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LIGNAN AND NORDITERPENE DILACTONE CONSTITUENTS OF *PODOCARPUS SALIGNA*

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Key Word Index—*Podocarpus saligna*; Podocarpaceae; roots; lignanolide; thujaplicatin methyl ether; norditerpene dilactone; salignone I; NOE difference spectroscopy.

Abstract—Thujaplicatin methyl ether and a new norditerpene dilactone named salignone I have been isolated from the roots of *Podocarpus saligna* and their structures determined by high field ^{1}H NMR and NOE difference spectroscopy.

INTRODUCTION

The observation of nuclear Overhauser enhancements (NOEs) in ^{1}H NMR spectroscopy provides information on the close spatial proximity of pairs of hydrogens which need not be coupled together, thus extending and enlarging upon the evidence for spatial connections and stereochemistry which can be gained from coupling constants alone [1]. Recently, the development of an FT NMR difference spectroscopy technique has made possible the reliable detection of NOEs down to levels well below 1% enhancement, thus considerably improving the power of the method [2–4]. Applications to numerous problems in the elucidation of structure and stereochemistry are now appearing in the literature [4–6].

We recently described the isolation and structure elucidation of several norditerpene dilactones from the leaves and stems of *Podocarpus saligna* from Chile [6, 7]. In continuation of our studies of this species we have isolated two polar compounds from a chloroform extract of the roots and here describe their identification and demonstrate how the application of NOE difference spectroscopy can play a crucial role in the structure elucidation process for natural products.

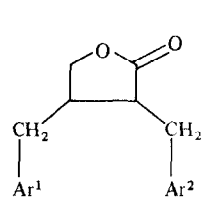
RESULTS AND DISCUSSION

A white solid was isolated from the roots of *P. saligna*, having ν_{max} 1765 cm^{-1} typical of a butyrolactone. The mass spectrum ([M]$^+$ 388) indicated a formula $C_{21}H_{24}O_7$, which was supported by the observation of 24 hydrogens in the 400 MHz ^{1}H NMR spectrum (Table 1). Analysis of the couplings visible in this spectrum established the presence of a butyrolactone ring substituted at the 2 and 3 positions by oxygenated benzyl functions. Thus, the partial structure **1** could be drawn. It was evident from the presence of a 2H singlet (δ6.32) that one of the two benzene rings was symmetrically trioxygenated, whereas the other ring was deduced to be 3,4 dioxygenated from the characteristic pattern of H-2 (δ6.41, *d*, $J_{2,6}$ = 2 Hz), H-5 (δ6.81, *d*, $J_{5,6}$ = 8 Hz) and H-6 (δ6.52, *dd*, $J_{6,5}$ = 8 Hz, $J_{6,2}$ = 2 Hz). It could be seen that three of the five oxygens on the aryl groups were methylated, the remaining two being present as hydroxyl groups. This leaves three remaining questions:

(a) How are the three methyl groups distributed amongst the ring oxygens? In view of the evidence for symmetry in the trioxygenated ring, the possible arrangements are **2** and **3** or **4** and **5** or **6** and **7**. The determination

Table 1. 400 MHz ^{1}H NMR spectrum of compound **8** in $CDCl_3$

Proton	δ (ppm)	Multiplicity	J (Hz)	NOE
A	6.81	*d*	8	B
B	6.52	*dd*	8,2	A
C	6.41	*d*	2	H, J, M, P
D	6.32	*s*		I, K, L, M, P
E	5.52	*br s*		
F	5.43	*s*		
G	4.18	*dd*	7,9	I, J, P (coupled)
H	3.90	*m*		B, C
I	3.83	*s*		D
J	3.80	*s*		D, G
K, L	3.21	*m*		D, M (coupled), P (-ve)
M, N, O	2.58	*m*		B, C, D
P	2.48	*m*		G (coupled), H (-ve), B, C, D



1

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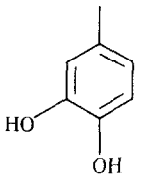

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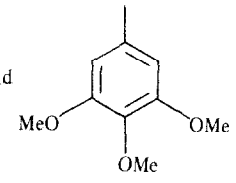

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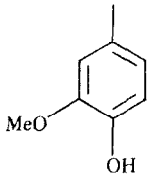

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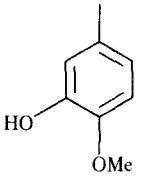

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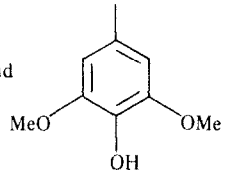

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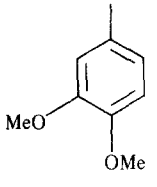

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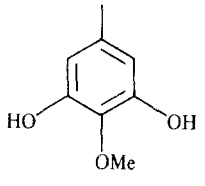

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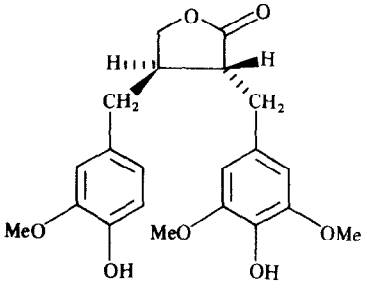

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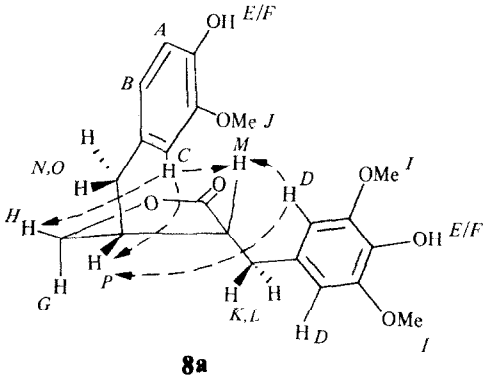


8a

of the placement of methyl groups on a set of oxygen functions is a frequently encountered problem in the structure elucidation of many classes of natural products and is one which is not normally solvable by conventional ^{1}H NMR techniques. However, application of NOE difference spectroscopy provides a simple and unambiguous solution. Thus, the observation of NOEs on the symmetrical pair of methyl groups *I* when irradiating the 2H singlet *D*, and vice versa, clearly establishes the *ortho* relationship between the two groups. Similarly, the NOE from *C* to *J* places these two groups *ortho* and identifies partial structures **4a** and **5** as the correct pair.

(b) How are the two benzyl groups placed on the butyrolactone ring? This question can be subdivided into

two parts: (i) Which is the 2-substituent and which the 3-substituent? (ii) Is the relationship *cis* or *trans*? The latter problem could, in principle, be solved by an analysis of the coupling constants, but because of the extensive coupling present in the saturated portion of the framework, the coupling between protons *P* and *M* is not readily identified, even at 400 MHz. The positional problem, like the problem in (a) above, is even less readily approached by conventional ^{1}H NMR techniques, since it involves determination of connectivities through quaternary carbons across which no couplings are discernible. Fortunately, these problems are solved simultaneously by an inspection of the NOEs observed by difference spectroscopy. Thus, when *H* is irradiated, the NOEs on the protons of the dioxygenated ring (*C*, *B*), but not the trioxygenated ring, help to place the former on the 3-position. Irradiation of *C* gives NOEs to *H*, *P* and *M*, whereas irradiation of *D* gives NOEs only to *P* and *M*, consistent with the *trans*-1-(trioxygenated benzyl), 2-(dioxygenated benzyl) arrangement, as illustrated in **8a**. It must be emphasized that the foregoing is only a partial analysis of all the NOE information. Examination of molecular models for possible structures is an essential feature of the full analysis of the data: only the model corresponding to one structure **8** is fully consistent with all of the NOEs observed (Table 1).

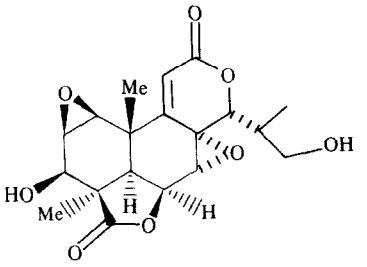
Compound **8**, named thujaplicatin methyl ether, has previously been isolated [8] from the heartwood of the western red cedar, *Thuja plicata*, and is notable as the first lignan to contain both a guaiacyl and a syringyl nucleus as well as being the first isolated from a conifer to contain a syringyl nucleus. It has not hitherto been observed in *Podocarpus* species. It is interesting to note that the proof of structure following the original isolation work rested on extensive use of chemical methods rather than on spectroscopy. Thus, nitrobenzene oxidation liberating vanillin and syringaldehyde identified the patterns of ring hydroxylation and methylation, and close comparison of synthesized isomers was used to establish the positional and geometric relationships around the butyrolactone ring.

The second compound isolated from the roots of *P. saligna* was a white solid with UV and IR characteristic of a Type B norditerpene dilactone [9]. The formula derived from high resolution EI-mass spectrum ([M]$^+$ 378; $C_{19}H_{22}O_8$) was supported by the observation of 22 hydrogen atoms in the 400 MHz ^{1}H NMR spectrum (Table 2). Analysis of the chemical shifts, coupling constants, decoupling experiments and NOE difference spectra lead to structure **9** for this compound. Again, the NOE data played a key role in the process of assembly of molecular fragments. Thus, the 20-Me, readily distinguishable from the 18-Me by its NOE with the 11-H, gives an NOE to one of the epoxide hydrogens, locating this at the 1-position. This is confirmed by the reciprocal NOE between 1α and 11. The orientation of the 1,2-epoxide group as β follows from the NOE between 1α and 5α underneath the A-ring. The 20-Me also gives an NOE to the epoxide hydrogen 7β, placing the two in a *cis* relationship. The other methyl group, 18-Me, gives NOEs to the adjacent hydrogens 3α, 5α and 6α, thus defining its environment and aiding in the recognition of these hydrogen atoms.

It will be noted that the quaternary methyl groups have provided a wealth of valuable structural information via the NOEs which they cascade onto their immediate environments (**9a**). This contrasts distinctly with the lack of information carried by such isolated, uncoupled groups in the conventional ^{1}H NMR spectrum and illustrates the power of the new technique in aiding structure elucidation. Again, it must be emphasized that it is important to relate all the available data (Table 2) to molecular models. When this is done, the series of overlapping and interlocking 3-dimensional relationships revealed by NOE difference spectroscopy leads logically to a single, unambiguous structure **9** for the compound.

Table 2. 400 MHz ^{1}H NMR spectrum of compound **9** in $CDCl_3$

Proton	δ (ppm)	Multiplicity	*J* (Hz)	Decoupling	NOE
11	6.83	*s*			1
17-OH	6.27	*br s*		17a, 17b	
6α	5.14	*dd*	$J_{6,5} = 5.0$ $J_{6,7} = 1.0$	5, 7	7
3-OH (+H_2O)	4.9				
14β	4.99	*d*	$J_{14,15} = 5.0$	15	7
3α	4.70	*dd*	$J_{3,2} = 6.0$ $J_{3,3\text{-OH}} = 5.0$	2	2
7β	4.46	*d*	$J_{7,6} = 1.0$		6
17a	4.39	*dm*	$J_{17a,17b} = 10$ $J_{17a,15} = 4.0$	15, 17b	7, 17b
17b	4.10	*m*	$J_{17b,17a} = 10$ $J_{17b,15} = 6$	15, 17a	15, 17a
1α	3.68	*d*	$J_{1,2} = 4.0$	2	5, 11
2α	3.57	*dd*	$J_{2,1} = 4.0$ $J_{2,3} = 6.0$	1, 3	3
15	2.32	*m*		16, 17a, 17b	7
5α	2.18	*d*	$J_{5,6} = 5.0$	6	1, 6, 18
20	1.59	*s*			1, 7, 11
18	1.42	*s*			3, 5, 6
16	1.38	*d*	$J_{16,15} = 6.7$		7



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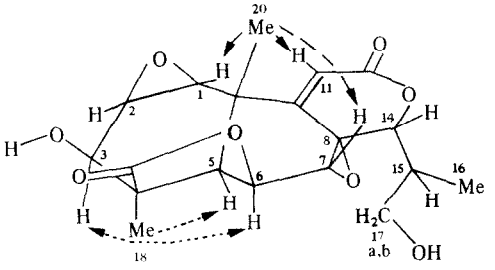

9a

EXPERIMENTAL

A $CHCl_3$ extract of the roots of *P. saligna* D. Don was purified by chromatography and fractions examined spectroscopically as described previously [6, 7].

Thujaplicatin Me ether (**8**). Mp 166–167°. Lit. [8]: 167–167.5°. $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1765. λ_{max}^{EtOH} nm: 260. EIMS: 388.1624, $C_{21}H_{24}O_7$ requires 388.1521. For ^1H NMR see Table 1.

Salignone I (**9**). Mp 111–112°. $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1770, 1720. λ_{max}^{EtOH} nm: 220. EIMS: 378.1325, $C_{19}H_{22}O_8$ requires 378.1313. For ^1H NMR see Table 2.

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