{14}O_6$ requires 374.0790. ^{1}H NMR (90 MHz, $CDCl_3$): δ 2.31 (3H, *s*, Me-7′), 2.45 (3H, *s*, Me-7), 6.93 (1H, *s*, H-3), 6.98 (2H, *s*, H-2′ and H-3′), 7.15 (1H, *d*, J = 1 Hz, H-6), 7.55 (1H, *d*, J = 1 Hz, H-8), 7.60 (1H, *s*, H-8′), 11.90 (1H, *s*, OH-5), 12.18 (1H, *s*, OH-5′). UV, IR, EIMS as reported [2, 3].

3′-Methoxydiospyrin (**3**). Yellow-orange needles from petrol (bp 40–60°)–$CHCl_3$, mp 220–225° subliming to give needle crystals, mp > 340°. UV λ_{max}^{MeOH} nm: 249, 291, 428; (log ε) 4.37, 4.19, 3.95; $\lambda_{max}^{MeOH+NaOH}$ nm: 228, 280, 545; IR ν_{max}^{KBr} cm^{-1}: 2910, 2850, 1665 (C=O), 1645 (C=O, hydrogen-bonded), 1609, 1450, 1380, 1310, 1265, 1250, 1229, 1100, 1050, 850, 820; ^{1}H NMR (90 MHz, $CDCl_3$): δ 2.31 (3H, *s*, Me-7′), 2.45 (3H, *s*, Me-7), 3.92 (3H, *s*, OMe-3′), 6.17 (1H, *s*, H-2′), 6.92 (1H, *s*, H-3), 7.13 (1H, *d*, J = 1 Hz, H-6), 7.54 (1H, *d*, J = 1 Hz, H-8), 7.58 (1H, *s*, H-8′), 11.88 (1H, *s*, OH-5), 12.00 (1H, *s*, OH-5′); EIMS: Found $[M]^+$ 404.0871; $C_{23}H_{16}O_7$ requires 404.0896; *m/z* (rel. int.): 404 $[M]^+$ (100), 403 (30), 386 (13), 361 (3).

Other compounds. 7-Methyljuglone [mp 123–124° (lit. [7] 125–126°)], lupeol [mp 215° (lit. [8] 215°)], betulin [mp 256–257° (lit. [9] 256–257°)] and betulinic acid [mp 314° (lit. [9] 300°)] were all characterized by direct comparison with authentic samples (UV, IR, ^{1}H NMR, EIMS, ORD and mmp).

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