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GLABRETAL TRITERPENOIDS FROM *DYSOXYLUM MUELLERI*

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Key Word Index—*Dysoxylum muelleri*; Meliaceae; dysoxin; triterpenoids; limonoid.

Abstract—A hexane extract of the wood of *Dysoxylum muelleri* has yielded 3α-cinnamoyl glabretal, 3α-benzoyl glabretal, 21,24-epoxy-23α,25-dihydroxy-4α,4β,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3α-cinnamoyl-7α-yl acetate, 21,24-epoxy,23α,25-dihydroxy-4α,4β,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3α-benzoyl-7α-yl acetate; 21,23-epoxy-21,24,25-trihydroxy-4α,4β,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3α-cinnamoyl-7α-yl acetate and 21,23-epoxy-21,24,25-trihydroxy-4α,4β,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3α-benzoyl-7α-yl acetate. A hexane extract of the bark yielded 6α-acetoxy-obacunone acetate.

INTRODUCTION

Dysoxylum muelleri is an Australian member of the Meliaceae family. Since the isolation of glabretal (**1c**) from *Guarea glabra* [1], compounds with the 14,18-cycloapoeuphane-type skeleton have been found in several Meliaeae species [2–5] and also in members of the Simaroubaceae [6] and Rutaceae [7]. These compounds are readily distinguished by the presence of a pair of doublets (J = 5.5 Hz) between δ 0.35 and 0.7 in the ^{1}H NMR spectrum ascribable to two protons at C-18 and a triplet ascribable to C-18 between δ 13 and 17 in the ^{13}C NMR spectrum [4]. Plant material was provided by Prof. D.A.H. Taylor, and a specimen is deposited in the Forest Herbarium, Oxford (DAHT 329).

RESULTS AND DISCUSSION

The wood and bark of *D. muelleri* were milled and extracted separately in a Soxhlet apparatus with refluxing hexane. The resulting extracts were separated over silica gel using column chromatography, yielding the dysoxins 1–3 from the wood and a limonoid from the bark.

Dysoxins 1–3 all possessed the 14,18-cycloapoeuphane-type nucleus indicated by a pair of doublets, each doublet integrating for one proton, at δ 0.35 and 0.69 (J = 5.5 Hz) in the ^{1}H NMR spectra. However, the compounds differed in the structures of the side chains. All three compounds occurred as the 7α-acetate, indicated by the H-7β broad one-proton singlet at δ 5.02 ($W_{1/2}$ = 8 Hz) and as a mixture of cinnamate and benzoate esters at C-3α. We were not able to separate the cinnamate from the benzoate ester using column chromatography. The ratio of the cinnamate to the benzoate ester could be estimated from the ratio of the integral of the H-3β broad singlet for the cinnamate ester, which differed slightly in chemical shift from that of the benzoate ester (δ 4.75 vs. 4.86). The sum of the integrals of the two H-3β resonances was equivalent to one proton. Thus, the components of each mixture will be referred to as (**a**) and (**b**).

Dysoxin 1a (**1a**), $C_{41}H_{54}O_6$ (cinnamate ester, 65%) and dysoxin 1b (**1b**) $C_{39}H_{52}O_6$ (benzoate ester, 35%) only differed from **1c** in the esters present at C-3α. The compounds possessed the melianone-type side chain, as in **1c**, as was evident by resonances at δ 5.42 (H-21), 3.86 (*m*, H-23) and a pair of doublets at δ 2.80 and 2.67 (J = 7.5 Hz) ascribable to H-24 in the two epimers. The hemiacetal ring opens and closes in solution; thus, a mixture of epimers is always present, resulting in complex ^{13}C NMR spectra. However, as one epimer is usually more stable, resonances due to the major and minor epimers can be deduced. Compounds **1a** and **1b** are thus 3α-cinnamoyl glabretal and 3α-benzoyl glabretal, respectively.

The ^{1}H NMR spectra of dysoxins **2a**, $C_{41}H_{56}O_7$ (cinnamate ester, 65%) and **2b**, $C_{39}H_{54}O_7$ (benzoate ester, 35%) indicated that these compounds differed from **1a** and **1b** only in the structure of the side chain. A double bond equivalence calculation indicated that there was only one ring in the side chain. Instead of the H-21 resonance at δ 5.42 in **1a** and **1b** and the C-21 resonance at δ 98, a pair of doublets was seen at δ 4.09 and 3.41 (each 1H, J = 12 Hz) corresponding to a triplet at δ 70.8 in the ^{13}C NMR spectrum. Thus a -CH_2-O group was present at C-21. The COSY spectrum indicated that H-24 (*d*, δ 2.84, J = 10 Hz) was coupled with H-23 (*m*, δ 3.85). H-23 was in turn coupled to the two H-22 protons (δ 1.48 and 1.95), which were in turn coupled to H-20 (δ 1.95). H-20 was

Table 1. ^{1}H NMR data for dysoxins **1(a, b)**, **2(a, b, c, d)** and **3(a, b, c, d)** (300 MHz) $CDCl_3$, δ rel. to TMS = 0, couplings (in parentheses) in Hz

	Compound						
Proton	**1a/b**	**2a/b**	**2c**	**2d**	**3a/b**	**3c**	**3d**
H-3β	4.86/4.75 *bs*	4.86/4.75 *bs*	4.86/4.75 *bs*	4.86/4.75 *bs*	4.86/4.75 *bt*	4.86/4.75 *bt*	4.86/4.75 *bt*
H-7β	5.03 *bt* ($W_{1/2}$ = 8)	5.03 *bt* ($W_{1/2}$ = 8)	5.02 *bt* ($W_{1/2}$ = 8)	5.03 *bt* ($W_{1/2}$ = 8)	5.03 *bt* ($W_{1/2}$ = 8)	5.02 ($W_{1/2}$ = 8)	5.03 ($W_{1/2}$ = 8)
H-18	0.69 d (5.5)	0.69 d (5.5)	0.69 d (5.5)	0.69 d (5.5)	0.69 d (5.5)	0.69 d (5.5)	0.69 d (5.5)
	0.35 *d* (5.5)	0.35 *d* (5.5)	0.35 *d* (5.5)	0.35 *d* (5.5)	0.35 *d* (5.5)	0.35 *d* (5.5)	0.35 *d* (5.5)
H-21	5.42 *m**	4.09 *d* (10)	4.09 *d* (10)	4.09 *d* (10)	5.32 *m**	6.17 *m**	6.14 *m**
		3.41 *d* (10)	3.48 *d* (10)	3.48 *d* (10)			
H-23	3.86 *m* ($W_{1/2}$ = 15)	3.85 *m* ($W_{1/2}$ = 15)	4.92 *m* ($W_{1/2}$ = 15)	4.92 *m* ($W_{1/2}$ = 15)	4.45 *m* ($W_{1/2}$ = 15)	4.50 *m* ($W_{1/2}$ = 15)	4.30 *m* ($W_{1/2}$ = 15)
H-24	2.80 *d* (7.5)	2.84 *d* (10)	3.14 *d* (10)	3.33 *d* (10)	3.14 *d* (7.5)	4.82 *d* (7.5)	5.37 *d* (7.5)
	(2.67 *d*)†						
3H-26, 27	1.25, 1.20	1.28, 1.23	1.20, 1.16	1.50, 1.45	1.28, 1.25	1.28, 1.18	1.56, 1.45
Me	1.10, 0.92,	1.14, 0.95,	1.13, 0.95,	1.20, 1.15,	1.10, 0.93,	1.10, 0.93,	1.10, 0.92,
	0.91, 0.81	0.94, 0.83	0.94, 0.84	1.14, 0.95	0.92, 0.83	0.90, 0.80	0.90, 0.81
O-CO-Me	2.05 *s*	2.05 *s*	2.05, 2.00 *s*	2.05, 2.00, 1.95 *s*	2.05 *s*	2.15, 2.06, 2.04 *s*	2.11, 2.06,
							2.04, 1.98
Cinnamate ester		Benzoate ester					
Proton		Proton					
2	6.44 *d* (16)	3, 7	8.04 *d* (7.6)				
3	7.66 *d* (16)	4, 5, 6	7.53 *m*				
5, 9	8.06 *d* (7.6)						
6, 8	7.44 *m*						
7	7.37 *m*						

*Superimposed H-21 doublets from major and minor epimers.

†H-24 for minor epimer.

coupled to the two H-21 protons (δ 4.09 and 3.41). From the above evidence it was not clear whether C-21 was linked to C-23 or C-24 to give a cyclic ether to satisfy the double bond equivalence calculation. Acetylation with acetic anhydride–pyridine yielded **2c**. The ^{1}H NMR spectrum indicated no shift of the H-21 protons while the H-24 resonance had shifted slightly from δ 2.84 to 3.15 and the H-23 multiplet had shifted from δ 3.85 to 4.95. Thus, C-21 and C-24 were joined via an ether linkage, and a hydroxyl group was situated at C-23. The molecular formula indicated that a further hydroxyl group had to be placed in the side chain. A singlet occurred at δ 74.2 in the ^{13}C NMR spectrum. This was assigned to C-25 and the hydroxyl group was placed at C-25. To confirm this, acetylation using acetic anhydride–pyridine–*N,N*-dimethylaminopyridine was performed yielding compound **2d**. An additional acetate group methyl proton resonance was observed at δ 1.95 in the ^{1}H NMR spectrum of **2d**, and H-24 was shifted downfield from δ 3.14 to 3.33. No other changes were noted in the ^{1}H NMR spectrum, so the placement of the extra hydroxyl group at C-25 was confirmed. This side chain has been found previously in bourjotinolone A [8], and comparison of the coupling constants and bandwidths confirmed the same stereochemistry as bourjotinolone A as shown in **2a** and **2b**. This is the first report of a compound with the 14,18-cyclopropane ring in which the bourjotinolone A-type side chain is present.

High resolution mass spectroscopy indicated that dysoxin **3a** had a molecular formula of $C_{41}H_{56}O_7$ (cinnamate ester, 65%) and dysoxin **3b**, $C_{39}H_{54}O_7$ (benzoate ester, 35%). The presence of a hemiacetal ring in the side chain as in **1a** and **1b** was indicated by a major peak in the ^{13}C NMR spectrum at δ 98 and a minor one at δ 102 ascribable to C-21 in the two epimeric forms. However, the molecular formula indicated only one ring present in the side chain, and an extra H_2O. Acetylation with acetic anhydride–pyridine resulted in the formation of two additional acetate groups, the acetate methyl group proton resonances appearing at δ 2.15 and 2.04, H-21 shifted downfield from δ 5.32 to 6.17 and H-24 from δ 3.14 to 4.82. After acetylation using *N,N*-dimethylaminopyridine a further acetate group methyl proton resonance appeared at δ 1.93. Thus, the 24,25-epoxide of glabretal has been opened in these compounds to give the 24,25-diol. Thus, structures **3a** and **3b** are assigned to dysoxin 3a and 3b, and **3c** and **3d** to the two acetylated derivatives. The ^{1}H and ^{13}C NMR resonances for dysoxins 1–3 were assigned using 2D NMR techniques and by comparison with published data for skimmiarepin A [7] and are given in Tables 1 and 2.

The wood yielded the known limonoid, 6α-acetoxyobacunol acetate (**4**). Its structure was determined using mass spectral and 2D NMR spectroscopy. This compound has been isolated previously from a Fijian *Dysoxylum* species, *D. richii* [9].

EXPERIMENTAL

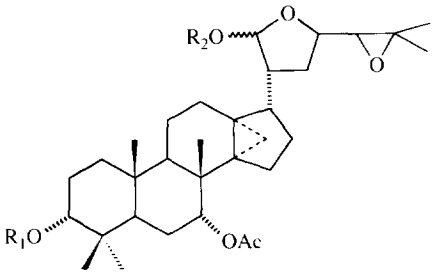
The wood (296 g) and bark (79.3 g) of *D. muelleri* was milled and extracted with refluxing hexane, yielding a wood (13.5 g) and a bark (4.6 g) extract. Re-

Table 2. ^{13}C NMR data for dysoxins **1a**, **2a** and **3a** (75 MHz), $CDCl_3$, δ rel. to TMS = 0

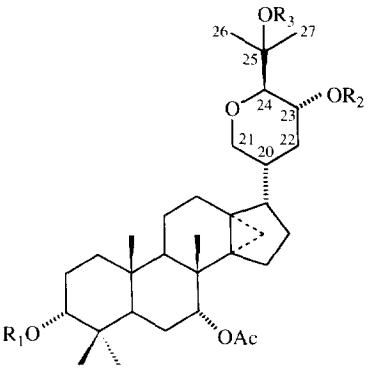
C	1a	2a	3a	C	1a	2a	3a
1	34.2 *t*	34.6 *t*	34.1 *t*	17	45.3 *d*	45.5 *d*	45.6 *d*
2	26.6 *t*	25.5 *t**	26.6 *t**	18	16.5 *t*	16.5 *t*	16.5 *t*
3	78.9 *d*	78.9 *d*	78.9 *d*	19	16.0 *q*	16.1 *q*	16.0 *q*
4	37.2 *s*†	37.3 *s*†	37.2 *s*†	20	49.6 *d*	46.0 *d*	49.0 *d*
5	42.4 *d*	42.4 *d*	42.4 *d*	21	98.3 *d*	70.8 *t*	98.0 *d*
6	24.5 *t*	24.1 *t*	26.2 *t*	22	29.6 *t*	36.8 *t*	29.6 *t*
7	76.8 *d*	76.3 *d*	76.4 *d*	23	78.4 *d*	64.9 *d*	78.6 *d*
8	38.3 *s*†	38.2 *s*†	38.1 *s*†	24	67.6 *d*	86.6 *d*	75.2 *d*
9	44.8 *d*	45.3 *d*	44.9 *d*	25	58.1 *s*	74.2 *s*	73.2 *s*
10	36.4 *s*†	36.4 *s*†	36.8 *s*	26	25.0 *q*	28.9 *q*‡	28.9 *q*‡
11	16.8 *t*	17.2 *t*	16.7 *t*	27	19.7 *q*	28.0 *q*‡	28.0 *q*‡
12	23.1 *t*	23.1 *t*	22.7 *t*	28	28.0 *q*	29.7 *q*‡	29.1 *q*‡
13	29.2 *s*	29.0 *s*	29.4 *s*	29	22.7 *q*	21.5 *q*	21.5 *q*
14	37.1 *s*†	37.1 *s*†	37.1 *s*†	30	19.2 *q*	20.0 *q*	19.6 *q*
15	25.0 *t**	26.2 *t**	26.5 *t**	OCOMe	27.8 *q*	27.8 *g*	27.8 *g*
16	27.7 *t**	27.7 *t**	26.7 *t**	OCOMe	165.4 s	165.4 s	165.4 s
Cinnamate ester				Benzoate ester			
1	169.9 *s*			1	166.5 *s*		
2	118.8 *d*			2	131.0 *s*		
3	144.3 *d*			3, 7	128.1 *d*		
4	134.5 *s*			4, 6	128.5 *d*		
5, 9	129.4 *d*			5	129.0 *d*		
6, 8	130.2 *d*						
7	132.8 *d*						

*,†,‡Values may be interchanged within the same column.

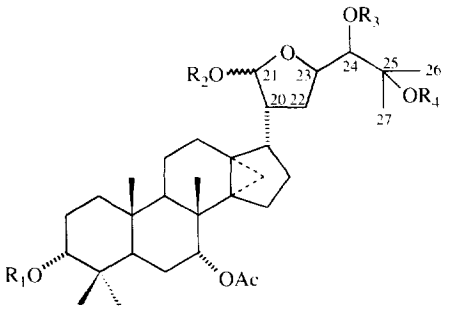
Resonance for the major ester (cinnamate) and major epimer have been reported.



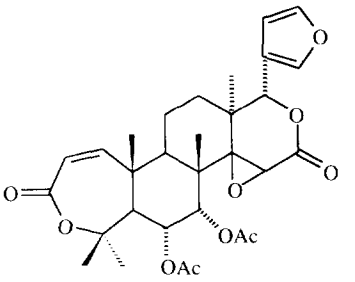
	R_1	R_2
1a	Cinn	H
1b	Benz	H
1c	H	H



	R_1	R_2	R_3
2a	Cinn	H	H
2b	Benz	H	H
2c	Cinn/Benz	Ac	H
2d	Cinn/Benz	Ac	Ac



	R_1	R_2	R_3	R_4
3a	Cinn	H	H	H
3b	Benz	H	H	H
3c	Cinn/Benz	Ac	Ac	H
3d	Cinn/Benz	Ac	Ac	Ac



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peated flash CC over silica gel (Merck 9385) using varying ratios of EtOAc and CH_2Cl_2 yielded mixts of **1a** and **1b**, **2a** and **2b**, and **3a** and **3b** from the wood and 6α-acetoxy-obacunone acetate from the bark. NMR spectra were recorded at room temp. on a Varian Gemini 300 MHz spectrophotometer in $CDCl_3$, IR spectra were recorded on a Mattison FTIR spectrophotometer and HRMS was performed on a Finnigan 1020 GC-MS spectrophotometer.

Dysoxin 1. Dysoxin 1 (50 mg) consisted of a mixt. of cinnamate (65%) and benzoate esters (35%) at C-3α.

Dysoxin 1*a* (**1a**). 21,23; 24,25-Diepoxy-21-hydroxy-4α,4β,8β - trimethyl - 14,18 - cyclo - 5α,13α,14α,17α - cholestan-3α-cinnamoyl-7α-yl acetate.

Dysoxin 1b (**1b**). 21,23; 24,25-Diepoxy-21-hydroxy-4α,4β,8β - trimethyl - 14,18 - cyclo - 5α,13α,14α,17α - cholestan - 3α - benzoyl - 7α - yl acetate. HRMS: $[M-H_2O]^+$ at *m/z* 642.3840 ($C_{41}H_{54}O_6$ requires 642.3922), EIMS: cinnamate ester: *m/z* 660 $[M]^+$, 642 $[M-H_2O]^+$; benzoate ester: *m/z* 634 $[M]^+$, 616 $[M-H_2O]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2900, 1730, 1700, 1530, 1460, 1370, 1260.

Dysoxin 2. Dysoxin 2 (35 mg) consisted of a mixt. of cinnamate (65%) and benzoate esters (35%) at C-3α.

Dysoxin 2*a* (**2a**). 21,24-Epoxy-23α,25-dihydroxy-4α,4β,8β - trimethyl - 14,18 - cyclo - 5α,13α,14α,17α - cholestan-3α-cinnamoyl-7α-yl acetate.

Dysoxin 2*b* (**2b**). 21,24-Epoxy-23α,25-dihydroxy-4α,4β,8β - trimethyl - 14,18 - cyclo - 5α,13α,14α,17α - cholestan-3α-benzoyl-7α-yl acetate. HRMS: $[M]^+$ at *m/z* 662.3424 ($C_{41}H_{58}O_7$ requires 662.4189). IR $\nu_{max}^{CHCl_3}$ cm $^{-1}$: 3400, 2900, 1730, 1700, 1500, 1460, 1360, 1260.

Dysoxin 3. Dysoxin 3 (40 mg) consisted of a mixt. of cinnamate (65%) and benzoate (35%) esters.

Dysoxin 3*a* (**3a**). 21,23-Epoxy-21,24,25-trihydroxy-4α,4β,8β - trimethyl - 14,18 - cyclo - 5α,13α,14α,17α - cholestan-3α-cinnamoyl-7α-yl acetate.

Dysoxin 3*b* (**3b**). 21,23-Epoxy-21,24,25-trihydroxy-4α,4β,8β - trimethyl - 14,18 - cyclo - 5α,13α,14α,17α -

cholestan-3α-benzoyl-7α-yl acetate. HRMS: $[M-H_2O]^+$ at m/z 660.3958 ($C_{41}H_{56}O_7$ requires 660.4028) (cinnamate ester); $[M-H_2O]^+$ at m/z 634.3864 ($C_{39}H_{54}O_7$ requires 634.3871) (benzoate ester). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2900, 1730, 1700, 1500, 1460, 1360, 1260.

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