Isolation and Structure Determination of Norditerpene Dilactones from *Podocarpus saligna* D. Don

By **Stephen A. Matlin** * and **M. Amelia Prazeres,** Chemistry Department, The City University, Northampton Square, London EC1V 0HB
John D. Mersh and **Jeremy K. M. Sanders,*** University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW
Magalis Bittner and **Mario Silva,*** Laboratorio de Productos Naturales, Departamento de Botanica, Universidad de Concepción, Concepción, Chile

The structures of three norditerpene dilactone derivatives isolated from *Podocarpus saligna* have been determined by high field ^{1}H n.m.r. and nuclear Overhauser enhancement difference spectroscopy; the utility of fast atom bombardment mass spectroscopy for this class of compounds has also been established.

Podocarpus species contain a variety of nor- and bisnorditerpene dilactones which show a range of interesting biological properties, including anti-tumour activity and insecticidal behaviour.[1] A closely related series of compounds has also been discovered in strains of *Aspergillus* fungi.[2] As recently pointed out,[1a] the compounds are generally difficult to purify, are often available only in small amounts, and are frequently troublesome to characterise without recourse to *X*-ray crystallography.

During examination of a chloroform extract of the leaves and stems of *Podocarpus saligna* D. Don from Chile, which is known to contain several nor- and bisnorditerpene dilactones,[1a,b,3] a number of compounds were isolated in 2-mg quantities by preparative h.p.l.c. on silica. Complete characterisation of three of these compounds by a combination of high field ^{1}H n.m.r. and nuclear Overhauser enhancement (n.O.e.) difference spectroscopy is described in this paper.

The observation of n.O.e.s has hitherto played a minor role in confirming details of the structures of norditerpene lactones. Those instances of n.O.e.s reported in the literature are shown in Figure 1. As in other cases where n.O.e.s have been measured by conventional integration techniques, only peak enhancements of 8—10% or more have been detected and this has been the major factor limiting the utilisation of n.O.e.s in structure elucidation. The powerful new method of n.O.e. difference spectroscopy makes possible the detection of peak enhancements at levels well below 1%. N.O.e. difference spectroscopy has been used to assign the spectra of steroids [4,5] and to determine the structures of new penicillins,[6,7] alkaloids,[8] and porphyrins.[9] It has also proved a powerful method for elucidation of conformation in compounds as diverse as simple ethers,[10] steroids, [5] alkaloids,[11] and peptide antibiotics.[12]

Three compounds which we isolated from *P. saligna* were classified as belonging to subgroup B of the norditerpene lactones (*i.e.*, 4,5-dihydropyran ring c)[1c] by virtue of their characteristic u.v. spectra ($\lambda_{max.}$ close to 220 nm). One of these compounds, m.p. 280—289 °C, showed a molecular ion at *m/e* 346 ($C_{18}H_{18}O_7$) in its e.i. mass spectrum. Its 400 MHz ^{1}H n.m.r. spectrum was recorded in [$^2H_5$]pyridine and extensive decoupling and n.O.e. difference measurements were performed, allowing the structure (1) to be assigned to it.

This structure notably contains an oxymethylene bridge on the β-face between C-7 and C-10. The two

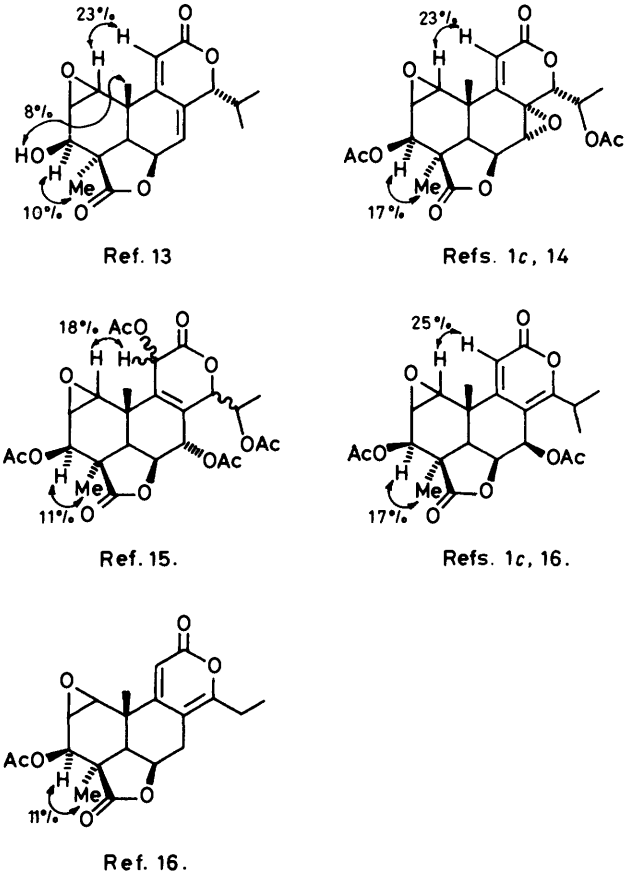
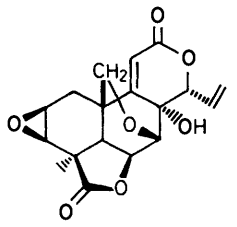
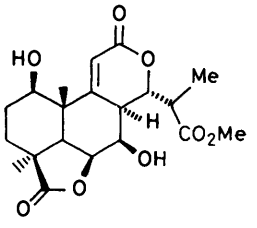


FIGURE 1. N.O.e.s reported to be observed by conventional methods

prochiral hydrogens of the bridging methylene group provide useful structural probes. The *pro-S* hydrogen (20*S*-H, δ 2.98) shows a long range (W) coupling to 5-H. It also has an n.O.e. interaction with 14-H, thus defining the α-orientation of the vinyl group attached to C-14.



(1)



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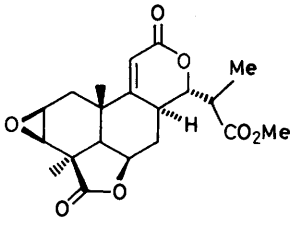


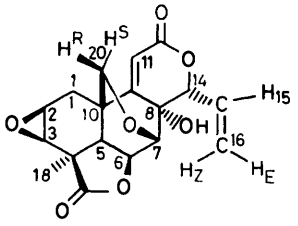
(3)

The α-orientation of the C-8 OH is revealed by its n.O.e.s with 6-H and 7-H.

The *pro-R* hydrogen, (20*R*-H, δ 4.46) receives an n.O.e. on irradiation of the 2,3-epoxy-group protons. Initially, this appears to suggest that the orientation of the epoxy-group is α. However, on examining molecular models, it becomes apparent that there is very little change in the spatial positions of the 2-H and 3-H protons between the α- and β-epoxy-group configurations and *both* isomers would be expected to show n.O.e.s to 20*R*-H. The α-epoxy-isomer should show slightly bigger n.O.e.s than the β-epoxy-isomer, but in the absence of both possible compounds for close comparison it was not possible to decide from the n.O.e. experiments which isomer we were dealing with. However, the stereochemistry of the epoxy-group could be determined from the observed coupling constants. In compounds containing a 2,3-epoxy-group with β-orientation,[17] the 1α- and 1β-protons both show characteristically small couplings (≤2.5 Hz) to the 2α-proton. No naturally occurring dilactone having a 2,3-epoxy-group with α-orientation has hitherto been isolated, but Hayashi *et al.*[18] have prepared two examples by the epoxidation of 2,3-olefinic dilactones. In both these compounds, the observed couplings (in [$^2H_5$]pyridine) were $J_{1\alpha,2\beta}$ 1.5 Hz and $J_{1\beta,2\beta}$ 6.0—6.5 Hz. These observations are consistent with the geometries revealed by molecular models, which show that in the 2,3-β-epoxide the 2α-proton sits gauche to both the 1α- and 1β-protons; whereas in the 2,3-α-epoxide the 2β-proton eclipses the

TABLE 1

400 MHz ^1H n.m.r. spectrum of compound (1) in [$^2H_5$]pyridine



(1)

Proton	δ (p.p.m.)	Multiplicity	*J* (Hz)	N.O.e.
1α	1.64	dd	$J_{1\alpha,1\beta}$ = 15.5	1β, 2, 5, 11
			$J_{1\alpha,2\alpha}$ = 1.5	
1β	1.75	dd	$J_{1\beta,1\alpha}$ = 15.5	1α, 2, 11, 20*S*
			$J_{1\beta,2\alpha}$ = 3.0	
2α	3.10 [a]	m	$J_{2\alpha,1\alpha}$ = 1.5	1α, 1β, 18, 20*R*
			$J_{2\alpha,1\beta}$ = 3.0	
			$J_{2\alpha,3\alpha}$ = 3.9	
3α	3.10 [a]	m	$J_{3\alpha,2\alpha}$ = 3.9	
5α	2.30	dd	$J_{5,6}$ = 7.3	1α, 6, 18
			$J_{5,20S}$ = 2.2	
6α	5.29 [b]	m	$J_{6,5}$ = 7.3	(5, 7, 18)
			$J_{6,7}$ = 3.7	
7α	4.27br	d	$J_{7,6}$ = 3.7	6, 14, 15
			$J_{7,11}$ = *c*	
8-OH	8.37br	s		5, 6, 15
11	5.90	s	$J_{11,7}$ = *c*	1α, 1β, 20*S*
14β	4.97br	d	$J_{14,15}$ = 8.0	7, 15, 16*Z*
15	6.13	ddd	$J_{15,14}$ = 8.0	14, 16*Z*(−ve), 16*E*
			$J_{15,16E}$ = 10.5	
			$J_{15,16Z}$ = 17.3	
16*Z*	5.29 [b]	dd	$J_{16Z,15}$ = 17.3	(15, 16*E*)
			$J_{16Z,16E}$ = 1.7	
16*E*	5.15	dd	$J_{16E,15}$ = 10.5	7, 15, 16*Z*
			$J_{16E,16Z}$ = 1.7	
18	1.10	s		3, 6
20*R*	4.46	d	$J_{20R,20S}$ = 9.8	20*S*
20*S*	2.98	dd	$J_{20S,5}$ = 2.2	1β, 11, 14, 20*R*
			$J_{20S,20R}$ = 9.8	

[a,b] Pairs of signals irradiated together since superimposed. [c] Unresolved coupling present.

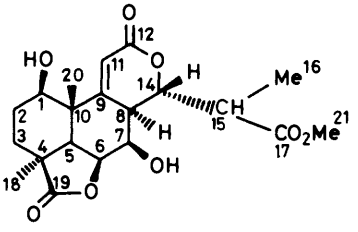
1β-proton and sits at a dihedral angle of about 120° to the 1α-proton, thus giving rise to two distinctly different coupling constants. As seen in Table 1, the observed coupling constants of 3 Hz and 1.5 Hz are consistent with a 2,3-epoxy-group having β-orientation and hence the structure of the compound is that shown in (1).

[$^2H_5$]pyridine and the results of decoupling and n.O.e. difference spectroscopy (Table 2) allow the structure of this compound to be assigned unambiguously as (2).

The CH(Me)CO_2Me side-chain was recognised by the presence of a characteristic doublet at δ 1.12 and a singlet at δ 3.33 for the two methyl groups. An n.O.e. at the

TABLE 2

400 MHz ^1H n.m.r. spectrum of compound (2) in [$^2H_5$]pyridine



(2)

Proton	δ (p.p.m.)	Multiplicity	J (Hz)	N.O.e.
1α	3.65	d	$J_{1\alpha,2\alpha}$ = 7	2α, 2β, 5, 11
1-OH	6.69br	s		1α, 2β, 11, 20
2α	1.67	ddt	$J_{2\alpha,1\alpha}$ = 7	
			$J_{2\alpha,2\beta}$ = 15	
			$J_{2\alpha,3}$ = 5	
2β	1.52	ddt	$J_{2\beta,2\alpha}$ = 15	1α, 1-OH, 3β
			$J_{2\beta,3\alpha}$ = 3	
			$J_{2\beta,3\beta}$ = 5	
3α	1.03	m		
3β	2.20	dt	$J_{3\beta,3}$ = 5	3α, 20
			$J_{3\beta,3\alpha}$ = 13	
5α	1.45	d	$J_{5,6}$ = 6	1α, 2α, 6, 8, 18
6α	4.78	m		5, 18
7α	4.78	m		8, 16
7-OH	7.80	d	$J_{7OH,7\alpha}$ = 2	14, 20
8α	3.05	dt	$J_{8,7}$ = 3	5
			$J_{8,11}$ = 3	
			$J_{8,14}$ = 11	
11	7·14	d	$J_{11,8}$ = 3	1α, 20
14β	4.78	m		15, 20, 21
15	3.05	dq	$J_{15,14}$ = 2	14, 16
			$J_{15,16}$ = 7	
16	1.12	d	$J_{16,15}$ = 7	8α/15, 14, 21
18	0.90	s		5, 6
20	1.70	s		1α, 2β, 3β, 7-OH, 11
21	3.33	s		

A sample isolated previously from *P. saligna* by Bittner and Silva has been characterised by Watson and Zabel using *X*-ray crystallography and its structure (1) was reported recently.[1b] Professor Watson kindly provided the residue of his *X*-ray sample and its 400 MHz ^1H n.m.r. spectrum proved to be identical with that of our own compound, confirming its assignment as (1), named salignone A. It can be seen that high field n.m.r. together with decoupling and n.O.e. difference spectroscopy is a technique which rivals *X*-ray crystallography in its power for structure elucidation.

The second compound isolated, m.p. 233—240 °C, showed a peak at highest mass m/z 376 ($C_{20}H_{24}O_7$) in its e.i. mass spectrum. However, since the ^1H n.m.r. spectrum revealed this compound to be a diol and to have a CH(Me)CO_2Me side chain, it was deduced that the true molecular ion (m/z 394, $C_{20}H_{26}O_8$) was not being seen, but was undergoing the expected fragmentations to the observed ions at m/z 376 (M − H_2O) and 307 [100%; M − CH(Me)CO_2Me]. The 400 MHz ^1H n.m.r. spectrum in C-20 methyl group on irradiation of 14-H places these two in a *cis* relationship and hence the C-14 side chain is α. The 7-OH group is defined as β by its n.O.e. on 14-H and 20-Me. The 1-OH group gives an n.O.e. at 20-Me, whilst the 1-H gives an n.O.e. to the 5α-H; hence the 1-OH group has a β-orientation.

This structure has not been reported in the literature, but we have been informed by Watson and Silva that a compound of this structure (m.p. 230—233 °C) has been identified by *X*-ray crystallography in their parallel work on *P. saligna* and was named salignone B by them.*

The third compound did not produce a molecular ion in its e.i. mass spectrum, even at 20 eV, showing as the

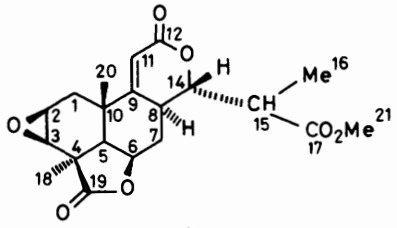
* A preliminary report on the identification of salignones A to H was presented by W. H. Watson in a lecture at the 12th International Symposium on the Chemistry of Natural Products, Tenerife, Canary Islands, Spain, 21—27th September 1980. The structure determination of salignones A and H is described in ref. 1*b* and that of salignone D in ref. 1*a*. Full details of the *X*-ray structures of the remainder of this series have not yet been published. Sellowin A was also found in the roots of *P. saligna*.

highest mass fragment an ion at m/z 289 ($C_{16}H_{17}O_5$). The presence of a CH(Me)CO_2Me side chain was recognised from the n.m.r. spectrum and suggested that the expected molecular ion would be at m/z 376 ($C_{20}H_{24}O_7$). The loss of substituents and consequent failure to produce observable molecular ions in the e.i. spectra is characteristic of the behaviour of norditerpene dilactones and has presented a serious obstacle to their spectroscopic characterisation. We have therefore made a preliminary evaluation of fast atom bombardment (f.a.b.) ionisation mass spectrometry. This is a new 'soft' ionisation technique which, like the related method of field desorption mass spectrometry, tends to produce intense pseudo-molecular ions ($M + 1$) with compounds which fail to give molecular ions by the e.i. method.[19] When the third compound isolated was submitted to f.a.b. mass spectrometry, a strong ion at m/z 377 was observed, in agreement with the formula deduced above. F.a.b. mass spectrometry is expected to play an important role in structure elucidation work in this field in the future.

Structure (3) was assigned to the compound on the basis of the 400 MHz ^{1}H n.m.r. data and n.O.e. difference spectroscopy results obtained (Table 3). The B-ring lacks oxygen substituents and shows a complex coupling pattern of the 6-H, 7-H, and 8-H protons. The presence of an epoxy-group on the A-ring was deduced from the appearance of characteristic signals at δ 3.04 and 3.10. The location of the epoxy-group at the 2,3-position follows from the observation of an n.O.e. between 3-H and the 18-Me group. The β-orientation of the 2,3-epoxy-group was defined by the coupling constants (*cf.*, salignone A) of 0 and 2.2 Hz between the 2α-H and the 1α-H respectively. The 400 MHz ^{1}H n.m.r. spectrum of this compound was also recorded in deuteriochloroform and some decoupling experiments were carried out on this solution (Table 3). Irradiation of H-5α caused H-6α to

TABLE 3

400 MHz ^{1}H n.m.r. spectrum of compound (3) in [$^2H_5$]pyridine and in deuteriochloroform



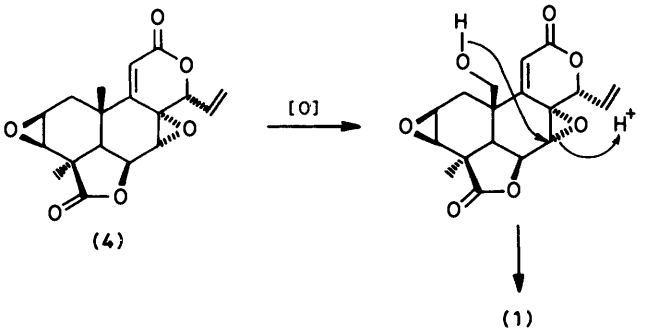
(3)

	In C_5D_5N				In $CDCl_3$			
Proton	δ (p.p.m.)	Multiplicity	J (Hz)	N.O.e.	Proton	δ (p.p.m.)	Multiplicity	J (Hz)
1α	1.35	d	$J_{1\alpha,1\beta}$ = 13.0 $J_{1\alpha,2\alpha}$ = 0	1β, 2, 11	1α	1.77	d	$J_{1\alpha,1\beta}$ = 14.5 $J_{1\alpha,2}$ = 0
1β	2.11	dd	$J_{1\beta,1\alpha}$ = 13.0 $J_{1\beta,2\alpha}$ = 2.2	1α, 2, 11, 20	1β	2.45	dd	$J_{1\beta,1\alpha}$ = 14.5 $J_{1\beta,2}$ = 2.5
2α	3.10	dd	$J_{2\alpha,1\alpha}$ = 0 $J_{2\alpha,1\beta}$ = 2.2 $J_{2\alpha,3\alpha}$ = 3.4	1α, 1β	2α	3.43	m	
3α	3.04	d	$J_{3\alpha,2\alpha}$ = 3.4	18	3α	3.20	d	$J_{3,2}$ = 3.8
5α	1.48	d	$J_{5,6}$ = 5.4	6, 8	5α	1.65	d	$J_{5,6}$ = 6
6α	4.9	m	$J_{6,5}$ = 5.4 $J_{6,7\alpha}$ = 4.4 $J_{6,7\beta}$ = 6.9		6α	4.98	ddd	$J_{6,5}$ = 6 $J_{6,7\alpha}$ = 10 $J_{6,7\beta}$ = 7.5
7α	2.35	m	$J_{7\alpha,6}$ = 4.4 $J_{7\alpha,7\beta}$ = 13.0 $J_{7\alpha,8}$ = 8.8 $J_{7\alpha,11}$ = 1.0	7β, (8 or 15 or both)	7α	2.48	ddd	$J_{7\alpha,6}$ = 10 $J_{7\alpha,7\beta}$ = 14 $J_{7\alpha,8}$ = 6
7β	1.63	m	$J_{7\beta,6}$ = 6.9 $J_{7\beta,7\alpha}$ = 13.0 $J_{7\beta,8}$ = 12.0	7α, 14, 20	7β	1.81	dt	$J_{7\beta,6}$ = 7.5 $J_{7\beta,7\alpha}$ = 14 $J_{7\beta,8}$ = 8
8α	2.82 [a]	m	$J_{8,7\alpha}$ = 8.8 $J_{8,7\beta}$ = 12.0 $J_{8,11}$ = 2.0 $J_{8,14}$ = 10.5	(5, 7α, 14, 16)	8α	2.90	m	$J_{8,7\alpha}$ = 6 $J_{8,7\beta}$ = 8 $J_{8,11}$ = 2.5 $J_{8,14}$ = 11.5
11	5.75	dd	$J_{11,7\alpha}$ = 1.0 $J_{11,8}$ = 2.0	1β	11	5.79	d	$J_{11,8}$ = 2.5
14β	4.20	dd	$J_{14,8}$ = 10.5 $J_{14,15}$ = 2.5	7β, 15	14β	4.33	dd	$J_{14,8}$ = 11.5 $J_{14,15}$ = 2.5
15	2.82 [a]	m	$J_{15,14}$ = 2.5 $J_{15,16}$ = 6·3	(5, 7α, 14, 16)	15	2.95	dq	$J_{15,14}$ = 2.5 $J_{15,16}$ = 7
16	1.18 [b]	d	$J_{16,15}$ = 6.3		16	1.37	d	$J_{16,15}$ = 7
18	1.15 [b]	s		1β, 3α, 5, 6, 7β, 15, 14	18	1.33	s	
20	1.20 [b]	s			20	1.50	s	
21	3.37	s			21	3.75	s	

[a,b] Groups of signals irradiated together since overlapping.

sharpen to a double doublet (7.5 and 10 Hz). Irradiation of 6α-H caused sharpening of 5α-H and 7β-H. Irradiation of 8α-H caused sharpening of 11-, 14-, 7α-, and 7β-H. Irradiation of 11-H caused 8-H to simplify and allowed the detection of a 6 Hz coupling in the latter ($J_{8,7\alpha}$) which was otherwise impossible to determine from the spectrum. A solvent effect is evident from comparison of the [$^2H_5$]pyridine and deteriochloroform spectra, the former giving downfield shifts of many of the signals. Conformational changes, especially about the B-ring, are indicated by alterations in the coupling constants around the 6-, 7-, and 8-positions.

Compound (3) is a new structure, not previously reported, for which we propose the name salignone J.



SCHEME

The use of [$^2H_5$]pyridine as solvent was important in the success of the n.O.e. experiments with hydroxy-protons: proton exchange was sufficiently inhibited to give separate, sharp signals for each different hydroxy-group and to allow the observation of n.O.e.s to and from these groups. Some saturation transfer between hydroxy-groups and water was observed, however, so exchange was not totally inhibited.

It is interesting that compounds (1)—(3) and all of the other compounds so far isolated from *P. saligna* [1,19] show a structural homogeneity, all belonging to the subgroup B type. Salignone A is unique amongst the norditerpene dilactones in being the first example to show oxidation of the 20-Me group. Biosynthetically, it appears to be derived by the hydroxylation of the bis-epoxide (4), sellowin B,[3] followed by internal attack of the hydroxymethyl-group on the ring-B epoxide (Scheme). Sellowin B has not yet been observed in *P. saligna*, but is known to occur in several other *Podocarpus* species.

EXPERIMENTAL

Preparative h.p.l.c. isolation of the norditerpene dilactones was carried out on columns of LiChroprep SI60 silica (15—25 μm) eluted with hexane–propan-2-ol–acetonitrile mixtures and monitored at 220 nm. Mass spectra were recorded on a high resolution MS30 (e.i.) in London and on an MS50 (f.a.b.) in Cambridge. ^1H n.m.r. spectra were recorded on Bruker WH400 spectrometers. N.O.e. difference experiments were carried out as described previously. Solution concentrations were less than 1 mg ml^{-1}.

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