

# Paesslerins A and B: Novel Tricyclic Sesquiterpenoids from the Soft Coral *Alcyonium paessleri*

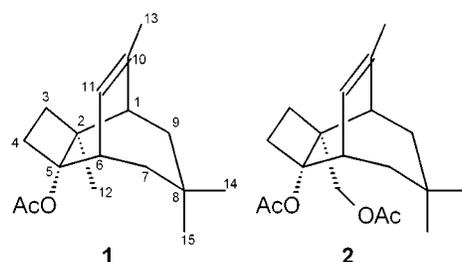
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## ABSTRACT



In the course of our search of new bioactive metabolites from marine invertebrates, paesslerins A and B, sesquiterpenoids with an unprecedented tricyclic skeleton, were isolated from the subAntarctic soft coral *Alcyonium paessleri* collected at a depth of 200 m near the South Georgia islands, and their structures were elucidated by spectroscopic techniques. These compounds show moderate cytotoxicity in preliminary assays.

The soft coral *Alcyonium paessleri*, collected from the South Georgia islands, is a rich source of novel sesquiterpenoids. In a previous paper,<sup>1</sup> we reported the isolation and structure elucidation of alcyopterosins A–O, 15 novel aromatic sesquiterpenoids of the rare illudalane class. These compounds, the first illudalane sesquiterpenoids reported from the marine environment, have some unusual structural features: four of these compounds are chlorinated, while another seven have a nitrate ester substituent, turning them into the first natural nitrate esters to be isolated and fully characterized.<sup>2</sup>

A second collection of *A. paessleri* in a search for minor metabolites afforded two cytotoxic sesquiterpenoids, paesslerins

A and B (1–2, Figure 1)) which have a novel tricyclic 2,8,8,10-tetramethyltricyclo[4.3.2.0<sup>2,5</sup>]undecane skeleton.

The pink soft coral (4 kg wet weight) was collected by deep-water netting (–200 m) during the 1997 campaign of the research vessel *BIP Dr. E. Holmberg* and kept frozen until used. A voucher specimen is preserved at the LABIC

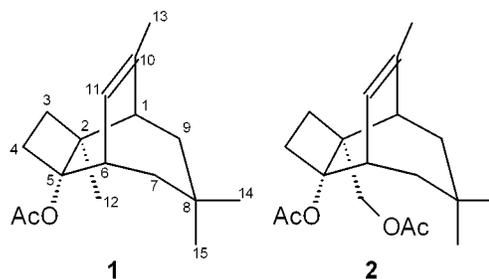


Figure 1. Paesslerins A and B (1–2).

(1) Palermo, J. A.; Rodríguez Brasco, M. F.; Spagnuolo, C.; Seldes, A. M. *J. Org. Chem.* **2000**, *65*, 4482–4486.

(2) There was a previous report of the presence of nitrate esters of linear alcohols as pheromone constituents of the moth *Bucculatrix thurberiella*, but the compounds were never isolated or fully characterized: Hall, D. R.; Beevor, P. S.; Campion, D. G.; Chamberlain, D. J.; Cork, A.; White, R. D.; Almaster, A.; Henneberry, T. J. *Tetrahedron Lett.* **1992**, *33*, 4811–4814.

**Table 1.**  $^1\text{H}$  (200 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  (50 MHz,  $\text{CDCl}_3$ ) NMR Data for Compounds **1–2**<sup>a</sup>

C atom	paesslerin A ( <b>1</b> )		paesslerin B ( <b>2</b> )	
	$\delta^{13}\text{C}$	$\delta^1\text{H}$ (mult, $J$ (Hz))	$\delta^{13}\text{C}$	$\delta^1\text{H}$ (mult, $J$ (Hz))
1	44.5	2.01 (1H; m)	40.0	2.20 (1H; dt, $J = 12.8, 7.1$ )
2	44.7		47.5	
3	31.6	2.07 (ddd; $J = 10.9, 7.8, 2.3$ ) 2.50 (1H; ddd; $J = 11, 11, 11$ )	32.7	2.14 (1H; ddd; $J = 11.0, 8.9, 2.5$ ) 2.61 (1H; ddd; $J = 11, 11, 11$ )
4	26.5	1.37 (m) 1.55 (m)	22.2	1.54 (1H; m) 1.74 (1H; ddd; $J = 11.4, 2.5$ )
5	80.1		79.2	
6	37.8	2.70 (1H; m)	37.8	2.71 (1H; m)
7	47.7	1.33 (dd; $J = 13.2, 4.7$ ) 1.77 (dd; $J = 13.2, 8.6$ )	41.2	1.37 (1H; dd; $J = 12.9, 3.6$ ) 1.80 (1H; dd; $J = 12.9, 8.9$ )
8	38.1		38.2	
9	41.8	1.27 (m) 1.61 (m)	47.5	1.25 (1H; dd; $J = 12.7, 7.1$ ) 1.62 (1H; dd; $J = 12.7, 12.7$ )
10	130.7		130.6	
11	130.3	5.31 (1H; bs)	130.3	5.34 (1H; bs)
12	21.5	1.13 (3H; s)	66.6	4.03 (dd; $J = 11.2, 0.9$ ) 4.42 (d; $J = 11.2$ )
13	18.1	1.61 (3H; dd; $J = 1, 0.7$ )	17.8	1.61 (3H; dd; $J = 2.2, 1.5$ )
14	30.5	0.96 (3H; s)	30.7	0.97 (3H; s)
15	31.1	1.03 (3H; s)	31.2	1.04 (3H; s)
$\text{CH}_3\text{COO}$	169.6		169.5	
$\text{CH}_3\text{COO}$	21.5	2.01 (3H; s)	21.5	2.00 (3H; s)
$\text{CH}_3\text{COO}$			171.3	
$\text{CH}_3\text{COO}$			21.0	2.09 (3H; s)

<sup>a</sup> Spectra were recorded at room temperature.  $\delta$  H values are in ppm relative to TMS.  $\delta$  C values were referenced to  $\text{CDCl}_3$  ( $\delta$ : 77.0).

collection (Universidad de Mar del Plata). The frozen soft coral was blended in EtOH and then extracted twice with  $\text{CH}_2\text{Cl}_2$ . The combined organic extracts were concentrated to an aqueous suspension and partitioned between EtOAc and water. The crude organic extract (32 g) was fractionated by vacuum flash chromatography on silica gel. The fraction eluted with cyclohexane/EtOAc (9:1) was subjected to HPLC (Rp-18, MeOH) and afforded among several known alcyopterisins 53 mg of compound **1** (0.0013% w/w of frozen tissue). HPLC separation (Rp-18, MeOH/ $\text{H}_2\text{O}$  (8:2)) of fraction 4 (cyclohexane/EtOAc (7:3)) gave 12 mg (0.0003% w/w of frozen tissue) of compound **2**.

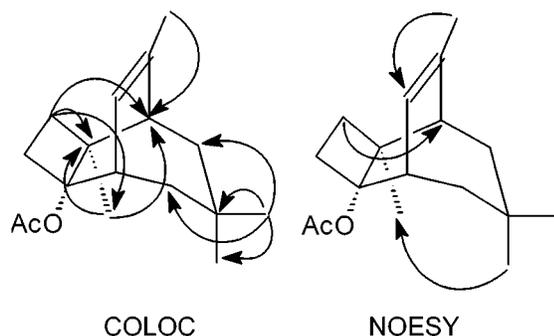
It was clear, after inspection of the NMR spectra (Table 1), that **1** was not an illudalane sesquiterpenoid. Compound **1** had the molecular formula  $\text{C}_{17}\text{H}_{26}\text{O}_2$  determined by HREIMS (calcd for  $\text{C}_{17}\text{H}_{26}\text{O}_2$  262.1933, found 262.1937), which indicated five double bond equivalents. Inspection of the NMR signals revealed the presence of an acetate and a single double bond which accounted for two of the unsaturations and determined a tricyclic skeleton. Four methyls were observable by  $^1\text{H}$  NMR, three of them on quaternary  $\text{sp}^3$  carbons, while the other was located on the double bond. All this information suggested the structure of an acetylated tricyclic sesquiterpenoid. A DEPT spectrum showed the presence of a single oxidized quaternary carbon, and the acetyl group was located on this position. A complete set of 2D NMR spectra (COSY, HETCOR, COLOC, and phase-sensitive NOESY) established the structure of the tricyclic

skeleton of **1**, as well as the relative stereochemistry and assignments of all resonances.

In the COLOC spectrum of **1**, two methyls were correlated with a quaternary carbon at  $\delta$  38.1 (C-8) and with a pair of methylenes at  $\delta$  47.7 (C-7) and  $\delta$  41.8 (C-9). Each of these methylenes was in turn correlated in the COSY spectrum with a different bridgehead proton (H-7 protons with H-6 ( $\delta$  2.70) and H-9 protons with H-1 ( $\delta$  2.01)). COSY correlations between H-11 ( $\delta$  5.31) and both bridgehead protons linked the double bond to C-6 and C-1 and closed one of the rings. An intense COSY correlation between the double bond methyl and the bridgehead proton at  $\delta$  2.70 due to a homoallylic coupling placed the methyl at C-10. This was supported by observed NOE correlations between H-11/H-6 and H<sub>3</sub>-13/H-1.

Another fragment that could be assembled from the COSY spectrum is an isolated  $\text{CH}_2\text{CH}_2$  chain, bound to the two remaining quaternary carbons in the molecule ( $\delta$  44.7 and 80.1). A proton belonging to one of these methylenes ( $\delta$  2.50) had an atypical "quartet" multiplicity due to three large (11 Hz) couplings, suggesting location on a small ring. This proton was correlated with the bridgehead carbon at  $\delta$  44.5, the quaternary carbon at  $\delta$  44.7, and the methyl carbon at  $\delta$  21.5 in the COLOC spectrum. On the other hand, this methyl correlated in the COLOC spectrum to both quaternary carbons ( $\delta$  44.7 and 80.1) as well as with the bridgehead carbon at  $\delta$  44.5. All these correlations placed the methyl with  $\delta$  H 1.13 at the quaternary carbon at  $\delta$  44.7, as well as

the methylene chain and the bridgehead carbon at  $\delta$  44.5. It was also evident that the quaternary carbons ( $\delta$  44.7 and 80.1) were vicinal. This left the quaternary carbon at  $\delta$  80.1 as the only available connection point for the bridgehead carbon at  $\delta$  37.8, and the other end of the  $\text{CH}_2\text{CH}_2$  chain, closing the two additional rings. A COLOC correlation between H-11 ( $\delta$  5.31) and C-5 ( $\delta$  80.1) supported this structure. All these correlations are shown in Figure 2, as



**Figure 2.** Some important correlations observed in the COLOC and NOESY spectra of **1**.

well as the relative stereochemistry established by NOESY correlations.

Interestingly, a similar four-membered ring was previously reported in 3-acetoxysterpurene isolated from the soft coral *Alcyonium acaule*,<sup>3</sup> and the  $^{13}\text{C}$  chemical shifts of these rings in both compounds were very similar. In the case of compound **2**, the  $^1\text{H}$  NMR spectrum showed that one of the quaternary methyls was absent, while an additional acetyl

(3) Cimino, G.; De Giulio, A.; De Rosa, S.; De Stefano, S. *Tetrahedron* **1989**, *45*, 6479–6484.

group and a pair of doublets from an oxygenated methylene could be observed. These features indicated that in **2** one of the methyls was oxidized to a primary alcohol and acetylated. Analysis of the NOESY spectrum showed that C-12 was the acetoxymethyl.

A literature search showed that this tricyclic framework is a novel sesquiterpenoid skeleton, for which the trivial name paesslerane is proposed. The paesslerane and illudalane carbocyclic skeletons may be biogenetically related by a protoilludane cation as a common precursor. Preliminary studies show that compounds **1** and **2** have moderate cytotoxicity against human tumor cell lines.

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**Supporting Information Available:** COSY, phase-sensitive NOESY, and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra for compounds **1** and **2**. HETCOR and COLOC and DEPT spectra for compound **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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