Pergamon

Tetrahedron 55 (1999) 12629–12636

TETRAHEDRON

Structures and Absolute Stereochemistry of Isocyanide and Isothiocyanate Amphilectenes from the Caribbean Sponge *Cribochalina* sp.

Maria Letizia Ciavatta,* Angelo Fontana, Raffaella Puliti, Gennaro Scognamiglio and Guido Cimino

Istituto per la Chimica di Molecole di Interesse Biologico,[1] Via Toiano 6, 80072 Arco Felice (Napoli) Italy

Received 4 June 1999; revised 3 August 1999; accepted 19 August 1999

Abstract: Three new diterpenes (**5-7**), based on amphilectene skeleton, were isolated from the Caribbean sponge *Cribochalina* sp.. All contain isocyanide and isothiocyanate functions and are epimers at C-7 of previously characterized diterpenoids. The structures of **5-7** were established by spectroscopic techniques. The sponge extract also showed the presence of the known metabolite **4**, together with the new bicyclic diterpene **8**, potential precursor of the co-occurring amphilectenes. For the first time, the absolute configuration of the amphilectene skeleton has been assigned by applying the modified Mosher's method on a derivative of the main compound **4**. The isocyanide **4** reduced the proliferation of lymphocytes T and B under various stimuli.

Marine natural products with isocyanide and isothiocyanate moieties have been reported from sponges and nudibranchs.[2,3] Within this group of compounds, diterpenes have been classified into three structural types. Those based on a highly substituted decalin system are called "kalihinols", e.g. **1**, (found in *Acanthella* sp.[4] and *A. cavernosa*[5]), whereas tricyclic or tetracyclic products such as **2** and **3**, fall in the class named amphilectenes and cycloamphilectenes, respectively (found in *Amphimedon* sp.,[6] *Hymeniacidon amphilecta*,[7] and *Halichondria* sp.[8]). Both diterpene classes have shown interesting *in vitro* biological activities as antimicrobial, cytotoxic and antimalarial agents.

Although many papers have been published on isocyanide and isothiocyanate amphilectenes, a complete NMR structure assignment of several diterpenoids based on this skeleton has been done, only recently, by Konig and coworkers.[9] However, they have not assigned the absolute stereochemistry.

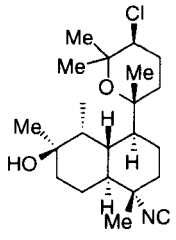
In our ongoing search for active natural metabolites from marine sources, we have isolated the amphilectene-like compounds **4-7** together with their putative precursor **8**. This paper describes the structure elucidation of the new compounds **5-8** and the detailed spectral characterization of the known metabolite **4**. Moreover we report the determination of the absolute stereochemistry of **4** *via* its semi-synthetic derivative **9**.

Results and Discussion.

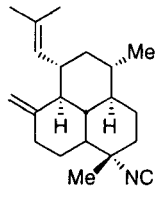
The sponge *Cribochalina* sp. (1 kg) was collected by scubadiving along the Caribbean coasts of Mexico during the spring 1995. The Et_2O soluble material from the acetone extract contained four compounds with R_f's ranging from 0.8 to 0.4 in light petroleum/Et_2O, 9:1.

0040-4020/99/$ - see front matter
PII: S0040-4020(99)00737-1

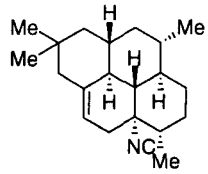
An aliquot (400 mg) of this extract was fractionated on silica gel with light petroleum/Et_2O gradient to yield 25 mg of **4**, 10 mg of **5**, 5 mg of **6** and 15 mg of **7**. Further purification of a less polar fraction gave **8** (3 mg) .



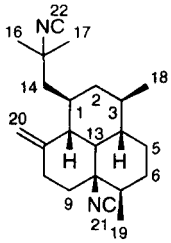
1



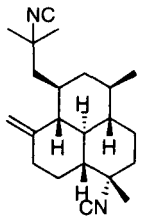
2



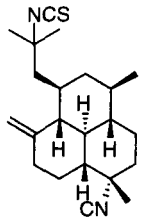
3



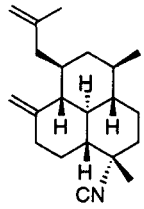
4



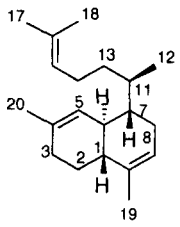
5



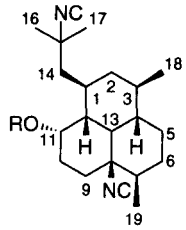
6



7



8



9 R = H
9a R = *S*-MTPA
9b R = *R*-MTPA

Compound **4** has been previously found in the sponge *Hymeniacidon amphilecta* by Wratten *et al.*[7] They reported an X-ray structure, but only a partial NMR assignment. Its ^{1}H-NMR spectrum exhibited signals for an exocyclic methylene (δ 4.86 and 4.64, H_2-20), two methyl doublets (δ 0.93, H_3-18; δ 0.97, H_3-19) and two downshifted broad methyl singlets (δ 1.43, H_3-16; δ 1.45, H_3-17). The strong IR band at 2130 cm^{-1} and the ^{13}C-NMR spectrum supported the presence of two isocyanide functions (δ 156.2, C-21; δ 154.3, C-22) linked to two quaternary carbons (δ 56.9, C-8; δ 56.5, C-15). The EIMS molecular peak at *m/z* 324, followed by the sequential loss of two units of cyanidric acid $(M - 27)^+$, was consistent with the molecular formula $C_{22}H_{32}N_2$. Although the majority of the signals were overlapped in the region between δ 1.8 and 2.3, 2D-NMR studies allowed all resonances to be assigned (Tables 1 and 2). The depicted structure of **4** was further confirmed by an accurate X-ray analysis on a single crystal obtained from *n*-hexane, this showed the same relative configuration of this compound with that previously reported.[7]

Table 1. ^{1}H-NMR data[a,b] for compounds **4-7**.

H	**4**	m *J* Hz	**5**	m *J* Hz	**6**	m *J* Hz	**7**	m *J* Hz
1	1.99	m	1.85	m	1.82	m	1.65	m
2	2.28	m	2.35	ddd 13.5,5.5,3.6	2.22	m	1.86	dt 13.5,7.3,7.3
	0.93	m	0.90	m	0.87	m	0.67	ddd13.5,11.8,11.8
3	1.10	m	1.15	m	1.16	m	1.15	m
4	1.15	m	1.28	m	1.25	m	1.25	m
5	1.99	m	2.00	m	2.00	m	2.00	m
	0.86	m	1.10	m	1.05	m	1.10	m
6	1.53	m	1.79	m	1.80	m	1.82	m
	1.43	m	1.52	m	1.54	m	1.58	m
7	1.39	m						
8			1.53	m	1.50	m	1.53	m
9	2.30	m	1.78	m	1.75	m	1.79	m
	1.32	m						
10	2.32	m	2.40	m	2.40	m	2.45	ddd 15.3,7.5,1.4
			2.25	m	2.28	m	2.24	m
11								
12	1.88	bt 10.5	1.65	bt 10.5	1.65	bt 10.5	1.63	m
13	1.08	m	1.25	m	1.18	m	1.20	m
14	2.05	d 15.0	2.12	d 14.8	2.10	d 14.0	2.62	d 15.0
	1.28	m	1.15	m	1.15	m	1.45	dd 15.0, 10.1
15								
16	1.43	bs	1.43	bs	1.43	bs	4.72	s
							4.63	s
17	1.45	bs	1.45	bs	1.44	bs	1.72	bs
18	0.93	d 6.0	0.92	d 6.0	0.92	d 6.2	0.89	d 6.4
19	0.97	d 6.2	1.40	bs	1.38	bs	1.41	bs
20	4.86	s	4.86	s	4.86	s	4.86	s
	4.64	s	4.64	s	4.64	s	4.63	s

a) Bruker AMX 500 MHz, $CDCl_3$; δ values are reported referred to $\underline{C}HCl_3$ (δ 7.26).

b) Assignments determined by ^{1}H-^{1}H COSY, HMQC experiments.

Table 2. ^{13}C-NMR data[a,b] for compounds **4-7**

C	**4**	*m*	**5**	*m*	**6**	*m*	**7**	*m*
1	33.1	d	34.1	d	34.1	d	34.8	d
2	40.8	t	43.4	t	43.4	t	41.4	t
3	35.4	d	39.5	d	39.6	d	39.8	d
4	42.5	d	39.8	d	39.9	d	39.9	d
5	29.6	t	24.6	t	24.6	t	24.6	t
6	29.8	t	33.8	t	33.8	t	33.9	t
7	40.7	d	59.7	s	59.7	s	59.8	s
8	56.9	s	42.2	d	42.2	d	42.3	d
9	39.5	t	21.6	t	21.6	t	21.6	t
10	33.4	t	33.8	t	33.7	t	33.5	t
11	149.7	s	148.2	s	148.5	s	148.4	s
12	45.9	d	49.6	d	49.6	d	49.3	d
13	55.4	d	45.4	d	45.6	d	45.6	d
14	45.7	t	44.9	t	45.8	t	42.1	t
15	56.5	s	56.8	s	60.7	s	144.4	s
16	29.8	q	29.4	q	29.4	q	109.2	s
17	31.8	q	31.4	q	31.5	q	22.5	q
18	19.7	q	19.4	q	19.5	q	19.4	q
19	15.6	q	28.9	q	28.9	q	29.0	q
20	106.2	s	107.3	s	107.0	s	106.5	s
21	156.2	bs	154.7	bs	154.8	bs	154.7	bs
22	154.3	bs	154.2	bs	126.8	bs		

a) Bruker AMX 500 MHz, $CDCl_3$; δ values are reported referred to $\underline{C}DCl_3$ (δ 77.0).

b) Assignments aided by DEPT sequence, HMQC and HMBC.

In the absence of atoms with strong anomalous scattering, no reliable evidence of the absolute stereochemistry was achieved. However, the stereochemistry at C-4 is certainly opposite to that of all other asymmetric carbons.

In order to establish the absolute stereochemistry, compound **4** was transformed into **9** by reductive ozonolysis in a one-pot reaction (see Experimental part). The obtaining of the compound **9** with the hydroxyl substituent at C-11 in the axial orientation is according to the crystallographic study of compound **4**, that showed the atomic crowding of the *Re* face due to the presence of the isobutyl chain in the equatorial orientation and the axial hydrogens at C-10 and C-12. Treatment of **9** with (*R*)- and (*S*)- MTPA chlorides in dry CH_2Cl_2 and DMAP gave the corresponding (*S*)-MTPA (**9a**) and (R)-MTPA (**9b**) esters. The chemical shift analysis[10] (δ_S - δ_R, Table 3) was consistent with the *S* configuration of C-11 of **9**. Accordingly, the complete absolute stereochemistry of **4** is to be assigned as 1*R*, 3*R*, 4*S*, 7*R*, 8*R*, 12*R*, 13*R*.

Table 3. Selected ^{1}H-NMR chemical shifts[a] and $\Delta\delta$[b] for the MTPA esters of **9**.

H	**9a**	**9b**	$\Delta\delta$
H-1	1.50	1.45	+ 0.05
H_3-16	1.44	1.42	+ 0.02
H_3-17	1.31	1.21	+ 0.10
H-12	1.85	1.83	+ 0.02
H_2-10	1.40	1.43	- 0.03
H_3-19	0.93	0.95	- 0.02

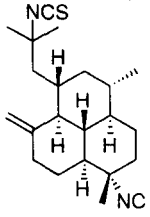
[a] Bruker AMX 500 MHz, $CDCl_3$, δ values are referred to $C\underline{H}Cl_3$ (δ 7.26); [b] $\Delta\delta$ ($\delta_{(S)\text{-ester}}$ - $\delta_{(R)\text{ester}}$) values are given in ppm.

Compound **5** differed from **4** only by the shift of the isocyanide function from C-8 to C-7. Accordingly, EIMS spectrum exhibited the molecular peak at *m/z* 324, followed by the sequential loss of two isocyanide groups which gave the MS fragments at *m/z* 297 and *m/z* 270. The ^{1}H-NMR data of **5** revealed the presence of one methyl doublet at δ 0.92 and three methyl singlets at δ 1.40, 1.43, 1.45. The structure of **5** was strongly supported by COSY correlations which showed coupling of H-8 (δ 1.53) with both H-13 (δ 1.25) and the methylene system at C-9 (δ 1.78), in turn coupled with the allylic hydrogens at C-10 (δ 2.40 and 2.25). The remaining resonances of **5** fell at chemical shifts very similar to those of **4**, except for the methylene protons at C-6 (δ 1.79 and 1.52) which were affected by the presence of the isocyanide group at C-7. However, 2D-NMR experiments allowed us to complete the structure assignment of **5** (Tables 1 and 2). In particular the HMBC correlations from C-15 to the signals at δ 1.43 and 1.45 (H_3-16 and H_3-17) proved to be very diagnostic to differentiate the chemical shift values of the methyls, as well as the long-range couplings of the downfield-shifted singlet at δ 1.40 (H_3-19) with the carbons at δ 42.2 (C-8) and 59.7 (C-7) unequivocally established the presence of the isocyanide group at C-7. The relative stereochemistry of **5** was inferred by 1D- and 2D- NOE experiments and by the analysis of the ^{1}H-NMR coupling constants. In fact, H-12 (δ 1.65) appeared as a broad triplet (*J*=10.5 Hz), thus suggesting a *trans* relationship between H-12 and H-1 (δ 1.85), and between H-12 and H-13 (δ 1.25). Strong NOE effects between H-4 (δ 1.28) and H-12 (δ 1.65), and between H-4 and H-8 (δ 1.53) give evidence for the 1,3-diaxial orientation of these protons and supported the all *trans* stereochemistry of the ring junctions of **5**. On the other hand, irradiation at H-13 (δ 1.25) increased the intensity of H-1 (δ 1.85) and H-3 (δ 1.15), thus supporting the axial orientation for these hydrogens and, therefore, the *cis* stereochemistry of the substituents at C-1 and C-3. Finally the NOE on CH_3-19 only showed effects with H_2-9.

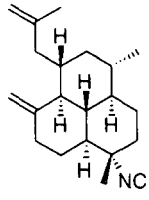
Compound **6** was very similar to **5**, with an isocyanide group replaced by an isothiocyanate one. This is according to the signal at δ 126.8 in the ^{13}C-NMR and the IR band at 2100 cm^{-1}. The molecular peak at *m/z* 356 and the loss of 59 *m/z* $(M\text{-}HNCS)^+$ confirmed the molecular formula $C_{22}H_{32}N_2S$. All the

spectroscopic data are reported in Tables 1 and 2 and in the experimental section. Compound **6** is closely related to the amphilectene **10** described by Konig and coworkers from the extract of the sponge *Cymbastela hooperi*.[9]

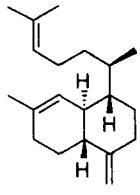
On the basis of the spectral data these differed only in the configuration at C-7. In fact, comparison of the NMR data for C-19 in **6** and **10** (δ 28.9 and 20.7, respectively) supported the equatorial orientation of C-19 in our compound. This configuration of **6** was further confirmed by the shielding of the axial protons at C-5 and C-13 due to the γ-gauche interaction with the NC group. Furthermore, the NOESY spectrum of **6** did not show any correlations between H_3-19 (δ 1.38) and H-13 (δ 1.18) that, on the contrary, should be diagnostic for the assignment of the stereochemistry in **10**.[9]



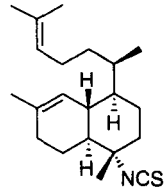
10



11



12



13

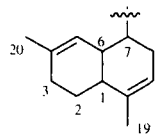
Compound **7** only had one isocyanide function (IR band at 2129 cm^{-1}, ^{13}C-NMR δ 154.7). This product differed from the others by the presence in the ^1H-NMR spectrum of two exomethylene groups (δ 4.86 and 4.64, H_2-20; 4.72 and 4.63, H_2-16) and of a vinylic methyl at δ 1.72 (H_3-17). In the COSY experiment this latter signal was coupled with the exomethylene at C-16 and was, furthermore, coupled with H_2-14 (δ 2.62 and 1.45) which in turn correlated with the methine at C-1 (δ 1.65). Other couplings were similar to those previously described for **5**. As described for **6**, compound **7** is also epimeric at C-7 with the product **11**, previously reported by Konig.[9] The structural analogies among **4** and the compounds **5-7**, allow one to suggest the 1*R*, 3*R*, 4*S*, 7*R*, 8*R*, 12*R*, 13*R* absolute configuration for these last compounds.

The extract of *Cribochalina* sp. also contained the terpene **8** which did not possess isocyanide or isothiocyanate functionalities. The ^1H-NMR spectrum of **8** showed one secondary methyl group (δ 0.81, H_3-12), four olefinic methyls (δ 1.64, H_3-18; 1.58, H_3-17, 1.69, H_3-19 and H_3-20), and three trisubstituted double bonds. EIMS and ^{13}C-NMR was consistent with the molecular formula $C_{20}H_{32}$. The broad doublet at δ 5.48 (H-5) was coupled to the methine proton at δ 2.02 (H-6) and showed long-range connectivities with the carbon at δ 17.6 (C-20), δ 30.8 (C-3), and δ 39.5 (C-1) in the HMBC experiment. Moreover, COSY couplings of H-1 (δ 1.93) were observed with the signals at δ 1.85 and 1.33 (H_2-2), both, in turn, correlated with the methylene at C-3 (δ 2.00-1.90). Consistent with the partial structure **a**, correlations were observed between the quaternary C-10 (δ 136.6) and the protons at δ 1.93 (H-1) and δ 1.69 (H_3-19). This latter signal was also allylically correlated with the vinyl proton at δ 5.40 (H-9), that had COSY interactions with the methylene group at δ 1.78 (H_2-8).

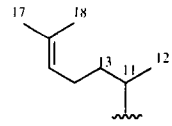
The unsaturated decaline system of fragment **a** was completed by couplings of the methine proton at δ 1.53 (H-7) with the signals at δ 1.78 (H_2-8) and δ 2.02 (H-6). The alkyl chain (fragment **b**) was defined on the basis of the correlations observed in the COSY and HMBC experiments (Table 4).

In fact, long-range interactions connected both C-12 (δ 13.3) and C-11 (δ 31.4) to the isolated resonance at δ 1.53 (H-7).

Compound **8** is very similar to the biflora-4,10(19), 15-triene **12**, first found in a termite soldier.[11]



a



b

Table 4. NMR data[a,b] for compound **8**

C	δ ^{1}H	m Hz	δ ^{13}C	m	long-range connectivities[c]
1	1.93	m	39.5	d	H_2-2,H-5,H-6,H-7,H-9,H_3-19
2	1.85	m	24.6	t	H_2-3, H-6,
	1.33	dddd 17.4,10.6,10.6,2.5			
3	2.00	m	30.8	t	H_2-2,H-5,H_3-20
	1.90				
4			134.3	s	H_2-3,H-6,H_3-20
5	5.48	bd 1.5	123.9	d	H_2-3,H_3-20
6	2.02	ddd 12.0,10.6,4.8	36.3	d	H_2-2,H-5,H-7
7	1.53	dddd 10.6,10.6,5.9,2.0	38.8	d	H-5,H-11,H_3-12,H_2-13
8	1.78	m	24.6	t	H-6,H-7
9	5.40	bs	121.4	d	H-1,H_2-8,H_3-19
10			136.6	s	H-1,H_2-8,H_3-19
11	1.78	m	31.4	d	H-7,H_3-12,H_2-13
12	0.81	d 6.8	13.3	q	H-7,H-11,H_2-13-
13	1.23	m	35.7	t	H_3-12,H_2-14,H-15,H_3-18
	1.18	m			
14	2.00	m	26.2	t	H_2-13
	1.83	m			
15	5.08	bt 1.6	125.0	d	H_2-13,H_2-14,H_3-17,H_3-18
16			130.9	s	H_2-14,H_3-17,H_3-18
17	1.58	bs	17.6	q	H-15,H_3-18
18	1.64	bs	25.7	q	H-15,H_3-17
19	1.69	bs	21.7	q	H-9
20	1.69	bs	17.6	q	H-5

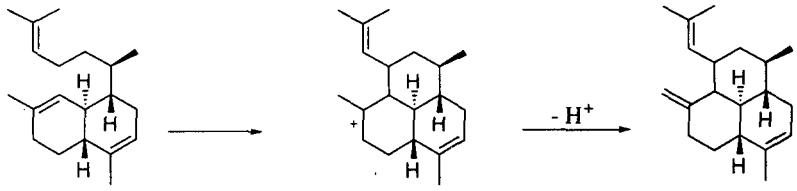
a) Bruker AMX 500 MHz, $CDCl_3$; δ values are reported referred to $\underline{C}HCl_3$ (δ 7.26).
b) Assignments determined by ^{1}H-^{1}H COSY, HMQC, DEPT sequence,
c) HMBC (J=10 Hz)

A similar carbon skeleton (**13**) has also been reported from an Adocidae sponge[12] and from *Cymbastela hooperi* by Konig and co-workers.[9] The relative stereochemistry of **8** was inferred on the basis of the coupling constants. The methine proton at H-7 (δ 1.53) showed two large coupling constants (J=10.6 Hz) attributable to the scalar correlations with the axial protons at C-6 and C-8. In fact, irradiation of the multiplet centered at δ 1.78 (H_2-8 and H-11) simplified the signal at δ 1.53 (H-7) to give a doublet with an apparent $J_{6,7}$ of 9.6 Hz. On the other hand, the axial proton at C-2 possessed by one geminal (J=17.4 Hz, H-2ax- H-2eq), one axial-equatorial (J= 2.5Hz, H-2ax-H-3eq) and two axial-axial (J=10.6, H-2ax-H-3ax and H-2ax-H-1ax) coupling constants. Finally the J-resolved 2D NMR experiment supported for H-6 the presence of two large (J=12.0 and 10.6 Hz) and one small (J=4.8 Hz) coupling constants. This data was in agreement with the axial orientation of H-1, H-6 and H-7. The structural similarities led us to suggest that **8** is the putative precursor of the amphilectene-compound (scheme 1).

Conclusion

Compounds that possess isocyanide or other correlated functionalities have been obtained from terrestrial and marine sources. Within marine organisms only sponges and opisthobranchs contain these metabolites. Only recently have amphilectene and cycloamphilectene-type diterpenes been completely described.

In this paper we have reported the isolation, the complete structural elucidation of new and known metabolites from the Caribbean sponge *Cribochalina* sp. and, for the first time, the absolute stereochemistry of this kind of diterpenoid.



Scheme 1

In preliminary assays, compound **4** showed a moderate activity against tumour cells, but an interesting antiproliferative effect on mononuclear blood cells stimulated both by allogenic feeders (ratio 1:2) and by phytohaemagglutinin.

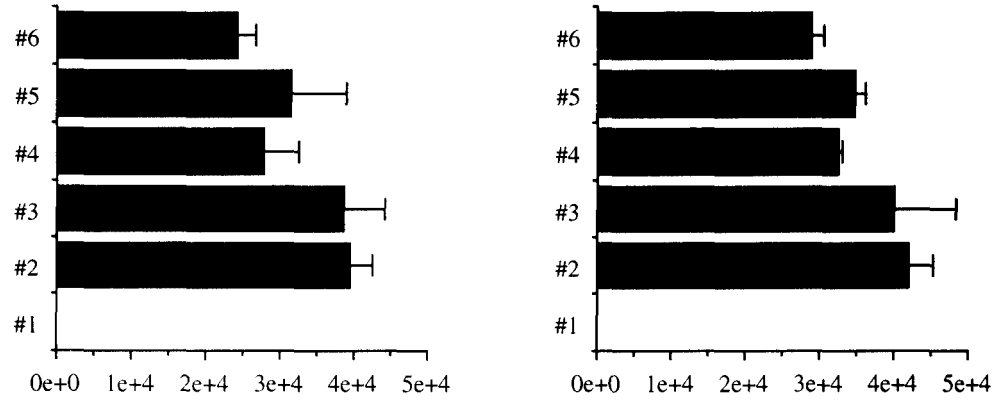


Fig. Immunosuppressive activity. #1. Normal; #2. Control; #3. 1mg/mL of **4**; #4. 5 mg/mL of **4**; #5. 10 mg/mL of **4**; #6. 20 mg/mL of **4**.

EXPERIMENTAL SECTION

General Experimental Procedures. Precoated TLC plates Merck Si gel 60 F254 were used for analytical TLC and Merck Kieselgel 60 powder was used for preparative column chromatography. The mass spectra were obtained from AEI MS-30 (EIMS). IR spectra were recorded in a liquid film on a BIORAD FTS 155 FTIR. Optical rotations were measured on a Jasco DIP 370 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX 500 (500 MHz) spectrometer.

Crystallographic study. The crystallographic work has been carried out using an Enraf-Nonius CAD4-F diffractometer and Enraf-Nonius S.D.P. (1985) software on a MicroVAX 3100 computer. Full matrix least-squares refinement with anisotropic temperature factors for non-hydrogen atoms converged at the discrepancy R factor=0.036 for 1924 observed reflections and 217 variables. Crystal data, atomic parameters, molecular geometry and structure factors have been deposited with the Supplementary Material.

Biological Material. The sponge was collected along the Caribbean coasts of Mexico at a depth of 10 meters, during the spring 1995. The sponge was classified by Prof. M. Pansini, University of Genova, Italy. A voucher specimen is deposited at ICMIB (CNR Arco Felice Naples, Italy).

Extraction and Isolation. The sponge (300 g dry weight) was extracted three times with acetone (1LX3). The ether soluble fraction was chromatographed on a Si-gel column using as eluant light petroleum ether with increasing amounts of Et_2O. The fractions containing compound **4** were obtained as a yellow oil but it was readily crystallized from *n*-hexane and was submitted to X-ray analysis. The other isocyanide compounds were obtained as amorphous powders. Only compound **8** was repurified on a silica-gel/$AgNO_3$ column using benzene/diethyl ether 95:5.

Compound **4** (25 mg, 0.077mM): obtained as white crystals from *n*-hexane m.p. 105-106° C; $[\alpha]_D$= -56 (c 1.5, $CHCl_3$); IR (liquid film) ν_{max}: 2926, 2857, 2131, 1637, 1450, 1374, 1181, 1096, 898 cm^{-1}; HREIMS: found 324.2578 (324.2565 calculated for $C_{22}H_{32}N_2$); EIMS at *m/z* 324 (48%), 297 (58%), 282 (85%), 255 (70%), 228 (76%), 201 (87%), 186 (100%), 159 (95%); ^{1}H- and ^{13}C-NMR data are reported in Tables 1 and 2; (Lit. data m.p. 105-106° C, $[\alpha]_D$ = -56).[7]

Compound **5** (10 mg, 0.031 mM): white solid; $[\alpha]_D$ = +26.5 (c 1.0, $CHCl_3$); IR (liquid film) ν_{max} : 2929, 2130, 1737, 1644, 1459, 1374, 1166, 880 cm^{-1}; HREIMS: found 324.2556 (324.2565 calculated for $C_{22}H_{32}N_2$); EIMS at m/z 324 (50%), 297 (60%), 282 (48%), 255 (30%), 228 (46%), 187 (100%), 159 (70%); ^{1}H- and ^{13}C-NMR data are reported in Tables 1 and 2.

Compound **6** (5 mg, 0.014 mM): white solid; $[\alpha]_D$ = + 42.9 (c 0.5, $CHCl_3$); IR (liquid film) ν_{max} : 2946, 2128, 2100, 1644, 1460, 1379, 1227, 1166, 888 cm^{-1}; HREIMS: found 356.2345 (356.2323 calculated for $C_{22}H_{32}N_2S$); EIMS at *m/z* 356 (5%), 297 (58%), 270 (10%), 159 (100%); ^{1}H- and ^{13}C-NMR data are reported in Tables 1 and 2.

Compound **7** (15 mg, 0.05 mM): white solid; $[\alpha]_D$= +65.0 (c 1.2, $CHCl_3$); IR (liquid film) ν_{max}: 2919, 2129, 1646, 1444, 1374, 890 cm^{-1}; HREIMS: found 297.2460 (297.2456 calculated for $C_{21}H_{31}N$); EIMS at *m/z* 297 (40%), 282 (90%), 270 (50%), 255 (100%), 228 (30%); ^{1}H- and ^{13}C-NMR data are reported in Tables 1 and 2.

Compound **8** (3 mg, 0.011 mM): oil; $[\alpha]_D^{20}$ = +51.4 (c 0.5, $CHCl_3$); HREIMS: found 272.2510 (272.2504 calculated for $C_{20}H_{32}$); EIMS at *m/z* 272 (30%), 216 (25%), 187 (40%), 159 (100%). ^{1}H- and ^{13}C-NMR data are reported in Table 4.

Reductive ozonolysis of **4**: compound **4** (8 mg, 0.024 mmol) was dissolved in 0.5 ml of MeOH and treated with 4 ml of a saturated solution of O_3 in MeOH (-78°C). After 30 min. 5 mg of $NaBH_4$ was added to the reaction and the solution was kept at -78°C for 30 min. and then allowed to warm to room temperature slowly. The organic solution was reduced at room temperature and the residue partitioned between $NaHCO_3$ and Et_2O. Purification on silica gel afforded 5 mg (0.015 mM, 65%) of **9**: white powder, $[\alpha]_D^{20}$ = -41.7 (c 0.5, $CHCl_3$); IR (liquid film) ν_{max}: 3446, 2927, 2130 cm^{-1}; HREIMS: found 328.2612 (328.2514 calculated for $C_{21}H_{32}N_2O$)^{1}H-NMR data ($CDCl_3$, δ in ppm): 4.13 (H-11), 3.48 (OH), 1.44 (CH_3-16 and CH_3-17), 0.99 (CH_3-19, d, *J*=6.0 Hz), 0.88 (CH_3-18, d, *J*=6.2 Hz); ^{13}C-NMR data ($CDCl_3$): 155.5, 153.1, 66.6, 63.6, 57.8, 45.9, 44.8, 44.0, 41.8, 40.4, 36.0, 32.5, 30.3, 29.9, 29.8, 29.5, 29.0, 28.9, 19.7, 15.6.

Immunosuppressive activity. Mononuclear blood cells were stimulated with allogenic feeders (ratio 1:2) and phytohaemoagglutinin (mitogen stimulus) at several concentrations of **4** in a 96-well flat-bottomed culture plate in serum for 72 h. Cultures were done in triplicate. Cultures were pulsed with 0.5 μCi [^{3}H]-thymidine and the proliferative response was assessed by determining radioactivity of [^{3}H]-thymidine incorporated.

Acknowledgements

Authors thank Dr. E. Mollo and Dr. G. Villani for collecting the sponge, Prof. M. Pansini for taxonomical characterization, and Dr. R. De Palma for pharmacological assays. EIMS spectra were provided by "Servizio di Spettrometria di Massa del CNR e dell' Universita' di Napoli"; NMR spectra were obtained from "Servizio NMR dell'Area CNR di Napoli", the staff of both of which is acknowledged.

References and Notes

1. Associated to National Institute for the Chemistry of Biological System (CNR).
2. Scheuer, P. J. *Acc. Chem. Res.*, **1992**, *25*, 433.
3. Faulkner, D.J. *Mar. Nat. Prod.*, **1998**, *15*, 113-158 and references cited therein.
4. Chang. C.W.J; Patra, A.; Roll. D.M.; Scheuer, P.J.; Matzumoto, G.M.; Clardy, J. *J. Am. Chem. Soc.* **1984**, *106*, 4644. Patra, A.; Chang, C.W.J.; Scheuer, P.J.; van Duyne, G.D.; Matsumoto, G.M.; Clardy, J.J. *J. Am. Chem. Soc.* **1984**, *106*, 7981. Chang, C.W.J.; Patra, A.; Baker, J.A.; Scheuer, P.J. *J. Am. Chem. Soc.* **1987**, *109*, 6119.
5. Omar, S.; Albert, C.; Fanni, T.; Crews, P. *J. Org. Chem.* **1988**, *53*, 5971.
6. Kazlauskas, R.; Murphy, P.T.; Blount, J.F. *Tetrahedron Lett.* **1980**, *21*, 315.
7. Wratten, J.S.; Faulkner, D.J.; Hirotsu, K.; Clardy, J. *Tetrahedron Lett.* **1978**, 4345.
8. Molinski, T.F.; Faulkner, D.J. *J. Org. Chem.* **1987**, *52*, 3334.
9. Konig, G.M.; Wright, A.D.; Angerhofer, C.K. *J. Org. Chem.* **1996**, *61*, 3259.
10. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
11. Wiemer, D.F.; Meinwald, J.; Prestwich, G.D.; Solheim, B.A.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 191.
12. Sharma, H.A.; Tanaka, J.; Higa, T.; Lithgow, A.; Bernardinelli, G.; Jefford, C.W. *Tetrahedron Lett.* **1992**, *33*, 1593.