Novel Anti-Inflammatory Spongian Diterpenes from the New Zealand Marine Sponge *Chelonaplysilla violacea*

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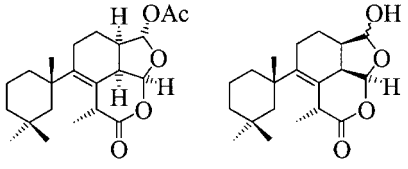
Six novel rearranged spongian diterpenes were isolated from the New Zealand sponge *Chelonaplysilla violacea*. The structures of the six metabolites were determined by extensive spectral analysis. Three of the new compounds possess anti-inflammatory activity. Pourewic acid A (**4**) and 15-methoxypourewic acid B (**5**) represent possible intermediates in previously described biogenetic pathways. Pourewanone (**3**) is the first example of a formate to be isolated from the marine environment.

Introduction

Spongian diterpenes are common, often biologically active, secondary metabolites isolated from sponges of the Dendroceratid and Dictyoceratid orders, or from nudibranchs that predate upon these sponges. Spongian diterpenes are so named as many of the early examples were isolated from the Dictyoceratid genus *Spongia*.[1] Many of the reported spongian diterpenes possess highly oxygenated and rearranged carbon skeletons, examples include tetrahydroaplysulfurin-1 (**1**)[2−4] and cadlinolide B (**2**).[4]



1 **2**

In the course of our NMR-directed sponge extract screening program, an extract of *C. violacea* collected using SCUBA at Stephens Island, Marlborough Sounds, New Zealand, was found to contain several compounds of interest. NMR-guided fractionation of extracts from 182 g of *C. Violacea*, using a combination of benchtop normal- and reversed-phase chromatography and culminating with RP-HPLC, resulted in the isolation of eight spongian diterpenes, two of which were the known metabolites **1** and **2**. Two of the remaining metabolites are related to **2** and are therefore reported as cadlinolides C (**7**) and D (**8**). Pourewanone (**3**) constitutes a new carbon skeleton whilst compounds **4**−**6** are lactone seco-acid derivatives of **2**. The names of the novel compounds **3**−**6** are derived from the Maori name for Stephens Island (Takapourewa).

Results and Discussion

Intense signals from the pseudomolecular ions of pourewanone (**3**) were observed in both the positive and negative ion HRESIMS modes, consistent with a molecular formula of $C_{20}H_{30}O_5$ (351.2160 $[M + H]^+$, Δ 1.8 ppm; 718.4527 $[2M + NH_4]^+$, Δ 0.3 ppm; 349.2019 $[M - H]^-$, Δ 0.5 ppm) and requiring six degrees of unsaturation. Signals of all 20 carbon atoms and all 29 protons attached to carbon atoms were observed in the ^{13}C and ^1H NMR spectra, respectively. Five ^{13}C resonances were observed at $\delta_C > 120$ ppm, attributable to sp^2-carbon atoms. A resonance at $\delta_C = 161.0$ ppm showed a one-bond correlation to a ^1H resonance at $\delta_H = 8.06$ ppm with a coupling constant of 225 Hz, diagnostic of a formate functionality.[5,6] Signals at $\delta_C = 198.1$, 135.6 and 167.3 ppm were assigned to the carbonyl, and the α- and β-carbon atoms of a fully substituted enone, which was supported by the observation of an absorbance at 248 nm in the UV spectrum, and consistant with a C=O stretch of 1665 cm^{-1} observed in the IR spectrum.[7] The remaining downfield carbon resonance at $\delta_C = 177.2$ ppm was assigned to an ester or carboxylic acid group. These functionalites accounted for all the oxygen atoms in the molecular formula; the remaining degrees of unsaturation therefore required a bicyclic structure.

Analysis of the COSY and HMBC spectra of **3** allowed the construction of three substructures. A 1,3,3-trimeth-

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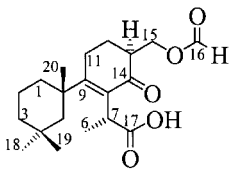
ylcyclohexyl ring was constructed from carbon atoms 1−5, 10, and 18−20. COSY correlations were observed between the ^{1}H resonances of C-1 (δ_C = 38.3, δ_H = 1.29, 1.96 ppm), C-2 (δ_C = 20.5, δ_H = 1.61, 1.61 ppm), and C-3 methylene groups (δ_C = 39.5, δ_H = 1.28, 1.28 ppm) which established the linear arrangement of these methylene groups. HMBC correlations were observed from the ^{1}H resonances of the C-18 methyl group (δ_C = 29.0, δ_H = 0.83 ppm) and the C-19 methyl group (δ_C = 31.4, δ_H = 0.95 ppm) to the ^{13}C resonances of each other, quaternary centre C-4 (δ_C = 31.8 ppm) and C-3 and C-5 methylene groups (δ_C = 49.6, δ_H = 1.38, 1.84 ppm), establishing the presence of a *gem*-dimethyl moiety between C-3 and C-5. HMBC correlations were also observed from the protons of the C-20 methyl group (δ_C = 28.8, δ_H = 0.83 ppm) to the C-1 and C-5 methylene groups, and the quaternary centre C-10 (δ_C = 41.0 ppm), thus completing the ring system.

A linear chain of four carbon atoms was established on the basis of COSY correlations observed between the resonances of their attached protons. In this way, the C-11 methylene group (δ_C = 27.7, δ_H = 2.63, 2.72 ppm) was connected to C-12 (δ_C = 25.1, δ_H = 1.74, 2.11 ppm) which in turn was connected to the C-13 methine group (δ_C = 44.7, δ_H = 2.56 ppm). 13-H correlated to the two protons of the C-15 oxymethylene group (δ_C = 62.9, δ_H = 4.26, 4.49 ppm). Finally, strong HMBC correlations were noted from both ^{1}H resonances of the C-15 oxymethylene group to the C-16 formate carbonyl group and from 16-H to C-15, establishing the placement of the formate residue and confirming its functionality.

The third substructure was constructed from observation of a strong COSY correlation between the ^{1}H resonances of the C-6 methyl group (δ_C = 15.0, δ_H = 1.37 ppm) and the C-7 methine group (δ_C = 39.0, δ_H = 3.84 ppm). HMBC correlations were observed from these protons to the ^{13}C resonances of each other, and also to that of the C-17 carbonyl group (δ_C = 177.1 ppm).

The final connectivity of pourewanone (**3**) was established from observation of several key HMBC correlations involving the enone system. A strong correlation was noted from the ^{1}H resonances of the C-20 methyl group to C-9 (δ_C = 167.3 ppm) to establish the linkage from the 1,3,3-trimethylcyclohexyl ring to the β-carbon atom of the enone. Both the protons of the C-6 methyl group and 7-H correlated to C-8 (δ_C = 135.6 ppm) while 7-H also correlated to the C-14 enone carbonyl group (δ_C = 198.1) which established the linkage of C-7 of the third substructure to the α-carbon atom of the enone. HMBC correlations from 13-H and both ^{1}H resonances of the C-15 oxymethylene group to C-14 confirmed the placement of the enone system. As all the degrees of unsaturation of **3** had been accounted for, the C-17 carbonyl group was established as a carboxylic acid centre, consistent with an OH stretch (3335 cm^{-1}) noted in the IR spectrum. Pourewanone (**3**) is to the best of our knowledge the first example of a natural product containing a formate functionality to be isolated from the marine environment.



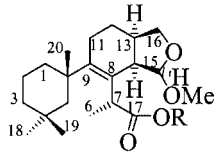
3

A single signal was observed for pourewic acid A (**4**) in the negative ion mode HRESIMS spectrum, appropriate for the molecular formula $C_{21}H_{34}O_4$ (349.2401 [M − H]$^-$, Δ 4.8 ppm), and requiring five degrees of unsaturation. Three ^{13}C resonances were observed at $\delta_C > 120$ ppm, attributable to sp^2-carbon atoms. Resonances at δ_C = 144.2 and 127.9 ppm were assigned to a nonconjugated tetrasubstituted double bond. The remaining downfield carbon resonance at δ_C = 178.8 ppm was assigned, as in **3**, to a carboxylic acid group, consistent with the two prominent stretching bands in the IR spectrum (OH: 3392; C=O: 1704 cm^{-1}). A ^{13}C resonance at δ_C = 111.0 ppm showed a one-bond correlation to a ^{1}H resonance at δ_H = 4.75 ppm, revealing the presence of an acetal. These functionalities accounted for all the oxygen atoms of the molecular formula and with no further evidence of double bonds, a tricyclic structure was required for **4**.

COSY and HMBC correlations (involving carbon atoms 1−5, 10, 18−20) of **4**, essentially identical to those observed for pourewanone (**3**), allowed the rapid construction of a 1,3,3-trimethylcyclohexyl ring. A linear chain of carbon atoms 11−13 and 16 was connected on the basis of COSY correlations observed between the resonances of their attached protons. 13-H also showed a COSY correlation to the C-14 methine group (δ_C = 48.8, δ_H = 2.71 ppm) which in turn correlated to the acetal centre (C-15). An HMBC correlation was noted from the ^{1}H resonance of the C-21 oxymethyl group (δ_C = 54.9, δ_H = 3.17 ppm) to C-15, and from 15-H to the C-21 methyl group, indicating the attachment of a methyl ether substituent to, and confirming the acetal nature of, C-15. Long-range W-coupling between 15-H and 16-H^b, along with HMBC correlations from 15-H to C-16 and from 16-H^a to C-15, revealed the presence of a tetrahydrofuran ring. As for **3**, a strong COSY correlation was observed between the proton resonances of 7-CH_3 and the methine group C-7, and HMBC correlations were observed from their protons to the ^{13}C resonances of each other, and also to that of the C-17 carbonyl group.

The final connectivity of pourewic acid A (**4**) was established from observation of several key HMBC correlations to the carbon atoms of the tetrasubstituted double bond. The ^{1}H resonances of the C-6 methyl group and 7-H showed HMBC correlations to the olefinic carbon atom C-8 (δ_C = 127.9 ppm). 7-H also correlated to C-14 and the second olefinic carbon atom C-9 (δ_C = 144.2 ppm), establishing the attachment of C-7 to olefinic carbon atom C-8, between C-9 and C-14. A strong HMBC correlation was observed from the protons of the C-20 methyl group to C-9, establishing the linkage of the 1,3,3-trimethylcyclohexyl ring to C-9. Observation of strong HMBC correlations

from 11-H^b to C-8, C-9 and C-10, confirmed the attachment of the 1,3,3-trimethylcyclohexyl ring to C-9, and also the placement of the olefin part. With all degrees of unsaturation accounted for, and the presence of an OH stretching band in the IR spectrum (3392 cm^{-1}), C-17 was assigned as a carboxylic acid. In order to confirm the presence of a carboxylic acid, a methyl ester derivative (**4a**) was prepared by treating pourewic acid A (**4**) with CH_2N_2. The chemical shifts of the methyl ester of **4a** are similar to those present in the previously reported methyl ester metabolite membranolide.[9]



4 R = H

4a R = Me

The relative stereochemistry of pourewic acid A (**4**) was determined from a combination of ^{1}H-^{1}H vicinal coupling constants and NOE correlations detected in both a ROESY and a series of gradient-enhanced 1D- NOESY experiments. A small dihedral angle was established between 14-H and 15-H from the small coupling constant measured between their respective resonances (ca. 2.7 Hz). NOE correlations were observed from 14-H to 13-H and 16-H^b, indicating that they are all on the same face of the molecule. A strong NOE was observed between 16-H^a and 12-H^b, and between 11-H^a and 13-H, indicating the spatial proximity of these two pairs of protons. Intense NOE correlations were observed between 7-CH_3 and 14-H, indicating that they are also on the same face of the molecule. Finally, NOE correlations were observed between the C-6 methyl group and the C-20 methyl group, 1-H^b and 7-H, and between 5-H^a and 11-H^b, allowing the relative stereochemistry of the molecule to be assigned as (7*R**,10*S**,13*S**,14*S**,15*R**) (Figure 1). The relative stereochemistry of C-7 and C-10 is consistent with that observed by X-ray diffraction for tetrahydroaplysulfurin-1.[2,3]

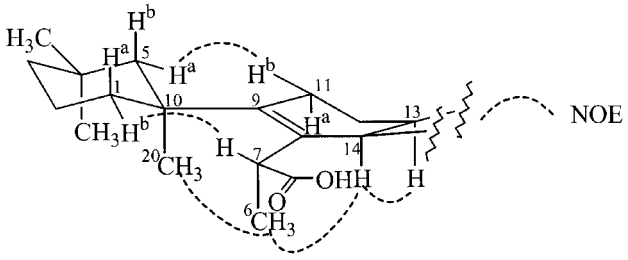


Figure 1. Selected NOE correlations establishing the relative stereochemistry of pourewic acid A (**4**)

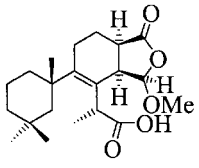
A pseudomolecular ion was detected for 15-methoxypourewic acid B (**5**) in the negative ion mode HRESIMS, suitable for the formula $C_{21}H_{32}O_5$ (363.2194 [M − H]$^-$, Δ

Table 1. ^{1}H NMR spectroscopic data (300 MHz, $CDCl_3$) for pourewanone (**3**), pourewic acid A (**4**), 15-methoxypourewic acid B (**5**), methylpourewate B (**6**), cadlinolide C (**7**) and cadlinolide D (**8**)

Position[a][b]	**3**		**4**		**5**		**6**		**7**		**8**	
	δ [ppm]	Multiplicity (*J* [Hz])	δ [ppm]	Multiplicity (*J* [Hz])	δ [ppm]	Multiplicity (*J* [Hz])	δ [ppm]	Multiplicity (*J* [Hz])	δ [ppm]	Multiplicity (*J* [Hz])	δ [ppm]	Multiplicity (*J* [Hz])
1-H^a	1.29	m	1.21	m	1.32	m	1.29	m	1.13	m	1.13	m
1-H^b	1.96	m	2.13	m	2.24	m	2.18	m	1.95	m	1.92	m
2-H^a	1.61	m	1.49	dt (13.2, 3.4)	1.59	m	1.51	m	1.51	m	1.51	m
2-H^b	1.61	m	1.85	m	1.59	m	1.83	m	1.51	m	1.51	m
3-H^a	1.28	m	1.18	t (3.2)	1.24	m	1.36	m	1.19	m	1.2	m
3-H^b	1.28	m	1.33	m	1.4	m	1.21	m	1.32	m	1.35	m
5-H^a	1.38	m	0.96	d (13.9)	1.07	m	1.00	m	1.26	d (13.7)	1.25	m
5-H^b	1.84	m	1.76	m	1.8	m	1.77	m	1.8	dt (13.7, 1.7)	1.78	m
6-H_3	1.37	d (6.8)	1.21	d (6.8)	1.27	d (6.8)	1.21	d (6.8)	1.40	d (7.3)	1.40	d (7.6)
7-H	3.84	q (6.8)	4.22	q (7.1)	4.32	q (7.0)	4.30	q (7.1)	4.18	q (7.6)	4.19	q (7.4)
11-H^a	2.63	dd (10.3, 4.6)	1.91	dd (11.7, 3.7)	1.63	m	1.47	m	1.93	m	2.07	m
11-H^b	2.72	t (3.9)	2.22	t (4.3)	2.31	m	2.34	dt (15.9, 2.9)	2.48	dt (16.1, 4.4)	2.35	m
12-H^a	1.74	m	1.24	m	1.41	m	1.30	m	0.92	m	1.19	m
12-H^b	2.11	m	1.63	dq (11.5, 3.7)	2.02	m	2.09	m	1.95	m	1.88	m
13-H	2.56	m	2.30	m	2.91	m	2.97	q (8.6)	2.65	m	2.40	m
14-H	–	–	2.71	dd (8.0, 2.7)	3.09	dd (10.0, 3.7)	2.97	dd (10.0, 3.9)	3.01	ddd (13.4, 5.6, 2.2)	3.17	br. t (7.1)
15-H^a	4.26	dd (11.5, 7.1)	4.75	d (2.9)	5.22	d (3.7)	5.41	d (3.3)	6.01	d (5.9)	5.97	d (6.3)
15-H^b	4.49	dd (11.2, 4.1)	–	–	–	–	–	–	–	–	–	–
16-H^a	8.08	s	3.76	d (8.5)	–	–	–	–	3.58	dd (10.0, 8.8)	4.92	d (3.2)
16-H^b	–	–	4.03	dd (8.3, 5.1)	–	–	–	–	4.18	t (8.2)	–	–
18-H_3[c]	0.83	s	0.87	s	0.91	s	0.85	s	0.76	s	0.76	s
19-H_3 [c]	0.95	s	0.84	s	0.92	s	0.87	s	0.90	s	0.90	s
20-H_3	1.28	s	1.03	s	1.13	s	1.05	s	1.13	s	1.13	s
21-H_3	–	–	3.17	s	3.27	s	3.71	s	–	–	3.45	s

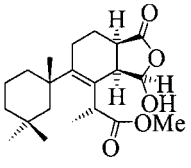
[a] Referenced to the residual solvent peak.[8] [b] Geminal protons are listed as ^{a} and ^{b} in order of ascending chemical shift; ^{a} or ^{b} do not imply any stereochemistry. [c] Assignment interchangeable.

4.8 ppm) and requiring six degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra were similar to those of **4**. The C-16 oxymethylene group of **4** is replaced by a lactone carbonyl group (δ_C = 179.2 ppm), as evidenced by a C=O stretch at 1780 cm^{-1}, observed in the IR spectrum, and an HMBC correlation from the acetal proton (δ_H = 5.22 ppm) of C-15. The ^{1}H chemical shifts of C-12 (δ_H = 2.02, 1.41 ppm) and C-13 (δ_H = 2.91 ppm) were similar to those reported for cadlinolide A which shares the same functionality at C-16.[4] All other ^{1}H and ^{13}C resonances and correlations were similar to those of **4** (see Tables 1 and 2), allowing the structure to be assigned as drawn. Similar ^{1}H-^{1}H coupling constants and NOE correlations were also observed, revealing the same relative stereochemistry to that of **4** (7*R**,10*S**,13*R**,14*R**,15*R**).



5

Methylpourewate B (**6**) was found to be an isomer of **5**, as evidenced by the pseudomolecular ions observed in both the positive and negative ion HRESIMS modes (365.2335 [M + H]$^+$, Δ 3.5 ppm; 363.2185 [M − H]$^-$, Δ 5.3 ppm), implying the same six degrees of unsaturation. The IR and NMR spectra were very similar to those of **5**, the only significant differences being the chemical shifts of the acetal (C-15) and the OCH_3 (C-21) signals. The oxymethyl protons resonate at a lower chemical shift (δ_H = 3.71) and also correlate to the carbonyl resonance at δ_C = 175.3 ppm (C-17), consistent with a methyl ester functionality. The ^{1}H and ^{13}C resonances of C-15, C-17, and C-21 are all similar to those of **4a** which shares the same functionality at these centres. Similar coupling constants and NOE correlations allowed the assignment of the same relative stereochemistry (7*R**,10*S**,13*R**,14*S**,15*R**).



6

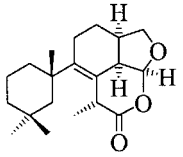
The molecular formula of cadlinolide C (**7**) was established as $C_{20}H_{30}O_3$ from a pseudomolecular ion observed in the positive ion mode HRESIMS (319.2260 [M + H]$^+$, Δ 2.6 ppm), requiring six degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **7** were very similar to those observed for **4**, including an oxymethylene group (C-16). No OH and only one C=O stretching band (1739 cm^{-1}) were observed in the IR spectrum. One degree of unsaturation was left unaccounted for after construction of the 1,3,3-

Table 2. ^{13}C NMR spectroscopic data ($CDCl_3$) for pourewanone (**3**), pourewic acid A (**4**), 15-methoxypourewic acid B (**5**), methylpourewate B (**6**), cadlinolide C (**7**) and cadlinolide D (**8**)

Position[a]	**3**[b] δ [ppm] (multiplicity)[d]	**4**[c] δ [ppm] (multiplicity)[d]	**5**[c] δ [ppm] (multiplicity)[d]	**6**[c] δ [ppm] (multiplicity)[d]	**7**[c] δ [ppm] (multiplicity)[d]	**8**[c] δ [ppm] (multiplicity)[d]
C-1	38.3 (t)	39.1 (t)	38.8 (t)	39.0 (t)	40.0 (t)	39.2 (t)
C-2	20.5 (t)	20.1 (t)	19.8 (t)	19.8 (t)	20.6 (t)	20.9 (t)
C-3	39.5 (t)	40.0 (t)	39.7 (t)	39.9 (t)	39.7 (t)	39.5 (t)
C-4	31.8 (s)	31.6 (s)	31.4 (s)	31.6 (s)	31.9 (s)	31.9 (s)
C-5	49.6 (t)	50.8 (t)	50.3 (t)	50.6 (t)	51.2 (t)	51.0 (t)
C-6	15.0 (q)	18.8 (q)	16.3 (q)	16.6 (q)	14.4 (q)	14.8 (q)
C-7	39.0 (d)	41.9 (d)	41.4 (d)	41.5 (d)	40.7 (d)	41.2 (d)
C-8	135.6 (s)	127.9 (s)	127.3 (s)	127.3 (s)	122.9 (s)	122.2 (s)
C-9	167.3 (s)	144.2 (s)	145.8 (s)	147.2 (s)	147.0 (s)	145.7 (s)
C-10	41.0 (s)	41.5 (s)	41.6 (s)	42.1 (s)	39.6 (s)	40.1 (s)
C-11	27.7 (t)	27.6 (t)	26.3 (t)	26.8 (t)	27.0 (t)	25.8 (t)
C-12	25.1 (t)	30.6 (t)	26.5 (t)	27.5 (t)	25.6 (t)	24.7 (t)
C-13	44.7 (d)	37.8 (d)	40.3 (d)	41.2 (d)	37.6 (d)	30.0 (d)
C-14	198.1 (s)	48.8 (d)	43.7 (d)	45.8 (d)	41.7 (d)	38.9 (d)
C-15	62.9 (t)	111.0 (d)	110.3 (d)	103.8 (d)	103.5 (d)	102.2 (d)
C-16	161.0 (d)	74.7 (t)	179.2 (s)	178.9 (s)	73.1 (t)	109.2 (d)
C-17	177.1 (s)	178.8 (s)	176.4 (s)	175.3 (s)	172.5 (s)	171.8 (s)
C-18[e]	29.0 (q)	26.6 (q)	26.9 (q)	26.8 (q)	27.6 (q)	28.1 (q)
C-19[e]	31.4 (q)	33.1 (q)	32.4 (q)	32.9 (q)	32.9 (q)	32.9 (q)
C-20	28.8 (q)	30.7 (q)	30.1 (q)	30.6 (q)	31.9 (q)	31.2 (q)
C-21	–	54.9 (q)	56.7 (q)	52.1 (q)	–	56.2 (q)

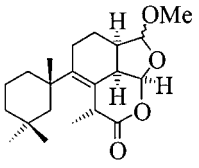
[a] Referenced to the residual solvent peak.[8] [b] 100 MHz. [c] 75 MHz. [d] Multiplicity determined by HSQC-DEPT experiment. [e] Assignment interchangeable.

trimethylcyclohexyl, cyclohexene, and tetrahydrofuran rings, and the carbonyl side chain. The absence of an OH stretching band implied that C-17 is attached to the C-15 acetal centre through a lactone linkage, accounting for the final degree of unsaturation. This assignment is supported by the de-shielded ^{1}H chemical shift of 15-H and the ^{13}C chemical shift of C-17 which is similar to that of cadlinolide B (**2**).[4] As previously, the stereochemistry of **7** could be assigned on the basis of observed NOE correlations and vicinal ^{1}H-^{1}H coupling constants. Cadlinolide C (**7**) is assigned the same relative stereochemistry (7*R**,10*S**,13*S**,14*S**,15*S**) as that proposed for cadlinolide B (**2**).[4]



7

Several pseudomolecular ions were observed in the positive ion HRESIMS mode, allowing the molecular formula of cadlinolide D (**8**) to be established as $C_{21}H_{32}O_4$ (349.2373 $[M + H]^+$, Δ 7.5 ppm; 371.2193 $[M + Na]^+$, Δ 2.1 ppm; 719.4483 $[2 M + Na]^+$, Δ 2.5 ppm), and requiring six degrees of unsaturation. As with **7**, only one significant stretching band was observed in the IR spectrum (C=O: 1744 cm^{-1}). A significant feature of the ^{1}H NMR spectrum of **8** was the 3:1 ratio observed between several closely related resonances, indicating the presence of two diastereomers. Several unsuccessful attempts were made to separate the two compounds, therefore the structural elucidation was carried out upon the mixture. It should be noted that cadlinolide B (**2**) was isolated as a similar diastereomeric mixture.[4]



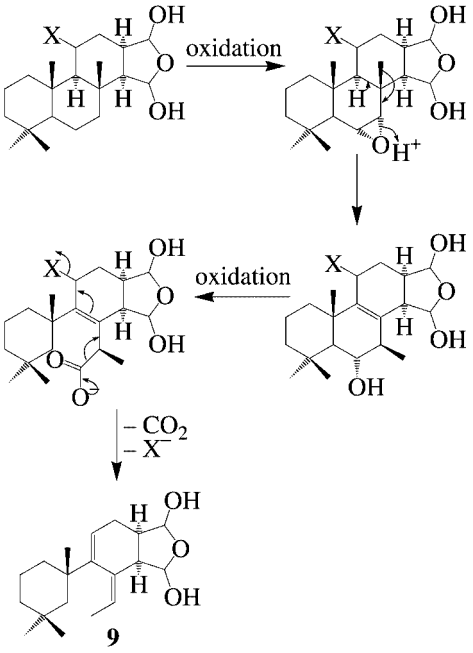
8

Most of the NMR resonances of **8** were very similar to those of **7**. The most significant difference between the two was the replacement of the oxymethylene group with a second acetal group (C-16: δ_C = 109.2, δ_H = 4.92 ppm). COSY and HMBC correlations were used to construct the 1,3,3-trimethylcyclohexyl and cyclohexene rings, and the carbonyl side chain. 13-H showed a strong COSY correlation to the oxymethine proton of the C-16 acetal whilst 14-H showed a similar correlation to the acetal proton of C-15. The absence of an OH stretch in the IR spectrum, coupled with the diagnostic chemical shifts of the C-15 (δ_C = 102.2 ppm) and C-16 methine groups, imply that these two acetal centres are linked through an ether bridge, completing a tetrahydrofuran ring. Finally, HMBC correlations were noted between the resonances of C-16 and the oxygenated C-21 methyl group (δ_C = 56.2, δ_H = 3.45 ppm), establishing the acetal nature of C-16. Again, the absence of an OH stretching band in the IR spectrum, coupled with the final degree of unsaturation, required that C-15 and C-17 be linked through a lactone bridge, finalising the connectivity of the molecule. The largest difference in chemical shift between the ^{1}H resonances of the diastereomers occurs at C-16, implying that this is the centre of epimerisation. This is the same position of epimerisation noted for cadlinolide B (**2**).[4]The relative stereochemistry of **8** was assigned as (7*R**,10*S**,13*R**,14*S**,15*S**), in a similar manner to that described previously.

The three stereogenic centres of pourewanone (**3**) are significantly isolated from each other and therefore it is difficult to relate the relative stereochemistry of the molecule. The remaining metabolites, however, all share the same relative stereochemistry as tetrahydroaplysulfurin-1 (**1**) and cadlinolide B (**2**) and **3** is therefore tentatively assigned as (7*R**,10*S**,13*S**).

As the sponge was extracted using MeOH, it is possible that the methyl ester of **6** and the methyl ketals of **4**, **5** and **8,** are artifacts of isolation. As no further sponge material was available, further experiments using an alternative solvent were not possible.

Pourewanone (**3**), pourewic acid A (**4**) and 15-methoxypourewic acid B (**5**) are all of biogenetic significance. The biogenesis of several previously reported rearranged spongian diterpenes based upon the gracilin carbon skeleton (**9**) is proposed to include the decarboxylation of a C-17 carboxylic acid to form a diene molecule (Figure 2).[10,11] No free C-17 carboxylic acid has been previously reported



9

Figure 2. Proposed biosynthesis of the gracilin carbon skeleton[10,11]

to support this premise until this study. One point to note is that in the suggested biogenetic syntheses, a leaving group is assumed to be found at C-11 of the carboxylic acid compounds throughout the pathway, assisting in the decarboxylation step.[10,11] No diterpenes isolated during this study possess any functionality at this position suggesting that addition of the leaving group occurs after the oxidative formation of the carboxylic acid.

Pourewanone (**3**) is the first example of the novel pourewane carbon skeleton. The formate of **3** is presumably formed by the oxidative cleavage of a $\Delta^{14,15}$ double bond. A C-15 alcohol could be dehydrated to form the $\Delta^{14,15}$ olefin which would then be oxidatively cleaved to form **3**. The oxidative cleavage of an olefin has been suggested in the biosynthesis of several natural products including vitamin A_1 from β-carotene[12] and also for the formation of the natural products muricenones A and B, and cyclomegistine, all three of which are supported by synthetic studies (Figure 3).[13−15]

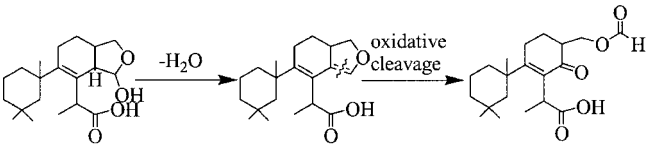


Figure 3. Proposed biogenesis of pourewanone (**3**)

Due to a paucity of available material, limited biological evaluation studies were carried out on the diterpenes isolated. Pourewic acid A (**4**) was inactive in both the HL-60 leukaemia cell line (10 μM maximum concentration) and in anti-microbial testing. Pourewic acid A (**4**), methylpourewate B (**6**) and cadlinolide C (**7**) were all submitted for anti-inflammatory evaluation. Each compound inhibited the production of superoxide by human peripheral blood neutrophils that had been stimulated with either fMLP (*N*-formyl-methionine-leucine-phenylalanine, IC_{50} = 74, 58, 13 μM respectively) or PMA (phorbol myristate acetate, IC_{50} = 77, 58, 13 μM respectively), indicating moderate anti-inflammatory activity.[16]

Conclusion

Six novel metabolites were isolated from a New Zealand marine sponge. Several of the new metabolites possess moderate anti-inflammatory activity as measured by the inhibition of superoxide production by human peripheral blood neutrophils when stimulated by PMA or fMLP. Three of the new metabolites are of biosynthetic importance. Two represent possible intermediates in a previously described pathway whilst pourewanone (**3**), the first example of a formate compound to be isolated from the marine environment, is formed by the oxidative cleavage of a double bond in a rearranged spongian diterpene skeleton.

Experimental Section

General Remarks: The ^{13}C NMR spectrum of pourewanone (**3**) was recorded using a Bruker Avance 400 spectrometer operating at 100 MHz. The ^{13}C NMR spectra of methylpourewate B (**6**) and cadlinolide C (**7**) were recorded using a Bruker Avance 300 spectrometer operating at 75 MHz. All other NMR spectra were recorded using a Varian Unity-Inova 300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C. All chemical shifts (δ) were referenced to the residual solvent peak.[8] HRESIMS were obtained using a PE Biosystem Mariner 5158 TOF mass spectrometer. Infrared spectra were recorded using a Bruker Tensor 27 spectrometer to ± 2 cm^{-1}. UV/Vis spectra were recorded using a Varian Cary 100 spectrometer to ± 1 nm. Optical rotations were recorded using a Perkin−Elmer 241 polarimeter. MPLC and HPLC were performed using a Rainin Dynamax SD-200 HPLC system coupled to a Rainin UV-1 detector. All solvents for MPLC either were analytical reagent grade or glass distilled before use. Solvents for HPLC were all analytical reagent grade. H_2O for MPLC and HPLC was glass-distilled and deionised using a MilliQ system. All solvent mixtures are reported as % v/v. RP-HPLC was performed using a Phenomenex Prodigy ODS column [semi-preparative (1.0 × 25 cm)]. TLC analyses were performed using Merck Kieselgel (Alufolien) 60 F_{254} plates. TLC plates were visualised by fluorescence quenching under UV light (λ = 254 nm), or by spraying with 50% $MeOH/H_2SO_4$ and then heating. Reversed-phase chromatography on PSDVB was performed using HP-20 (Mitsubishi), or HP-20S (Supelco). Kieselgel 60 (230−400 mesh ASTM) was used for normal-phase chromatography.

Isolation of Diterpene Metabolites from *Chelonaplysilla violacea* (MNP0979): *C. violacea* (MNP0979, a voucher sample (13RAK 59A) is kept at the Marine Natural Products Laboratory, Victoria University of Wellington) (182 g) was extracted with MeOH. The extract was purified on a column of poly(styrene-divinyl-benzene) (PSDVB) (HP-20), using 300-mL portions of (1) 20% Me_2CO/H_2O, (2) 40% Me_2CO/H_2O, (3) 60% Me_2CO/H_2O, (4) 80% Me_2CO/H_2O and (5) Me_2CO. Fraction 3 was then purified on PSDVB (HP-20S) using a gradient elution profile of increasing amounts of Me_2CO in H_2O. Repeated injections using various mixtures of MeCN and H_2O on RP-HPLC resulted in the isolation of cadlinolide C (**7**) (80% $MeCN/H_2O$, 4.50 mL/min, retention time 12.23 min, 2.4 mg), cadlinolide D (**8**) (70% $MeCN/H_2O$, 4.50 mL/min, retention time 24.62 min, 0.8 mg), cadlinolide B (**2**) (60% $MeCN/H_2O$, 4.50 mL/min, retention time 17.42 min, 1.4 mg) and 15-methoxypourewic acid B (**5**) (60% $MeCN/H_2O$, 4.50 mL/min, retention time 19.91 min, 1.2 mg). Fraction 4 from the HP-20 purification was separated into two major fractions using several silica columns, eluted with mixtures of CH_2Cl_2 and MeOH (from 0−10% MeOH). Repeated injection on RP-HPLC resulted in the final purification of pourewanone (**3**) (80% $MeCN/H_2O$, 4.50 mL/min, retention time 5.61 min, 2.2 mg), methylpourewate B (**6**) (80% $MeCN/H_2O$, 4.50 mL/min, retention time 8.11 min, 3.0 mg), tetrahydroaplysulfurin-1 (**1**) (80% $MeCN/H_2O$, 4.50 mL/min, retention time 10.98 min, 1.8 mg) and pourewic acid A (**4**) (70% $MeCN/H_2O$, 4.50 mL/min, retention time 19.69 min, 2.9 mg).

Tetrahydroaplysulfurin-1 (1): White solid. $[\alpha]_D^{20}$ = +16.6 (*c* = 0.40, CH_2Cl_2) [ref. values $[\alpha]_D$ = +169 (*c* = 1.0), $[\alpha]_D$ = +65 (*c* = 1.5, $CDCl_3$); all other spectroscopic data are in agreement with those previously reported.[2−4]

Cadlinolide B (2): White solid. $[\alpha]_D^{20}$ not available as diastereomeric mixture; all other spectroscopic data are in agreement with those previously reported.[4]

Pourewanone (3): White solid. $[\alpha]_D^{20} = +22.6$ ($c = 0.35$, CH_2Cl_2). UV (CH_2Cl_2): $\lambda_{max.} = 248$ nm ($\varepsilon = 14425$). IR (film): $\tilde{\nu}_{max.} = 3335$, 2929, 1725, 1665, 1456, 1366, 1307, 1186 cm^{-1}. NMR spectroscopic data see Tables 1 and 2. HRESIMS: obsd. $m/z = 351.2160$ $[M + H]^+$, 349.2019 $[M - H]^-$, $C_{20}H_{30}O_5$ requires 351.2166, Δ 1.8 ppm; 349.2021, Δ 0.5 ppm.

Pourewic Acid A (4): White solid. $[\alpha]_D^{20} = -10.8$ ($c = 0.86$, CH_2Cl_2). IR (film): $\tilde{\nu}_{max.} = 3392$, 2927, 1704, 1455, 1221, 1096, 1060 cm^{-1}. NMR spectroscopic data see Tables 1 and 2. HRESIMS: obsd. $m/z = 349.2401$ $[M - H]^-$, $C_{21}H_{34}O_4$ requires 349.2384, Δ 4.8 ppm.

15-Methoxypourewic Acid B (5): Pale yellow solid. $[\alpha]_D^{20} = -368.6$ ($c = 0.23$, CH_2Cl_2). IR (film): $\tilde{\nu}_{max.} = 3347$, 2927, 1778, 1707, 1456, 1204 cm^{-1}. NMR spectroscopic data see Tables 1 and 2. HRESIMS: obsd. $m/z = 363.2194$ $[M - H]^-$, 366.2337 [M (− 3 H + 3 D) − H]$^-$, 368.2504 [M (− 3 H + 3 D) + H]$^+$, $C_{21}H_{32}O_5$ requires 363.2177, Δ 4.8 ppm; 366.2365, Δ 7.9 ppm; 368.2504, Δ 3.7 ppm.

Methylpourewate B (6): Pale yellow solid. $[\alpha]_D^{20} = -81.8$ ($c = 0.70$, CH_2Cl_2). IR (film): $\tilde{\nu}_{max.} = 2947$, 1762, 1735, 1455, 1206, 1124 cm^{-1}. NMR spectroscopic data see Tables 1 and 2. HRESIMS: obsd. $m/z = 365.2335$ $[M + H]^+$, 363.2185 $[M - H]^-$, $C_{21}H_{32}O_5$ requires 365.2323, Δ 3.5 ppm; 363.2166, Δ 5.3 ppm.

Cadlinolide C (7): Pale yellow solid. $[\alpha]_D^{20} = +27.3$ ($c = 0.68$, CH_2Cl_2). IR (film): $\tilde{\nu}_{max.} = 2945$, 1739, 1455, 1228, 1026 cm^{-1}. NMR spectroscopic data see Tables 1 and 2. HRESIMS: obsd. $m/z = 319.2260$ $[M + H]^+$, $C_{20}H_{30}O_3$ requires 319.2268, Δ 2.6 ppm.

Cadlinolide D (8): Off-white solid. $[\alpha]_D^{20}$ not available as diastereomeric mixture. IR (film): $\tilde{\nu}_{max.} = 2946$, 1744, 1456, 1206, 1028 cm^{-1}. NMR spectroscopic data see Tables 1 and 2. HRESIMS: obsd. $m/z = 349.2399$ $[M + H]^+$, 371.2201 $[M + Na]^+$, $C_{21}H_{32}O_4$ requires 349.2373, Δ 7.5 ppm; 371.2193, Δ 2.1 ppm.

Methylation of Pourewic Acid A (4): Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) (1 g) was treated with KOH (5 g dissolved in 18 mL 44% H_2O/EtOH) to generate CH_2N_2, which was dissolved in Et_2O. A solution of pourewic acid A (**4**) (0.8 mg dissolved in 1 mL CH_2Cl_2) was treated with excess CH_2N_2 for 4 h. The solvent was removed under vacuum to yield a mixture of compounds. The mixture was dissolved in 50% CH_2Cl_2/MeOH (1 mL) which was passed through an amino column (0.5 × 1.5 cm) that had been pre-equilibrated with 50% CH_2Cl_2/MeOH (5 mL). The column was washed with 50% CH_2Cl_2/MeOH (4 mL) which was collected together with the eluent of loading. The column was then eluted with 5% AcOH/47.5% CH_2Cl_2/47.5% MeOH (5 mL). The sample containing the eluent of loading and the column washings was concentrated to dryness under reduced pressure to yield methylpourewate A (**4a**) (0.7 mg).

Methyl Pourewate A (4a): White amorphous powder. IR (film): $\tilde{\nu}_{max.} = 2928$, 1732, 1455, 1366, 1096, 909, 732 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): $\delta_H = 0.88$ (s, 3 H, 19-H), 0.92 (s, 3 H, 18-H), 0.97 (m, 1 H, 5-H^a), 1.02 (s, 3 H, 20-H), 1.18 (m, 1 H, 3-H^a), 1.20 (d, $J = 6.8$ Hz, 3 H, 6-H), 1.24 (m, 1 H, 12-H^a), 1.26 (m, 1 H, 1-H^a), 1.38 (m, 1 H, 3-H^b), 1.49 (m, 1 H, 2-H^b), 1.65 (m, 1 H, 12-H^b), 1.84 (m, 1 H, 5-H^b), 1.86 (m, 1 H, 11-H^a), 1.94 (m, 1 H, 2-H^a), 2.18 (m, 1 H, 1-H^b), 2.20 (m, 1 H, 11-H^b), 2.34 (q, $J = 7.6$ Hz, 1 H, 13-H), 2.65 (dd, $J = 8.1$, 2.4 Hz, 1 H, 14-H), 3.24 (s, 3 H, methoxy 21-H), 3.67 (s, 3 H, methyl ester 22-H), 3.76 (dd, $J = 8.8$, 3.4 Hz, 1 H, 16-H^a), 4.02 (dd, $J = 8.5$, 6.4 Hz, 1 H, 16-H^b), 4.18 (q, $J = 4.4$ Hz, 1 H, 7-H), 4.62 (d, $J = 2.4$ Hz, 1 H, 15-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta_C = 16.1$ (C-6) 20.0 (C-2), 26.2 (C-18), 27.7 (C-11), 30.9 (C-20), 31.2 (C-12), 31.6 (C-4), 33.3 (C-19), 38.0 (C-13), 39.2 (C-1), 40.1 (C-3), 41.6 (C-10), 42.0 (C-7), 49.0 (C-14), 50.9 (C-5), 51.7 (methyl ester C-22), 54.6 (methoxy C-21), 74.9 (C-16), 110.8 (C-15), 128.2 (C-8), 143.9 (C-9), 174.7 (C-17) ppm. HRESIMS: obsd. $m/z = 333.2417$ $[M - OMe]^+$, $C_{21}H_{33}O_3$ requires 333.2424, Δ 2.1 ppm.

Supporting Information: NMR spectra for compounds **3**−**8** are available, see the footnote on the first page of this article.

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