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Tetrahedron Letters 49 (2008) 2200–2203
Tetrahedron Letters

Axiplyns A–E, new sesquiterpene isothiocyanates from the marine sponge *Axinyssa aplysinoides*

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Received 2 December 2007; revised 31 January 2008; accepted 1 February 2008
Available online 7 February 2008

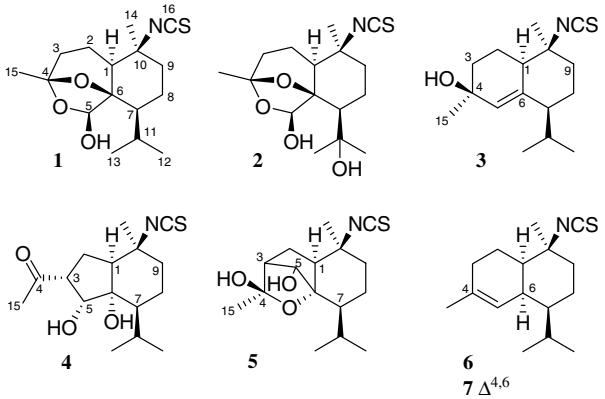
Abstract

Five new isothiocyanate sesquiterpenes, designated axiplyns A–E (**1**–**5**) have been isolated, together with two known isothiocyanate sesquiterpenes (**6**, **7**), from the sponge *Axinyssa aplysinoides* collected at Misali Island, Tanzania. Axiplyns **4** and **5** embody a new indane sesquiterpene skeleton, and compounds **1**, **2**, and **5** contain unprecedented ring systems, namely a 6,8-dioxabicyclo[3.2.1]octane and a 2-oxabicyclo[2.2.1]heptane. Axiplyns A, B, and C are potent brine shrimp toxins with LD_{50} values between 1.5 and 1.8 μg/mL.

Keywords: Marine sponge; Sesquiterpenoid; Isothiocyanate; NMR

In a continuing discovery program for bioactive compounds from marine invertebrates,[1,2] several sponge extracts were screened for brine shrimp toxicity. Consequently, we encountered potent activity from an *Axinyssa aplysinoides* species collected at Misali Island, southwest of Pemba Island, Tanzania.[3] The genus *Axinyssa* (order Halichondrida, family Halichodriidae) is characterized by the occurrence of sesquiterpenes containing unusual nitrogenous functional groups, such as formamide, isonitrile, thiocyanate, and isothiocyanates.[4–6] Interestingly, many of these nitrogenous functional group-containing terpenes have been found to possess different biological activities.[5,7,8] We herewith report the isolation and structure elucidation of five new sesquiterpenoid isothiocyanate metabolites as well as their brine shrimp bioassays.

Homogenized *A. aplysinoides* (12.0 g, dry weight), was extracted with EtOAc–MeOH–H_2O (5:5:1). The extract (500 mg) was then subjected to solvent-partitioning, that is, aq MeOH against hexane and CH_2Cl_2. The CH_2Cl_2 fraction (186 mg) was chromatographed on Sephadex LH-20, eluted with hexane–MeOH–$CHCl_3$ (2:1:1) to afford a complex cytotoxic mixture. Sequential VLC on silica gel led to the isolation of five new compounds, axiplyns A–E (**1**–**5**, 0.04–0.017% dry weight)[9–12] and two known compounds (1*R*,6*S*,7*S*,10*S*)-10-isothiocyanato-4-amorphene (**6**) and 10-isothiocyanato-4,6-amorphadiene (**7**), previously isolated from the Fijian sponge *Axinyssa fenestratus*.[5] Compounds **6** and **7** were identified by comparison of their spectroscopic data including optical rotations with literature data. Assuming common biosynthesis, the absolute configuration of **6** suggested the configurations of C-1, 7, and 10 for all five axiplyns.



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0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.02.005

Axiplyn A (**1**) was isolated as a colorless oil and showed a molecular ion peak at *m/z* 311 $[M]^+$ in the EIMS. The presence of 16 carbon signals in the ^{13}C NMR spectrum was consistent with the molecular formula of $C_{16}H_{25}NO_3S$, which was established by HREIMS (*m/z* 311.1555, calcd for $C_{16}H_{25}NO_3S$, 311.1555), indicating five degrees of unsaturation. From the formula, the IR (2100 cm^{-1}) and ^{13}C NMR spectrum (δ_C 133.6 ppm) of the compound was suggested to be a sesquiterpene isothiocyanate. Examination of the ^{13}C NMR revealed, in addition to the NCS group, three low field oxygen carrying carbons at δ_C 106.0 s (ketal), 95.9 d (hemiacetal), and 86.6 s (ethereal carbon) implying a tricyclic structure. The COSY spectrum revealed the presence of a three spin system as shown in Figure 1. HMBC correlations (Table 1 and Fig. 1) established the complete planar structure of **1**. Key starting points for interpretation of the CH correlations were those of the four methyl groups and the hydroxyl group depicted in Figure 1.

The relative stereochemistry of **1** was determined mainly by the analysis of NOE experiments and was found to fit the configuration of the known 10-isothiocyanato-4-amorphene.[5] Accordingly, the isopropyl as well as the Me-14 (δ_C 28.6 ppm) groups were determined to be trans diequatorial orientated (inspection of literature data indicates characteristic carbon chemical shifts as a function of the methyl stereochemistry namely, ca. 20 and ca. 30 ppm for axial and equatorial methyls, respectively).[5] The configuration of the other four chiral centers of **1** was determined by NOESY correlations. NOEs between the 1,3-oriented, H-1, 5, and 7 established their axial stereochemistry, and thence the configuration of C-1, C-5, and C-6 (Fig. 1). The latter configuration also indicated the β positioning of the C4–C6 oxygen bridge, hence, establishing the configuration of C-4 (Table 2).

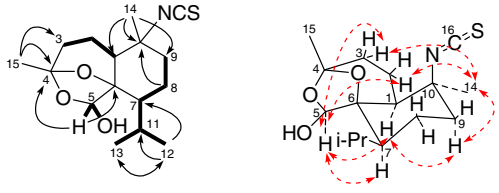


Fig. 1. Key COSY (▬), HMBC (⌒), and NOESY (⌒) correlations of axiplyn A (**1**).

The spectral data of axiplyn B (**2**) indicated a close structural similarity to compound **1**. The EIMS peak at *m/z* 327 indicated the presence of an additional oxygen atom in the molecule (HREIMS *m/z* 327.1505 calcd for $C_{16}H_{25}NO_4S$, 327.1504). The major differences in the NMR spectra of **2** in comparison to **1** appeared in the ^{13}C NMR spectrum, which showed a singlet carbon resonating at δ_C 79.7 ppm, and a shift of the isopropyl methyl groups to δ_C 23.8 q and 29.4 ppm, suggesting the presence of an additional hydroxyl group. According to the ^1H NMR spectrum, where all the methyl groups appeared as singlets, in addition to both Me-12 and Me-13 giving HMBC correlations to the 79.7 ppm singlet, the position of the additional hydroxyl was determined as C-11 and established the structure of compound **2**. Similar NOEs to those observed for **1** pointed to the same stereochemistry.

The CIMS of axiplyn C (**3**) exhibited a $[M+H-H_2O]^+$ ion at *m/z* 262.[13] The molecular formula was determined

Table 1
NMR data of axiplyn A (**1**)[a]

Position	δ_C	δ_H[b] (*J* in Hz)	COSY[c]	HMBC (H to C)	NOE
1	49.1 d	1.41 m	2	2, 5, 6	2a, 2b, 3a, 9a
2	18.4 t	1.76 m	1, 3a, 3b	1, 3, 4, 10	1, 3a, 3b
3	33.6 t	a 1.59 m	2, 3b	1, 2, 15	
		b 1.75 m	2, 3a		
4	106.0 s				
5	95.9 d	5.82 d (7.7)	5-OH	4, 6	
6	86.6 s				
7	50.5 d	1.42 m	8a, 8b, 11	6, 8	8a, 8b, 11, 12, 13
8	22.3 t	a 1.57 m	7, 8b, 9a, 9b	6, 7, 10, 11	7, 9a, 9b, 11, 12
		b 1.80 m	7, 8a, 9a, 9b		
9	41.4 t	a 1.60 m	8a, 8b, 9b	7, 10	1, 7, 8a, 8b, 14
		b 2.11 dd (13.3, 4.1)	8a, 8b, 9a		
10	64.2 s				
11	27.1 d	2.24 br sep (6.7)	7, 12, 13	6, 7, 8, 12, 13	7, 8a, 8b, 12, 13
12	21.1 q	0.92 d (6.7)	11	7, 11, 13	7, 8a, 8b, 11, 13
13	24.9 q	1.06 d (6.7)	11	7, 11, 12	7, 11, 12
14	28.6 q	1.45 s		1, 9, 10	9a, 9b
15	24.2 q	1.51 s		3, 4	
16	133.6 s				
5-OH		2.47 d (7.7)	5		

[a] Data were recorded in $CDCl_3$ at 400 and 100 MHz for ^1H and ^{13}C NMR, respectively.
[b] The CH correlations were assigned by an HSQC spectrum.
[c] a and b denote the upfield and downfield resonances, respectively, of a geminal pair.

Table 2
^{13}C NMR data of axiplyns A–E (**1**–**5**)[a] and **6**

Position	1	2	3	4	5	6
1	49.1 d	47.1 d	47.6 d	55.9 d	48.8 d	42.0 d
2	18.4 t	18.6 t	20.0 t	27.3 t	25.5 t	23.9 t
3	33.6 t	34.2 t	31.5 t	51.7 d	42.6 d	27.4 t
4	106.0 s	108.1 s	77.7 s	214.0 s	104.0 s	137.0 s
5	95.9 d	99.2 d	126.5 d	72.5 d	72.4 d	118.3 d
6	86.6 s	81.5 s	142.9 s	81.8 s	86.9 s	35.8 d
7	50.5 d	43.9 d	47.6 d	49.5 d	48.0 d	48.4 s
8	22.3 t	23.5 t	22.3 t	18.8 t	19.0 t	22.8 t
9	41.4 t	39.9 t	39.9 t	39.6 t	39.3 t	42.5 t
10	64.2 s	63.8 s	66.5 s	64.7 s	66.6 s	61.2 s
11	27.1 d	79.7 s	27.0 d	24.8 d	24.8 d	28.8 d
12	21.1 q	23.8 q	17.7 q	18.0 q	19.3 q	20.5 q
13	24.9 q	29.4 q	22.1 q	24.2 q	24.2 q	21.6 q
14	28.6 q	28.5 q	25.8 q	29.6 q	29.3 q	28.8 q
15	24.2 q	23.3 q	25.6 q	32.3 q	24.7 q	24.4 q
16[b]	133.6 s	135.4 s	130.0 s	131.9 s	132.0 s	126.0 s

[a] Data were recorded in $CDCl_3$ at 100 MHz.
[b] Detection of the isothiocyanato carbon was usually by HMBC and not from the ^{13}C, due to the small amount of material available and long relaxation time.

by HRMS of the $[M+H-H_2O]$ peak and the ^{13}C-resonances to be $C_{16}H_{25}NOS$. A planar structure agreeing with axiplyn C was previously described by Alvi et al.;[5] however, the latter 10-isothiocyanato-5-amorphen-4-ol, isolated from the sponge *A. fenestratus*, exhibited different chemical shifts in both the ^{1}H and the ^{13}C spectra. The major changes were in the chemical shift of C-4, δ_C 77.7 ppm for **3**, in contrast to δ_C 69.0 s, and δ_H 5.60 ppm for the vinyl singlet H-5, as opposed to δ_H 6.24 s, and the existence of a highly shielded hydroxyl proton in the spectrum of **3** at 8.08 ppm. The considerably low field shift of the hydroxyl proton obligates the hydroxyl group to be close to the isothiocyanate group creating a hydrogen bond with the nitrogen, as seen in Figure 2. To enable the latter hydrogen bond, the decalin system has to possess a pseudo-cis conformation (the cyclohexene ring being a twisted boat) and the NCS and OH groups to be pseudo-axial, both on the β side of the molecule.[14]

The final material that was isolated from the sponge extract was a 1:1 mixture ($CDCl_3$, NMR) of two inseparable compounds (**4** and **5**). Both **4** and **5** share the same molecular ion peak at *m*/*z* 311 $[M]^+$ in the EIMS. Comprehensive 2D NMR analysis enabled the structure determination of both **4** and **5**. Analyzing the signals in both 1D and 2D NMR established those belonging to axiplyn D (**4**), disclosing the same substituted cyclohexane with the isopropyl and isothiocyanate moieties as in axiplyns A–C (**1**–**3**). The

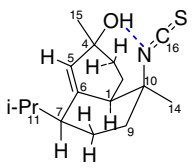


Fig. 2. Spatial structure of axiplyn C (**3**) (– – – hydrogen bond).

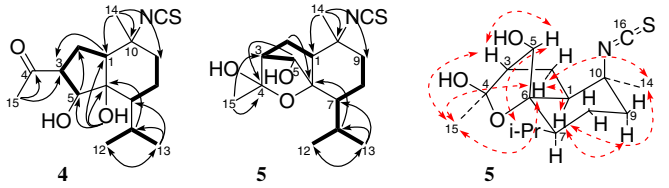
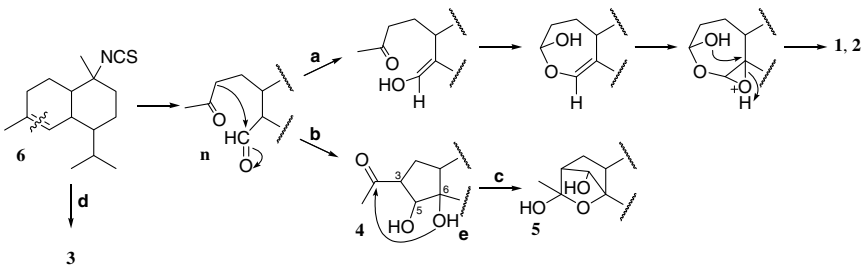


Fig. 3. Key COSY (▬) and HMBC (⌒) correlations of axiplyns D (**4**) and E (**5**) and key NOESY (⌒) correlations for axiplyn E (**5**).

changes were in the second half of the molecule, containing a methyl ketone (δ_C 214.0 s, 32.3 q, δ_H 2.28 s), a secondary hydroxyl [δ_C 72.5 d, δ_H 4.61 t and 3.27 d (CHOH)] and a quaternary hydroxyl carbon [δ_C 81.8 s, δ_H 3.55 s (OH)].[14] In view of the five degrees of unsaturation of **4**, the above functionalities (isothiocyanate and methyl ketone) implied a bicyclic structure. The structure determination of the second ring was achievable from the COSY and HMBC experiments as shown in Figure 3. Namely, axiplyn D (**4**) is composed of a substituted indane, where the cyclopentane ring carries two hydroxyls (on C-5 and C-6) and the methyl ketone on C-3. Subtracting the resonances of **4** from the mixture afforded the signals for axiplyn E (**5**). The major difference between **4** and **5** was the disappearance of the methyl ketone, which in **5** is changed into a hemiketal methyl group [δ_C 104.0 s, 86.9 s, 24.7 q, δ_H 1.40 s, 5.50 br s (OH)].[15] The tricyclic system of **5** was determined using COSY and HMBC experiments (Fig. 3).

The relative stereochemistry of axiplyn D (**4**) was determined by the analysis of the coupling constants of the protons on the carbon atoms carrying the functional groups and NOE cross-peaks. The starting point was the *trans* diequatorial isopropyl methylcyclohexane moiety, see above. An NOE between H-1 and 6-OH established the *cis* ring fusion, and thence the C-6 configuration. An NOE cross-peak between H-5, that has to be pseudo-axial (δ_H 4.61 t, $J_{3,5} = J_{5,OH} = 8.5$ Hz), and H-8β determined the α-configuration of the 5-OH group, and the $J_{3,5}$-value and NOE between H-5 and H-3 established the 3β-H, pseudo-axial configuration. Since axiplyn E (**5**) is in equilibrium with **4**, the relative stereochemistry of **4** also defined the stereochemistry of **5**, except for C-4. NOE measurements of the rigid axiplyn E (**5**) molecule confirmed its stereochemistry as shown in Figure 3. The isopropyl as well as the Me-14 (δ_C 29.3 ppm, vida supra) groups were again determined to be trans diequatorial orientated.[5] The configuration of the other four chiral centers was also confirmed by NOE correlations. Namely, an NOE between Me-14 and H-1 determined the latter axial configuration, an NOE between the isopropyl group and H-5 determined that the oxygen bridge of the hemiketal group was α-directed, thus establishing the configuration of C-6, and an NOE between Me-15 and H-3 directed this methyl to the α-side of the molecule.

Comparing the structures of **4** and **5** shows the close relationship between the two, that is, axiplyn **5** is the hemiketal of **4**, the 6-hydroxy group of **5** closes a six-membered



Scheme 1. Suggested biogenesis of the axiplyns. (a) Enolization of the aldehyde and closure of a hemiketal; (b) aldol condensation; (c) hemiketalization; (d) oxidation with allylic rearrangement; (e) the stage of hydroxylation on C-6 is unknown.

hemiketal with the methyl ketone of **4**. Indeed, a comparison between the proton-NMR of the same sample, in different solvents, exhibited changes in the ratio of **4** and **5**, that is, in $CDCl_3$ (1:1), C_6D_6 (2:1), and pyridine-d_5 (5:3) for **4** and **5**, respectively, demonstrating the equilibrium between the two and explaining the failure to separate the mixture.

A suggested biogenesis for **1**–**5**, shown in Scheme 1, starts with 10-isothiocyanato-4-amorphene (**6**). Oxidative cleavage of the C4–C5 double bond in **6** will give keto aldehyde **n**, which sequentially will result in two different routes (a and b) towards compounds **1**, **2**, **4**, and **5**. One route, (a) by cyclization of the enol–aldehyde–OH group with the ketone will afford hemiketals **1** and **2**, and the other, (b) consists of a ring contraction via an aldol condensation (C3–C5 bond formation) giving compounds **4** and **5**. Oxidation of **6**, involving allylic rearrangement (d), will result in compound **3**.

Axiplyns A–C (**1**–**3**) were tested for toxicity to brine shrimp larvae (*Artemia salina*), and were found to be active with the LD_{50} values of 1.6 μg/mL, 1.5 μg/mL, and 1.8 μg/mL, respectively.[16] Axiplyns embody two unprecedented ring systems namely a 7-hydroxy-6,8-dioxabicyclo-[3.2.1]octane (in **1** and **2**) and a 3,7-dihydroxy-2-oxabicyclo[2.2.1]heptane in **5**. In addition, axiplyns **4** and **5** contain a newly substituted indane skeleton sesquiterpene.

Supplementary data

General experimental procedures, ^1H and ^{13}C NMR spectra, and ^1H NMR data for axiplyns A–E (**1**–**5**). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.02.005.

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2. Sorek, H.; Rudi, A.; Aknin, M.; Gaydou, E.; Kashman, Y. *Tetrahedron Lett.* **2006**, *47*, 7237–7239.
3. The sponge *Axinyssa aplysinoides* was collected at Misali Island, southwest of Pemba Island, Tanzania (5° 13′ 60 S, 39° 36′ 0 E) (5th December, 2004). Due to the rich variety of marine life around the island, it has received official recognition and is now called Misali Island Marine Conservation Area. A voucher specimen has been deposited at the Zoological Museum, Tel Aviv University, Israel (ZMTAU PO 25472). The collection site is a steep reef, an almost vertical wall, at a depth of 16–28 m characterized by an extremely rich fauna of sponges that cover, in some sites, most of the reef surface. *A. aplysinoides* was collected from a sandy patch at a depth of 20 m.
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9. Axiplyn A (**1**): colorless oil, $[\alpha]_D^{23}$ +13 (*c* 0.15, CH_2Cl_2); ^1H and ^{13}C NMR data, see Table 1; IR (CH_2Cl_2) ν_{max} 3554, 2100, 1260, 1140, 950 cm^{-1}; EIMS *m/z* 311 $[M]^+$, HREIMS *m/z* 311.1555 (calcd for $C_{16}H_{25}NO_3S$, 311.1555).
10. Axiplyn B (**2**): colorless oil, $[\alpha]_D^{23}$ +31 (*c* 0.13, CH_2Cl_2); ^{13}C NMR data, see Table 2, ^1H NMR data, see Supplementary data; IR (CH_2Cl_2) ν_{max} 3480, 2105, 1260, 1138, 956 cm^{-1}; EIMS *m/z* 327 $[M]^+$, HREIMS *m/ z* 327.1505 (calcd for $C_{16}H_{25}NO_4S$, 327.1504).
11. Axiplyn C (**3**): colorless oil, $[\alpha]_D^{23}$ +40 (*c* 0.15, CH_2Cl_2); ^{13}C NMR data, see Table 2, ^1H NMR data, see Supplementary data; IR (CH_2Cl_2) ν_{max} 3561, 2103 cm^{-1}; CIMS *m/z* 262 $[M+H-H_2O]^+$, HRCIMS *m/z* 262.1554 (calcd for $C_{16}H_{24}NS$, 262.1551).
12. Axiplyns D and E (**4**, **5**): colorless oil, ^{13}C NMR data, see Table 2, ^1H NMR data, see Supplementary data; IR (CH_2Cl_2) ν_{max} 3420, 2860, 2100, 1625, 1205, 1120 cm^{-1}; EIMS *m/z* 311 $[M]^+$, HREIMS *m/z* 311.1557 (calcd for $C_{16}H_{25}NO_3S$, 311.1555).
13. Loss of water in the MS of **3** was indicated by the ^{13}C NMR spectrum requiring an oxygen atom in the molecule.
14. The β-side denotes the direction of the isopropyl group.
15. The hydroxyl protons were observed in the ^1H NMR spectrum in C_6D_6.
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