Two New Sesquiterpene Isothiocyanates from the Marine Sponge Acanthella klethra

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J. Nat. Prod., **1992**, 55 (5), 633-638• DOI:
10.1021/np50083a012 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 5, pp. 633-638, May 1992

TWO NEW SESQUITERPENE ISOTHIOCYANATES FROM THE MARINE SPONGE *ACANTHELLA KLETHRA*

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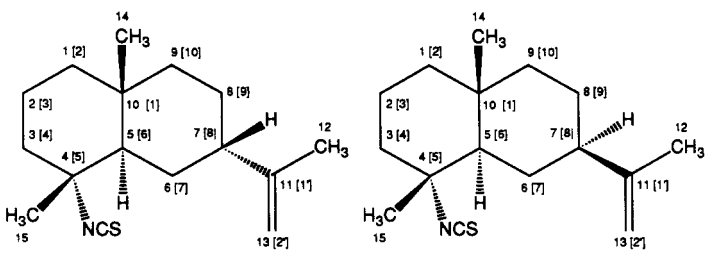
ABSTRACT.—From the lipophilic extract of the marine sponge *Acanthella klethra*, two new sesquiterpene isothiocyanates have been isolated and characterized as (1*R*,5*R*,6*R*,8*S*)-dec[4.4.0]ane-1,5-dimethyl-8-(1′-methylethenyl)-5-isothiocyanate [**1**] and (1*R*,5*R*,6*R*,8*R*)-dec[4.4.0]ane-1,5-dimethyl-8-(1′-methylethenyl)-5-isothiocyanate [**2**]. The structures of **1** and **2** were deduced from X-ray and spectroscopic (nmr, ir, and ms) data.

Sponges of the genus *Acanthella* have previously been shown to be rich sources of terpenes having various nitrogen-containing functionalities (1,2). On our recent collecting trip to the Great Barrier Reef, Australia, we were able to collect a species from this genus, the bright orange sponge *Acanthella klethra* Pulitzer-Finali (Axinellidae). The lipophilic extract of our sample of this sponge yielded two new sesquiterpenes **1** and **2** and three previously reported sesquiterpenes **3** (3), **4** (3), and **5** (4). Each of the compounds contained either an isonitrile or an isothiocyanate moiety.

RESULTS AND DISCUSSION

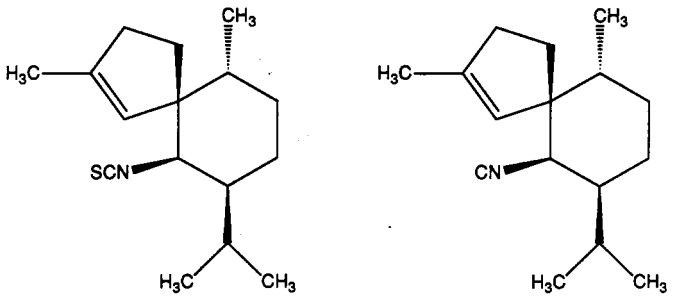
The CH_2Cl_2 solubles obtained from the freeze-dried sponge *A. klethra* were fractionated by vlc using Si gel. The fraction that eluted with pure hexane was further purified by hplc on normal phase silica. From this separation we obtained the previously reported sesquiterpenes **3** (3), **4** (3), and **5** (4) as well as very minor amounts of two new sesquiterpenes, **1** and **2**.

Compound **1** had the molecular formula $C_{16}H_{25}SN$ as deduced by ms. The ir spectrum indicated the presence of an isothiocyanate function (2140 cm^{-1}); this contention was supported by peaks at *m/z* 230, 205, and 204 in the mass spectrum of **1** for $[M-HS]^+$, $(M-SCN]^+$, and $[M-HSCN]^+$, respectively, accounting for two of the degrees of unsaturation implied by the molecular formula. Further, the presence of two resonances in the ^{13}C-nmr spectrum of **1** for an *exo*-methylene group [146.1 (s), 111.3 (t) ppm] and the absence of any further sp^2 resonances indicated **1** to be a bicyclic molecule. From the ^1H-^1H COSY spectrum it was possible to elaborate two major proton spin systems, fragments 1 and 2 (Scheme 1). Remaining after the ^1H-^1H spin anal-



1 **2**

The numbers in brackets are used for systematic naming



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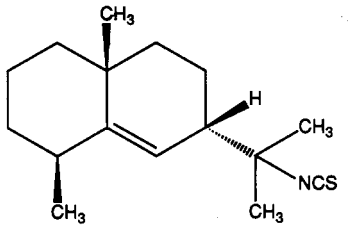

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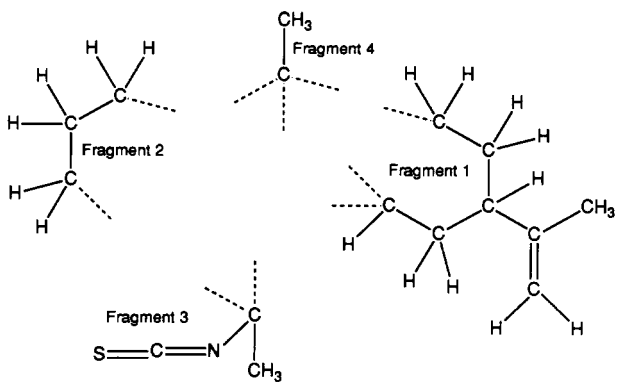
ysis were two tertiary methyl groups, two quaternary carbons, and an isothiocyanate function, which required assignment. Clearly from the ^{13}C-nmr data the carbon with resonance at 65.0 (s) ppm must bear the isothiocyanate. As we had no evidence for a *gem*-dimethyl grouping it was evident that each tertiary methyl group must reside on separate quaternary carbons, giving rise to fragments 3 and 4 (Scheme 1). The four elaborated fragments (Scheme 1) can combine in a number of ways, but when the ^{13}C-nmr data for **1** were compared with those for other compounds containing similar structural elements (5,6) it was clear that **1** had a eudesmane carbon skeleton. The four chiral centers within **1** were assigned from the results of nOe experiments and from ^{13}C-nmr and ^{1}H-nmr literature comparisons. Thus, the ring junction was assigned as trans on the basis of the ^{13}C-nmr chemical shifts for C-5 and C-10 (5–7) as well as the absence of an observable nOe between the C-15 methyl group protons and the proton at C-5. The remaining two centers, C-4 and C-7, were proposed on the basis of literature comparisons of ^{13}C-nmr and ^{1}H-nmr data for compounds containing comparable stereocenters. Thus, the stereochemistry at C-4 was deduced from comparisons made with data presented by Nanayakkara *et al.* (8) for a series of hydroxylated eudesmane derivatives, while that for C-7 was suggested as the opposite of that proposed by Nanayakkara *et al.*



SCHEME 1

(8) and Bagchi *et al.* (9) on the basis of the 10 ppm difference in the chemical shift for C-7 in the comparable compounds.

As **1** was crystalline, we undertook a single crystal X-ray crystallographic analysis of it. The results of this investigation (see Figure 1 and Experimental) not only proved our original deductions to be correct but also allowed us to deduce the absolute stereochemistry for **1**. Compound **1** is systematically named (1*R*,5*R*,6*R*,8*S*)-dec[4.4.0]ane-1,5-dimethyl-8-(1′-methylethenyl)-5-isothiocyanate. It must be noted at this point that the numbering system used for the naming of **1** is different from that used in discussing and reporting experimental details for **1**. The reason for this is that there appears to be some precedent for the numbering of this type of skeleton in a non-systematic way (5,6).

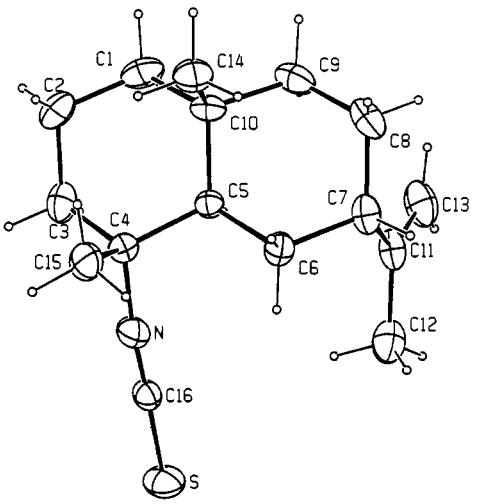


FIGURE 1. The molecular structure of **1**.

Compound **2** had the molecular formula $C_{16}H_{25}SN$ by ms. Three of the five degrees of unsaturation implied by the molecular formula were accounted for by an isothiocyanate moiety (2065 cm^{-1}, $[M-HS]^+$, $[M-SCN]^+$) and an *exo*-methylene grouping [149.9 (s), 108.6 (t) ppm], indicating **2** to be bicyclic. Comparison of all the spectroscopic data for **2** with those for **1**, and the results of a 2D ^1H-^1H COSY and a proton-detected 2D long range ($J = 10$ Hz) ^1H-^{13}C COSY (HMBC) experiment (Table 1), revealed that the two molecules were very similar. Further, from detailed comparison of the two sets of ^{13}C- and ^1H-nmr data with 2D nmr data for **2**, it was possible to conclude that the two molecules were virtually identical except in the region of C-8 to C-5. This observation indicated that the stereochemistry at either C-5 or C-7 or at both centers was different in **2** from that in **1**. Further comparisons of the ^{13}C-nmr data for **2** with those for compounds containing similar structures (8–10) clearly revealed the difference between **2** and **1** to be only at C-7; in **2** the olefinic side chain is β. Compound **2** is thus (1*R*,5*R*,6*R*,8*R*)-dec[4.4.0]ane-1,5-dimethyl-8-(1′-methylethenyl)-5-isothiocyanate. Once again the numbering used for the naming of **2** is different from that used for the discussion/experimental sections for the same reason as outlined for **1**.

Literature data (8–11) together with the current spectroscopic data for **1** and **2** suggest that the ^1H- and ^{13}C-nmr resonances of H-7 and C-7 are diagnostic for the stereochemistry at C-7 of the eudesmane skeleton. Thus α protons at C-7, in eudesmanes, are considerably shielded (δ 1.98 for **2**) when compared to β protons at C-7 (δ

TABLE 1. ^{1}H-nmr (300 MHz, $CDCl_3$) and ^{13}C-nmr (75.5 MHz, $CDCl_3$) Data for Compound **2**.

Position	^{13}C	^{1}H	Long range correlations (J 10 Hz)
1	40.4 (t)	1.14 (m), 1.41 (m)	
2	18.9 (t)	1.52 (m)	
3	42.0 (t)	1.75 (m), 2.03 (m)	
4	65.3 (s)		
5	53.2 (d)	1.52 (m)	
6	26.8 (t)	1.43 (m), 1.53 (m)	
7	46.0 (d)	1.98 (m)	
8	27.6 (t)	1.32 (m), 1.78 (m)	
9	44.7 (t)	1.21 (m), 1.44 (m)	
10	34.6 (s)		
11	149.9 (s)		
12	21.1 (q)	1.77 (s)	*21.1*, 149.9, 108.6, 46.0
13	108.6 (t)	4.73 (s)	*108.6*, 46.0, 21.1
14	19.1 (q)	0.90 (s)	*19.1*, 53.2, 44.7, 40.4, 34.6
15	22.1 (q)	1.32 (s)	*22.1*, 65.3, 53.2, 42.0
16	Not obsd.		

2.47 for **1**). For the ^{13}C-nmr resonances a similar effect is observed; with a β side chain, C-7 resonates at δ 46.0 in **2** while with an α side chain the equivalent resonance is at δ 39.2 in **1**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—X-ray data were collected on an Enraf-Nonius CAD4 diffractometer using graphite-monochromated CuKα radiation. The HMBC spectrum of **2** was measured using a Bruker AMX 500 spectrometer with associated software. The other procedures have been described by König *et al.* (12).

MATERIALS.—All sponge materials were collected by divers, using SCUBA, from the vicinities of Phantom and Pelorus Islands, Queensland, Australia. The animals were collected from a depth of 9–12 m during September 1990 and deep frozen. A voucher specimen is deposited with the Museum of Tropical Queensland, 74-84 Flinders Street, Townsville Q4810, Australia; museum number G25006.

EXTRACTION AND ISOLATION.—Deep frozen sponge tissue was freeze-dried. Dry tissue (70.7 g) was extracted with CH_2Cl_2 (2.5 liters) and then with MeOH (3 liters). From both extracts the $CHCl_2$ solubles (3.1 g) were taken, combined, and chromatographed over silica with petroleum ether containing increasing proportions of EtOAc as eluent; ten fractions each of approximately 90 ml were obtained. Tlc and ^{1}H-nmr analysis of these fractions revealed that fractions 1 and 2, eluted with pure hexane, were most interesting. Hplc separation of these two fractions combined afforded three previously reported compounds **3**, **4**, and **5** and two new sesquiterpene metabolites.

(1R,5R,6R,8S)-Dec[4.4.0]ane-1,5-dimethyl-8-(1'-methylethenyl)-5-isothiocyanate [**1**].—Compound **1** (1.7 mg, 0.0024%): white crystalline solid, recrystallized from Et_2O; mp 62.3°; $[\alpha]^{25}D$ +142.9° (c = 0.035, $CHCl_3$); ir ν max 2910, 2140, 1440, 1100, 895 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 0.94 (s, 3H, H-14), 1.06 (m, 1H, H-9), 1.10 (m, 1H, H-1), 1.31 (s, 3H, H-15), 1.37 (m, 1H, H-1), 1.37 (m, 1H, H-9), 1.57 (m, 2H, H-2), 1.60 (m, 1H, H-6), 1.61 (m, 1H, H-5), 1.70 (m, 1H, H-8), 1.71 (m, 1H, H-3), 1.79 (s, 3H, H-12), 1.83 (m, 1H, H-8), 2.02 (m, 1H, H-3), 2.02 (m, 1H, H-6), 2.47 (br s, 1H, H-7), 4.88 (br s, 1H, H-13), 4.97 (br s, 1H, H-13); ^{13}C nmr (75.5 MHz, $CDCl_3$) 18.8 (q, C-14), 18.8 (t, C-2), 21.9 (q, C-15), 22.8 (q, C-12), 23.4 (t, C-8), 24.3 (t, C-6), 35.2 (s, C-10), 39.2 (d, C-7), 40.4 (t, C-1), 40.6 (t, C-9), 41.8 (t, C-3), 47.5 (d, C-5), 65.0 (s, C-4), 111.3 (t, C-13), 146.1 (s, C-11) ppm; hreims *m/z* 263.1677 ($C_{16}H_{25}SN$ requires 263.1709); eims *m/z* (% rel. int.) $[M]^+$ 263 (4), 248 (3), 230 (7), 206 (24), 205 (96), 204 (58), 189 (25), 161 (20), 149 (89), 135 (56), 123 (78), 109 (81), 95 (79), 81 (100).

(1R,5R,6R,8R)-Dec[4.4.0]ane-1,5-dimethyl-8-(1'-methylethenyl)-5-isothiocyanate [**2**].—Compound **2** (1.5 mg, 0.0022%): clear oil; $[\alpha]^{25}D$ +180.0° (c = 0.025, $CHCl_3$); ir ν max 2910, 2065, 1445, 855 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 1; hreims *m/z* 263.1751 ($C_{16}H_{25}SN$ requires 263.1709);

eims *m/z* (% rel. int.) $[M]^+$ 263 (2), 230 (2), 205 (90), 189 (11), 149 (33), 135 (30), 123 (38), 109 (53), 95 (63), 81 (65).

Compounds **3** (15 mg, 0.02%), **4** (85 mg, 0.1%), and **5** (5 mg, 0.007%) were identified by comparison of their spectroscopic and physical data with those previously published (3,4).

SINGLE CRYSTAL X-RAY ANALYSIS OF **1**.[1]—Crystal data: $C_{16}H_{25}NS$, MW = 263.1709, orthorhombic, space group $P2_12_12_1$, $a = 7.354$ (1), $b = 13.059$ (2), $c = 16.485$ (2) Å, $V = 1583.2$ (6) Å^3, $Z = 4$, $Dc = 1.105$ gcm^{-3}, $\lambda = 1.54184$ Å, μ(CuKα) = 16.3 cm^{-1}, T = 300 K. Intensity data were measured using $\omega = 2\theta$ scans of variable rate from a colorless crystal of dimensions $0.25 \times 0.50 \times 0.55$ mm having a maximum transmission coefficient of 99.82%. Of the 1855 unique data ($\theta < 75°$), 1821 had intensities greater than zero and were used in the refinement. Data reduction included corrections for background, Lorentz, polarization, and absorption by Ψ scans (min. trans. coeff. 71.2%). Refinement was carried out by full-matrix least squares based on F, with weights $w = \sigma^{-2}(F_o)$. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$. The error of fit was 2.781. Non-hydrogen atoms were refined anisotropically, while hydrogen atoms were located by difference maps and refined isotropically. Final $R = 0.036$, $R_w = 0.048$ for 264 variables. Maximum and minimum residual densities were 0.12 and -0.16 eÅ^{-3}. The mirror-image structure was refined under identical conditions to $R = 0.047$; $R_w = 0.064$; thus the absolute configuration is confirmed to be that shown by the ORTEP representation (Figure 1). All calculations were carried out using the MolEN programs (123). Final atomic coordinates are listed in Table 2.

TABLE 2. Final Atomic Coordinates and Equivalent Isotropic Thermal Parameters for **1**.

Atom	*x*	*y*	*z*	B_{eq} (Å^2)[a]
S	−0.02160 (9)	0.43266 (4)	0.38284 (4)	7.63 (1)
N	0.2274 (3)	0.2800 (2)	0.37152 (9)	6.64 (4)
C-1	0.7195 (3)	0.1576 (2)	0.2901 (2)	6.93 (6)
C-2	0.6882 (3)	0.1588 (2)	0.3807 (1)	6.88 (5)
C-3	0.5304 (4)	0.2280 (2)	0.4025 (1)	6.12 (5)
C-4	0.3554 (3)	0.1966 (1)	0.3586 (1)	4.48 (3)
C-5	0.3883 (2)	0.1909 (1)	0.26591 (9)	3.79 (3)
C-6	0.2184 (3)	0.1657 (1)	0.2175 (1)	4.57 (3)
C-7	0.2479 (3)	0.1822 (2)	0.1257 (1)	5.40 (4)
C-8	0.4149 (4)	0.1229 (2)	0.0991 (1)	6.83 (5)
C-9	0.5820 (3)	0.1442 (2)	0.1505 (1)	6.67 (5)
C-10	0.5521 (2)	0.1231 (1)	0.2416 (1)	4.96 (4)
C-11	0.2437 (3)	0.2944 (2)	0.1016 (1)	5.70 (4)
C-12	0.0828 (4)	0.3543 (2)	0.1296 (1)	7.42 (6)
C-13	0.3658 (4)	0.3372 (2)	0.0535 (1)	7.66 (6)
C-14	0.5230 (4)	0.0068 (2)	0.2534 (2)	6.39 (5)
C-15	0.2677 (3)	0.1023 (2)	0.3968 (1)	5.97 (4)
C-16	0.1227 (3)	0.3443 (1)	0.3757 (1)	4.70 (3)

[a] $B_{eq} = (8\pi^2/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$.

ACKNOWLEDGMENTS

We thank Dr. Jane Fromont of the Sir George Fisher Center for Tropical Marine Studies, James Cook University, Townsville Q4811, Australia, for performing all taxonomy; Dr. John Coll and his research group, Department of Chemistry and Biochemistry, James Cook University, for providing technical assistance in collection of sponge samples; Dr. E. Zass, Department of Organic Chemistry, ETH Zürich, for performing literature searches; Mr. O. Greter and Dr. W. Amrein, ETH Chemistry Department Mass Spectral Service, for recording all mass spectra and making all accurate mass measurements; and Dr. Heinz Rüegger, Laboratory for Inorganic Chemistry, ETH Zurich, for measuring the HMBC spectrum of **2**. We would also like to thank Prof. Klaus Fischer, Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803-1804, for several useful suggestions concerning this study.

[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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Received 25 September 1991