(2*R*,3*R*,7*Z*)-2-Aminotetradec-7-ene-1,3-diol, a New Amino Alcohol from the Caribbean Sponge *Haliclona vansoesti*

C. Devijver,[†] M. Salmoun,[†] D. Daloze,[†] J. C. Braekman,*[,†] W. H. De Weerdt,[‡] M. J. De Kluijver,[‡] and R. Gomez[‡]

Laboratory of Bio-organic Chemistry, Department of Organic Chemistry, Faculty of Sciences, CP 160/07, University of Brussels, 50 Avenue F.D. Roosevelt, B-1050 Brussels, Belgium, and Institute for Systematics and Population Biology, Zoologisch Museum, University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands

Received February 14, 2000

The Caribbean marine sponge *Haliclona vansoesti* was found to contain a high amount (5% dry wt) of a new analogue of 4-sphingenine, (2*R*,3*R*,7*Z*)-2-aminotetradec-7-ene-1,3-diol (**1**). Its structure was determined by detailed spectroscopic analysis. The relative configuration was deduced from the NMR data of the corresponding acetonide **5**, while the absolute configuration was secured via protection of the primary alcohol and amino groups, and esterification of the secondary alcohol with Mosher's reagent.

In our screening for biologically active and significant metabolites from sponges, the CH_2Cl_2-soluble fraction of the MeOH extract of the Caribbean sponge *Haliclona vansoesti* n. sp. (Haposclerida, Chalinidae) was found to be toxic against nauplii of the brine shrimp *Artemia salina* (LD_{50} = 35 mg/L). In this paper we report the structure determination, including the absolute configuration, of the major compound [**1**, (2*R*,3*R*,7*Z*)-2-aminotetradec-7-ene-1,3-diol] of this toxic fraction. The amino alcohol **1** represents about 5% of the dry weight of the sponge and was isolated as a translucent lac after two successive chromatographies of the CH_2Cl_2 extract on Si gel.

HREIMS of compound **1** gave a molecular ion at *m*/*z* 243.2190, while CIMS showed a $(M + H)^+$ quasimolecular ion at *m*/*z* 244 as the major ion. This indicated the formula $C_{14}H_{29}NO_2$ (calcd 243.2198) for **1**. The HREIMS showed also fragment ions at *m*/*z* 226.2168 ($C_{14}H_{28}NO$, calcd 226.2171) and 212.2011 ($C_{13}H_{26}NO$, calcd 212.2014) corresponding to the loss, from the molecular ion, of HO and CH_3O, respectively. Moreover, a large IR band spreading out from 3300 to 3100 cm^{-1}, together with the formation of the triacetyl compound **2** ($\nu_{C=O}$ at 1742 and 1659 cm^{-1}; ^1H and ^{13}C NMR data in Table 1) indicated, the presence in **1** of one secondary and one primary hydroxyl group, and of one $CHNH_2$ group. From the ^1H and ^{13}C NMR data it could also be deduced that the remaining carbon atoms of **1** consisted of one terminal methyl group, one disubstituted double bond, and eight methylene groups. The two alcohols and the primary amine could be readily juxtaposed as represented in **1** based on the COSY data, while a cis configuration was assigned to the double bond based on the ^{13}C chemical shifts (28.0 and 27.6 for **1** and 27.9 and 27.4 for **2**) of the adjacent methylenes.[1] The position of the double bond was established by an HMBC experiment. Indeed, clear long-range correlations were observed for **1** and **2** between H-3 and C-4 and C-5, as well as between H-7 and C-5 and C-6. The location of the double bond was further confirmed by treating triacetate **2** with dimethyl disulfide and a catalytic amount of iodine.[2] The EIMS of the resulting derivative **3** showed two characteristic fragment ions at *m*/*z* 318 and 145 Da, compatible with the cleavage of the C_8-C_7 bond. In addition, the detailed analysis of the 1D and 2D NMR spectra ($^1H-^1H$ COSY, HMQC, HMBC) of compounds **1** and **2** led to the assignments reported in Table 1 and to the conclusion that the structure of the natural compound is (7*Z*)-2-aminotetradec-7-ene-1,3-diol.

Table 1. NMR Data of Compounds **1** and **2** ($CDCl_3$, 600 and 150.87 MHz, *J* in Hz)

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
(CH_3CONH)HC-2				5.61, br d, 10
(CH_3COO)H_2C-1			171.4	
(CH_3COO)HC-3			171.1	
(CH_3CONH)HC-2			170.6	
HC-8	131.3	5.34, m	131.5	5.36, m
HC-7	129.6	5.31, m	129.3	5.27, m
HC-3	69.9	3.75, m	72.9	5.07, m
H_2C-1	60.8	3.88; 3.73, m	64.0	4.03, m
HC-2	58.7	3.25, m	50.8	4.39, m
H_2C-12	32.5	1.25, m	32.4	1.25, m
H_2C-4	34.0	1.48; 1.52, m	31.5	1.56; 1.60, m
H_2C-10	30.4	1.30, m	30.3	1.32, m
H_2C-11	29.7	1.25, m	29.7	1.25, m
H_2C-9	28.0	2.00, m	27.9	1.97, m
H_2C-6	27.6	2.02, m	27.4	2.02, m
H_2C-5	26.1	1.53, m	25.9	1.35, m
(CH_3CONH)HC-2			23.9	2.00, s
H_2C-13	23.3	1.25, m	23.3	1.25, m
(CH_3COO)HC-3			21.6	2.07, s
(CH_3COO)HC-1			21.4	2.04, s
H_3C-14	14.8	0.87, t, 7	14.8	0.87, t, 7

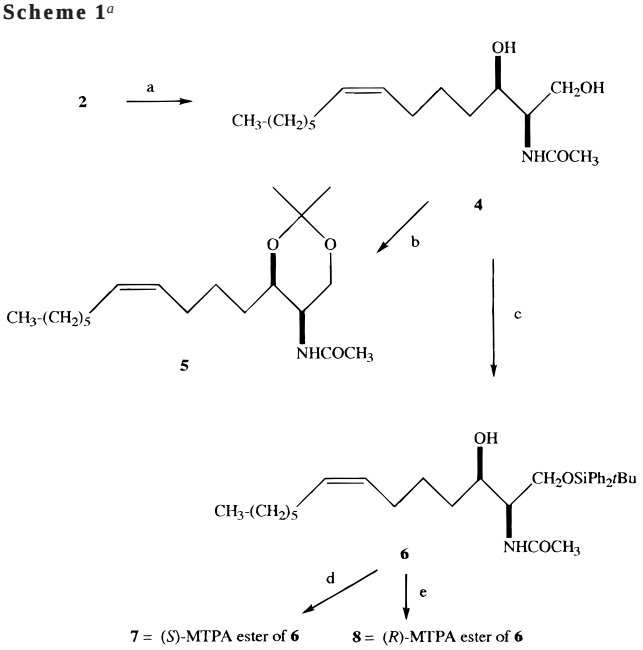
The relative configuration of **1** was deduced from the ^1H NMR spectrum of acetonide **5** prepared from diol **4** as described in Scheme 1. Indeed, the $^1H-^1H$ COSY spectrum of **5** permitted the signals of H_2-1, H-2, H-3, and NH (Table 2) to be readily assigned. Moreover, the measured coupling constants were only compatible with the preferred conformation **9** for acetonide **5**, corresponding to a *threo* configuration for **1**. On the contrary, the equivalent coupling constants reported by Mancini et al.[3] for the preferred conformation **10**, corresponding to the *erythro* configuration of an analogue of compound **1**, are very different. The *threo* configuration for **1** was further confirmed by NOE difference experiments on **5**. Irradiation of H_{eq}-1 (δ 3.71) produced an enhancement to H_{ax}-1 and H-2, while irradiation of H_{ax}-1 (δ 4.05) produced an enhancement to H_{eq}-1, H-2, and H-3.

The absolute configuration of compound **1** was determined by preparing both Mosher's esters[4,5] **7** and **8** of the silyl ether **6** derived from diol **4** (Scheme 1). Positive $\Delta\delta$

* To whom correspondence should be addressed. Tel.: 32.02.6502961. Fax: 32.02.6502798. E mail: braekman@ulb.ac.be.
[†] University of Brussels.
[‡] University of Amsterdam.

10.1021/np000081c CCC: $19.00
Published on Web 06/28/2000

Scheme 1[a]



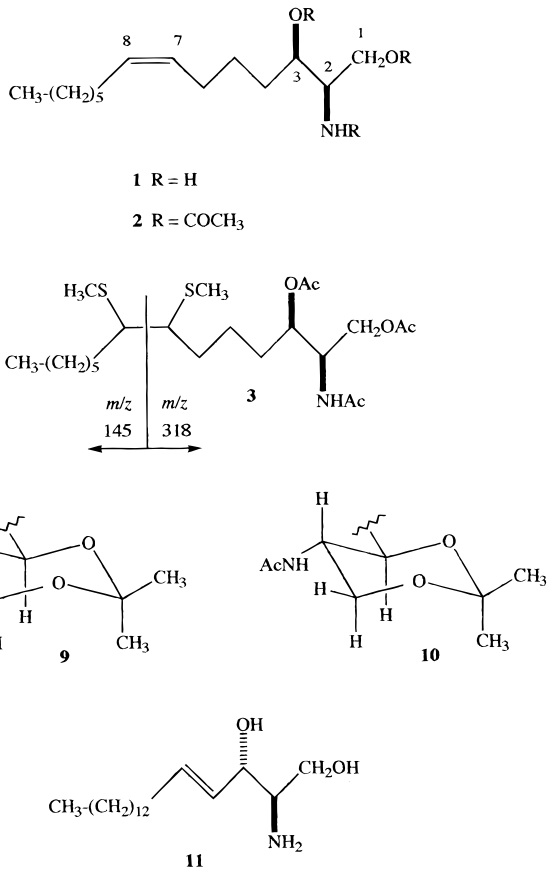
[a] (a) MeOH, Na_2CO_3, 1 h, room temperature; (b) acetone, $CuSO_4$, 8 h, reflux; (c) *tert*-butylchlorodiphenylsilane, pyridine, 24 h, room temperature; (d) (*R*)-MTPACl, pyridine, CCl_4, 24 h, room temperature; (e) (*S*)-MTPACl, pyridine, CCl_4, 24 h, room temperature.

Table 2. Partial ^{1}H NMR Data of **5** ($CDCl_3$, 600 MHz)

position	δ_H	multiplicity	*J*
H_{eq}-1	3.71	dd	12.0, 1.8
H_{ax}-1	4.05	dd	12.0, 2.0
H-2	3.86	dddd	9.5, 1.8, 2.0, 2.0
H-3	3.93	m	
NH	6.15	br d	9.5

($\delta S - \delta R$) were observed for some methylenes of the hydrocarbon chain, while significant negative values were observed for H_2C-1 and NH, indicating the (*R*)-configuration at C-3. Because the *threo* configuration was established between C-2 and C-3, the absolute configuration of **1** is thus 2*R*,3*R*. Compound **1** was found to be toxic against nauplii of the brine shrimp *A. salina* (LD_{50} = 9 mg/L) and can thus be considered as responsible, at least in part, of the toxicity of the sponge.

Structurally, compound **1** is related to the sphingosine derivatives, such as 4-sphingenine (**11**), sphinganine, and phytosphingosine, long known as central structural elements of the sphingolipids, which are important constituents of the lipid portion of the cell membranes in all groups of organisms. More recently, several sphingosine derivatives have been isolated from marine organisms as secondary metabolites. Examples of these metabolites are the crucigasterins from the tunicate *Pseudodistoma crucigaster*,[6] the coriacenins from the sponge *Clathrina coriacea*,[7] and the epimeric aliphatic amino alcohols, (2*S*,3*S*)- and (2*S*,3*R*)-2-aminotetradeca-5,7-dien-3-ol, from the sponge *Xestospongia* sp.[8] By analogy with the biosynthesis of 4-sphingenin (**11**),[9] we may postulate that the formation of (2*R*,3*R*,7*Z*)-2-aminotetradec-7-ene-1,3-diol (**1**) requires a C_{12} fatty acid and L-serine. But, as compounds **1** and **11** differ by their configuration at C-3, we must postulate that the reduction of the intermediary ketones proceeds along diastereomeric pathways.



Experimental Section

General Experimental Procedures. HREIMS were performed on a Micromass Autospec 3F instrument. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 and 150.87 MHz, respectively, using a Varian Unity 600 instrument. Some ^{1}H NMR spectra were recorded at 250 MHz on a Bruker WM 250 spectrometer using TMS as internal standard. The IR spectra were obtained on a Bruker IFS 25 instrument as a film on a NaCl disk. The optical rotations were measured on a Perkin-Elmer 141 polarimeter (Na-vapor lamp) in a 10-cm cell at room temperature. Thin-layer chromatography (TLC) analyses were performed on 0.25-mm Polygram Si gel SILG/UV_{254} precoated plates (Macherey Nagel) and column chromatographies over Si gel (MN Kieselgel 0.04−0.063 mm), using the flash technique.

Animal Material. The sponge consists of thick cushions, with a loose, cavernous structure with large, circular to elliptical oscula on slightly raised elevations, with raised, transparent rims. The surface is smooth, and the consistency crisp and fragile. Characteristically, the choanosome is white, and the ectosome light purple and semitransparent. The ectosomal skeleton is a delicate, tangential, subisotropic reticulation, loosely lying on the choanosomal skeleton, which is also a subisotropic reticulation of a denser structure than the ectosome, but with many subectosomal and choanosomal spaces. The spicula are slightly curved, hastate oxeas, 120−220 × 3.5−10.5 μm. The specimens were collected in May 1998, off Curaçao, on reef-slope localities, growing on dead corals at 37−52 m depth. This material was assigned to a new species, *Haliclona vansoesti*, recently described by De Weerdt et al.[10] The species belongs to the subgenus *Halichoclona* of the genus *Haliclona* (class Demospongiae, order Haploscleridae, family Chalinidae). It occurs in the Caribbean, where it was found in coral-reef environments in Curaçao, St. Vincent, Martinique, and Jamaica at 2−52 m depth. The material is housed in the Zoological Museum of the University of Amsterdam (ZMA), catalogued as ZMA POR. 13391−95, voucher numbers 98/CU/JUN04/MK/124, 125, 144, 181, and 191.

Extraction, Isolation, and Spectral Properties. Specimens of *H. vansoesti* (38 g dry wt) stored in MeOH were exhaustively extracted with MeOH. The MeOH extract was evaporated in vacuo and the residue (23 g) partitioned between H_2O and CH_2Cl_2. The organic phase was evaporated to dryness

in vacuo to obtain a gum (5.5 g). Part of this gum (0.44 g) was flash chromatographed over a Si gel column using as eluent the mixture CH_2Cl_2/MeOH (98:2 to 70:30). The fraction containing the major compound (visualized by TLC by spraying with Dragendorff's reagent) was further flash chromatographed over a Si gel column using as eluent the mixture hexane/acetone/NH_4OH (80:20:0.5). This led to (2*R*,3*R*,7*Z*)-2-aminotetradec-7-ene-1,3-diol (**1**; 160 mg; 5.26% dry wt) as a translucent lac homogeneous in TLC: $[\alpha]^{20}_{589}$ 19.7° (*c* 0.46, $CHCl_3$); IR (film) broad band between 3300 and 3100 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HMBC correlations [δ_H (δ_C)] 5.34 (30.4, 28.0, 27.6), 5.31 (28.0, 27.6, 26.1), 3.88/3.73 (69.9, 58.7), 3.75 (58.7, 34.0, 26.1), 3.25 (69.9, 60.8), 2.02 (131.3, 129.6, 34.0, 26.1), 2.00 (131.3, 129.6, 30.4), 0.87 (32.5, 23.3); HREIMS *m*/*z* 244.2275 (4, $[M+H]^+$, calcd for $C_{14}H_{30}NO_2$, 244.2276), 243.2190 (1, M^+, calcd for $C_{14}H_{29}NO_2$, 243.2198), 226.2168 (2, calcd for $C_{14}H_{28}NO$, 226.2171), 212.2011 (17, calcd for $C_{13}H_{26}NO$, 212.2014), 60 (100); CIMS *m*/*z* 244 $[M+H]^+$.

Acetylation of 1. Compound **1** (20 mg) was treated at room temperature during 24 h with a 1:1 mixture of Ac_2O and pyridine (1 mL). After addition of water (2 mL) and evaporation to dryness in vacuo, the solid residue was purified by flash chromatography over a Si gel column using the mixture hexane/acetone (8:2), affording triacetate **2** (15 mg): amorphous solid homogeneous in TLC and GC (OV1 capillary column, T = 220°); IR (film) 3295, 1742, 1659, 1369 and 1235 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HMBC correlations [δ_H (δ_C)] 5.61 (170.6, 50.8), 5.36 (30.3, 27.9), 5.27 (25.9, 27.4), 5.07 (171.1, 64.0, 31.5, 25.9), 4.39 (170.6, 72.9, 64.0, 31.5), 4.03 (171.4, 72.9, 50.8), 2.07 (171.1, 72.9), 2.04 (171.4, 64.0), 2.02 (131.5, 129.3, 31.5, 25.9), 2.00 (170.6, 50.8), 1.97 (131.5, 129.3, 30.3), 1.56 (72.9, 50.8, 27.4, 25.9), 1.35 (72.9, 31.5, 27.4), 1.32 (29.7), 0.87 (32.4, 23.3); EIMS *m*/*z* 369 (21, M^+), 309 (17), 224 (30), 144 (40), 112 (46), 102 (100).

Determination of Double-Bond Position. A hexane solution (350 μL) of triacetate **2** (0.3 mg) was treated with 500 μL of DMDS and ethereal iodine (3 mg iodine in 200 μL Et_2O) in a sealed microreactor vial. The reaction mixture was kept at room temperature for 20 h and then diluted with hexane (500 μL). Iodine was removed by shaking with 10^{-1} M $Na_2S_2O_3$. The organic phase was evaporated to dryness, yielding the DMDS adduct **3** that was dissolved in hexane (250 μL) and analyzed by GC−MS: EIMS *m*/*z* 463 (2, M^+), 416 (4), 391 (3), 368 (3), 318 (7), 258 (18), 165 (17), 145 (27), 102 (54), 84 (57), 81 (54), 69 (93), 55 (100).

Selective Hydrolysis of 2. To a solution of triacetate **2** (60 mg) in absolute MeOH (1 mL) was added anhydrous Na_2CO_3 (20 mg). The mixture was stirred at room temperature during 1 h. Then, the solution was filtered, the solvent evaporated, and the solid residue chromatographed over Si gel (eluent, CH_2Cl_2 with increasing amount of MeOH) to give diol **4** (38 mg; yield 82%): amorphous solid; IR (film) 1652 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) 6.35 (1H, d, *J* = 8 Hz), 5.34 (2H, m), 3.90 (2H, m), 3.78 (2H, m), 2.04 (3H, s), 2.00 (4H, m), 1.60−1.20 (12H, m), 0.88 (3H, t, *J* = 7 Hz); EIMS *m*/*z* 285 (6, M^+), 254 (12), 236 (13), 212 (10), 194 (13), 102 (27), 85 (100).

Acetonide 5. To the monoacetate **4** (13 mg) dissolved in anhydrous acetone was added anhydrous $CuSO_4$ (25 mg). The solution was refluxed for 8 h, filtered, and evaporated to dryness. The resulting solid residue was chromatographed over florisil (eluent, hexane with increasing amount of acetone) to give acetonide **5** (7 mg, yield 47%): amorphous solid; ^1H NMR ($CDCl_3$, 600 MHz) 6.15 (1H, br d, *J* = 9.5 Hz), 5.32 (2H, m), 4.05 (1H, dd, *J* = 12, 2 Hz), 3.93 (1H, m), 3.86 (1H, dddd, *J* = 9.5, 1.8, 2, 2 Hz), 3.71 (1H, dd, *J* = 12, 1.8 Hz), 2.03 (3H, s), 2.00 (4H, m), 1.46 (3H, s), 1.41 (3H, s), 0.87 (3H, t, *J* = 7 Hz); EIMS *m*/*z* 325 (1, M^+), 310 (14), 267 (21), 143 (6), 130 (10), 85 (100).

Selective Protection of the Primary Alcohol of 4. To a cold (0 °C) solution of **4** (41 mg) in dry pyridine (1 mL) was syringed dropwise *tert*-butylchlorodiphenylsilane (50 μL, 1.3 equiv), and the mixture was stirred for 24 h at room temperature under a nitrogen atmosphere. The solution was evaporated to dryness and then diluted with ether (15 mL). The ether solution was washed successively with saturated $KHSO_4$, water, and brine and dried over Na_2SO_4. After filtration and evaporation of the solvent, the solid residue was chromatographed over Si gel (eluent, CH_2Cl_2 with increasing amount of MeOH) to give compound **6** (33 mg, yield 44%): amorphous solid; IR (film) 3340, 1649 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) 7.7−7.3 (10H, m), 6.03 (1H, d, *J* = 8 Hz), 5.33 (2H, m), 4.00 (1H, m), 3.86 (3H, m), 2.03 (4H, m), 1.94 (3H, s), 1.07 (9H, s), 0.88 (3H, t, *J* = 7 Hz); EIMS *m*/*z* 523 (1, M^+), 480 (8), 466 (79), 454 (12), 448 (14), 388 (27), 284 (27), 240 (19), 199 (100), 180 (19), 135 (31).

Preparation of the Mosher's Esters of 6. Batches of (*R*)-MTPACl and (*S*)-MTPACl were prepared starting from the corresponding commercial acids (*S*)-MTPA (50 mg) and (*R*)-MTPA (50 mg), respectively, using the procedure described previously.[5] To a solution of (*R*)-MTPACl in freshly distilled pyridine (300 μL) and dry CCl_4 (300 μL) was added a solution of **6** (16 mg) in the same mixture of solvent (300 μL). The resulting solution was stirred at room temperature under a nitrogen atmosphere for 24 h. After addition of water (5 mL), the aqueous solution was extracted with CH_2Cl_2 (3 × 5 mL), the organic phase was evaporated to dryness, and the solid residue was chromatographed over Si gel (eluent, hexane with increasing amount of EtOAc) to give the (*S*)-MTPA ester **7** (8 mg, yield 53%). The corresponding (*R*)-MTPA ester **8** was prepared following the same procedure but starting from (*S*)-MTPACl.

Compound 7: ^1H NMR ($CDCl_3$, 250 MHz) 7.7−7.3 (10H, m), 5.52 (H-3, m), 5.36/5.29 (H-7 and H-8, m), 5.27 (NH, d, *J* = 8 Hz), 4.27 (H-2, m), 3.54 (H-1_a, dd, *J* = 10, 5 Hz), 3.40 (H-1_b, dd, *J* = 10, 7 Hz), 3.47 (OCH_3, s), 1.80 ($COCH_3$, s), 1.06 (9H, s), 0.87 (3H, t, *J* = 7 Hz); EIMS *m*/*z* 729 (1,M^+), 696 (7), 682 (67), 670 (6), 448 (100), 240 (26), 199 (60), 189 (39), 135 (26).

Compound 8: ^1H NMR ($CDCl_3$, 250 MHz) 7.7−7.3 (10H, m), 5.47 (H-3, m), 5.46 (NH, d, *J* = 8 Hz), 5.34/5.23 (H-7 and H-8, m), 4.27 (H-2, m), 3.66 (H-1_a, dd, *J* = 10, 5 Hz), 3.53 (H-1_b, dd, *J* = 10, 7 Hz), 3.46 (OCH_3, s), 1.79 ($COCH_3$, s), 1.08 (9H, s), 0.88 (3H, t, *J* = 7 Hz); EIMS identical to that of **7**.

Acknowledgment. This research was supported by the European Community (Project MAS3-CT97-0144) and the National Fund for Scientific Research of the French Community of Belgium. We thank Dr. M. Luhmer for the NMR spectra and Mr. C. Moulard for the mass spectra.

References and Notes

(1) Barabas, A.; Botar, B. A.; Gocan, A.; Popovici, N.; Hodosan, F. *Tetrahedron* **1978**, *34*, 2191−2194.
(2) Leclercq, S.; Braekman, J. C.; Kaisin, M.; Daloze, D.; Detrain, C.; de Biseau, J. C.; Pasteels, J. M. *J. Nat. Prod.* **1997**, *60*, 1143−1147, and cited references.
(3) Mancini, I.; Guella, G.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1994**, *77*, 51−58.
(4) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(5) Leclercq, S.; Thirionet, I.; Broeders, F.; Daloze, D.; Vander Meer, R.; Braekman, J. C. *Tetrahedron* **1994**, *50*, 8465−8478.
(6) Jares-Erijman, E. A.; Bapat, C. P.; Lithgow-Bertelloni, A.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 5732−5737.
(7) Casapullo, A.; Fontana, A.; Cimino, G. *J. Org. Chem.* **1996**, *61*, 7415−7419.
(8) Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 366−369.
(9) Luckner, M. *Secondary Metabolism in Microorganisms, Plants, and Animals*; Springer-Verlag: Berlin, 1990, p 152.
(10) De Weerdt, W. H.; De Kluijver, M. J.; Gomez, R. *Beaufortia* **1999**, *49*, 47−54.

NP000081C