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The Valdivones, Anti-inflammatory Diterpene Esters from the South African Soft Coral *Alcyonium valdivae*+

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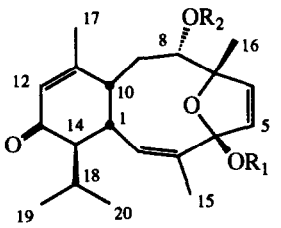
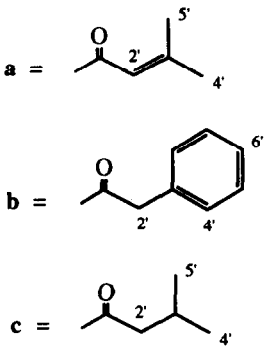
Abstract: The South African soft coral *Alcyonium valdivae* contains five diterpene esters, valdivones A (**1**) and B (**2**), the corresponding methoxy ketals (**3**) and (**4**), and dihydrovaldivone A (**5**). Although the producing organisms are unrelated taxonomically, the valdivones are most closely related to the sarcodictins (e.g. **6**) from the stoloniferan coral *Sarcodictyon roseum*. Valdivones A (**1**) and B (**2**) inhibit chemically-induced inflammation in the mouse ear assay.

Marine invertebrates from the "Wild Coast" of South Africa, which lies within the Republic of Transkei, have not previously been studied by natural product chemists. We were recently able to survey marine invertebrates from this region and found a rather disappointing incidence of bioactivity in the 90 sponges, tunicates and soft coral specimens obtained. A secondary screening of the ^{1}H NMR spectra of the crude extracts led us to investigate the soft coral *Alcyonium valdivae*. The interesting peaks in the ^{1}H NMR spectra were associated with two diterpene esters, valdivones A (**1**) and B (**2**). The corresponding methyl ketals **3** and **4**, which presumably arise by addition of the extraction solvent, and dihydrovaldivone A (**5**) were isolated as minor constituents.

The combined organic-soluble material from a methanolic extract of *A. valdivae* was chromatographed on silica gel using a hexane/ethyl acetate solvent gradient to obtain two fractions with interesting ^{1}H NMR spectra. Repeated chromatography of the more polar fraction gave valdivones A (**1**, 40 mg, 0.097% dry wt.) and B (**2**, 28 mg, 0.068% dry wt.) while the less polar fraction yielded 4-O-

+ Dedicated to Professor Sir Derek Barton on the occasion of his 75th birthday.





1 R_1 = H R_2 = **a**
2 R_1 = H R_2 = **b**
3 R_1 = Me R_2 = **a**
4 R_1 = Me R_2 = **b**
5 R_1 = H R_2 = **c**

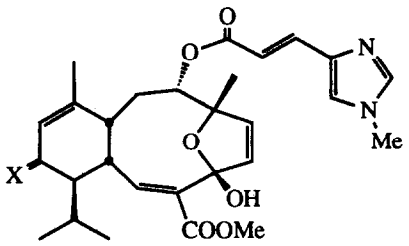
methyl valdivone A (**3**, 1 mg, 0.0024% dry wt.) and 4-O-methyl valvidone B (**4**, 1 mg, 0.0024% dry wt.). Dihydrovaldivone A (**5**, 2 mg, 0.0049% dry wt.), which shows almost identical chromatographic behaviour to that of valdivone A (**1**), was isolated after repeated HPLC separations.

Valvidone A (**1**) was obtained as colorless needles, mp. 89–91°C, of molecular formula $C_{25}H_{34}O_5$. Valvidone B (**2**) crystallized as colorless needles, mp. 171–173°C, of molecular formula $C_{28}H_{34}O_5$. Comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** suggested that the two compounds were the dimethylacrylate [δ ($CDCl_3$) 5.68 (br s, 1 H, H-2'), 1.90 (s, 3 H, H-4'), 2.18 (s, 3 H, H-5')] and phenylacetate [δ ($CDCl_3$) 7.21–7.27 (m, 5 H, phenyl), 3.61 (s, 2 H, H-2')] esters, respectively, of the same diterpene alcohol of molecular formula $C_{20}H_{28}O_4$. The structure of the common diterpene portion of the valdivones was elucidated by analysis of the ^1H and ^{13}C NMR spectra with the aid of the COSY, DEPT, HMQC and HMBC experiments (see Table 1); the ^1H NMR spectrum of **1** was recorded in C_6D_6 solution after it was found that key signals (H-14, H-18, H-5') overlapped in the $CDCl_3$ spectrum.

The COSY experiment revealed the contiguous sequence of coupled signals from H-8 to the isopropyl group attached at C-14 and showed the coupling between H-1 and H-2 and between H-5 and H-6. The H-5 and H-6 signals at δ 6.09 (s, 2H) in $CDCl_3$ or at 5.76 (d, 1 H, J = 6 Hz) and 5.87 (d, 1 H, J = 6 Hz) in C_6D_6 were coupled only to each other and the chemical shifts and coupling constant are appropriate for the β-hydrogens on a 2,2,5,5-tetrasubstituted dihydrofuran ring. Long-range couplings between H-2 and CH_3-15 and from H-12 to CH_3-17 were also observed. The HMBC experiment (Table 2) was instrumental in assembling the diterpene carbon skeleton and allowed no alternative structure. In particular, the multiple correlations from the H-1 signal, the correlations from the methyl groups, and correlations to the C-4 and C-7 signals can be used to define the ring system. The correlation between H-8 and C-1' requires that the ester be attached at C-8.

Table 1. ^{13}C [50 MHz, δ (mult.)] and ^{1}H NMR [500 MHz, δ (mult., *J* in Hz)] data for valdivones A (**1**) and B (**2**).

	valdivone A (**1**)				valdivone B (**2**)			
C#	δ_C ($CDCl_3$)		δ_H (C_6D_6)		δ_C ($CDCl_3$)		δ_H ($CDCl_3$)	
1	37.6	(d)	4.55	(ddd, 9.5, 4.5, 2)	37.6	(d)	4.21	(ddd, 9.5, 4.5, 2)
2	127.6	(d)	5.29	(dd, 9.5, 1.5)	127.6	(d)	4.97	(br d, 9.5)
3	135.6	(s)			135.6	(s)		
4	113.0	(s)			113.0	(s)		
5	131.1	(d)	5.76	(d, 6)	131.3	(d)	6.08	(d, 6)
6	134.0	(d)	5.87	(d, 6)	133.7	(d)	5.95	(d, 6)
7	90.2	(s)			90.0	(s)		
8	80.3	(d)	4.93	(dd, 7, 1)	81.7	(d)	4.58	(br d, 7)
9	31.2	(t)	1.78	(br d, 15)	31.0	(t)	1.53	(br d, 15)
			1.66	(ddd, 15, 11, 7)			1.46	(ddd, 15, 12, 7)
10	40.9	(d)	3.27	(br d, 11, 4.5)	40.9	(d)	3.00	(br d, 12)
11	160.0	(s)			159.3	(s)		
12	126.3	(d)	5.86	(br s)	126.5	(d)	5.67	(br s)
13	203.1	(s)			202.7	(s)		
14	59.4	(d)	2.19	(dd, 10.5, 2)	59.4	(d)	1.86	(m)
15	21.7	(q)	1.61	(br s, 3 H)	21.7	(q)	1.69	(s, 3 H)
16	25.8	(q)	1.31	(s, 3 H)	25.6	(q)	1.32	(s, 3 H)
17	22.9	(q)	1.43	(br s, 3 H)	22.7	(q)	1.73	(s, 3 H)
18	26.9	(d)	1.81	(m, 10.5, 7)	26.9	(d)	1.88	(m)
19	21.4	(q)	1.02	(d, 3 H, 7)	21.4	(q)	0.87	(d, 3 H, 7)
20	22.2	(q)	1.01	(d, 3 H, 7)	22.1	(q)	1.03	(d, 3 H, 7)
1'	165.9	(s)			171.0	(s)		
2'	115.7	(d)	5.67	(br s)	41.7	(t)	3.61	(s, 2 H)
3'	158.2	(s)			133.6	(s)		
4'	27.5	(q)	1.45	(s, 3 H)	129.1	(2d)		
5'	20.3	(q)	2.09	(s, 3 H)	128.7	(2d)	7.21–7.27	(m, 5 H)
6'					127.4	(d)		



6 X = H_2
7 X = O

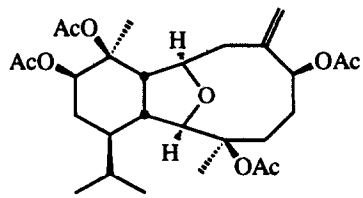


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Table 2. ^{1}H–^{13}C correlations from the HMBC experiment on valdivone A (**1**).

^{1}H signal	^{13}C correlations
H-1	C-2 C-3 C-10 C-11 C-13 C-14 C-18
H-2	C-3 C-4 C-14 C-15
H-5	C-4 C-6 C-7
H-6	C-4 C-5 C-7
H-8	C-6 C-7 C-9 C-10 C-16 C-1'
H-9	C-1 C-7 C-8 C-10
H-10	C-1 C-9 C-11 C-12
H-12	C-10 C-14 C-17
H-14	not observed
H-15	C-2 C-3 C-4
H-16	C-6 C-7 C-8
H-17	C-10 C-11 C-12
H-18	not observed
H-19	C-14 C-18 C-20
H-20	C-14 C-18 C-19
H-2'	C-1' C-3' C-4' C-5'
H-4'	C-2' C-3' C-5'
H-5'	C-2' C-3' C-4'
OH	C-3 C-4 C-5

A search of the marine natural products literature[2] uncovered a series of diterpenes, exemplified by sarcodictyin A (**6**),[3] that had the same carbon skeleton and the identical dihydrofuran ring system. The most closely related structure, the ketone **7**, was obtained by oxidation of the corresponding alcohol, sarcodictyin C.[4] The coupling constants in the ^{1}H NMR spectrum of **7** are nearly identical to those of valdivones A (**1**) and B (**2**), which implies the same stereochemistry at C–1, C–8, C–10, and C–14. The stereochemistry was confirmed by selected NOEDS experiments. Irradiation of the H–9β signal in $CDCl_3$ solution resulted in enhancement of the H–16, H–10, and H–8 signals while irradiation of the H–8 signal caused enhancement of the H–1, H–10, and H–16 (weak) signals. Owing to the overlap of the H–14 and H–18 signals in $CDCl_3$ solution, the H–1 signal was irradiated in C_6D_6 solution to obtain enhancements of the H–18 and H–9β signals. These data support the structural and stereochemical assignments that were assumed by comparison of the ^{1}H NMR data of valdivones A (**1**) and B (**2**) with those of ketone **7**.

4–O–Methyl valdivone A (**3**) and 4–O–methyl valvidone B (**4**) were isolated as very minor constituents and were identified essentially on the basis of their ^{1}H NMR spectra that each contained a methoxy signal at δ 3.18 (for **3**) or 3.16 (for **4**) in place of the hydroxyl proton signal. The remaining signals in the ^{1}H NMR spectra of methyl ketals **3** and **4** were almost identical to the corresponding signals in hemiketals **1** and **2**, respectively. The methyl ketals **3** and **4** are considered to be artifacts of the isolation procedure that involved extraction with methanol.

Dihydrovaldivone A (**5**) is a minor compound that was separated with difficulty from valdivone A (**1**). Inspection of the ^{1}H NMR spectrum revealed the presence of an isovalerate ester, which gave rise to signals at δ 0.96 (d, 6 H, J = 6 Hz, H–4',5'), 2.10 (m, 1 H, J = 6 Hz, H–3'), and 2.21 (d, 2 H, J = 6 Hz, H–2'), and which replaced the dimethylacrylate ester in valdivone A (**1**). A COSY experiment was used to locate all of the remaining ^{1}H NMR signals for **5**, which were at almost identical chemical shifts and showed similar coupling constants to the corresponding signals for **1**.

The carbon skeleton of the valdivones was first encountered in eunicellin (**8**) from the gorgonian *Eunicella stricta*[5] and later in metabolites from both gorgonians and soft corals.[6] The major difference between the eunicellin–type compounds and the sarcodictyins and valdivones is the location of the ether ring. The close relationship between the valdivones and the sarcodictyins is all the more remarkable because the producing organisms, *Alcyonium valdivae* (Order Alcyonacea) and *Sarcodictyon roseum* (Order Stolonifera) belong to different orders of the class Alcyonaria.

Valdivones A (**1**) and B (**2**) show fairly strong inhibition of chemically–induced inflammation in the mouse ear assay[7] [**1**, 93% inhibition at 50 μg/ear; **2**, 72% inhibition at 50 μg/ear] but do not strongly inhibit bee venom phospholipase A_2 [**1** and **2**, 43% inhibition at 16 μg/ml]. The valdivones are inactive against a standard panel of bacteria and fungi.

Experimental Section

Collection, extraction and isolation: The yellow soft coral *Alcyonium valdivae* [SIO collection # 92-046; California Acadamy of Sciences catalog # CASIZ 087152] was collected by hand using SCUBA (-15 m) from Coffee Bay, Transkei, South Africa, and was immediately frozen. After 3 months at -20°C, the sample (41.1 g dry wt.) was sonicated in methanol (3 x 250 mL) and each batch of methanol was decanted and evaporated. The combined extracts were partitioned between water (25 mL) and hexane (2 x 25 mL), dichloromethane (25 mL), and ethyl acetate (25 mL). Each of the organic extracts was dried and evaporated to obtain the hexane (690 mg), dichloromethane (400 mg), and ethyl acetate (20 mg) fractions. Analysis of the organic extracts by TLC and ^{1}H NMR spectroscopy revealed similar interesting components in all fractions and they were therefore combined to yield a brown gum.

The combined extracts were chromatographed on silica gel using a gradient elution from hexane to ethyl acetate. The fraction eluting with 40% ethyl acetate in hexane contained a mixture of valdivones A and B that appeared to co-crystallize. The two components were separated by rechromatography on silica to obtain valdivone A (**1**, 40 mg, 0.097% dry wt.) and valdivone B (**2**, 28 mg, 0.068% dry wt.). Both compounds were obtained as colorless needles from 1:1 ethyl acetate/hexane. The mother liquor from the recrystallization of valdivone A (**1**) was subjected to repeated HPLC on silica using 20% ethyl acetate in hexane as eluant to obtain dihydrovaldivone A (**5**, 2 mg, 0.0049% dry wt.), which is slightly less polar. The fraction eluting with 10% ethyl acetate in hexane contained a mixture of 4-O-methyl valdivone A (**3**, 1 mg, 0.0024% dry wt.) and 4-O-methyl valvidone B (**4**, 1 mg, 0.0024% dry wt.), that were separated and purified by HPLC on silica using 20% ethyl acetate in hexane as eluant.

Valdivone A (1): colorless needles, mp. 89-91°C; $[\alpha]_D$ = 94.4° (c 0.39, $CHCl_3$); IR (KBr) 3420, 2950, 1720, 1652, 1450 cm^{-1}; UV ($CHCl_3$) 254 nm (ε 7650); ^{1}H NMR ($CDCl_3$) δ 6.09 (br s, 2 H, H-5, H-6), 5.71 (br s, 1 H, H-12), 5.68 (br s, 1 H, H-2'), 4.98 (br d, 1 H, *J* = 9.5 Hz, H-2), 4.65 (br d, 1 H, *J* = 7 Hz, H-8), 4.28 (ddd, 1 H, *J* = 9.5, 4.5, 2 Hz, H-1), 3.07 (br d, 1 H, *J* = 12 Hz, H-10), 2.18 (s, 3 H, H-5'), 1.90 (s, 3 H, H-4'), 1.89 (m, 2 H, H-14, H-18), 1.75 (s, 3 H, H-17), 1.74 (s, 3 H, H-15), 1.63 (br d, 1 H, *J* = 15 Hz, H-9), 1.44 (ddd, 1 H, *J* = 15, 12, 7 Hz, H-9), 1.43 (s, 3 H, H-16), 1.06 (d, 3 H, *J* = 7 Hz, H-19), 0.87 (d, 3 H, *J* = 7 Hz, H-20); ^{1}H NMR (C_6D_6) see Table 1.; ^{13}C NMR ($CDCl_3$) see Table 1.; FABMS, *m/z* (int, %), 415 (87), 397 (42), 314 (19), 297 (26), 83 (100); HRFABMS, Obsd. *m/z* = 415.2465 $(MH)^+$, $C_{25}H_{35}O_5$ requires 415.2485.

Valdivone B (2): colorless needles, mp. 171-173°C; $[\alpha]_D$ = 79.4° (c 0.57, $CHCl_3$); IR (KBr) 3427, 3030, 1728, 1652, 1370 cm^{-1}; UV ($CHCl_3$) 242 nm (ε 11660); ^{1}H NMR ($CDCl_3$) see Table 1.; ^{13}C NMR ($CDCl_3$) see Table 1.; FABMS, *m/z* (int, %), 451 (100), 433 (56), 314 (19), 297 (14), 91 (74); HRFABMS, Obsd. *m/z* = 451.2484 $(MH)^+$, $C_{28}H_{35}O_5$ requires 451.2485.

4-O-Methyl valdivone A (3): oil; IR ($CHCl_3$) 2930, 1720, 1650 cm^{-1}; UV ($CHCl_3$) 248 nm (ε 2510); ^1H NMR ($CDCl_3$) δ 6.13 (d, 1 H, *J* = 6 Hz, H-5), 5.96 (d, 1 H, *J* = 6 Hz, H-6), 5.69 (br s, 2 H, H-12,2'), 5.06 (br d, 1 H, *J* = 9.4 Hz, H-2), 4.65 (br d, 1 H, *J* = 6 Hz, H-8), 4.27 (ddd, 1 H, *J* = 9.4, 5, 2 Hz, H-1), 3.18 (s, 3 H, O-Me), 3.08 (m, 1 H, H-10), 2.16 (br s, 3 H, H-5'), 1.91 (s, 3 H, H-4'), 1.89 (m, 1 H, H-14), 1.76 (s, 3 H, H-15), 1.66 (s, 3 H, H-17), 1.42 (s, 3 H, H-16), 1.05 (d, 3 H, *J* = 6 Hz, H-20), 0.86 (d, 3 H, *J* = 6 Hz, H-19); HRMS, Obsd. *m/z* = 428.2555, $C_{26}H_{36}O_5$ requires 428.2563.

4-O-Methyl valdivone B (4): oil; IR ($CHCl_3$) 2970, 1730, 1655 cm^{-1}; UV ($CHCl_3$) 251 nm (ε 1830); ^1H NMR ($CDCl_3$) δ 7.27 (br m, 5 H), 6.02 (d, 1 H, *J* = 6 Hz, H-5), 5.95 (d, 1 H, *J* = 6 Hz, H-6), 5.70 (br s, 1 H, H-12), 5.03 (br d, 1 H, *J* = 9 Hz, H-2), 4.60 (br d, 1 H, *J* = 6 Hz, H-8), 4.19 (ddd, 1 H, *J* = 9, 5, 2 Hz, H-1), 3.65 (s, 2 H, H-2'), 3.16 (s, 3 H, O-Me), 2.99 (m, 1 H, H-10), 1.86 (m, 1 H, H-14), 1.70 (s, 3 H, H-15), 1.65 (s, 3 H, H-17), 1.30 (s, 3 H, H-16), 1.02 (d, 3 H, *J* = 6 Hz, H-20), 0.86 (d, 3 H, *J* = 6 Hz, H-19); HRMS, Obsd. *m/z* = 464.2569, $C_{29}H_{36}O_5$ requires 464.2563.

Dihydrovaldivone A (5): oil; IR ($CHCl_3$) 2970, 1730, 1660 cm^{-1}; UV ($CHCl_3$) 248 nm (ε 2270); ^1H NMR ($CDCl_3$) δ 6.10 (s, 2 H, H-5,6), 5.72 (br s, 1 H, H-12), 4.98 (br d, 1 H, *J* = 9 Hz, H-2), 4.61 (br d, 1 H, *J* = 6 Hz, H-8), 4.25 (ddd, 1 H, *J* = 9, 5, 2 Hz, H-1), 3.03 (m, 1 H, H-10), 2.21 (d, 2 H, *J* = 6 Hz, H-2'), 2.10 (m, 1 H, *J* = 6 Hz, H-3'), 1.74 (s, 6 H, H-15, H-17), 1.45 (s, 3 H, H-16), 1.07 (d, 3 H, *J* = 6 Hz, H-20), 0.96 (d, 6 H, *J* = 6 Hz, H-4',5'), 0.86 (d, 3 H, *J* = 6 Hz, H-19); ^{13}C NMR ($CDCl_3$) δ 202.9, 159.6, 135.5, 133.9, 131.2, 127.7, 126.5, 113.0, 90.1, 81.2, 59.5, 43.5, 40.9, 37.6, 31.3, 26.8, 25.8 (2C), 22.8, 22.3, 22.2, 21.7, 21.4 (2 signals not observed); HRMS, Obsd. *m/z* = 416.2557, $C_{25}H_{36}O_5$ requires 416.2563.

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