Tetrahedron Vol. 43, No. 22, pp. 5381 to 5388, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00

NITROGENOUS SESQUITERPENES FROM THE MARINE SPONGE *ACANTHELLA ACUTA*: THREE NEW ISOCYANIDE-ISOTHIOCYANATE PAIRS.

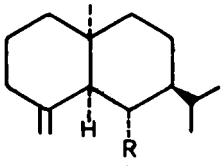
Luciano Mayol[a], Vincenzo Piccialli[b] and Donato Sica[b*]

a. Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, Via L.Rodinò 22, I-80138 Napoli (Italy).
b. Dipartimento di Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone 16, I-80134 Napoli (Italy).

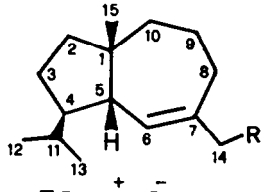
(*Received in UK* 14 *September* 1987)

Abstract. - The isolation and the structure elucidation of six new nitrogenous sesquiterpenes (3-8) from the marine sponge *Acanthella acuta* are described. A complete ^{1}H- and ^{13}C-NMR assignment for the new metabolites, which form three new isocyanide-isothiocyanate pairs, was accomplished with the aid of carbon-proton shift correlated 2D-NMR experiments.

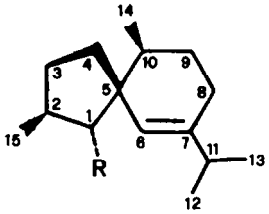
Recently we have described the isolation of the sesquiterpene isocyanide 1, the major metabolite from the sponge *A. acuta*[1], along with its corresponding isothiocyanate 2[2]. Several minor metabolites carrying the rare $-N^{+}\equiv C^{-}$ and $-N=C=S$ functionalities are present in the methanol extract of this organism; the present paper deals with the structure determination of six of these compounds (3-8). Spectral evidence clearly indicated that the new terpenoids were based on three different carbon skeletons in such a way to form three isocyanide-isothiocyanate couples, the difference within each couple being confined to the nature of the functional group.



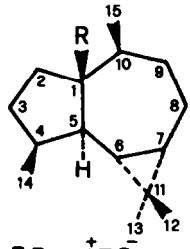
1 R = $N^{+}\equiv C^{-}$
2 R = N=C=S



7 R = $N^{+}\equiv C^{-}$
8 R = N=C=S



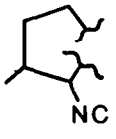
3 R = $N^{+}\equiv C^{-}$
4 R = N=C=S



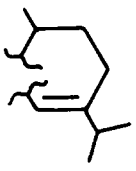
5 R = $N^{+}\equiv C^{-}$
6 R = N=C=S

This relationship was chemically confirmed through the conversion of each isonitrile into the corresponding isothiocyanate by treatment with sulphur at 120°C. Most of the structural work was carried out on the more abundant isonitriles 3, 5, 7.

Compound 3 was isolated as an optically active oil ($[\alpha]_D$ -31.5, c 1.2, $CHCl_3$), from the chloroform soluble material of the methanol extract of the sponge **A.acuta**, collected in the Bay of Napoli during the Spring 1985. Its molecular formula was established as $C_{16}H_{25}N$ by elemental analysis, high resolution mass spectroscopy and carbon and proton count from the respective NMR spectra. The ^{13}C-NMR spectrum contained fifteen distinct signals attributable to sixteen carbons, namely three non protonated carbons, five methines, four methylenes and four methyls (Table 1) as deduced from DEPT[3] (Distortionless Enhancement by Polarization Transfer) experiments and ^{1}H-NMR spectrum which displayed four methyl doublets at δ 0.90 (3H, J= 6.6 Hz), 0.96 (6H, J= 6.6 Hz), and 1.14 (3H, J= 6.6 Hz). The methyls resonating at δ 0.96 were shown to belong to an isopropyl group since their signal collapsed into a singlet by irradiation at δ 2.13 (1H, b septet, J= 6.6 Hz). The ^{13}C-NMR signals at δ 153.4 (-$\overset{|}{C}$-) and 71.7 (1:1:1 triplet, J= 6.1 Hz, -$\overset{|}{C}$H), suggested the presence in the molecule of a $-N^{+}\equiv C^{-}$ function which was confirmed by IR (ν_{max} 2135 cm^{-1}), MS (m/z 204, M^+-HCN) and ^{1}H-NMR spectrum which contained a broad doublet at δ 3.15 (J= 8.8 Hz) due to the proton geminal to the $-N^{+}\equiv C^{-}$ group. Furthermore the ^{13}C- and ^{1}H-NMR spectra showed the resonances pertinent to a trisubstituted double bond [^{1}H: δ 5.05 (1H, bs); ^{13}C: δ 125.9 (-$\overset{|}{C}$H), 143.7 (-$\overset{|}{C}$-)]. From the above data arises that 3, having five degrees of unsaturation, must possess a carbobicyclic skeleton. Moreover, taking into account that its ^{13}C-NMR spectrum contained only one sp^3-quaternary carbon atom (δ 48.9) and that the proton spectrum did not exhibit any methyl singlet resonance, the two rings had to be linked through a spiranic carbon. The good proton dispersion in the 500 MHz ^{1}H-NMR spectrum of 3 allowed to determine confidently the partial structures A and B, by spin decoupling experiments.



A



B

The only atom in the molecule of 3 not accounted for by these structural elements is the above mentioned quaternary sp^3-carbon which, evidently, connects moieties A and B as drawn in formula 3.

Support for such a structure came from the one-bond ^{13}C-^{1}H correlation[4], which permitted the assignment of all the protonated carbons in the ^{13}C-NMR spectrum of 3, and ^{13}C-^{1}H long-range correlation[5] (COLOC), which definitely proved the proposed structure on the basis of a set of correlation peaks via ^{2}J and ^{3}J listed in Table 1.

Due to the particular geometry of the molecule, the overall relative stereochemistry of 3 could not be derived solely from nOed's experiments which, however, settled the configuration at the chiral centers C-1 and C-2 (Table 3). The configuration at the remaining chiral centers (C-5 and C-10) was assigned on the basis of the values of the vicinal coupling constants of 10-H with 9-H_2 (6.6 and 3.0 Hz) which established the pseudoaxial orientation of 10-Me and a series of ^{1}H-NMR spectra performed in the presence of variable amounts of Eu(fod)$_3$. The most significant result arising from this experiment was the remarkable Europium shifts suffered by both 10-H and 10-Me which, by examination of molecular models, could be explained

Table 1. ^{1}H- and ^{13}C-NMR data for 3[a].

Position	δ_C	δ_H (J)	$^{1}H/^{13}C$ long range correlation
1	71.7[b]	3.15 bd (8.8)	
2	42.8	2.20 m	4-Hb
		Ha 1.85 m	
3	30.5		4-Hb
		Hb 1.27 dddd (10.0,9.5,9.5,9.0)	
		Ha 1.75 ddd (13.2,9.0,9.0)	
4	33.8		3-Hb,6-H
		Hb 1.41 m	
5	48.9		1-H, 6-H, Me-14
6	125.9	5.05 bs	1-H
7	143.7		
8	23.6	1.91 bdd (6.0,6.0)	6-H
		Ha 1.70 dddd (13.2,6.0,6.0,3.0)	
9	27.9		8-H, Me-14
		Hb 1.41 m	
10	32.4	1.98 ddq (6.6,6.6,3.0)	6-H, Me-14
11	34.9	2.13 b septet (6.6)	6-H, Me-12,Me-13
12	21.4	0.96 d (6.6)	
13	21.4	0.96 d (6.6)	
14	16.7	0.90 d (6.6)	
15	19.0	1.14 d (6.6)	1-H, 3-Hb
16	153.4		

a. The assignments are based on ^{13}C-^{1}H shift correlated 2D-NMR spectroscopy.

b. The signal appear as a 1:1:1 triplet (J= 6.1 Hz) due to the coupling with the nitrogen atom.

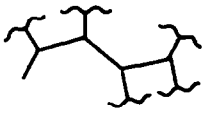
only assuming that the chirality at C-5 and C-10 is the one depicted in formula 3.

Compound 5, $[\alpha]_D$ -13.7 (c 1.4, $CHCl_3$), is a colourless crystalline solid, m.p. 65-66°C, having the molecular formula $C_{16}H_{25}N$ [elemental analysis, HRMS and ^{13}C-NMR (Table 2)]. The presence of the $-N^{+}\equiv C^{-}$ group was easily deduced from the IR absorption at 2135 cm^{-1} and the mass spectrum (m/z 204, M^{+}-HCN) and supported by the ^{13}C-NMR spectrum which also indicated it to be linked to a fully substituted sp^3-carbon [δ 156.5 (-$\overset{|}{\underset{|}{C}}$-), 65.7 (broad signal, -$\overset{|}{\underset{|}{C}}$-)]; this was confirmed by the ^{1}H-NMR spectrum which did not show any resonance for protons geminal to the $-N^{+}\equiv C^{-}$ group (all the signals are confined in the region 0.7-2.2 ppm).

Taking into account that the elemental formula of 5 implies three further degrees of unsaturation, in addition to the two accounted for by the $-N^{+}\equiv C^{-}$ group, and that in its ^{13}C-NMR spectrum no sp^2-carbon signals are present, it could be inferred that the molecule must incorporate three rings.

Double resonance experiments readily established the presence of the segment C in the molecule which must also incorporate the structural elements -$\overset{|}{\underset{|}{C}}$-$N^{+}\equiv C^{-}$, -$\overset{|}{\underset{|}{C}}$H-$CH_3$, -$\overset{|}{\underset{|}{C}}(CH_3)_2$ and

four methylenes as resulted by inspection of both ^{1}H- and ^{13}C-NMR spectra. The above fragments could be arranged as drawn in formula 5 on the basis of the following criteria. The $-\overset{|}{C}-N^{+}\equiv C^{-}$ group is to be linked to C-5 since 5-H resonated at δ 1.64 as a double doublet (J= 11.0 and 11.0 Hz), with each line further split into a 1:1:1 triplet (J= 2.9 Hz) due to the coupling with the nitrogen atom. Furthermore the $-\overset{|}{C}(CH_3)_2$ residue was required to join C-6 and C-7, thus forming a cyclopropyl ring, on account of the chemical shift of the quaternary carbon atom (δ 20.2, C-11) and those of 6-H and 7-H (δ 0.79 and 0.74, respectively).



C

Although a complete analysis of the remaining proton signals could not be accomplished, owing to the complexity of their spin system and the partial overlapping of some signals, spin decoupling experiments, assisted by one-bond ^{13}C-^{1}H shift correlated spectrum, which allowed the location of all the CH_ACH_B systems in the ^{1}H-NMR spectrum, gave evidence that 7-H, 8-H_2, 9-H_2, and 10-H are contiguous in the given order and not coupled with the protons of the remaining two CH_2 groups which, in turn, were seen to be adjacent as well. These data suggested for the new compound the structure 5 (devoid of stereochemistry) which also seems to be quite plausible on a biogenetic ground. To put these conclusions on a firm basis the aromatization of 5 was performed by treatment with Se at 280°C which afforded guaiazulene identified by comparison with an authentic specimen.

The overall relative stereochemistry of 5 was assigned on the basis of nOed's data (Table 3) and measuring the Europium induced shifts in the ^{1}H-NMR spectrum. Particularly the configuration of the chiral centers C-4, C-5, C-6 and C-7 was determined by observation of nuclear Overhauser effects between 5-H and 13-H_3, 6-H and 14-H_3 and 5-H and 4-H. The cis relationship among 15-H_3, 14-H_3, 6-H, 7-H and the $-N^{+}\equiv C^{-}$ group resulted from the remarkable LIS suffered by these protons when a set of ^{1}H-NMR spectra of 5 were performed in the presence of increasing amounts of Eu(fod)$_3$. The spatial proximity between 6-H and the funcional group could be also responsible for the consistent upfield shift observed for this proton (6-H) in the isothiocyanate 6 (see experimental). During the drawing up of the present paper we became acquainted with the finding of the same metabolite from Braekman's group. The Belgique researchers determined the structure devoid of the chirality at C-1 and C-10 through the chemical correlation with palustrol[6].

Isonitrile 7 was isolated as an optically active oil $[\alpha]_D$ +44.0 (c 1.0, $CHCl_3$) and was shown to be an isomer of 3 and 5 by elemental analysis, HRMS and ^{13}C-NMR data. The isonitrile function was evident from IR (ν_{max} 2130 cm^{-1}), MS (m/z 204, M^{+}-HCN) and ^{13}C-NMR [δ 48.7 ($-CH_2$), 1:1:1 triplet, J= 6.6Hz] spectra. On the other hand the presence of a trisubstituted double bond and, consequently, a carbobicyclic skeleton for 7, was deduced from both the ^{1}H-[δ5.55 (1H, bd, J= 2.2 Hz)] and ^{13}C-NMR [δ 134.0 ($-\overset{|}{C}H$), 132.9 ($-\overset{|}{C}-$)] spectra (Table 2). The marked downfield shift of the broad two-proton singlet at δ 3.90 indicated that the protons in question (14-H_2) were allylic as well as geminal to the $-N^{+}\equiv C^{-}$ group. This deduction was supported by the observation of a long-range coupling between 6-H and 14-H_2. Therefore, since three further allylic protons were observed in the ^{1}H-NMR spectrum of 7, the partial structure D could be readily derived by spin decoupling experiments.

The remaining part of the molecule incorporates three methyls, four methylenes, two methynes and a quaternary carbon atom as deduced from DEPT experiments. Two methyl doublets are

Table 2. ^{1}H- and ^{13}C-NMR data for 5 and 7[a].

Position	5		7	
	δ_C	δ_H (J)	δ_C	δ_H (J)
1	65.7[b]		42.8	
		Ha 2.09 bdd (12.5,6.6)		Ha 1.36[f] m
2	39.9		42.1	
		Hb 1.45 m		Hb 1.28[f] m
		Ha 1.94 m		Ha 1.80[f] m
3	32.4		24.7	
		Hb 1.54 m		Hb 1.36[f] m
4	34.8	2.16 m	50.5	1.76[f] m
5	47.2	1.64 ddt (11.0,11.0,2.9)	51.1	2.16 dd (10.3, 5.8)
6	24.2	0.79 dd (9.6,9.6)	134.0	5.55 bd (2.2)
7	27.0	0.74 ddd (9.6,9.6,6.6)	132.9	
		Ha 1.89 m		Ha 2.03 dd (14.7,14.7)
8	24.4		30.6	
		Hb 1.02 m		Hb 2.25 dd (14.7,6.0)
9	32.6	Ha and Hb 1.51 m	21.0	Ha and Hb 1.50[f] m
				Ha 1.90[f] m
10	45.4	1.31 m	45.3	
				Hb 1.39[f] m
11	20.2		31.6	1.58[f] m
12	28.5	1.06	18.9[c]	0.82[d] d (6.6)
13	15.7	0.99	21.6[c]	0.85[d] d (6.6)
14	18.0	1.00 (7.3)	48.7[e]	3.90 bs
15	18.3	1.02 (7.3)	19.1	0.75 s
16	156.5		not observed	

a. The assignments are based on ^{13}C-^{1}H shift correlated 2D-NMR spectroscopy.

b. The signal appear broadened due to the coupling with the nitrogen atom.

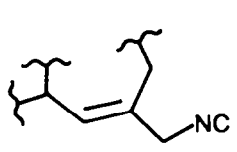
c-d. Values with identical superscript within each column may be interchanged.

e. The signal appear as a 1:1:1 triplet (J= 6.6 Hz) due to the coupling with the nitrogen atom.

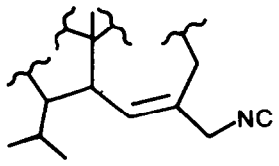
f. Values deduced from the ^{13}C-^{1}H shift correlated 2D-NMR spectrum.

present in the ^{1}H-NMR spectrum of 7 at δ 0.85 (J= 6.6 Hz) and 0.82 (J= 6.6 Hz) and they were shown to belong to an isopropyl group [by irradiation at δ 1.58 both doublets collapsed into singlets]. The third methyl group resonated as a singlet at δ 0.75 thus indicating it to be linked to the only quaternary carbon atom of the molecule (δ 42.8, C-1). The carbon-proton shift correlated 2D-NMR spectrum via ^{1}J allowed the location in the ^{1}H-NMR spectrum of all the couples of signals due to geminal protons and the signal pertinent to the remaining methyne group (δ 1.76, 4-H). Spin decoupling work indicated that this group is comprised by C-5 and

C-11. Furthermore, since 5-H did not show any other vicinal coupling, apart from those with 4-H and 6-H, C-5 must be linked to the fully substituted carbon (C-1). Thus, the partial structure D could be extended to E.



D



E

At this stage only four methylene groups are to be accounted for to complete the gross structure for the metabolite under investigation. Extensive spin-decoupling experiments corroborated by 2D-NMR data showed that they form two separate couples of consecutive $-CH_2-$ groups one of them contiguous to 8-H_2 and the other one to 4-H. Therefore, structure 7 is the only one in which the above data can be logically accomodated.

The nOed's data (Table 3) indicated that the relative configuration for the novel isocyanide is the same reported for mintsulphide[7] and aphanamol I and II[8], the only other metabolites based on this skeleton reported in literature. On the other hand the NMR data of 7, when comparable with those of aphanamols, are in good agreement.

Inspection of the molecular model (Dreiding) of 7 indicated that the two methylene groups 8-H_2 and 9-H_2 are spatially oriented in such a way to form two dihedral angles in the range of 80-100° (8-Ha with 9-Hb and 8-Hb with 9-Ha). This agrees with the small values, if any, of the coupling constants within each couple of the above protons thus accounting for the multiplicities observed for 8-Ha and 8-Hb.

Table 3. Results of nOed's experiments on compounds 3, 5 and 7

3		5		7	
Signal irradiated	Signal enhanced	Signal irradiated	Signal enhanced	Signal irradiated	Signal enhanced
1-H	6-H	5-H	4-H	6-H	14-H_2
6-H	1-H		13-H_3		4-H
	11-H	13-H_3	8-Ha	14-H_2	6-H
15-H_3	1-H	14-H_3	6-H		8-Ha
	3-Ha	6-H	14-H_3	15-H_3	5-H
					11-H
					10-Ha
					10-Hb

It is noteworthy that 7 is the only sesquiterpene isonitrile so far isolated in which the $-N^{+}\equiv C^{-}$ group is located on a methylene carbon. The fact that also in aphanamols the same carbon is functionalized suggests they could derive from a common biogenetic precursor[9].

The isomeric isothiocyanates 4, 6 and 8 (molecular formula $C_{16}H_{25}NS$ deduced from

HRMS and ^{13}C-NMR spectroscopy) were isolated from the less polar fraction of the methanol extract of A.acuta. The presence in each of them of the -N=C=S functionality was readily derived from the inspection of both the IR and MS spectra and confirmed by NMR data. A detailed analysis of the spectral properties of the individual metabolites (see experimental) clearly indicated a close structural relationship with isocyanides 3, 5 and 7, respectively, thus suggesting they were the corresponding isothiocyanates. This hypothesis was corroborated by comparing the spectroscopic and chromatographic properties of 4, 6 and 8 with those of the synthetic isothiocyanates obtained from 3, 5 and 7, by reaction with sulphur at 120°C.

EXPERIMENTAL

General methods. EIMS were determined at 70 eV on a Kratos MS 80 mass spectrometer. IR spectra were recorded on a Perkin-Elmer Model 399 spectrophotometer. ^{1}H-NMR experiments were performed on a Bruker WM-500 spectrometer and ^{13}C-NMR spectra were recorded on a Bruker WM-250 spectrometer in $CDCl_3$ solutions. Proton chemical shifts are referenced to the residual chloroform signal (7.24 ppm); carbon-13 chemical shifts are referenced to the solvent (77.0 ppm). The multiplicity of ^{13}C-NMR resonances was determined by DEPT experiments which were performed using polarization transfer pulses of 90° and 135° obtaining in the first case only signals for -^{1}CH groups and in the other case positive signals for -^{1}CH and -^{1}CH$_3$ and negative ones for -^{1}CH$_2$ groups. Polarization transfer delays were adjusted to an average ^{1}C-H coupling of 135 Hz. The ^{2}shift correlations with polarization transfer via ^{1}J coupling were carried out adjusting the fixed delays to give maximum polarization for J_{C-H} = 135 Hz. The long-range heteronuclear correlation was performed with maximum polarization for 6.25 Hz, leading to ^{2}J and ^{3}J spots in the same spectrum[5]. ^{1}H-nOed's were run at 250 MHz in degassed $CDCl_3$ solutions. Optical rotations were taken on a Perkin-Elmer Model 141 polarimeter using a 10 cm microcell. High performance liquid chromatographies were performed on a Varian 2010 apparatus equipped with a differential refractometer using a Hibar LiChrosorb Si 60 (10 x 250 mm) column and n-hexane/diethyl ether solvent mixtures as eluent.

Extraction and isolation. Fresh sponge A.acuta was collected in the Bay of Napoli in April 1985. A voucher specimen is deposited in our laboratory in the Dipartimento di Chimica Organica e Biologica dell'Università di Napoli. Freshly collected animals (48 g, dry weight after extraction) were extracted three times with MeOH at room temperature for three days. The combined lipid extracts were concentrated under reduced pressure to obtain an aqueous suspension which was extracted with $CHCl_3$ (4 x 200 mL). The organic phase was dried over sodium sulphate and the solvent evaporated to yield a dark red oil (6.3 g) which was chromatographed on a silica gel column eluted under a slight N_2 pressure with a solvent gradient system from light petroleum to diethyl ether through benzene. The early eluted fractions (eluent light petroleum-C_6H_6, 8:2) containing mixtures of isothiocyanates, were combined and further separated by HPLC (eluent n-hexane-Et_2O, 97:3) affording compounds 4 (8 mg), 6 (7 mg), and 8 (6 mg) in pure form.

The isothiocyanates had the following HPLC retention times in minutes on the above column (n-hexane-Et_2O 97:3, flow rate 1 mL/min.): 4 (18.8), 6 (20.2), 8 (23.0). The fractions of the original chromatogram eluted slightly after the isothiocyanates (eluent light petroleum-C_6H_6, 2:8) gave mixtures of isocyanides which, combined, were subjected to a further HPLC separation (eluent n-hexane Et_2O, 97:3) affording pure samples of 3, 5, and 7 (35, 25 and 20 mg, respectively). The HPLC retention times in minutes for these compounds (above conditions) were: 3 (36.4), 5 (37.4), 7 (49.0). Elemental analyses 3: found C 83.11%, N 6.02%, H 10.93%; 5: found C 83.09%, N 6.00%, H 10.91%; 7: found C 83.08%, N 6.02%, H 10.93% ($C_{16}H_{31}N$ requires C 83.05%, N 6.05%, H 10.89%). The NMR data for compounds 3, 5, and 7 are reported in Tables 1-3. Compounds 4, 6 and 8 showed the following spectral features:

4, colourless oil, $[\alpha]_D$ -12.9 (c 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2100 cm^{-1}; HRMS, M$^+$ found 263.1721 (calc. for $C_{16}H_{25}NS$ 263.1709); ^{1}H-NMR ($CDCl_3$) δ 5.05 (1H, bs, 6-H), 3.32 (1H, d, J= 8.8 Hz, 1-H), 2.19 (1H, m, 2-H), 2.13 (1H, b septet, J= 6.6 Hz, 11-H), 2.02 (1H, m, 10-H), 1.91 (2H, bt, J= 6.6 Hz, 8-H$_2$), 1.85 (1H, m, 3-Ha), 1.73 (1H, ddd, J= 13.2, 8.8 and 8.8 Hz, 4-Ha), 1.70 (1H, m, 9-Ha), 1.40 (1H, m, 9-Hb and 4-Hb), 1.27 (1H, m, 3-Hb), 1.12 (3H, d, J= 6.6 Hz, Me-15), 0.96 (6H, d, J= 6.6 Hz, Me-12 and Me-13), 0.90 (3H, d, J= 6.6 Hz, Me-14); ^{13}C-NMR ($CDCl_3$) δ 143.8 (C-7), 126.2 (C-6), 75.0 (C-1), 49.1 (C-5), 42.6 (C-2), 35.0 (C-11), 34.0 (C-4), 32.5 (C-10), 30.4 (C-3), 28.2 (C-9), 23.6 (C-8), 21.4 (C-12 and C-13), 19.4 (C-15), 16.8 (C-14), C-16, not observed.

6, colourless oil, $[\alpha]_D$ -32.8 (c 0.7, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2100 cm^{-1}; HRMS, M$^+$ found 263.1712 (calc. for $C_{16}H_{25}NS$ 263.1709); ^{1}H-NMR ($CDCl_3$) δ 2.19 (1H, m, 4-H), 2.07 (1H, m, 1-Ha), 1.92 (2H, m, 3-Ha and 8-Ha), 1.76 (1H, dd, J= 11.0 and 11.0 Hz, 5-H), 1.07 (3H, s, Me-13), 1.04 (1H, m, 8-Hb), 1.01 (3H, d, J= 6.6 Hz, Me-15), 1.00 (3H, d, J= 6.6 Hz, Me-14), 0.99 (3H, s, Me-12), 0.75 (1H, ddd, J= 9.6, 9.6 and 5.9 Hz, 7-H), 0.67 (1H, dd, J= 11.0 and 9.6 Hz, 6-H); ^{13}C-NMR ($CDCl_3$) δ 125.5 (C-16), 79.8 (C-1), 49.1 (C-10), 47.2 (C-5), 39.7 (C-2), 34.7

(C-4), 33.1 (C-3 or C-9), 32.8 (C-9 or C-3), 28.8 (C-13), 27.2 (C-7), 24.5 (C-8), 24.3 (C-6), 20.4 (C-11), 18.6 (C-15), 18.2 (C-14), 15.6 (C-12).

8, colourless oil, $[\alpha]_D$ +36.2 (c 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2100 cm^{-1}; HRMS, M^+ found 263.1720 (calc. for $C_{16}H_{25}NS$ 263.1709); ^{1}H-NMR ($CDCl_3$) δ 5.51 (1H, bd, J= 2.2 Hz, 6-H), 3.98 (2H, bs, 14-H_2), 0.85 (3H, d, J= 6.6 Hz, Me-12 or Me-13), 0.82 (3H, d, J= 6.6 Hz, Me-13 or Me-12), 0.75 (3h, s, Me-15); ^{13}C-NMR ($CDCl_3$) δ 134.1 (C-6), 132.8 (C-7), 51.2 (C-5), 50.3 (C-4), 45.4 (C-10), 42.8 (C-1), 42.0 (C-2), 31.6 (C-11), 30.5 (C-8), 24.6 (C-3), 21.6 (C-12 or C-13), 20.9 (C-9), 19.1 (C-15), 18.8 (C-13 or C-12), C-16, not observed.

Treatment of 3 with sulphur to obtain 4. A mixture of 3 (15 mg) and excess of S was heated at 120°C for 16 h. After cooling, 40-70° light petroleum was added and the solution was filtered and taken to dryness. The residue purified by TLC (silica gel, n-hexane, uv) afforded 10 mg of an oily product whose spectral and chromatographic properties were identical to those of natural 4.

Treatment of 5 and 7 with sulphur to obtain 6 and 8, respectively. Compounds 5 (10 mg) and 7 (8 mg) were treated with S by the procedure used for 3, thus obtaining 3.5 mg of 6 and 3 mg of 8, respectively, identical to the natural 6 and 8.

Dehydrogenation of 5. A mixture of 12 mg of 5 and 24 mg of Se was heated at 280°C for 30 min. After cooling, 40-70° light petroleum was added and the filtered solution was extracted with 60% aq. H_2SO_4. The acid phase was diluted with water and extracted with 40-70° light petroleum. After washing with H_2O the organic phase was taken to dryness to give guaiazulene, identified by comparison of its properties with those of an authentic specimen.

REFERENCES AND NOTES

1. Minale et al. [L.Minale, R.Riccio and G.Sodano Tetrahedron 30, 1341 (1974)] reported acanthellin-1 as the major constituent of the isocyanide fraction from A.acuta. We actually found acanthellin-1 in our collection but as a minor metabolite comprising less than 0.04% of the methanol extract. We think that this is most likely due to seasonal variations.
2. P.Ciminiello, S.Magno, L.Mayol and V.Piccialli J.Nat.Prod. in the press.
3. M.R.Bendall, D.M.Doddrell, D.T.Pegg, W.E.Hull: DEPT-Bruker-Information Bullettin, Bruker Analytshe Masstechnik, Karlsruhe 1982.
4. A.Bax and G.Morris J.Magn.Res. 42, 501 (1981).
5. H.Kessler, C.Griesinger, J.Zarbock and H.R.Loosli J.Magn.Res. 57, 331 (1984).
6. J.C.Braekman, D.Daloze, F.Deneubourg, J.Huysecom and G.Vandevyver Bull.Soc.Chim.Belg., in the press.
7. T.Yoshida, S.Muraki, K.Takahashi, T.Kato, C.Kabuto, T.Suzuki, T.Uyehara and T.Ohnuma J.C.S. Chem.Comm. 512 (1979).
8. M.Nishizawa, A.Inoue, Y.Hayashi, S.Sastrapradja, S.Kosela and T.Iwashita J.Org.Chem. 49, 3660 (1984).
9. Niwa et al. [M.Niwa, M.Iguchi and S.Yamamura Tetrahedron Lett. 4291 (1979)] reported a biomimetic reaction of epoxygermacrene-D leading to the formation of two compounds based on the same skeleton as that of compound 7.

Acknowledgments. This work was supported by "Ministero della Pubblica Istruzione (Italy)". We thank Stazione Zoologica (Napoli) for supplying the sponge.