Cladocoran A and B: Two Novel γ-Hydroxybutenolide Sesterterpenes from the Mediterranean Coral *Cladocora cespitosa*

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The novel terpenoids, cladocoran A (**1**) and B (**2**), showing a γ-hydroxybutenolide end group, have been isolated from the Mediterranean Anthozoan *Cladocora cespitosa*. The unprecedented skeleton of cladocoran was elucidated by spectroscopic methods and chemical conversion. The absolute stereochemistry of the secondary alcohol at C-18 was assigned by the advanced Mosher's method applied to an oxidized derivative of **1**.

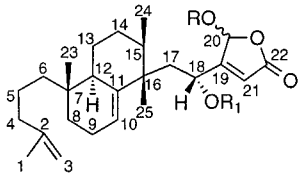
Introduction

Marine organisms have attracted considerable attention as a source of novel natural products with unique structures and biological activities.[2−4] In particular, terpenes[4] show an unrivaled example of chemical diversity and physiologically interesting properties.[5] Despite the great interest in marine organisms, only a few studies concern corals of the order Madreporaria.[2] Recently, we have isolated two novel sesterterpenoids from the colonial Anthozoan *Cladocora cespitosa* (L.), cladocoran A (**1**) and B (**2**). In this paper we wish to describe the identification and the remarkable chemical reactivity of **1** and **2**, occurring as the main metabolites of the diethyl ether soluble fraction from the acetone extract of the coral.

Results and Discussion

C. cespitosa (L.) is a Mediterranean organism living along the Italian and Spanish coasts. The Et_2O extract (720 mg) of the coral was chromatographed on a silica gel column, affording several fractions, one of which showed two UV-absorbing products positive to a spot-reaction with cerium sulfate. Further purification of this fraction gave the novel γ-hydroxybutenolides **1** (30 mg, R_f 0.6 petroleum/diethyl ether, 4:6) and **2** (12 mg, R_f 0.2 petroleum/diethyl ether, 4:6).

Treatment of cladocoran A (**1**) with CH_2N_2 in diethyl ether gave quantitatively the optically active methyl ester **3**, ($[\alpha]_D = -42.0°$). Compound **3** exhibited an EIMS molecular ion at *m/z* 472 corresponding to the molecular formula $C_{29}H_{44}O_5$ (HREIMS *m/z* 472.3165). The carbon resonances at δ 169.4 and 166.4 and the IR absorptions at 1744 and 1722 cm^{-1} indicated two ester functions, one of which was assigned to a β,β′-disubstituted α,β-



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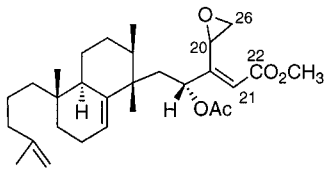
2 R = H; R_1 = H
5 R = Ac; R_1 = Ac



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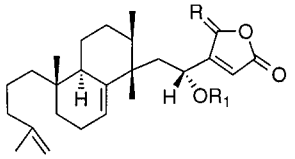

4 R = H,H; R_1 = Ac
6 R = CH_2; R_1 = H

unsaturated moiety on the basis of the proton signal at δ 5.94 and the UV band centered at 224 nm. The ^1H NMR spectrum of **3** was poorly defined in $CDCl_3$ due to signal overlapping (Table 1). However, it was satisfactorily resolved when measured in C_5D_5N. The resonances at δ 6.22 (H-21), 5.56 (H-10), 4.87 (H-3a), and 4.83 (H-3b) in the ^1H NMR (C_6D_5N) spectrum, as well as the six ^{13}C NMR signals in the olefinic region, implied two trisubstituted double bonds and one exomethylene group that accounted for three of the eight unsaturations required. The broad signal at δ 4.85 (H-20) was coupled to the methylene protons at δ 3.26 (H-26a) and 3.11 (H-26b) and showed long-range connectivities with the carbons at δ 160.8 (C-19), 117.7 (C-21), and 47.5 (C-26) in the HMBC experiments (Figure 1). Moreover, consistent with partial structure **c**, diagnostic correlations were also observed between the olefinic carbon at δ 160.8 (C-19) and the protons at δ 6.22 (H-21) and 4.96 (H-18). The

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Table 1. ^{1}H and ^{13}C NMR Data[a] of Cladocoran A Methyl Ester (3) in C_5D_5N and $CDCl_3$

	C_5D_5N			$CDCl_3$	
position	H[b]	C[c]	HMBC	H[b]	C[c]
1	1.73 (br s, 3H)	22.6 q	H-3a, H-3b, H-4	1.72 (br s, 3H)	22.6 q
2		146.4 s	H-1, H-3a, H-3b, H-4, H-5		146.5 s
3	4.83 (s, 1H)	110.2 t	H-1, H-4	4.69 (s, 2H)	109.3 t
	4.87 (s, 1H)				
4	2.10 (m, 2H)	38.9 t	H-1, H-3a, H-3b, H-5, H-6b	2.02 (m, 2H)	38.5 t
5	1.50 (m, 2H)	21.9 t		1.43 (m, 2H)	21.5 t
6	1.35 (m, 1H)	39.8 t	H-4, H-5, H-23	1.23 (m, 1H)	39.4 t
	1.63 (m, 1H)			1.43 (m, 1H)	
7		34.4 s	H-5, H-6a, H-6b, H-8, H-12, H-23		34.1 s
8	1.33 (m, 2H)	30.2 t	H-6a, H-9, H-10	1.30 (m, 2H)	29.3 t
9	1.95 (m, 2H)	23.4 t	H-8, H-10	1.98 (m, 2H)	23.1 t
10	5.56 (br t, 1H)	119.6 d	H-8	5.43 (br t, 1H, 3.4)	119.2 d
11		144.2 s	H-9, H-12, H-13, H-15, H-25		143.8 s
12	2.19 (m, 1H)	43.3 d	H-6a, H-6b, H-10, H-13a, H-23	1.99 (m, 1H)	43.0 d
13	1.05 (ddd, 1H, 5.5, 5.5, 12.5)	29.1 t	H-12, H-14	1.05 (ddd, 1H, 3.8, 4.2, 12.9)	28.7 t
	1.80 (bdd, 1H, 12.5, 3.1)			1.80 (m, 1H)	
14	1.47 (m, 2H)	31.5 t	H-13, H-15, H-24	1.35 (m, 1H)	31.2 t
				1.56 (m, 1H)	
15	1.22 (m, 1H)	44.9 d	H-12, H-13a, H-13b, H-14, H-24	1.23 (m, 1H)	44.6 d
16		42.9 s	H-10, H-14, H-15, H-17a, H-17b, H-24, H-25		42.2 s
17	1.38 (dd, 1H, 3.0, 14.6)	37.1 t	H-18, H-25	1.30 (m, 1H)	36.7 t
	2.20 (m, 1H)			2.05 (m, 1H)	
18	4.96 (m, 1H)	69.3 d	H-17a, H-20, H-21	4.71 (d, 1H, 7.0)	69.1 d
19		160.8 s	H-17a, H-17b, H-18, H-20, H-21, H-26a, H-26b		160.6 s
20	4.85 (br s, 1H)	50.2 d	H-18, H-21, H-26a, H-26b	4.57 (t, 1H, 3.5)	49.7 d
21	6.22 (s, 1H)	117.7 d	H-18	5.94 (s, 1H)	116.9 d
22		166.5 s	H-21, MeCOO		166.4 s
23	0.81 (s, 3H)	22.9 q	H-6b	0.79 (s, 3H)	22.7 q
24	0.84 (d, 3H, 7.0)	16.7 q	H-15	0.83 (d, 3H, 6.7)	16.4 q
25	1.28 (s, 3H)	25.9 q	H-15, H-17a, H-17b	1.16 (s, 3H)	25.5 q
26	3.11 (t, 1H, 4.6)	47.5 t		3.03 (d, 2H, 3.5)	47.2 t
	3.26 (dd, 1H, 4.6, 2.2)				
MeCOO	3.65 (s, 3H)	51.4 q		3.71 (s, 3H)	51.4 q
MeCO	2.03 (s, 3H)	20.9 q		2.01 (s, 3H)	21.2 q
		169.9 s			169.4 s

[a] Assignments aided by ^{1}H COSY, ^{1}H−^{1}H decoupling, and HMQC experiments. [b] 500 MHz. [c] 125 MHz.

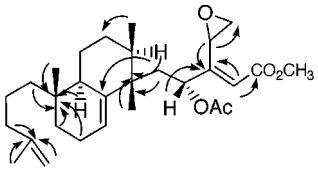
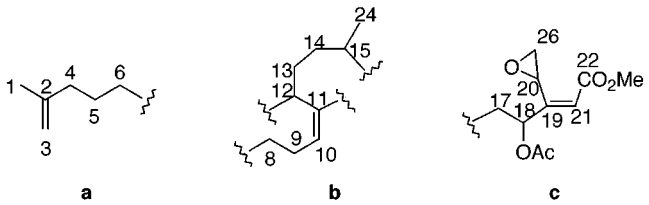


Figure 1. HMBC for compound **3**.

Chart 1



acetoxy function, suggested by the sharp singlet at δ 2.03 in the ^{1}H NMR spectrum, was assigned at C-18 (δ 69.3) on the basis of the downfield shift (δ 4.96) of the carbinol proton, whereas the COSY cross-peaks allowed linkage C-18 to C-17 (δ 1.38 and 2.20). The structure of the C1−C6 side chain (partial structure **a**) was defined by the presence of the C3−C5 system in the TOCSY spectrum and by the $^{2,3}J_{C,H}$ correlations of the quaternary carbon at δ 146.4 (C-2) with the protons at δ 2.10 (H_2-4), 1.73 (H_3-1), and 1.50 (H_2-5) (Figure 1). A more complex analysis was required for the elucidation of the central bicyclic system (partial structure **b**) (Chart 1). Irradiation of the broad triplet at δ 5.56 (H-10) indicated an allylic coupling with the signal at δ 2.19 (H-12) and a vicinal coupling with that at δ 1.95 (H_2-9). This latter resonance revealed a further interaction with the methylene group centered at δ 1.33 (H_2-8). On the other hand, TOCSY data connected clearly the carbon chain from C_{12} to C_{15} and confirmed the results of the COSY experiment which showed cross-peaks between the adjacent protons H-12/H_2-13, H_2-13/H_2-14, and H_2-14/H-15. Double resonance and COSY experiments also indicated coupling between the methyl doublet at δ 0.84 (H_3-24) and the methine at δ 1.22 (H-15). The HMBC spectrum was diagnostic in closing the bicyclic system and in linking the partial structures **a**, **b**, and **c**. The quaternary carbon at δ 34.4 (C-7) showed long-range correlations with the protons at δ 2.19 (H-12), 1.63 (H-6a), 1.35 (H-6b and H_2-8), 1.50 (H_2-5), and 1.95 (H_2-9), as well as with the methyl group at δ 0.81 (H_3-23) (Figure 1). In the same way, the methine proton at δ 1.22 (H-15) was correlated to the quaternary carbons at δ 42.9 (C-16) and 144.2 (C-11), and the signal at δ 2.19 (H-12) to the C-11 (δ 144.2). Finally, cross-peaks from C-16 (δ 42.9) to the methyl at δ 1.28 (H_3-25) and the methylene protons at C-17 (δ 1.38 and 2.20) connected **b** and **c**, and completed the skeletal assignment (Table 1).

The relative stereochemistry of the bicyclic system in **3** was established by analysis of vicinal coupling constants ($^3J_{vic}$) and of NOEs (Figure 2) in three different NOESY experiments. The proton at C-12 showed an axial−axial ($J_{H-12/H-13a}$ = 12.5 Hz) and an axial−equatorial ($J_{H-12/H-13b}$ = 5.5 Hz) coupling constant with the methylene group at C-13, whereas H-15 occurred as a broad signal exhibiting two small couplings with H_2-14

Table 2. ^{1}H and ^{13}C NMR Data[a] of Compounds 1 and 2

	1			2	
position	H[b]	C[c]	HMBC	H[b]	C[c]
1	1.72 (br s, 3H)	22.4 q		1.70 (br s, 3H)	22.3 q
2		146.9 s	H-1		146.8 s
3	4.70 (s, 2H)	109.5 t	H-1	4.65 (s, 1H)	109.8 t
				4.71 (s, 1H)	
4	2.02 (m, 2H)	38.3 t	H-1	2.01 (m, 2H)	38.3 t
5	1.40 (m, 2H)	21.5 t		1.45 (m, 2H)	21.3 t
6	1.05 (m, 1H)	39.2 t	H-23	1.05 (m, 1H)	38.8 t
	1.40 (m, 1H)			1.40 (m, 1H)	
7		34.1 s	H-9, H-23		33.9 s
8	1.30 (m, 2H)	29.3 t	H-23	1.25 (m, 1H)	29.0 t
				1.35 (m, 1H)	
9	1.95 (m, 2H)	22.9 t	H-8	2.02 (m, 2H)	22.7 t
10	5.55 (br t, 1H)	120.5 d	H-9, H-17, H-25	5.64 (br t, 1H)	119.7 d
11		143.3 s	H-25		143.4 s
12	1.58 (m, 1H)	44.1 d	H-23	1.58 (m, 1H)	43.5 d
13	1.08 (m, 1H)	29.7 t		1.10 (m, 1H)	29.3 t
	1.80 (m, 1H)		H-24	1.82 (m, 1H)	
14	1.35 (m, 1H)	31.3 t		1.35 (m, 1H)	31.2 t
	1.61 (m, 1H)		H-24, H-25	1.60 (m, 1H)	
15	1.30 (m, 1H)	44.7 d	H-24, H-25	1.30 (m, 2H)	44.8 d
16		42.8 s	H-24, H-25		42.6 s
17	1.16 (m, 1H)	34.7 t	H-25	1.47 (m, 1H)	37.1 t
	1.72 (m, 1H)			2.05 (m, 1H)	
18	5.19 (d, 1H, 10.6)	67.5 d	H-17, H-21	4.54 (d, 1H, 10.5)	66.4 d
19		168.4 s	H-18		170.0 s
20	6.05 (br s, 1H)	97.5 d	H-18, H-21	6.11 (br s, 1H)	97.1 d
21	5.95 (s, 1H)	118.4 d	H-18	6.01 (s, 1H)	117.2 d
22		169.0 s	H-20, H-21		171.0 s
23	0.82 (s, 3H)	23.3 q		0.83 (s, 3H)	23.7 q
24	0.88 (d, 3H, 7.0)	16.4 q		0.86 (d, 7.0, 3H)	16.4 q
25	1.16 (s, 3H)	24.7 q		1.26 (s, 3H)	25.7 q
MeCO	2.08 (s, 3H)	20.9 q			
		171.0 s	H-18, Me		

[a] Assignments aided by ^{1}H COSY, ^{1}H−^{1}H decoupling, and HMQC experiments. [b] 500 MHz. [c] 125 MHz.

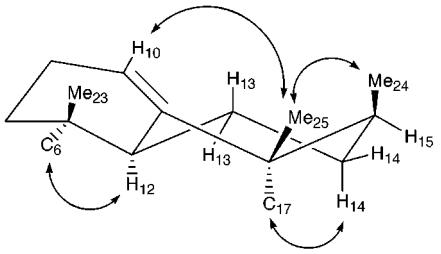


Figure 2. Selected NOE for compound **3**.

(Figure 2). A strong enhancement (16%) was experienced by CH_3-25 when H-10 had been irradiated, and a cis relationship between CH_3-25 and CH_3-24 was attributed on the basis of a cross-peak in the NOESY spectrum. A weak but very diagnostic NOE between H-12 and the methylene hydrogens at C-6 defined the relative stereochemistry of the alkyl chain at C-7. We assigned all the methyl groups as β on biogenetic grounds, but it is impossible to exclude the possibility that the orientation of CH_3-23 is opposite to that of CH_3-24 and CH_3-25.

Once the novel skeleton of **3** was elucidated, the structure of the natural precursor **1** was easy to assign (Table 2). Cladocoran A (**1**) is an amorphous colorless oil, $[\alpha]_D = -25.8°$. Its molecular formula was determined to be $C_{27}H_{40}O_5$ by HREIMS (444.2875) and differs from that of **3** by the loss of 28 Da. The missing units were those of the ester methyl group and of the methylene moiety in the oxirane ring. The ^{13}C NMR in $CDCl_3$ (Table 2) contained 27 signals including seven quaternary carbons and five methyl, nine methylene, six methine groups, of which one was a vinylic carbon (δ 118.4, C-21), one a hemiacetal carbon (δ 97.5, C-20), and another a hydroxyl-bearing carbon (δ 67.5, C-18) (Table 2). The assignment of dehydrodecaline system resonances as well as those of the side chain in compound **3** were enterely supported by 2D-NMR experiments and confirmed by comparison with data in **1**. In the same way, the relative stereochemistry of the ring substituents in cladocoran A (**1**) was determined to be the same as that found in **3** by NOESY and monodimensional NOE experiments. The remaining NMR data of **1**, which greatly differed from those of **3**, suggested the presence of a γ-hydroxybutenolide inserted in a molecular arrangement related to that of secomanoalide (**7**)[8] or of luffariellin B (**8**).[9] In fact, the HMBC spectrum revealed long-range correlations between H-21 and C-22 (δ 168.2), C-19 (δ 170.1) and C-20 (δ 97.5), as well as cross-peaks of C-19 with H-20 and H-18. This last proton also showed COSY correlations both with the methylene protons at δ 1.72 and 1.16 (Hs-17) and with olefin hydrogen at δ 5.95 (H-21). Finally, as occurred in **3**, the long-range correlations of H_2-17 with C-16 (δ 42.8) and CH_3-25 (δ 24.7) allowed the side chain to be linked to C-16 and completed the unambiguous structural characterization of the sesterterpene **1**.

It is obvious that the γ-hydroxy γ-lactone end moiety of cladocoran A (**1**) arises from the intramolecular addition of the carboxylic acid to an aldehyde group at C-20

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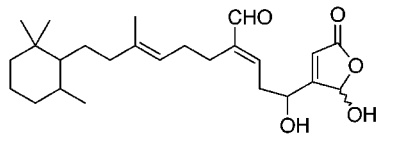

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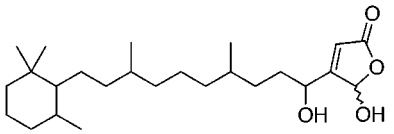

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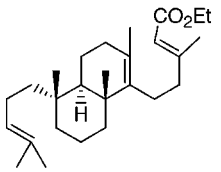

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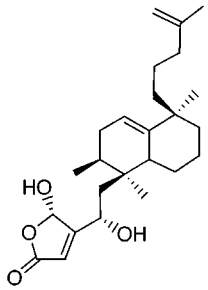

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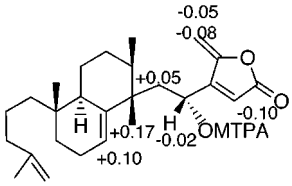

Figure 3. $\Delta\delta$ observed for MTPA [$\delta_{S\ \text{ester}} - \delta_{R\ \text{ester}}$] derivatives of **6**. Spectra were recorded in $CDCl_3$ at 25 °C.

Chart 2

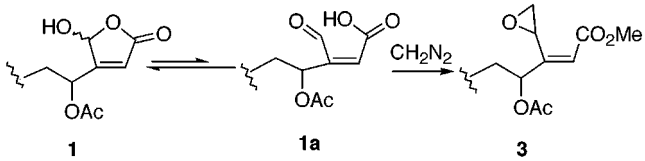

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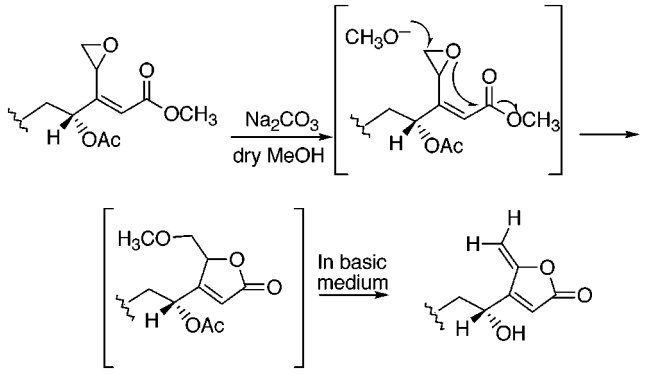
Chart 3



(Chart 2). As suggested by the small signal at δ 9.41 in the ^{1}H NMR spectrum (see Supporting Information), the cyclic structure seems to be in equilibrium with the open form **1a**. Our efforts to isolate the native aldehyde were fruitless, but the presence of this compound explains satisfactorily the formation of compound **3** (Chart 2). Additionally, the reduction[10] of **1** with $NaBH_4$ in MeOH gave the γ-butenolide **4** as the major product (see Experimental Section). The lactone **4** had the molecular formula $C_{27}H_{40}O_4$ (EIMS *m/z* 428) and showed an AB system (δ 4.71 and 4.82, J = 16.5) for H_2-20, as well as the expected upfield-shift of C-20 (δ 70.8) in the ^{13}C NMR spectrum (see Experimental Section).

Cladocoran A (**1**) occurs in the organic extract as a mixture of α and β epimers at C-20. In C_5D_5N (see Supporting Information), the signals of H-20 for the α and β diasteroisomers appeared resolved and of similar intensities. This was confirmed by GC−MS and HPLC analysis of the mixture of epimeric alcohols **5a** and **5b** obtained by acetylation of **1** (see Experimental Section).

The absolute configuration of the cladocoran skeleton at C-18 was deduced by Mosher's method.[10,11] As the hydrolysis of the acetyl function of **1** in alkaline or acidic medium gave an unexpectedly complex mixture of products, the absolute stereochemistry was established by MTPA esterification of the derivative **6**. This latest compound resulted from treatment of the product **3** with Na_2CO_3 in dry MeOH. It is likely that **6** is formed by a transesterification involving the secondary alcohol derived from the opening of the oxirane ring (Chart 3). Reaction of **6** with (*R*)- and (*S*)-MTPA chlorides in dry pyridine yielded the corresponding (*S*)-MTPA (**6a**) and (*R*)-MTPA (**6b**) esters. The *R* absolute configuration of C-18 was assigned on the basis of the $\Delta\delta$ values[11,12] calculated by the ^{1}H NMR and COSY spectra of **6a** and **6b** in $CDCl_3$ (Figure 3).

Cladocoran B (**2**) was isolated as a colorless oil. Its molecular weight was determined at *m/z* 402, corresponding to the molecular formula $C_{25}H_{38}O_4$ (HREIMS 402.2774 *m/z*). All spectral data for **2** were very similar to those of **1**, except for the absence of the acetyl group at C-18 (Table 2). The upfield shift of H-18 (δ 4.54 ppm) in **2** supported the proposed structure of cladocoran B (**2**). This assignment was further confirmed by the acetylation of **2** with acetic anhydride in pyridine to give **1**.

Conclusion

The extracts of the colonial organism *C. cespitosa* contain the unique sesterterpenes **1** and **2** featuring the γ-hydroxybutenolide group previously associated with phospholipase A_2 inhibition.[16−19] Although characterized by a novel carbon skeleton, cladocorans share more than

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one analogy with the aldose reductase inhibitor dyside-apaluanic acid (**9**)[13] and the protein phosphatase inhibitor dysidiolide (**10**),[20] both recently isolated from the organic extracts of sponges *Dysidea*.

Regarding the origin of cladocorans, some doubts still remain about the actual organism able to synthesize them. The cladocoran skeleton, as previously stated, is closely related to compounds typically found in sponges. Moreover, the calcareous exoskeleton of the *Cladocora* collected at Aguilas offers hospitality to a population of green algae and, very likely, other organisms, such as bacteria or encrusting sponges. Extracts of the coral from other Mediterranean sites (south of Spain and southeast of Italy) do not contain **1** and **2**, thus supporting the thesis of their origin from symbionts or sponges.

Experimental Section

Collection. The colonial Anthozoan *C. cespitosa* (L.) was collected off Aguilas (south of Spain) at a depth of 20 m, in September 1995. The colonial organisms (250 g dry weight), frozen immediately, were transferred to ICMIB and stored at −20 °C until the analysis day. A voucher specimen is deposited in our institute.

Extraction and Isolation. The frozen animals were chopped three times, and extracted with acetone (3 × 500 mL). The acetone extracts were filtered and reduced in vacuo to an aqueous layer (ca. 400 mL) and partitioned with diethyl ether (4 × 300 mL). At once, the ethereal fractions were evaporated in vacuo to yield ca. 720 mg of extract. A silica gel column (200 g) was packed with light petroleum ether and eluted with increasing amounts of diethyl ether. Fractions obtained from light petroleum ether/diethyl ether 6:4 (100 mg) and light petroleum ether/diethyl ether 3:7 (60 mg) were submitted to another purification on silica gel column (15 g), achieving pure cladocoran **1** (30 mg) and **2** (12 mg).

Cladocoran A (**1**): pale yellow oil; $[\alpha]^{20}_D = -25.8°$ (*c* 0.4, $CHCl_3$); UV λ_{max} (EtOH) 224 (ϵ 8400); IR ν_{max} (liquid film) 3367, 2935, 2865, 1762, 1753, 1645, 1452, 1375, 1228, 1135, 888, 764; EIMS, *m*/*z* (%) 444 (3, M^+), 429 (3, $M^+ - 15$), 384 (25, $M^+ - 60$), 301 (20, $M^+ - 60 - C_6H_{11}$), 257 (90), 213 (90); HREIMS, *m*/*z* 444.2887 ($C_{27}H_{44}O_5$ requires 444.2875); ^{1}H and ^{13}C NMR data, see Table 2.

Cladocoran B (**2**): pale yellow oil; $[\alpha]^{20}_D = -59.9°$ (*c* 0.6, $CHCl_3$); UV λ_{max} (EtOH) 224 (ϵ 8700); IR ν_{max} (liquid film) 3364, 2934, 2873, 1748, 1649, 1456, 1139, 954, 890, 759; EIMS, *m*/*z* (%) 402 (3, M^+), 384 (3, $M^+ - H_2O$), 366 (3, $M^+ - 2\ H_2O$), 301 (50, $M^+ - H_2O - C_6H_{11}$), 259 (70), 201 (65), 176 (100); HREIMS, *m*/*z* 402.2774 ($C_{25}H_{38}O4$ requires 402.2769); ^{1}H and ^{13}C NMR data see Table 2.

Cladocoran A Methyl Ester (**3**). To 10 mg of cladocoran A (**1**) was added 5 mL of diazomethane in diethyl ether. After 0.5 h the diethyl ether was evaporated in vacuo and **3** was quantitatively obtained: pale yellow oil; $[\alpha]^{20}_D - 42.0°$ (*c* 0.2, $CHCl_3$); UV λ_{max} (EtOH) 224 nm (ϵ 12 000); EIMS, *m*/*z* (%) 472 (5, M^+), 457 (5), 387 (15), 329 (20), 311 (30), 258 (80), 213 (100); HREIMS, *m*/*z* 472.3165 ($C_{29}H_{44}O_5$ requires 472.3188); ^{1}H and ^{13}C NMR data see Table 1.

Reduction of Cladocoran A (**1**) **in 4.** A 5 mg portion of compound **1** was treated with MeOH and $NaBH_4$ as previously described:[10] oil; $[\alpha]^{20}_D = -38.3$ (*c* 0.2, $CHCl_3$); IR ν_{max} (liquid film) 2926, 2857, 1792, 1753, 1645, 1451, 1375, 1227, 1151, 1027, 895, 772; EIMS, *m*/*z* (%) ^{1}H NMR data ($CHCl_3$, 500 MHz) δ 1.70 (s, H_3-1), 4.63 (s, 1H, H-3a), 4.70 (s, 1H, H-3b), 2.02 (m, 2H, H-4), 1.40 (m, 2H, H-5), 1.05 (m, 1H, H-6a), 1.39 (m, 1H, H-6b), 1.25 (m, 1H, H-8a), 1.35 (m, 1H, H-8b), 1.98 (m, 2H, H_2-9), 5.50 (br t, 1H, H-10), 1.58 (m, 1H, H-12), 1.11 (m, 1H, H-13a), 1.82 (m, 1H, H-13b), 1.23 (m, 1H, H-14a), 1.61 (m, 1H, H-14b), 1.31 (m, 1H, H-15), 1.70 (m, 1H, H-17a), 1.95 (m, 1H, H-17b), 5.39 (d, 10.6, 1H, H-18), 4.71−4.82 (ABq, J = 16.5 Hz, H_2-20), 5.89 (br s, 1H, H-21) 0.82 (s, 3H, H-23), 0.87 (d, 7.1, 3H, H-24), 1.12 (s, 3H, H-25), 2.08 (s, 3H, CH_3CO); ^{13}C data (125 MHz, $CDCl_3$) 22.3 (C-1), 145.9 (C-2), 109.9 (C-3), 38.3 (C-4), 21.4 (C-5), 39.1 (C-6), 33.9 (C-7), 29.1 (C-8), 23.7 (C-9), 120.5 (C-10), 143.4 (C-11), 43.8 (C-12), 29.5 (C-13), 31.6 (C-14), 44.7 (C-15), 42.5 (C-16), 35.4 (C-17), 5.39 (C-18), 169.7 (C-19), 70.8 (C-20), 115.5 (C-21), 169.7 (C-22), 22.9 (C-23), 16.4 (C-24), 24.4 (C-25), 20.9 (CH_3CO).

Treatment of 1 with Ac_2O. To 3 mg of cladocoran A (**1**) was added Ac_2O in dry pyridine and stirred at rt overnight affording a mixture, **5a** and **5b**. The mixture of **5a** and **5b** was injected on a Spherisorb SW5 HPLC column (*n*-hexane/ethyl acetate, 95:5, flow 0.8 mL/min, detector UV 254 nm) and two main peaks in 1:1 ratio were observed.

MTPA Esters. In order to establish the absolute stereochemistry of C-18, cladocoran A methyl ester (**3**) was first submitted to methanolysis (MeOH anhydrous and Na_2CO_3 overnight) and then treated with (*R*)-(−)-MTPA chloride and (*S*)-(+)-MTPA chloride to yield *S*- and *R*-esters of **6**, respectively.

***S*-ester ^{1}H NMR data**: 5.97 (H-21), 5.65 (H-18), 5.49 (H-10), 5.19 (H-20a), 4.99 (H-20b), 0.82 (H-24), 1.05 (H-25).

***R*-ester ^{1}H NMR data**: 6.07 (H-21), 5.67 (H-18), 5.36 (H-10), 5.24 (H-20a), 5.07 (H-20b), 0.87 (H-24), 0.88 (H-25).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**, **1a**, **2**, and **5a,b** (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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