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NORDITERPENE DILACTONES FROM *PODOCARPUS SALIGNA*

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Key Word Index—*Podocarpus saligna*; Podocarpaceae; rimuene; nubigenol; norditerpene dilactones; salignones K, L and M.

Abstract—Rimuene and nubigenol have been identified in *Podocarpus saligna* for the first time, and the structures of three new norditerpene dilactones, salignones K, L and M, isolated from this species have been elucidated.

INTRODUCTION

Podocarpus saligna D. Don is one of three *Podocarpus* species growing in Chile, the other two being *P. andina* and *P. nubigena*. Norditerpene dilactones [1–5] are widespread in the Podocarpaceae and we recently reported [6] the structure elucidation of three norditerpenes, salignone A (**1**), salignone B (**2**) and salignone J (**3**) from *P. saligna*. This work describes the further investigation of chemical constituents of *P. saligna*, including the structure elucidation of three more new norditerpene dilactones.

RESULTS AND DISCUSSION

Successive extractions of leaves and stems of *P. saliga* were made with petrol (60–80°), ethyl acetate and chloroform. Chromatography of the petrol extract on a silica column furnished three principal fractions, the least polar containing the hydrocarbon *n*-nonacosane ($[M]^+$ at *m/z* 408, $C_{29}H_{60}$), which has previously been observed in a benzene extract of *P. saligna* [7]. The mass spectrum of this fraction also showed weaker peaks at *m/z* 422 and 436, suggesting the presence of the homologous $C_{30}H_{62}$ and $C_{31}H_{64}$ hydrocarbons. The second fraction was identified by TLC, GLC, ^{1}H NMR and mass spectral fragmentation patterns [8–11] as rimuene (**4**). This diterpene has previously been observed in *P. andina* and *P. nubigena* [12], as well as in other *Podocarpus* species [13, 14], but has not hitherto been reported in *P. saligna*. The most abundant and most polar component isolated from the petrol extract was identified as hydroquinone by TLC, IR, ^{1}H NMR and mmp. The presence of hydroquinone in *P. saligna* has not been reported previously.

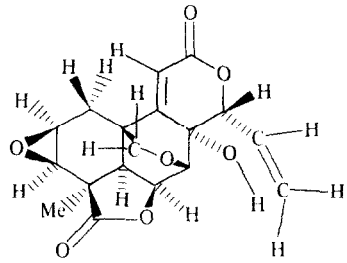
The ethyl acetate extract of *P. saligna* yielded a dihydrochalcone identified as nubigenol (**5**) by mp, ^{1}H NMR, EIMS and conversion to the corresponding pentaacetate as described previously [15]. Nubigenol was first isolated from *P. nubigena* [15] and this is the first record of its observation in *P. saligna*.

The chloroform extract was fractionated by chromatography on a silica gel column followed by preparative TLC on silica gel plates. Since this failed to give pure components, attention was directed to the use of HPLC, which has proved to be a powerful technique for the analysis and purification of natural products [16, 17] but had not hitherto been applied to norditerpene dilactones. In view of the short wavelength chromophore which these compounds possess (Type B dilactones show λ_{max} close to 220 nm), a variable wavelength detector was employed. It was found that a silica gel column combined with a mobile phase of hexane–isopropanol–acetonitrile (85:12:3) gave sharp peaks with a minimum of tailing. Each band isolated from preparative TLC of the chloroform extract was analysed using this HPLC system and then purified by larger scale injections of up to 1–2 mg on the analytical column. Collected fractions were re-analysed by HPLC to check purity and submitted to IR, UV, MS and high field ^{1}H NMR, leading to the identification of three new norditerpene dilactones.

The three new compounds all had λ_{max} values at 220–221 nm, characteristic of Type B norditerpene dilactones. Their structures (**6–8**) were determined by analysis of their 400 MHz ^{1}H NMR spectra (Table 1). Compound **6**, named salignone K, showed many similarities to compound **3** [6] in its ^{1}H NMR spectrum, the differences being accomodated by the presence of an additional 7β-hydroxyl group. Compound **7**, named salignone L, differs from compound **3** by the presence of an additional 1α-hydroxyl group. All of the A-ring hydroxyepoxides so far reported in the literature have a 1,2β-epoxy, 3β-hydroxy arrangement and the NMR data reported for about a dozen compounds of this type [1, 18–27] show that they all have chemical shifts and coupling constants for the A-ring protons which are distinctly different to those of salignone L **7**. The ^{1}H NMR spectrum (Table 1) of the third new dilactone (**8**), named salignone M, showed it to have a vinyl group at C-14, a 7,8-epoxy group and a 2β-hydroxyl group. The pattern of signals for the A-ring protons in **8** closely resembled that reported [21] for 1-deoxy-2-hydroxy-nagilactone A and the position of the hydroxyl group was confirmed by double irradiation experiments.

EXPERIMENTAL

Mps were taken on a micro hot-stage apparatus and are uncorr. ^{1}H NMR spectra were recorded on a Brucker WH400 in



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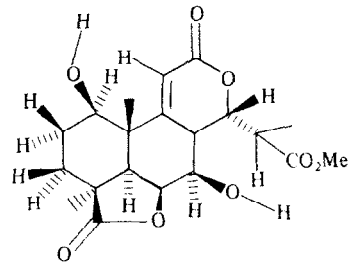

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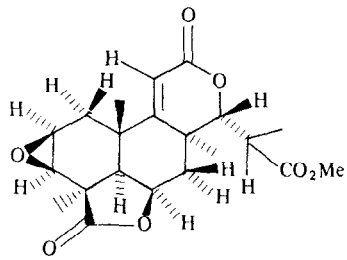

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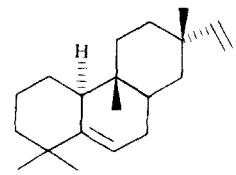

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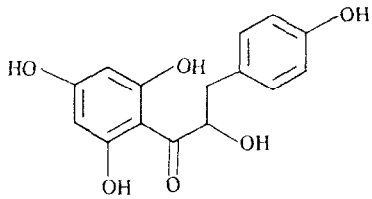

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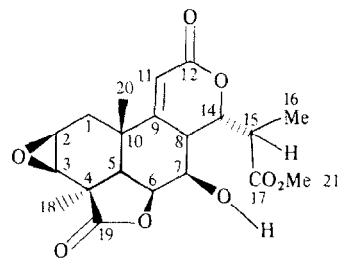

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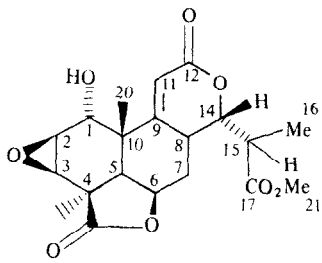

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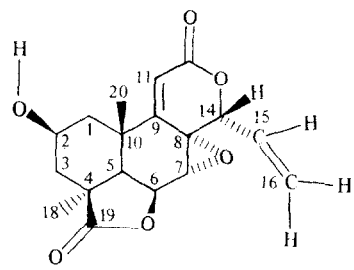

8

$CDCl_3$ with TMS as internal standard. The HPLC employed a 25 cm × 4.5 mm i.d. column of Hypersil 5 μm spherical silica, and a Cecil 2012 variable wavelength detector set at 222 nm.

Salignone K (**6**). Mp 233–242°; λ_{max} (EtOH) 220 nm, log ε 4.04; $[M]^+$ at *m/z* 392, $C_{20}H_{24}O_8$. See Table 1 for ^1H NMR.

Salignone L (**7**). Mp 222–225°; λ_{max} (EtOH) 220 nm, log ε 4.03; $[M]^+$ at *m/z* 392, $C_{20}H_{24}O_8$. See Table 1 for ^1H NMR.

Salignone M (**8**). Mp 270° (dec); λ_{max} 221 nm; $[M]^+$ at *m/z* 332, $C_{18}H_{20}O_4$. See Table 1 for ^1H NMR.

Acknowledgements—We thank Dr. B. Mann of the SERC High Field NMR Service, Sheffield University, for the ^1H NMR

Table 1. 400 MHz ^{1}H NMR spectra of salignones K, L and M in $CDCl_3$

	Salignone K (6)		Salignone L (7)		Salignone M (8)	
Proton	δ	J (Hz)	δ	J (Hz)	δ	J (Hz)
1α	1.73	$J_{1\alpha,1\beta} = 15$ $J_{1\alpha,2} = 0$			2.31 *dd*	$J_{1\alpha,1\beta} = 14$
1β	2.47	$J_{1\beta,1\alpha} = 15$ $J_{1\beta,2} = 2.1$	4.07 *bs*	$J_{1,2} \leqslant 2$	2.38 *dd*	$J_{1\alpha,2} = 10$ $J_{1\beta,1\alpha} = 14$ $J_{1\beta,2} = 10$
2α	3.41 *m*		3.39 *bd*	$J_{2,1} \leqslant 2$ $J_{2,3} = 4$	4.06 *m*	
3α	3.20 *d*	$J_{3,2} = 3.6$	3.33 *d*	$J_{3,2} = 4$	1.95 *dd*	$J_{3\alpha,2\alpha} = 5$ $J_{3\alpha,3\beta} = 14$
3β					2.18 *dd*	$J_{3\beta,2\alpha} = 10$ $J_{3\beta,3\alpha} = 14$
5α	1.67 *d*	$J_{5,6} = 5.7$	Hidden by H_2O		1.90 *d*	$J_{5,6} = 5$
6α	4.87 *dd*	$J_{6,5} = 5.7$ $J_{6,7\alpha} = 7.9$	4.95 *m*		4.93 *dd*	$J_{6,5} = 5$ $J_{6,7} = 2$
7α	4.78 *dd*	$J_{7\alpha,6} = 7.9$ $J_{7\alpha,8} = 4.0$	2.45 *m*			
7-OH	2.50 *bs*					
7β			2.34 *m*	$J = 8$ $J_{7\beta,7\alpha} = 15$	3.73 *d*	$J_{7,6} = 2$
8α	3.03 *ddd*	$J_{8,7\alpha} = 4.0$ $J_{8,11} = 2.9$ $J_{8,14} = 11.4$	2.85 *m*			
11	5.83 *d*	$J_{11,8} = 2.9$	6.72 *d*	$J_{11,8} \leqslant 3$	6.09 *s*	
14β	4.62 *dd*	$J_{14,8} = 11.4$ $J_{14,15} = 2.1$	4.31 *dd*	$J_{14,8} = 12$ $J_{14,15} = 2$	5.00 *d*	$J_{14,15} = 8$
15	3.10 *dq*	$J_{15,14} = 2.1$ $J_{15,16} = 7.1$	2.94 *m*		5.83 *ddd*	$J_{15,14} = 8$ $J_{15,16Z} = 10$ $J_{15,16E} = 17$
16	1.42 *d*	$J_{16,15} = 7.1$	1.36 *d*	$J_{16,15} = 6$		
16*Z*					5.53 *d*	$J_{16Z,15} = 10$
16*E*					5.51 *d*	$J_{16E,15} = 17$
18	1.55 *s*		1.25 *s*		1.31 *s*	
20			1.49 *s*		1.39 *s*	
21	3.73 *s*		3.73 *s*			

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