

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

Hagit Sorek<sup>a</sup>, Ayellet L. Zelikoff<sup>a</sup>, Yehuda Benayahu<sup>b</sup>, Yoel Kashman<sup>a,\*</sup>

<sup>a</sup> School of Chemistry, Tel-Aviv University, Ramat Aviv 69978, Israel

<sup>b</sup> Department of Zoology, Tel-Aviv University, Ramat Aviv 69978, Israel

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

Available online 7 February 2008

### Abstract

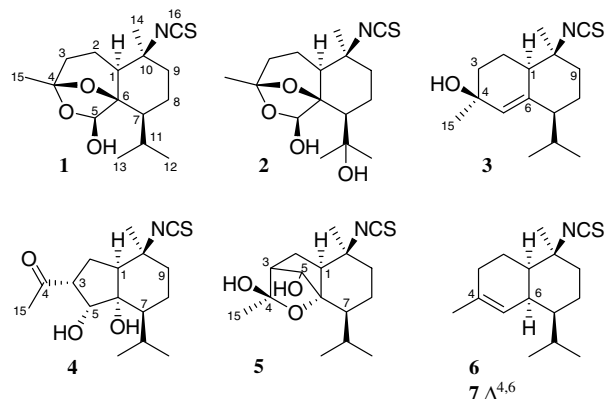
Five new isothiocyanate sesquiterpenes, designated axiplies A–E (1–5) have been isolated, together with two known isothiocyanate sesquiterpenes (6, 7), from the sponge *Axinyssa aplysinoides* collected at Misali Island, Tanzania. Axiplies 4 and 5 embody a new indane sesquiterpene skeleton, and compounds 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. Axiplies A, B, and C are potent brine shrimp toxins with LD<sub>50</sub> values between 1.5 and 1.8 µg/mL. © 2008 Elsevier Ltd. All rights reserved.

**Keywords:** Marine sponge; Sesquiterpenoid; Isothiocyanate; NMR

In a continuing discovery program for bioactive compounds from marine invertebrates,<sup>1,2</sup> 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.<sup>3</sup> 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.<sup>4–6</sup> Interestingly, many of these nitrogenous functional group-containing terpenes have been found to possess different biological activities.<sup>5,7,8</sup> 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<sub>2</sub>O (5:5:1). The extract (500 mg) was then subjected to solvent-partitioning, that is, aq MeOH against hexane and CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> fraction (186 mg) was chromatographed on Sephadex LH-20, eluted with hexane–MeOH–CHCl<sub>3</sub> (2:1:1) to afford

a complex cytotoxic mixture. Sequential VLC on silica gel led to the isolation of five new compounds, axiplies A–E (1–5, 0.04–0.017% dry weight)<sup>9–12</sup> 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*.<sup>5</sup> 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 axiplies.



\* Corresponding author. Tel.: +972 3 6408419; fax: +972 3 6409293.  
E-mail address: [kashman@post.tau.ac.il](mailto:kashman@post.tau.ac.il) (Y. Kashman).

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  $^{13}\text{C}$  NMR spectrum was consistent with the molecular formula of  $\text{C}_{16}\text{H}_{25}\text{NO}_3\text{S}$ , which was established by HREIMS ( $m/z$  311.1555, calcd for  $\text{C}_{16}\text{H}_{25}\text{NO}_3\text{S}$ , 311.1555), indicating five degrees of unsaturation. From the formula, the IR ( $2100\text{ cm}^{-1}$ ) and  $^{13}\text{C}$  NMR spectrum ( $\delta_{\text{C}}$  133.6 ppm) of the compound was suggested to be a sesquiterpene isothiocyanate. Examination of the  $^{13}\text{C}$  NMR revealed, in addition to the NCS group, three low field oxygen carrying carbons at  $\delta_{\text{C}}$  106.0 s (ketal), 95.9 d (hemiacetal), and 86.6 s (etheral 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-amor-

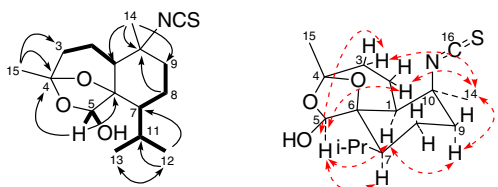


Fig. 1. Key COSY (—), HMBC (—), and NOESY (---) correlations of axiptyn A (**1**).

phene.<sup>5</sup> Accordingly, the isopropyl as well as the Me-14 ( $\delta_{\text{C}}$  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).<sup>5</sup> 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  $\beta$  positioning of the C4–C6 oxygen bridge, hence, establishing the configuration of C-4 (Table 2).

The spectral data of axiptyn 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  $\text{C}_{16}\text{H}_{25}\text{NO}_4\text{S}$ , 327.1504). The major differences in the NMR spectra of **2** in comparison to **1** appeared in the  $^{13}\text{C}$  NMR spectrum, which showed a singlet carbon resonating at  $\delta_{\text{C}}$  79.7 ppm, and a shift of the isopropyl methyl groups to  $\delta_{\text{C}}$  23.8 q and 29.4 ppm, suggesting the presence of an additional hydroxyl group. According to the  $^1\text{H}$  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 axiptyn C (**3**) exhibited a  $[M+H-H_2O]^+$  ion at  $m/z$  262.<sup>13</sup> The molecular formula was determined

Table 1  
NMR data of axiptyn A (**1**)<sup>a</sup>

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}^b$ ( $J$ in Hz)	COSY <sup>c</sup>	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 b 1.75 m	2, 3b 2, 3a	1, 2, 15	
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 b 1.80 m	7, 8b, 9a, 9b 7, 8a, 9a, 9b	6, 7, 10, 11	7, 9a, 9b, 11, 12
9	41.4 t	a 1.60 m b 2.11 dd (13.3, 4.1)	8a, 8b, 9b 8a, 8b, 9a	7, 10	1, 7, 8a, 8b, 14
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		

<sup>a</sup> Data were recorded in  $\text{CDCl}_3$  at 400 and 100 MHz for  $^1\text{H}$  and  $^{13}\text{C}$  NMR, respectively.

<sup>b</sup> The CH correlations were assigned by an HSQC spectrum.

<sup>c</sup> a and b denote the upfield and downfield resonances, respectively, of a geminal pair.

Table 2  
<sup>13</sup>C NMR data of axiptylins A–E (1–5)<sup>a</sup> 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 <sup>b</sup>	133.6 s	135.4 s	130.0 s	131.9 s	132.0 s	126.0 s

<sup>a</sup> Data were recorded in CDCl<sub>3</sub> at 100 MHz.

<sup>b</sup> Detection of the isothiocyanato carbon was usually by HMBC and not from the <sup>13</sup>C, due to the small amount of material available and long relaxation time.

by HRMS of the [M+H–H<sub>2</sub>O] peak and the <sup>13</sup>C-resonances to be C<sub>16</sub>H<sub>25</sub>NOS. A planar structure agreeing with axiptylin C was previously described by Alvi et al.;<sup>5</sup> however, the latter 10-isothiocyanato-5-amorphen-4-ol, isolated from the sponge *A. fenestratus*, exhibited different chemical shifts in both the <sup>1</sup>H and the <sup>13</sup>C spectra. The major changes were in the chemical shift of C-4, δ<sub>C</sub> 77.7 ppm for **3**, in contrast to δ<sub>C</sub> 69.0 s, and δ<sub>H</sub> 5.60 ppm for the vinyl singlet H-5, as opposed to δ<sub>H</sub> 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.<sup>14</sup>

The final material that was isolated from the sponge extract was a 1:1 mixture (CDCl<sub>3</sub>, NMR) of two inseparable compounds (**4** and **5**). Both **4** and **5** share the same molecular ion peak at *m/z* 311 [M]<sup>+</sup> 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 axiptylin D (**4**), disclosing the same substituted cyclohexane with the isopropyl and isothiocyanate moieties as in axiptylins A–C (1–3). The

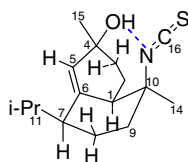


Fig. 2. Spatial structure of axiptylin C (**3**) (--- hydrogen bond).

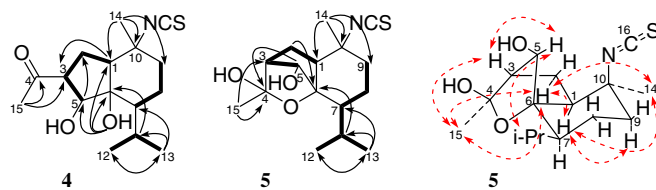
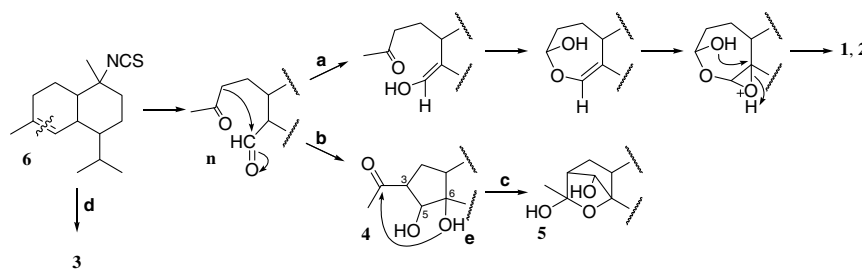


Fig. 3. Key COSY (—) and HMBC (↷) correlations of axiptylins D (**4**) and E (**5**) and key NOESY (---) correlations for axiptylin E (**5**).

changes were in the second half of the molecule, containing a methyl ketone (δ<sub>C</sub> 214.0 s, 32.3 q, δ<sub>H</sub> 2.28 s), a secondary hydroxyl [δ<sub>C</sub> 72.5 d, δ<sub>H</sub> 4.61 t and 3.27 d (CHOH)] and a quaternary hydroxyl carbon [δ<sub>C</sub> 81.8 s, δ<sub>H</sub> 3.55 s (OH)].<sup>14</sup> 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, axiptylin 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 axiptylin 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<sub>C</sub> 104.0 s, 86.9 s, 24.7 q, δ<sub>H</sub> 1.40 s, 5.50 br s (OH)].<sup>15</sup> The tricyclic system of **5** was determined using COSY and HMBC experiments (Fig. 3).

The relative stereochemistry of axiptylin 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<sub>H</sub> 4.61 t, *J*<sub>3,5</sub> = *J*<sub>5,OH</sub> = 8.5 Hz), and H-8β determined the α-configuration of the 5-OH group, and the *J*<sub>3,5</sub>-value and NOE between H-5 and H-3 established the 3β-H, pseudo-axial configuration. Since axiptylin 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 axiptylin E (**5**) molecule confirmed its stereochemistry as shown in Figure 3. The isopropyl as well as the Me-14 (δ<sub>C</sub> 29.3 ppm, *vide supra*) groups were again determined to be *trans* diequatorial orientated.<sup>5</sup> 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, axiptylin **5** is the hemiketal of **4**, the 6-hydroxy group of **5** closes a six-membered



Scheme 1. Suggested biogenesis of the axiptylins. (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  $\text{CDCl}_3$  (1:1),  $\text{C}_6\text{D}_6$  (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**.

Axiptylins A–C (**1–3**) were tested for toxicity to brine shrimp larvae (*Artemia salina*), and were found to be active with the  $\text{LD}_{50}$  values of 1.6  $\mu\text{g}/\text{mL}$ , 1.5  $\mu\text{g}/\text{mL}$ , and 1.8  $\mu\text{g}/\text{mL}$ , respectively.<sup>16</sup> Axiptylins 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, axiptylins **4** and **5** contain a newly substituted indane skeleton sesquiterpene.

### Supplementary data

General experimental procedures,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, and  $^1\text{H}$  NMR data for axiptylins 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.

### References and notes

- Sorek, H.; Rudi, A.; Benayahu, Y.; Kashman, Y. *Tetrahedron Lett.* **2007**, *48*, 7691–7694.
- Sorek, H.; Rudi, A.; Akin, M.; Gaydou, E.; Kashman, Y. *Tetrahedron Lett.* **2006**, *47*, 7237–7239.
- The sponge *Axinyssa aplysinoides* was collected at Misali Island, southwest of Pemba Island, Tanzania ( $5^\circ 13' 60\text{ S}$ ,  $39^\circ 36' 0\text{ E}$ ) (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.
- Marcus, A. H.; Molinski, T. F.; Fahy, E.; Faulkner, D. J.; Xu, C.; Clardy, J. *J. Org. Chem.* **1989**, *54*, 5184–5186.
- Alvi, K. A.; Tenenbaum, L.; Crews, P. *J. Nat. Prod.* **1991**, *54*, 71–78.
- He, H.; Salva, J.; Catalos, R. F.; Faulkner, D. J. *J. Org. Chem.* **1992**, *57*, 3191–3194.
- Tada, H.; Yasuda, F. *Chem. Pharm. Bull.* **1985**, *33*, 1941–1945.
- Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *52*, 2359–2368.
- Axiptylin A (**1**): colorless oil,  $[\alpha]_{\text{D}}^{23} +13$  ( $c$  0.15,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; IR ( $\text{CH}_2\text{Cl}_2$ )  $\nu_{\text{max}}$  3554, 2100, 1260, 1140, 950  $\text{cm}^{-1}$ ; EIMS  $m/z$  311  $[\text{M}]^+$ , HREIMS  $m/z$  311.1555 (calcd for  $\text{C}_{16}\text{H}_{25}\text{NO}_3\text{S}$ , 311.1555).
- Axiptylin B (**2**): colorless oil,  $[\alpha]_{\text{D}}^{23} +31$  ( $c$  0.13,  $\text{CH}_2\text{Cl}_2$ );  $^{13}\text{C}$  NMR data, see Table 2,  $^1\text{H}$  NMR data, see Supplementary data; IR ( $\text{CH}_2\text{Cl}_2$ )  $\nu_{\text{max}}$  3480, 2105, 1260, 1138, 956  $\text{cm}^{-1}$ ; EIMS  $m/z$  327  $[\text{M}]^+$ , HREIMS  $m/z$  327.1505 (calcd for  $\text{C}_{16}\text{H}_{25}\text{NO}_4\text{S}$ , 327.1504).
- Axiptylin C (**3**): colorless oil,  $[\alpha]_{\text{D}}^{23} +40$  ( $c$  0.15,  $\text{CH}_2\text{Cl}_2$ );  $^{13}\text{C}$  NMR data, see Table 2,  $^1\text{H}$  NMR data, see Supplementary data; IR ( $\text{CH}_2\text{Cl}_2$ )  $\nu_{\text{max}}$  3561, 2103  $\text{cm}^{-1}$ ; CIMS  $m/z$  262  $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$ , HRCIMS  $m/z$  262.1554 (calcd for  $\text{C}_{16}\text{H}_{24}\text{NS}$ , 262.1551).
- Axiptylins D and E (**4, 5**): colorless oil,  $^{13}\text{C}$  NMR data, see Table 2,  $^1\text{H}$  NMR data, see Supplementary data; IR ( $\text{CH}_2\text{Cl}_2$ )  $\nu_{\text{max}}$  3420, 2860, 2100, 1625, 1205, 1120  $\text{cm}^{-1}$ ; EIMS  $m/z$  311  $[\text{M}]^+$ , HREIMS  $m/z$  311.1557 (calcd for  $\text{C}_{16}\text{H}_{25}\text{NO}_3\text{S}$ , 311.1555).
- Loss of water in the MS of **3** was indicated by the  $^{13}\text{C}$  NMR spectrum requiring an oxygen atom in the molecule.
- The  $\beta$ -side denotes the direction of the isopropyl group.
- The hydroxyl protons were observed in the  $^1\text{H}$  NMR spectrum in  $\text{C}_6\text{D}_6$ .
- Meyer, B. N.; Ferrigni, J. E.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31–34.