NAPHTHOQUINONES AND TRITERPENES FROM SOME ASIAN DIOSPYROS SPECIES

MUHAMAD BIN ZAKARIA*, J A D JEFFREYS*, PETER G WATERMAN† and SHOU-MING ZHONG

Phytochemistry Research Laboratories, Department of Pharmacy and *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow G1 1XW, UK

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Abstract—Investigation of the stem bark, wood and/or fruit of nine Asian *Diospyros* spp (Ebenaceae) has revealed a range of triterpenes and naphthoquinones typical of the genus In addition to lupeol, betulin and betulinic acid, which were present in all samples, seven other triterpenes were isolated and identified Six of the nine species also yielded naphthoquinones In two species these were based on 2-methyljuglone (plumbagin) and in the other four on 7-methyljuglone

INTRODUCTION

In a recent paper [1] we reported the occurrence of a number of naphthoquinones, naphthoquinone dimers and triterpenes in the stem barks and/or woods of seventeen African species of Diospyros L (Ebenaceae) We now report the results of an investigation of the stem barks, woods and, in one case, fruits, of nine Diospyros species of Asian origin, eight from Malaysia and one (D lotus) from China

RESULTS AND DISCUSSION

All compounds isolated from or detected in the Diospyros samples examined are listed in Table 1 As in the previous study three triterpenes, lupeol, betulin and betulinic acid were detected in all plant parts investigated [1] Seven further triterpenes were identified from three of the species investigated. The olean-14-ene derivatives taraxerol (1) and taraxerone (2) were isolated from D lotus and characterized by direct comparison with authentic material Both are relatively common in the genus and 1 has previously been reported from D lotus [2] Taraxeryl acetate (3) was obtained from two other species, D maingayi and D singaporensis, and its identity confirmed by conversion to 1 Acetyl oleanolic acid (4), an olean-12ene, was also isolated from D lotus and identified by direct comparison with authentic material Whilst the corresponding alcohols are both relatively common in the genus neither 3 nor 4 appears to have been noted previously

The remaining three triterpenes were of the urs-12-ene series differing in the level of oxidation of the C-17 methyl and thus giving a series comparable to lupeol, betulin and betulinic acid α -Amyrin (5) and ursolic acid (7), from *D* maingayi and *D* lotus respectively, have been recorded previously from *Diospyros* and identity was confirmed by direct comparison with authentic material The 17-hydro-

xymethyl derivative, uvaol (6), which had not previously been found in the genus, was isolated from both Dmaingayi and D lotus The ¹H NMR and EIMS complied with the structure 6 which was confirmed by partial synthesis of 6 from 3β -acetyl methyl ursolate (8) by lithium aluminium hydride reduction

Naphthoquinones were found in six of the nine species In both D stamang and D wallicht the naphthoquinones found were plumbagin (9), its reduced form isoshinanolone (10) and the 6-6' dimer elliptinone (11) Compound 9 was identified by direct comparison and 10 and 11 on the basis of the close agreement of spectral data to those already published It was notable that for both species 9 and 10 occurred in stem bark and wood whereas 11 was restricted to the bark. In the other naphthoquinone producing taxa compounds were based on 7methyljuglone (12) which was itself found in D ismaili and D sumatrana Shinanolone (13), a reduced form of 12 was obtained from D maingayi together with the common 6-8' dimer isodiospyrin (14), the latter also being found in Dlotus D ismaili yielded two somewhat rarer dimers, the 2-3' linked rotundiquinone (15) and the 3-8' linked neodiospyrin (16) as well as a novel coumarinylnaphthoguinone, ismailin (17) [3] Both 12 and 14 were identified by direct comparison with authentic material and 13, 15 and 16 on the basis of close agreement with literature data Neither the dimer diosindigo-A, reported from D maingayi and D sumatrana [4], nor the numerous dimers reported from D lotus [5], were detected

The secondary metabolites produced by these nine species show considerable overlap with those recorded in the African taxa [1] In both groups lupeol, betulin and betulinic acid occur throughout but, due mainly to D*lotus* and D maingayi, the diversity of triterpenes appears to be greater among the Asian group There also appears to be greater diversity among the naphthoquinones produced by Asian species, nine compounds from nine taxa in comparison with eight from the seventeen African taxa The close correspondence between the two geographic groups is further illustrated by the presence of ismailin (17) in D ismaili and the corresponding plumbagin adduct canaliculatin in the African D canaliculata [1]

[†]Author to whom communications should be addressed



EXPERIMENTAL

Plant material Plant samples were collected in 1980 and 1981 and were sun dried or oven dried below 60° Details of sources are given in Table 1

General isolation and purification methods Powdered samples were extracted in a Soxhlet with petrol (bp 40–60°), then CHCl₃ and, finally, MeOH Extracts were concd under red pres and examined by TLC (silica gel) Compounds were generally separated by CC over silica gel eluting with either petrol or CHCl₃ followed by either of those solvents containing increasing amounts of EtOAc Further purification of fractions from the column was achieved by prep TLC using the same materials as for CC Where naphthoquinones were present all operations were performed in subdued light and the columns wrapped in aluminium foil

Isolation and identification of individual substances compounds from D ismaili Concn of the petrol extract of the wood (272 g)followed by CC eluting with CHCl₃ gave 12 (14 mg), lupeol (30 mg), 15 (2 mg), 16 (3 mg), betulin (4 mg) and betulinic acid (10 mg) Concn of the CHCl₃ extract gave 17 (1 45 g) and prep TLC of the supernatant gave further 17 (35 mg), 15 (6 mg) and 16 (7 mg) Similar treatment of the CHCl₃ extract of the fruits (40 g) gave 12 (15 mg), 15 (18 mg) and 16 (14 mg)

7-Methyljuglone (12) Orange needles from petrol (bp 40-60°), mp 124-125° (lit [6] 124-125°), identical with authentic sample (UV, IR, ¹H NMR, mmp)

Rotundiquinone (15) Red needles from CHCl₃ subliming at 235° with decomp at 320° Found $[M]^+$ 374 0783, C₂₂H₁₄O₆ requires 374 0790 UV λ_{max}^{EtOH} nm 254, 272 sh, 435, (+ NaOH) 285, 550 ¹H NMR (CDCl₃, 90 MHz) δ 2 46 (6H, s, Me-7, Me-7'), 6 99 (1H, s, H-2'), 7 02 (1H, s, H-3), 7 13 (2H, br s, H-6, H-6'), 7 50 (2H, br s, H-8, H-8'), 11 71 (1H, s, OH-5'), 11 81 (1H, s, OH-5)—in agreement with lit [7] EIMS m/z (rel int) 374 (100), 357 (17), 317 (10), 187 (8), 134 (9), 106 (22)

Neodiospyrin (16) Red needles from CHCl₃, decomp 205–210° Found $[M]^+$ 374 0776, C₂₂H₁₄O₆ requires 374 0790 UV $\lambda_{\text{max}}^{\text{max}}$ nm 250 sh, 290, 350 sh, 428, (+ NaOH) 290, 530 ¹H NMR (CDCl₃, 90 MHz) δ 2 30 (3H, s, Me-7'), 2 47 (3H, s, Me-7), 6 62 (1H, s, H-2), 6 79, 6 93 (2H, ABq, J = 10 Hz, H-2', H-3'), 7 12 (1H, br s, H-6), 7 27 (1H, s, H-6'), 7 53 (1H, br s, H-8), 11 75 (1H, s, OH-5), 12 28 (1H, s, OH-5')—in agreement with [7] EIMS *m/z* (rel int) 374 (100), 331 (8), 187 (7), 134 (6), 106 (24) *Ismailin* (17) See ref [3]

Compounds from D lotus Petrol and CHCl₃ extracts of the wood (240 g) were conc and subjected to prep TLC eluting with CHCl₃ to give 14 (20 mg) and a mixture of triterpenes Prep TLC of the triterpenes eluting with toluene–EtOAc-HOAc (48 1 1) gave 6 (25 mg) Prep TLC of the conc petrol extract of the bark (400 g) gave, on elution with petrol containing increasing EtOAc,

5 R = OH, R₁ = Me
6 R = OH, R₁ = CH₂OH
7 R = OH, R₁ = COOH
8 R = OAc, R₁ = COOMe

14 (34 mg), 1 (15 mg), 4 (10 mg) and 7 (13 mg) Repeated prep TLC of mixed fractions eluting with toluene-EtOAc-HOAc gave 14 (16 mg), 2 (5 mg), 1 (25 mg) and 4 (5 mg)

Isodiospyrin (14) Orange needles from CHCl₃, mp 228–231° (lit [5] 229–230°), identical with authentic sample (UV, IR, ¹H NMR, mmp)

Taraxerol (1) Needles from MeOH, mp 280–282° (ltt [8] 285°), $[\alpha]_{D}^{20} + 3°$ (c 0 01, CHCl₃) (ltt [2] + 3°) Found [M]⁺ 426 3855, C₃₀H₅₀O requires 426 3861, IR, ¹H NMR, TLC identical with authentic sample

Taraxerone (2) Plates from MeOH–CHCl₃, mp 240–245° (ltt [9] 245°), $[\alpha]_{D}^{20} + 10^{\circ}$ (c 005, CHCl₃) (ltt [8] + 12°) Found $[M]^+$ 424 3712, C₃₀H₄₈O requires 424 3705, IR, ¹H NMR, TLC identical with authentic sample

Uvaol (6) Identical with material from D maingayi (see below)

Acetyl oleanolic acid (4) Needles from MeOH, mp 265–267° (lit [10] 265–267°), $[\alpha]_D^{20} + 69°$ (c 0 01, CHCl₃) (lit [10] + 60°) Found [M]⁺ 498 3700, C₃₂H₅₀O₄ requires 498 3709 ¹H NMR (CDCl₃, 90 MHz) δ 0 77, 0 86, 0 87, 0 88, 0 97, 1 08 (6 × Me), 2 02 (3H, s, Ac), 4 49 (1H, dd, J = 8 Hz, 4 Hz, H-3), 5 22 (1H, m, H-12) TLC identical with authentic material





IO R = Me, R, = H I3 R = H, R, = Me

II (9)-(9) linked 6-6'

9 R = Me, R, = H

14 (12)-(12) linked 6-8'

15 (12)-(12) linked 2-3

16 (12)-(12) linked 3-8'



Table 1 Distribution of naphthoquinones and triterpenes in the Diospyros species examined

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D ismaili Ng	Genting	M	11	1	4	1		1	1	1	1	1	Ì	1	1	5	'	I	e	4	2
	Highlands	ír,	+	+	+	I	Ι	T	Ì		1	1	'	1	1	ا يو		4	S S	5	1
D lotus L	Nanjing	B	+	+	+	10	1	1	Ś	' 	1	-	-	1	1	1	-	ŝ	1 	1	-i
		M	+	+	+	+	Ι	T	1		0	1		1	1	1		1	r T	ı I	1
D maingayi (Hiern) Bakh	Tolok Datok	æ	8	8	80	1	1	3	1	9	2	1	' I	1	1	1	-	' 0	1	1	1
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	Highlands	M	+	+	+	I	I	I	Ì	1	1	1	•	'	1	1	1	1	1	I I	1
D wallach Williams	Kepong	B	8	10	50	I	I	Ι	Ì		1	H	0	e e	0	1	1	•	1	1	1
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B = stem bark, W = wood, F = ripe fruit, + = present (TLC) but not isolated, I = lupeol, b = betulin, ba = betulinic acid

Ursolic acid (7) Needles from MeOH, mp 285–290° (lit [11] 286–291°), $[\alpha]_D^{00} + 60°$ ($c \ 0 \ 02, \ C_5H_5N$) (lit [11] + 66°) Found [M]⁺ 456 3599, $C_{30}H_{48}O_3$ requires 456 3603 Identity confirmed by direct comparison with authentic sample (IR, ¹H NMR, TLC) and by synthesis of acetyl derivative of 7, mp 270–272° from MeOH (lit [2] 275°)

Compounds from D maingayi The combined petrol and CHCl₃ extracts of the bark (100 g) were coned and subjected to CC eluting with CHCl₃ and then CHCl₃ + MeOH to give lupeol (17 mg), 3 (3 mg), 5 (6 mg), 6 (90 mg), 14 (12 mg), betulin (50 mg) and betulinic acid (80 mg) Similar treatment of the wood (207 g) gave lupeol (2 mg), 6 (5 mg), 14 (3 mg), betulin (10 mg), betulinic acid (15 mg) and 13 (5 mg)

Taraxeryl acetate (3) Needles from MeOH, mp 301–302° (ltt [12] 303–304°), $[\alpha]_D^{20} + 10°$ (c 0 31, CHCl₃) Found $[M]^+$ 468 3990, $C_{32}H_{52}O_2$ requires 468 3967 IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 2910, 1720, 1635 ¹H NMR (CDCl₃, 90 MHz) $\delta 2 02$ (3H, s, Ac), 448 (1H, dd, J = 8 Hz, 4 Hz, H-3 α), 5 53 (1H, dd, J = 8 Hz, 4 Hz, H-14) Acid hydrolysis yielded taraxerol (1), identical in all respects with authentic material

 α -Amyrin (5) Needles from MeOH, mp 183–184° (lit [13] 184–185°), $[\alpha]_D^{20} + 80°$ (c 0 35, CHCl₃) (lit [13] + 80°) Found [M]⁺ 426 3848, C₃₀H₅₀O requires 426 3861 Identical with an authentic sample (IR, ¹H NMR, mmp, TLC)

Uvaol (6) Needles from MeOH, mp 224–225° (lt [13] 222–224°), $[\alpha]_D^{20} + 72°$ (c 011, CHCl₃) (ltt [13] + 72°) Found [M]⁺ 442 3800, C₃₀H₅₀O₂ requires 442 3810 IR v_{Mar}^{KBr}cm⁻¹ 3460, 2920, 1480, ¹H NMR (CDCl₃, 90 MHz) δ 3 24 (1H, dd, J = 9 Hz, 7 Hz, H-3 α), 3 19, 3 53 (2H, ABq, J = 13 Hz, CH₂OH), 5 18 (1H, dd, J = 5 Hz, 4 Hz, H-12) A sample of acetyl methyl ursolate (8, 100 mg) in dry Et₂O was refluxed with LiAlH₄ (30 mg) for 1 hr Normal work up gave a product (62 mg) identical in all respects to 6

Shinanolone (13) Needles from petrol (bp 40–60°), mp 108° (lit [2] 110–111°) Found $[M]^+$ 192 0760, $C_{11}H_{12}O_3$ requires 192 0786 UV λ_{max}^{MeOH} nm 268, 333 IR ν_{max}^{KBr} cm⁻¹ 3560, 2920, 1635 ¹H NMR (CDCl₃, 90 MHz) δ 1 95–2 30 (2H, m, CH₂-2), 2 31 (3H, s, Me-7), 2 70–3 00 (2H, m, CH₂-3), 4 40 (1H, dd, J = 9 Hz, 6 Hz, H-1), 6 59, 6 66 (2H, ABq, J = 2 Hz, H-6, H-8), 12 37 (1H, s, OH-5) EIMS m/z (rel int) 192 (100), 170 (80), 148 (76)

Compounds from D stamang Combined petrol and $CHCl_3$ extracts of the bark (329 g) were subjected to CC and eluted with CHCl₃ and then CHCl₃ with EtOAc to give 9 (440 mg) followed by a mixture Prep TLC of the mixture with CHCl₃ gave more 9 (30 mg), 11 (150 mg) and lupeol (56 mg) Prep TLC of later fractions from the column gave 10 (9 mg), betulin (17 mg) and betulinic acid (7 mg)

Plumbagin (9) Yellow needles from petrol (bp 40–60°), mp 73° (lit [5] 75°) Identical in all respects (UV, IR, mmp, ¹H NMR, ¹³C NMR) with authentic sample

¹H NMR (CDCl₃, 90 MHz) $\delta 2 23$ (6H, d, J = 2 Hz, Me-2, Me-2'), 6 85 (2H, q, J = 2 Hz, H-3, H-3'), 7 73 (4H, br s, H-7, H-7', H-8, H-8') 12 48 (2H, s, OH-5, OH-5') EIMS m/z (rel int) 374 (53), 357 (7), 331 (5), 278 (6)

Isoshinanolone (10) Needles from petrol (bp 40–60°), mp 165° (decomp) (lit [5] 160° decomp) UV λ_{max}^{MeOH} nm 261, 334 IR ν_{max}^{MEO} cm⁻¹ 2920, 2845, 1642, 1623 ¹H NMR (CDCl₃, 90 MHz) δ 1 16 (3H, d, J = 6 Hz, Me-2), 2 20–3 20 (2H, m, CH₂-3), 2 44 (1H, m, H-2), 4 75 (1H, d, J = 3 Hz, H-1), 6 94 (2H, m, H-6, H-8), 7 49 (1H, dd, J = 10 Hz, 10 Hz, H-7), 12 41 (1H, s, OH-5) Compound 10 (5 mg) dissolved in CHCl₃ (2 ml) and C₅H₅N (1 ml) was treated with CrO₃ and stirred for 24 hr The reaction mixture was diluted with H₂O and extracted into Et₂O to give an oxidation product identical in all respects to 9

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