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New Communesin Derivatives from the Fungus *Penicillium* sp. Derived from the Mediterranean Sponge *Axinella verrucosa*

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The ethyl acetate extract of *Penicillium* sp., derived from the Mediterranean sponge *Axinella verrucosa*, yielded the known compound communesin B (**1**) and its new congeners communesins C (**2**) and D (**3**), as well as the known compounds griseofulvin, dechlorogriseofulvin, and oxaline. All structures were unambiguously established by 1D and 2D NMR and MS data. In several bioassays performed on different leukemia cell lines, the communesins exhibited moderate antiproliferative activity.

In contrast to research on terrestrial fungi, which started 60 years ago with the discovery of penicillin, that on marine fungi is a relatively new field. Microorganisms, in general, have provided mankind with more than 100 of the most important antibiotics currently utilized in medicine. Extensive studies on terrestrial fungi have been decreasing as the discovery rate of novel metabolites decreases.[1] Recently, many natural products chemists have shifted their attention to marine fungi, as the oceans offer a broad and diverse population of unique and previously unrecognized microorganisms. In our search for new natural products from sponge-derived fungi, the known metabolite communesin B (**1**)[2] (Figure 1) and its new derivatives communesins C (**2**) and D (**3**) were isolated from a strain of *Penicillium* sp. derived from the sponge *Axinella verrucosa*, together with known *Penicillium* metabolites griseofulvin,[3] dechlorogriseofulvin,[4] and oxaline.[5] The communesins were first isolated from a strain of *Penicillium* sp. associated with the marine alga *Enteromorpha intestinalis*.[2] Since the discovery of penicillin, the genus *Penicillium* has yielded to date 660 compounds of diverse chemical structures exhibiting various types of biological activities.[6]

Communesin B (**1**) was isolated as a white amorphous powder. Its structure was established on the bases of EIMS, ^{1}H, ^{13}C, COSY, HMBC, HMQC, and ROESY NMR data. The ^{1}H and ^{13}C NMR data were identical to those previously reported in the literature.[2]

Communesin C (**2**), which was isolated as a white amorphous powder, gave the pseudomolecular ion peak at *m*/*z* 495 $[M + H]^+$ in (+)-ESIMS, which is 14 amu less than that of **1**. HREIMS data established the molecular formula as $C_{31}H_{34}N_4O_2$. Moreover, the mass spectra showed a strong ion peak at *m*/*z* 424, also corresponding to $[M + H - 71]^+$, due to the loss of the dimethyl epoxide moiety, as with **1**. The ^{1}H NMR spectrum of **2** was comparable with that of **1** (Table 1). The only difference was the absence of the *N*-methyl group which was observed at δ 2.78 in **1**. Slight changes in the chemical shifts of H-6 and of the aromatic H-14 signals to lower fields were also exhibited in **2** due to the loss of the shielding effect of the *N*-methyl group. The 2D ^{1}H−^{1}H COSY spectrum showed that all the spin systems found in **1** were retained in **2**. It was therefore clear that the new derivative **2** is the *N*-demethyl derivative of **1**.

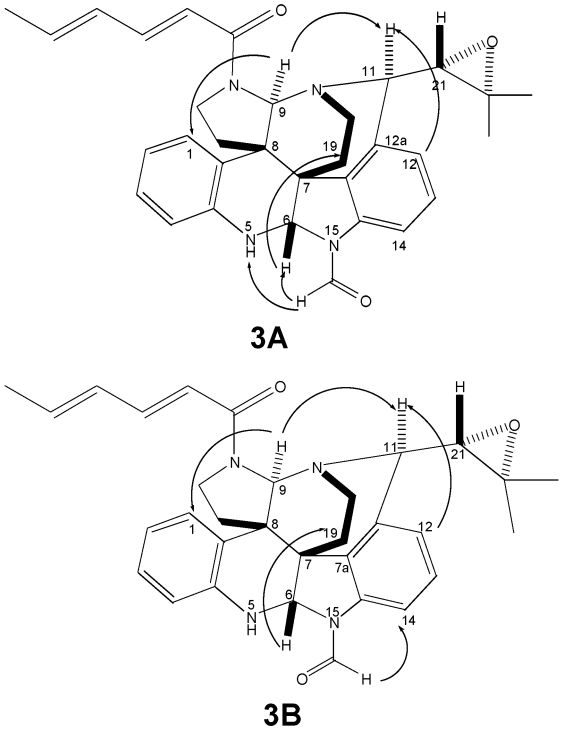

Figure 1. Important NOEs found in the ROESY spectra of **3A** and **3B**.

Communesin D (**3**) was isolated as a white amorphous powder. Its (+)-ESIMS spectra showed a base peak at *m*/*z* 523 $[M + H]^+$, which is 28 amu larger than **2**. Its molecular formula was established as $C_{32}H_{34}N_4O_3$ through HREIMS. The fragmentation pattern of the pseudomolecular ion at *m*/*z* 523 by MS/MS generated the daughter ion peak at *m*/*z* 495 $[M + H - 28]^+$, which could be ascribed to the loss of

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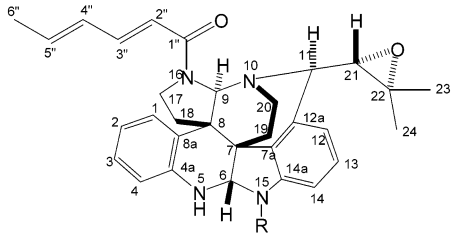
Table 1. NMR Data of the Compounds **1**−**3** Measured at 500 MHz in DMSO-d_6

	communesin B (**1**)			communesin C (**2**)		communesin D (**3A**)				communesin D (**3B**)			
atom no.	C_δ[a]	H_δ	mult., *J* (Hz)	H_δ	mult., *J* (Hz)	C_δ[b]	H_δ	mult., *J* (Hz)	HMBC (δ_H to δ_C)	C_δ[b]	H_δ	mult., *J* (Hz)	HMBC (δ_H to δ_C)
1	122.9 d	6.61	m	6.57	bm	120.0 d	6.68	m		120.0 d	6.68	m	
2	119.7 d	6.61	m	6.57	bm	120.1 d	6.68	m	C-4	120.1 d	6.68	m	C-4
3	127.4 d	6.94	m	6.90	m	128.7 d	6.96	m	C-4	128.7 d	6.96	m	C-4
4	117.1 d	6.79	bd 8.2	6.79	bd 7.7	120.0 d	6.84	bd 8.0	C-2, C-3, C-8a	120.0 d	6.81	bd 8.0	C-2, C-8a
4a	145.3 s					143.0 s				143.0 s			
5		4.75	s	5.99	s		6.72	s	C-7, C-8a		6.09	s	C-7, C-8a
6	83.4 d	4.65	s	4.83	s	73.5 d	5.60	s	C-4a, C-8	76.0 d	5.50	s	C-4a, C-14a, C-19
7	52.6 s					51.1 s				50.9 s			
7a	133.6 s					136.0 s				139.6 s			
8	53.4 s					53.9 s				53.6 s			
8a	133.4 s					131.1 s				131.8 s			
9	80.5 d	5.14	s	5.12	s	79.9 d	5.28	s	C-8a, C-20	89.9 d	5.28	s	C-8a, C-20
11	66.8 d	4.20	d 9.5	4.18	d 9.2	65.0 d	4.40	d 9.5	C-11, C-20	65.0 d	4.41	d 9.0	C-11, C-20
12	113.3 d	6.09	d 7.6	6.10	d 7.7		6.64	d 7.6	C-7a, C-11, C-13, C-14		6.64	d 7.7	C-7a, C-11, C-13, C-14
12a	137.5 s					138.9 s				138.9 s			
13	129.9 d	6.82	dd 8.2, 7.6	6.74	dd 8.2, 7.7	135.5 d	6.69	dd 8.2, 7.6	C-12, C-12a	132.0 d	6.69	dd 8.2, 7.6	C-12, C-12a
14	102.8 d	5.91	d 8.2	6.03	d 8.2	112.9 d	7.60	d 8.2	C-7a,	107.5 d	7.09	d 8.2	C-7a, C-14a
15				5.82	s (N*H*)								
15a	29.9 q	2.78	s				8.70	s (C*H*O)	C-6, C-14a		9.05	s (C*H*O)	C-6
14a	152.3 s					141.0 s				140.9 s			
17A	45.4 t	3.72	dt 11.4, 8.2	3.73	m		3.78	m			3.78	m	
17B		2.73	m	2.78	m		2.82	m			2.82	m	
18A	31.5 t	2.73	m	2.78	m		2.82	m			2.82	m	
18B		1.74	m	1.78	m		1.80	m	C-9		1.80	m	C-9
19A	38.9 t	2.31	dd 12.6, 8.8	2.27	m	37.6 t	2.49	m		37.6 t	2.48	m	
19B		2.12	dt 12.6, 9.5	2.09	m		2.23	bm			2.23	bm	
20A	37.1 t	3.30	dd 15.8, 9.5	*c*		36.0 t	*c*		C-11	36.0 t	HDO		C-11
20B		3.20	ddd 15.8, 9.5, 8.8	*c*			*c*		C-11		HDO		C-11
21	65.6 d	2.90	d 9.5	2.88	d 9.2	63.4 d	2.92	d 9.5	C-11	63.4 d	2.92	d 9.0	C-11
22	61.6 s					60.0 s		s		60.0 s			
23	25.0 q	1.56	s	1.55	s	25.0 q	1.62	s	C-21, C-22, C-24	25.0 q	1.62	s	C-21, C-22, C-24
24	20.8 q	1.32	s	1.32	s	20.8 q	1.32	s	C-21, C-22, C-23	20.8 q	1.32	s	C-21, C-22, C-23
1″	170.4 s					167.2 s				167.2 s			
2″	122.5 d	6.48	d 15.2	6.53	d 15.1	122.1 d	6.55	d 15.5	C-4″	122.1 d	6.55	d 15.5	C-4″
3″	142.1 d	7.06	dd 15.2, 9.5	7.07	dd 15.1, 10.1		7.10	dd 15.5, 10.4	C-2″		7.10	dd 15.5, 10.4	C-2″
4″	130.9 d	6.14	dd 14.5, 9.5	6.16	m	131.1 d	6.18	m		131.1 d	6.18	m	
5″	137.7 d	6.14	dq 14.5, 5.7	6.16	m	136.9 d	6.18	m	C-6″	136.9 d	6.18	m	C-6″
6″	18.8 q	1.82	d 5.7	1.81	d 5.4	19.0 q	1.84	d 5.1	C-4″, C-5″	19.0 q	1.84	d 5.1	C-4″, C-5″

[a] Carbon NMR spectra were taken in MeOD. [b] Carbon assignments were determined indirectly from the HMBC, which did not allow a complete assignment. [c] Signals underneath water peak.



R = CH_3 communesin B (**1**)
R = H communesin C (**2**)
R = CHO communesin D (**3**)

a carbonyl group. The EIMS spectra of **3** also showed an ion peak at *m/z* 451 corresponding to $[M + H - 71]^{+\bullet}$ as in **1**, which again signified the loss of a dimethyl epoxide moiety. This fragmentation pattern is characteristic for these compounds. The ^{1}H NMR spectrum of **3** was also very similar to both **1** and **2**. As in **2**, the most prominent difference from **1** was the loss of the N-CH_3 signal. Furthermore an additional proton signal at δ 8.70 was observed in **3**, which corresponded to a formyl proton attached at N-15. The possible substitution could occur only at N-15 as a significant change in resonance was observed for H-6 and the aromatic protons H-12 and H-14 of the indoline moiety. The ^{1}H signal of H-6 was shifted ca. 1 ppm to low field at δ 5.55 when compared with that of **1** due to the deshielding effect of the formamide function in **3**. The doublet signals for the aromatic protons H-12 and H-14 from the indoline ring were also shifted to lower field, due to the resonance-enhanced electron-withdrawing effect of the formaldehyde substituent at N-15. The COSY spectrum showed that all the spin systems found in **1** and **2** were retained in **3**, as there were no changes in the resonance patterns of the respective spin systems. The HMBC spectra confirmed the attachment of the formyl substituent at N-15, as correlations were observed between the formyl proton with both C-14a and C-6, while N*H*-5 gave cross-peaks with C-7 and C-8a (Table 1 and Figure 2). It was therefore unambiguously established that **3** is the formamide derivative of communesin C.

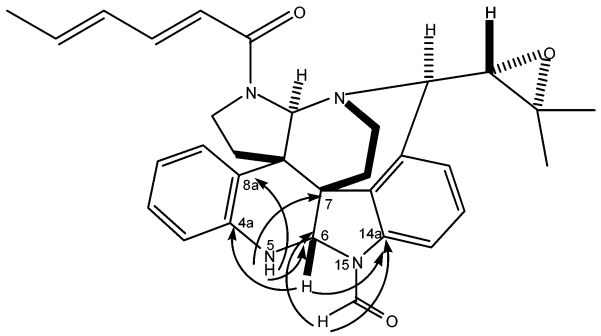


Figure 2. Important long-range correlations found in the HMBC spectra of **3**.

An interesting feature in the NMR spectra of **3** in DMSO-d_6 showed the presence of two stereoisomers of this compound, which differed in the orientation of the *N*-formyl function. DMSO-d_6 allowed the slow interconversion of the two isomers, which caused the appearance of two signals for each of the formyl protons as well as those of N*H*-5, H-6, and H-14. They resonated at different chemical shifts due to the deshielding effect of the formyl carbonyl. The co-occurrence of the two isomers was confirmed from the 2D ROESY spectrum of **3**, which showed correlations between C*H*O and H-6 for isomer **3A**, while C*H*O correlated with H-14 for isomer **3B**. The relative stereochemistry of the chiral centers (C-6, C-7, C-8, C-9, and C-11) of **3A** and **3B** was identical with **1**, as deduced from the ROESY data of **3** shown in Figure 2. Important NOEs were observed between H-1 and H-9, H-9 and H-11, H-6 and H-19A, and H-12 and H-11. The stereochemistry at C-21 was identical with that of communesin B, following Murata's *J*-based configurational analysis,[7] and protons at C-21 and C-11 were *anti*, as indicated by their coupling constant of 9.0 Hz. NOE between the geminal methyl groups of the epoxide system and H-12 showed that the epoxide was oriented *syn* to N-10. These data were consistent with the already published data on nomofungin, a closely related alkaloid isolated from an endophytic fungus of *Ficus microcarpa* L.[8] The absolute stereochemistry of nomofungin was assigned using the exciton chirality method parallel to *J*-based configurational analysis. The reported NMR data of nomofungin was essentially identical with those of communesin B.[2,8a] Recently, through a synthetic approach, it was confirmed that the structure of nomofungin is identical with that of communesin B.[9] Nomofungin was reported to disrupt microfilaments in mammalian cells.[8]

Since the known communesins A and B were described to inhibit proliferation of mouse leukemic cells P-388,[2] we investigated whether the isolated communesins **1**−**3** would also be growth inhibitory to other tumor cell lines. They were tested on a series of human leukemia cell lines by the MTT assay and through radioactive thymidine incorporation. All three compounds exhibited a moderate antiproliferative activity (Table 2). Communesins B (**1**) and D (**3**) were found to be most active toward the human acute B cell lymphoblastic leukemia SUP-B15 with ED_{50} values of 7.2 and 9.0 μg/mL, respectively. Conversely, communesin C was observed to be most active on the human acute T lymphoblastic leukemia MOLT-3 with an ED_{50} of 8.6 μg/mL. Furthermore, all three communesin derivatives were active against brine shrimp, *Artemia salina*, with LC_{50} values of 0.30 and 0.57 μg/mL for compounds **1** and **3**, respectively, and 1.96 μg/mL for compound **2**.

After a decade since the isolation of the first communesins from an algal-derived *Penicillium* species,[2] we were able to find new derivatives from the same fungal genus derived from a sponge. This again exemplifies the chemical diversity of fungal metabolites occurring in the marine environment.

Table 2. Antiproliferative Activity of the Communesin Congeners on Different Leukemia Cell Lines after 48 h through MTT Assay

	ED_{50} (μg/mL)		
cell line[a]	communesin B (**1**)	communesin C (**2**)	communesin D (**3**)
U-937	10.4	11.3	13.1
THP-1	11.4	13.1	16.2
NAMALWA	9.9	8.2	14.6
L-428	>20	inactive	inactive
MOLT-3	8.1	8.6	9.9
SUP−B15	7.2	10.8	9.0

[a] NAMALWA, Burkitt's lymphoma; THP-1, monocytic leukemia; U-937, histiocytic leukemia; L-428, Hodgkin's lymphoma; MOLT-3, T-cell leukemia; SUP-B15, B-cell leukemia.

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a Perkin-Elmer model 341 LC polarimeter. 1D and 2D NMR spectra (chemical shifts in ppm) were recorded on Bruker AMX 300, ARX 400, or DRX 500 NMR spectrometers using standard Bruker software. Mass spectra (ESIMS) were recorded on a ThermoFinnigan LCQ Deca ion trap mass spectrometer. High-resolution EIMS were recorded on a Finnigan MAT 8430 and on an Intectra AMD 402. For HPLC analysis, samples were injected into a HPLC system equipped with a photodiode-array detector (Gynkotek, München, Germany). Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with Eurospher 100-C_{18}, 5 μm (Knauer, Berlin, Germany). Separation was achieved by applying a linear gradient from 100% H_2O (with 0.2% TFA) to 100% MeOH over 40 min. Compounds were purified by semipreparative HPLC, which was conducted on a Merck Hitachi LaChrome L-7100 pump and a Merck Hitachi LaChrome L-7400 UV detector. Chromatograms were recorded on a Merck Hitachi D-2000 Chromato-Integrator. Separation columns (300 × 8 mm, i.d.) were prefilled with Eurospher 100-C_{18}, 7 μm (Knauer, Berlin, Germany). TLC was performed on precoated TLC plates with Si gel 60 F_{254} and Si gel RP-18 F_{254} (Merck, Darmstadt, Germany). Compounds were detected by UV absorbance or by fluorescence at 254 and 366 nm. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements.

Isolation and Cultivation of the Fungi. The fungus *Penicillium* sp. was isolated from the sponge *Axinella verrucosa* collected during an expedition in the Mediterranean Sea near the island of Elba, Italy. The taxonomic identification of the sponge was carried out by Dr. R. Van Soest, and a voucher specimen (ZMA.POR 15866) was deposited at the Department of Coelenterates and Porifera, Zoologisch Museum, University of Amsterdam.

The fungus was grown in a 1 L culture medium consisting of yeast extract (3 g/L), malt extract (3 g/L), peptone (5 g/L), glucose (10 g/L), and sea salt (24.4 g/L).The pH of the medium was adjusted to 7.2−7.4 using 0.1 N NaOH or HCl prior to inoculation. After 21 days of incubation at room temperature, 300 mL of EtOAc was added to the culture broth and stored in polyethylene flasks at −80 °C until extraction. A voucher strain (no. E00-12/3) was deposited at the Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven. The taxonomic identification of the strain was effected by the standard method of Pitt,[10] as described before.[11]

Isolation of the Secondary Metabolites. The culture broth and mycelium were extracted exhaustively with EtOAc, and the resulting total dried extract (440.7 mg) was then partitioned between MeOH and cyclohexane. The MeOH extract was chromatographed over Sephadex using 100% MeOH as eluent. From five resulting fractions, fractions 2 and

3 were further separated over a silica column with CH_2Cl_2/MeOH (95:5) and yielded the communesin congeners **1** (12.54 mg), **2** (1.0 mg), and **3** (1.0 mg) and the known compound oxaline (2.35 mg). Fractions 4 and 5 yielded griseofulvin (33 mg) and dechlorogriseofulvin (1.0 mg) after purification through preparative reversed-phase HPLC.

Communesin B (1): white amorphous powder; $[\alpha]_D$ −58° (*c* 0.10, MeOH); UV λ_{max} (MeOH) nm 205, 271; ^{1}H NMR and ^{13}C NMR data, see Table 1; (+)ESIMS *m*/*z* 509.3 $[M + H]^+$; MS/MS *m*/*z* 437.5 $[(M - ((CH_3)_2C{-}O{-}CH)) + H]^+$; EIMS (70 eV) *m*/*z* $[M]^+$ 508 (45), 437 (15), 254 (65), 198 (25), 149 (35), 95 (82), 92 (64), 71 (80), 58 (85), 45 (100), 32 (65).

Communesin C (2): white amorphous powder; $[\alpha]_D$ −30° (*c* 0.038, MeOH); UV λ_{max} (MeOH) nm 206, 271; ^{1}H NMR data, see Table 1; (+)ESIMS *m*/*z* 495.3 $[M + H]^+$; MS/MS *m*/*z* 423.5 $[(M - ((CH_3)_2C{-}O{-}CH) + H]^+$; EIMS (70 eV) *m*/*z* $[M]^+$ 494 (20), 423 (20), 368(40), 313 (48), 254 (52), 236 (60), 95 (96), 83 (100), 71(88); HREIMS *m*/*z* 494.2682 (calcd for $C_{31}H_{34}N_4O_2$, 494.2682).

Communesin D (3): white amorphous powder; $[\alpha]_D$ +23.3° (*c* 0.039, MeOH); UV λ_{max} (MeOH) nm 206, 267; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; (+)ESIMS *m*/*z* 523.3 $[M + H]^+$; MS/MS *m*/*z* 495.4 $[(M - CO) + H]^+$, MS^n *m*/*z* 423.5 $[(495.4 - ((CH_3)_2C{-}O{-}CH)) + H]^+$; EIMS (70 eV) *m*/*z* $[M]^+$ 522 (45), 451 (5), 400 (8), 254(68), 199 (28), 149 (30), 95 (79), 92 (54), 71 (70), 58 (87), 45 (100), 32 (62); HREIMS *m*/*z* 522.2621 (calcd for $C_{32}H_{34}N_4O_3$, 522.2631).

Evaluation of Biological Activity. Antiproliferative activity was examined using several human leukemia cell lines as described earlier.[12] Activity against brine shrimp, *Artemia salina*, was determined as previously outlined.[13]

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References and Notes

(1) Biabani, M. A. F.; Laatsch, H. *J. Prakt. Chem./Chem.-Ztg.* **1998**, *340*, 589.
(2) Numata, A.; Takahashi, C.; Ito, Y.; Takada, T.; Kawai, K.; Usami, Y.; Matsumura, E.; Imachi, M.; Ito, T.; Hasegawa, T. *Tetrahedron Lett.* **1993**, *34*, 2355−2358.
(3) (a) Grove, J. F. *J. Chem. Soc.* **1952**, 3949−3967. (b) MacMillan, J. *J. Chem. Soc.* **1953**, 1697.
(4) (a) MacMillan, J. *Chem. Ind.* **1951**, 719. (b) McMaster, W. J. *J. Chem. Soc.* **1960**, 4628−46231.
(5) Nagel, D. W.; Pachler, K. G. R.; Steyn, P. S.; Vleggaar, R.; Wessels, P. L. *Tetrahedron* **1976**, *32*, 2625−2631.
(6) *AntiBase 2002*, a database for rapid structure identification of microbial metabolites; Wiley-VCH Verlag Berlin GmbH, Chemical Concepts Division: Weinheim, 2002.
(7) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.
(8) (a) Ratnayake, A. S.; Yoshida, W. Y.; Mooberr, S. L.; Hemscheidt, T. K. *J. Org. Chem.* **2001**, *66*, 8717−8721. (b) Ratnayake, A. S.; Yoshida, W. Y.; Mooberr, S. L.; Hemscheidt, T. K. *J. Org. Chem.* **2003**, *68*, 1640.
(9) (a) May, J. A.; Zeidan, R. K.; Stoltz, B. M. *Tetrahedron Lett.* **2003**, *44*, 1203−1205. (b) Crawley, S. L.; Funk, R. L. *Org. Lett.* **2003**, *5*, 3169−3171.
(10) Pitt, J. I. *A Laboratory Guide to Common Penicillium Species*, 2nd ed.; CSIRO, Division of Food Processing: North Ryde, Australia, 1988.
(11) Edrada, R. A.; Heubes, M.; Brauers, G.; Wray, V.; Berg, A.; Gräfe, U.; Wohlfarth, M.; Muehlbacher, J.; Schaumann, K.; Sudarsono; Bringmann, G.; Proksch, P. *J. Nat. Prod.* **2002**, *65*, 1598−1604.
(12) Bohnenstengel, F. I.; Steube, K. G.; Meyer, C.; Nugroho, B. W.; Hung, P. D.; Kiet, L. C.; Proksch, P. *Z. Naturforsch.* **1999**, *54c*, 55−60.
(13) Balbin-Oliveros, M.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; van Soest, R. W. M. *J. Nat. Prod.* **1998**, *61*, 948−952.

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