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New Metabolites from Sponge-Derived Fungi *Curvularia lunata* and *Cladosporium herbarum*^{||}

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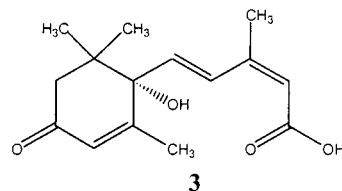
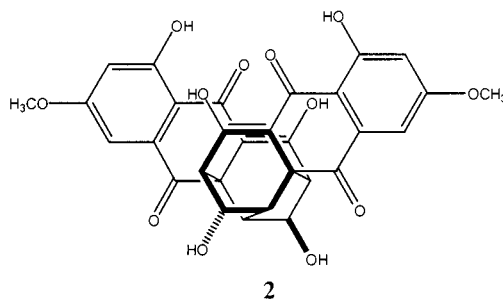
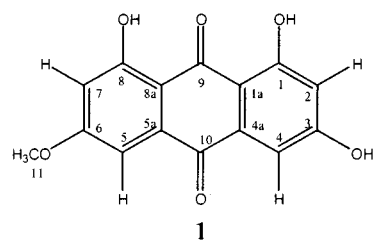
The fungus *Curvularia lunata*, isolated from the marine sponge *Niphates olemda*, yielded the new 1,3,8-trihydroxy-6-methoxyanthraquinone, which we named lunatin (**1**), the known modified bisanthraquinone cytoskyrin A (**2**), and the known plant hormone (+)-abscisic acid (**3**). Both anthraquinones were found to be active against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Two strains of the fungus *Cladosporium herbarum*, isolated from the sponges *Aplysina aerophoba* and *Callyspongia aerizusa*, respectively, yielded two new α -pyrones, herbarin A (**4**) and herbarin B (**5**), the known compound citreoviridin A (**6**), and the new phthalide herbaric acid (**7**). All structures were unambiguously established by 1D and 2D NMR and MS data.

Marine microorganisms such as bacteria, fungi, and blue-green algae have proven to be a rich source of new biologically active secondary metabolites.^{1–3} In our search for new natural products from sponge-associated fungi, a new anthraquinone (**1**) was isolated from *Curvularia lunata*, whereas two new α -pyrones (**4**, **5**) and a new phthalide (**7**) were obtained from two strains of *Cladosporium herbarum* isolated from the sponges *Aplysina aerophoba* and *Callyspongia aerizusa*, respectively.

Fungal cultures of *C. lunata* were grown from a tissue sample of the marine sponge *Niphates olemda*, which was collected in Indonesia. The EtOAc extract from the culture broth and mycelia of the fungus afforded the new anthraquinone lunatin (**1**), the known bisanthraquinone cytoskyrin A⁴ (**2**), and the known plant hormone (+)-abscisic acid⁵ (**3**).

Compound **1** was obtained as an orange-colored amorphous powder. Its molecular formula was determined by HREIMS to be C₁₅H₁₀O₆. Its structure was established through 1D (¹H, ¹³C, and DEPT) and 2D (COSY and HMBC) NMR (Table 1). The ¹H NMR spectrum showed two phenolic protons at δ 12.31 and 12.22, two pairs of *meta*-coupled protons at δ 7.14 and 6.82 ($^4J = 2.5$ Hz) and at δ 7.09 and 6.56 ($^4J = 2.3$ Hz), and a methoxyl group at δ 3.91. The ¹³C NMR and DEPT spectra revealed 15 carbon atoms: two quinone carbonyls, twelve aromatic carbons, and one methoxyl. Of the aromatic carbons, four were unsubstituted, and of the substituted carbons, four were oxygen-bound. The proposed structure of **1** and the assignment of NMR signals were corroborated by the HMBC spectrum. Furthermore, the position of the methoxyl group was confirmed by an NOE experiment which showed selective enhancement of the signals for H-5 and H-7 after irradiation at δ 3.91 (H-11 signal).

A lichen metabolite with a proposed structure identical to that of **1** was recently reported.⁶ However, important



differences in chemical shift data were observed such as the ¹H NMR data for H-4, the coupling constant between H-2 and H-4, and the ¹³C NMR data for C-1a, C-2, and C-4. In addition, a disparity in the chemical solution behavior of the two compounds, in which **1** was insoluble in CHCl₃, which was the solvent used for the measurement of the NMR spectra of the lichen metabolite, also proved that the two compounds are not identical. The lichen metabolite could thus possibly be an isomeric compound, although this aspect needs further clarification.

Compound **2**, which was isolated as a yellow amorphous powder, was identified on the basis of IR, HREIMS, [α]_D, ¹H, ¹³C, HMBC, and HMQC NMR data. This known cytotoxic bisanthraquinone was originally reported from

^{||} Dedicated to Dr. Ludger Witte, who tragically passed away last year.

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Table 1. NMR Data for Lunatin (**1**) in DMSO-*d*₆ at 600 MHz

position	¹³ C	¹ H (m, <i>J</i> in Hz)	HMBC correlations
1	164.4 (s)		
1a	108.3 (s)		
2	108.1 (d)	6.56 (d, 2.3)	C1, C1a, C3
3	165.9 (s)		
4	109.3 (d)	7.09 (d, 2.3)	C1a, C3, C10
4a	134.6 (s)		
5	107.4 (d)	7.14 (d, 2.5)	C7, C8a, C10
5a	134.8 (s)		
6	165.5 (s)		
7	106.6 (d)	6.82 (d, 2.5)	C5, C6, C8, C8a
8	164.1 (s)		
8a	109.6 (s)		
9	188.4 (s)		
10	181.1 (s)		
11	56.2 (q)	3.91 (s)	C6
C3-OH			
C8-OH		12.31 (s)	
C1-OH		12.22 (s)	

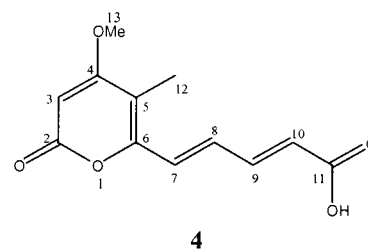
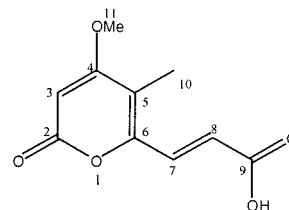
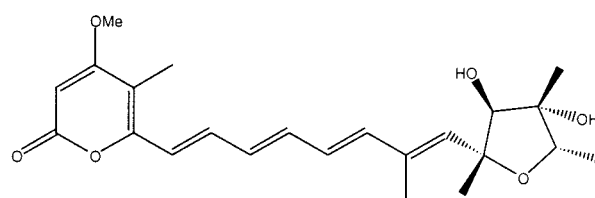
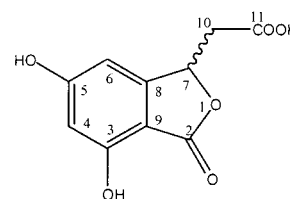
the endophytic fungus *Cytospora* sp. isolated from the plant *Conocarpus erecta*.⁴

Both anthraquinones **1** and **2** were found to be active against *S. aureus*, *E. coli*, and *B. subtilis* but had no activity against *C. albicans* (Table 4).

Extensive studies on the chemistry and biosynthesis of anthraquinonoids have clarified that modified bisanthraquinones are derived from the dimerization of monomeric anthraquinones, followed by partial hydrogenation.^{7,8} Our report on the co-isolation of the monomeric anthraquinone **1** and the modified bisanthraquinone **2** provides additional proof to this claim.

Two strains of *C. herbarum* were isolated from the sponges *A. aerophoba* collected in the Mediterranean Sea and *C. aerizusa* collected in Indonesia. The fungal strain associated with *A. aerophoba* yielded two new α -pyrones, which we name herbarins A (**4**) and B (**5**), and the known compound citreoviridin A⁹ (**6**).

The structures of **4** and **5** were elucidated through comparison of their NMR data with those of **6**, since all compounds were found to be structurally related, as initially suggested by the similarity in their UV spectra. Compound **4** has the molecular formula C₁₂H₁₂O₅, as determined by HRESIMS. When compared with that of **6**, the ¹³C NMR spectrum of **4** (see Table 2) showed an additional carboxylic carbon, only eight olefinic carbons, one methoxyl and one methyl carbon. The tetrahydrofuran moiety present in **6** was found to be absent in the structure of **4**. The DEPT spectrum of **4** revealed that five of the olefinic carbons are unsubstituted, and of the three substituted carbons, two are connected to oxygens. The presence of an α -pyrone ring was confirmed by HMBC, as were the attachments of the methoxyl, methyl, and unsaturated

**4****5****6****7**

side chain to C-4, C-5, and C-6, respectively, and the connectivity of the carboxylic acid moiety in the diene side chain (Table 2). The coupling constants between H-7 and H-8 (³*J* = 15.0 Hz) and between H-9 and H-10 (³*J* = 15.2 Hz) indicated an all-*trans* configuration for the diene moiety, as in **6**.

Compound **5** was obtained as a white amorphous powder having the formula C₁₀H₁₀O₅, as determined by HREIMS. The ¹³C and ¹H NMR spectra of **5** were similar to those of **4** (see Table 2). The signals corresponding to the α -pyrone nucleus and the methyl and the methoxyl groups were also found in **5**. The difference in the spectra of both compounds lies only in the absence of two olefinic carbons and two olefinic protons, as concluded from the ¹³C and ¹H NMR

Table 2. NMR Data for Herbarins A and B (**4** and **5**) in MeOD

position	herbarin A (600 MHz)			herbarin B (300 MHz)	
	¹³ C	¹ H (m, <i>J</i> in Hz)	HMBC correlations	¹³ C	¹ H (m, <i>J</i> in Hz)
2	172.6 (s)			172.1 (s)	
3	90.6 (d)	5.74 (s)	C2, C5	91.9 (d)	5.81 (s)
4	165.9 (s)			165.1 (s)	
5	112.9 (s)			110.5 (s)	
6	154.3 (s)			152.6 (s)	
7	126.7 (d)	7.03 (d, 15.0)	C6, C8, C9	125.3 (s)	7.50 (d, 15.4)
8	133.4 (d)	7.22 (dd, 15.0, 11.4)	C6, C7, C9	132.3 (d)	6.64 (d, 15.4)
9	144.3 (d)	7.50 (dd, 11.4, 15.2)	C8, C10, C11	169.0 (s)	
10	127.9 (d)	6.20 (d, 15.2)	C8, C11	9.3 (q)	2.13 (s)
11	169.7 (s)			57.5 (q)	3.97 (s)
12	9.1 (q)	2.09 (s)	C4, C5, C6		
13	57.4 (q)	3.95 (s)	C4		

Table 3. NMR Data for Herbaric Acid (7) in DMSO- d_6 at 600 MHz

position	^{13}C	^1H (m, J in Hz)	HMBC correlations
2	171.4 (s)		
3	167.1 (s)		
4	103.9 (d)	6.30 (d, 1.6)	C2, C3, C5, C9
5	159.7 (s)		
6	101.7 (d)	6.42 (dd, 1.6, 0.9)	C3, C4, C7, C10
7	78.2 (d)	5.68 (br dd, 7.9, 5.2)	C8, C10, C11
8	154.8 (s)		
9	104.5 (s)		
10 A	40.4 (t)	2.91 (dd, 5.2, 16.5)	C7, C8, C11
10 B		2.73 (dd, 7.9, 16.5)	C7, C8, C11
11	173.0 (s)		

Table 4. Zones of Inhibition (in mm) in Agar-Plate Diffusion Assay

compound (μg)	<i>S. aureus</i>	<i>E. coli</i>	<i>HBI-101</i>	<i>B. subtilis</i>	<i>C. albicans</i>
lunatin (1)					
5	8.5	9.0	8.0	7.5	n.a. ^a
10	10.0	11.0	10.5	9.0	n.a.
cytoskyrin A (2)					
5	8.5	9.0	8.0	8.0	n.a.
10	10.0	11.0	9.0	12.0	n.a.

^a Not active.

data of **5**, indicating the shortening of the olefinic side chain attached to C-6 of the α -pyrone ring. The coupling constant of 15.4 Hz between H-7 and H-8 revealed the *trans* configuration of these two protons.

Compounds **4** and **5** showed activity against *Artemia salina*. Compound **4** gave mortality rates of 85% and 75% at 100 μg and 50 μg dose levels, respectively, while compound **5** gave 80% and 65% mortality rates at 100 μg and 50 μg dose levels, respectively. Compound **6** did not show any activity toward *A. salina* but showed growth inhibitory activity in the insecticidal assay with *Spodoptera littoralis* (7% and 33% growth rate for concentrations of 250 and 100 ppm, respectively). Agar diffusion assay showed that none of the α -pyrone congeners (**4**, **5**, and **6**) exhibited significant antibiotic activity.

The second strain of *C. herbarum* isolated from the Indonesian sponge *C. aerizusa* had been previously reported to yield two new macrolides, pandangolides 3 and 4, the known compounds pandangolide 2, cladospolide B, and iso-cladospolide B, and the furan carboxylic acid derivatives: sumiki's acid plus its new acetyl congener, acetyl sumiki's acid. In this paper, we report on the isolation of a further new compound from the same fungal extract, for which we propose the name herbaric acid (**7**).

Compound **7** was obtained as a brown, amorphous powder. The molecular formula was deduced as $\text{C}_{10}\text{H}_7\text{O}_6$ by HREIMS. Its structure was established through 1D (^1H and ^{13}C) and 2D (COSY, HMQC, and HMBC) NMR (Table 3). The ^1H NMR and COSY spectra showed two *meta*-coupled aromatic protons at δ 6.42 and 6.30 ($^4J = 1.6$ Hz) and a methylene at δ 2.91 and 2.73 with a geminal coupling constant of 16.5 Hz, which further coupled to an aliphatic proton at δ 5.68 ($^3J = 5.2$ and $^3J = 7.9$ Hz, respectively). The ^{13}C NMR showed 10 carbon atoms: two carboxyl carbons (173.0 and 171.4); six aromatic carbons, two of which are oxygen-substituted; an oxygen-substituted tertiary carbon; and one methylene group. The proposed structure of herbaric acid and the assignment of NMR signals were both confirmed by HMQC and HMBC. An apparent long-range coupling between H-6 and H-7 ($^4J = 0.9$ Hz) was also confirmed through a homonuclear spin decoupling experiment, where irradiation at δ 5.68 (ex-

pected H-7 signal) gave an NOE effect on H-6. EIMS spectra provided additional proof of the proposed structure, with strong ion peaks at m/z 178 and 165 corresponding to the loss of $[\text{HCOOH}]^+$ and $[\text{CH}_2\text{COOH}]^+$, respectively.

Compound **7** is the 5-hydroxyl derivative of the known toxin iso-ochracinic acid previously isolated from *Alternaria kikuchiana*, a parasite responsible for the black spot disease on Japanese pears.¹¹ It is also related to acetophthalidin, a cytotoxic metabolite produced by *Aspergillus fumigatus*, isolated from a sea sediment, which has an acetyl group at the C-7 position.¹² When tested against *A. salina* and human leukemia cell line HL-60 (data not shown), **7**, however, showed no activity.

It is interesting to note that the two strains of *C. herbarum* isolated from two different sponges differ completely with regard to the secondary compounds produced. Whereas the strain isolated from *A. aerophoba* yielded mainly pyrone derivatives (**4**–**6**), the fungal strain isolated from the sponge *C. aerizusa* produced mainly macrocyclic lactones.¹⁰ Since both fungal strains were cultivated under the same conditions, the chemical differences found are likely to be caused by different fungal genotypes.

Experimental Section

General Experimental Procedures. ^1H NMR and ^{13}C NMR spectra (chemical shifts in ppm) were recorded at 300 K on Bruker DPX 300, ARX 400, and AVANCE DMX 600 NMR spectrometers. Mass spectra were measured on Finnigan MAT 8430, Finnigan MAT TSQ-7000, and Finnigan MAT Inco 50 mass spectrometers. Specific rotations were measured on a Perkin-Elmer Model 341 LC polarimeter. For HPLC analysis, samples were injected into an HPLC system coupled to a photodiode-array detector (Gynkotek, Munich, Germany). Routine detection was at 254 nm in aqueous MeOH. The separation column (125 \times 4 mm, i.d.) was prefilled with Eurospher C₁₈ (Knauer, Berlin, Germany).

Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on TLC plates precoated with Si 60 F₂₅₄ (Merck, Darmstadt, Germany). The compounds were detected by their UV absorbance at 254 nm and by spraying the TLC plates with anisaldehyde reagent.

Isolation and Cultivation of Fungi. All sponge samples were collected by scuba diving. *N. olemda* (De Laubenfels, 1954) and *C. aerizusa* (Desqueyroux-Faúndez, 1984) were collected in the Bali Bata National Park in Indonesia, and voucher specimens from these sponges are kept under the registration numbers ZMA POR. 13438 and ZMA POR. 13437, respectively, in the Zoological Museum, Amsterdam, The Netherlands. *A. aerophoba* (Riedl, 1983) was collected in the Mediterranean Sea close to Banyuls-sur-Mer (France) as previously described.¹³ Under sterile conditions, a piece of tissue from the inner part of the freshly collected sponges was cut and inoculated on malt agar slants consisting of 15 g/L agar and 24.4 g/L artificial sea salt mixture. The inoculated agar slants were incubated at 27 $^\circ\text{C}$, and from these, pure fungal cultures were isolated after repeated inoculation on fresh malt agar plates. The isolated fungi were identified as *C. lunata* and *C. herbarum* by the Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands. Mass cultivation of the fungi (20 L) was carried out in Erlenmeyer flasks in malt extract broth consisting of 1.5% malt extract in distilled water supplemented with 2.44% of SERA artificial sea salt mixture at pH 7.6–7.8. After incubation at room temperature without shaking for a period of 32 days for *C. lunata*, or 41 and 35 days for *C. herbarum* isolated from *C. aerizusa* and *A. aerophoba*, respectively, the fungal biomass was separated from the culture broth, and both the mycelium and the broth were extracted with ethyl acetate.

Isolation of Secondary Metabolites. The EtOAc extract (3.47 g) of *C. lunata* was chromatographed over Sephadex LH-

20 with 100% Me₂CO as eluent. From the TLC and HPLC chromatograms of the eluates, four fractions were obtained. Fraction 1 was purified using a C₁₈ Lobar reversed-phase column with MeOH–H₂O (70:30) to give (+)-abscisic acid (101.2 mg) (**3**). From fraction 2, the new anthraquinone lunatin (5.2 mg) (**1**) was obtained after purification over a diol column using EtOAc–Me₂CO (9:1). Fraction 3 gave lunatin and cytoskyrin A (21.3 mg) (**2**) after preparative HPLC (Merck, Darmstadt, Germany) on a Eurospher C₁₈ (300 × 8 mm, i.d.) column using the following gradient: 0 min, 60% MeOH; 10 min, 80% MeOH; 15 min, 80% MeOH; 20 min, 100% MeOH.

The EtOAc crude extract of *C. herbarum* (10.1 g) isolated from the sponge *Aplysina aerophoba* was initially separated by step-gradient vacuum liquid chromatography on silica gel using different ratios of CH₂Cl₂ and MeOH. The nonpolar fractions, which were eluted with 95%, 90%, and 80% CH₂Cl₂ were combined and chromatographed over a Sephadex LH-20 column with MeOH as eluent. Thirteen fractions were obtained. Fractions 5 and 10 were separately chromatographed over a C₁₈ reversed-phase column using MeOH–H₂O (7:3) as eluent and yielded compounds **4** (6.6 mg) and **5** (5.8 mg), respectively, after purification using gradient C₁₈ reversed-phase preparative HPLC with the following gradient: 0 min, 20% MeOH; 15 min, 65% MeOH; 20 min, 100% MeOH. Fraction 8 yielded compound **6** (51.6 mg), after further separation over a normal-phase C₁₈ column using CH₂Cl₂–MeOH (95:5).

The EtOAc extract of the *C. herbarum* strain isolated from *C. aerizusa* (7.0 g) was chromatographed over a C₁₈ reversed-phase column using MeOH–H₂O (64:36) as eluent. Five fractions were obtained. Herbaric acid (4.1 mg) was isolated from fraction 1 after further separation over a C₁₈ Lobar reversed-phase column with H₂O–MeOH (85:15) and purified by preparative HPLC on a Eurospher C₁₈ (300 × 8 mm, i.d.) column with the gradient of 10% MeOH at 0 min to 100% at 35 min. From fraction 1 was also obtained sumiki's acid as described in a previous paper.¹⁰ Fractions 2–5 yielded pandangolides 2, 3, and 4, cladospolide B, iso-cladospolide B, and acetyl sumiki's acid, as previously described.¹⁰

Lunatin (1): ¹H and ¹³C NMR data, see Table 1; EIMS *m/z* 286 [M]⁺ (100), 257 (11), 243 (26), 228 (11), 215 (20); HREIMS *m/z* 286.0474 (calcd for C₁₅H₁₀O₆, 286.0477).

Herbarin A (4): ¹H and ¹³C NMR data, see Table 2; (+)-ESIMS *m/z* 511 [2M + K]⁺ (13), 495 [2M + Na]⁺ (24), 275 [M + K]⁺ (23), 259 [M + Na]⁺ (75), 237 [M + H]⁺ (100); (–)ESIMS *m/z*, 235 [M – H]⁺ (100), 191 (18), 113 (38); HRESIMS *m/z* 237.0737 [M + H]⁺ (calcd for C₁₂H₁₃O₅, 237.0763).

Herbarin B (5): ¹H NMR and ¹³C NMR data, see Table 2; (+)ESIMS *m/z* 443 [2M + Na]⁺ (100), 265 [M + Na + MeOH] (84), 233 [M + Na]⁺ (56), 211 [M + H]⁺ (25); HREIMS *m/z* 210.0524 (calcd for C₁₀H₁₀O₅, 210.0525).

Herbaric acid (7): [α]_D –27° (c 0.184, MeOH); ¹H NMR and ¹³C NMR data, see Table 3; EIMS *m/z* 224 [M]⁺ (46), 178 [M – H₂CO₂]⁺ (100), 165 [M – CH₂COOH]⁺ (83), 150 (15), 137 (38); HREIMS *m/z* 224.0317 (calcd for C₁₀H₇O₆, 224.0321).

Agar Plate Diffusion Assays. Susceptibility disks (5.5 mm) were impregnated with 1 mg of extract or 5 and 10 μg of the pure isolated compounds and placed on agar plates with the test bacterium: *B. subtilis* 168, *S. aureus* ATCC 25923,

E. coli ATCC 25922, *E. coli* HBI 101, and *C. albicans*. The plates were checked for inhibition zones after 24 h of incubation at 37 °C.

Insect Bioassays. The chronic feeding assays were carried out with larvae of the polyphagous pest insect *Spodoptera littoralis* (Noctuidae, Lepidoptera). The larvae were from a laboratory colony reared on artificial diet under controlled conditions at 26 °C as described previously.¹⁴ Feeding studies were conducted with neonate larvae (*n* = 20 for each treatment) that were kept on a diet containing extracts or compounds under study. After an 8-day exposure survival and weights of surviving larvae were recorded and compared with controls that had been exposed to a diet treated with solvent (MeOH) only.

Brine Shrimp Lethality Assay. Eggs of *Artemia salina* (Dohse, Aquaristik GmbH, Bonn, Germany) were hatched in a small tank filled with artificial seawater which was prepared with a commercial salt mixture (Sera Sea-Salt, Aquaristik GmbH, Bonn, Germany) and distilled water. After 48 h, 20 nauplii were transferred into 10 mL tubes containing 50 and 100 μg of the compound and 20 μL of DMSO. Artificial seawater was added to make 5 mL. Control vials containing only DMSO were also prepared. The percent mortality at each dose level, including the control, was determined after 24 h.

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References and Notes

- (1) Faulkner, J. D. *Nat. Prod. Rep.* **2001**, *18*, 1–49.
- (2) Davidson, B. S. *Curr. Opin. Biotech.* **1995**, *6*, 284–291.
- (3) Fenical, W.; Jensen, P. R. *Annu. Rev. Microbiol.* **1994**, *48*, 559–584.
- (4) Brady, S. F.; Singh, M. P.; Janso, J. E.; Clardy, J. *Org. Lett.* **2000**, *2*, 4047–4049.
- (5) Arai, K.; Shimizu, S.; Miyajima, H.; Yamamoto, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2870–2872.
- (6) Ivanova, V.; Schlegel, R.; Gräfe, U. *Pharmazie* **2000**, *55*, 785–786.
- (7) Takeda, N.; Seo, S.; Ogihara, Y.; Sankawa, U.; Iitaka, I.; Kitagawa, I.; Shibata, S. *Tetrahedron* **1973**, *29*, 3703–3719.
- (8) Seo, S.; Sankawa, U.; Ogihara, Y.; Iitaka, Y.; Shibata, S. *Tetrahedron* **1973**, *29*, 3721–3726.
- (9) Sakabe, N.; Goto, T.; Hirata, Y. *Tetrahedron* **1977**, *33*, 3077–3081.
- (10) Jadulco, R.; Proksch, P.; Wray, V.; Sudarsono; Berg, A.; Gräfe, U. *J. Nat. Prod.* **2001**, *64*, 527–530.
- (11) Kameda, K.; Namiki, M. *Chem. Lett.* **1974**, 1491–1492.
- (12) Cui, C. B.; Ubukata, M.; Kakeya, H.; Onose, R.; Okada, G.; Takahashi, I.; Isono, K.; Osada, H. *J. Antibiot.* **1996**, *49*, 216–219.
- (13) Brauers, G.; Ebel, R.; Edrada, R. A.; Wray, V.; Berg, A.; Gräfe, U.; Proksch, P. *J. Nat. Prod.* **2001**, *64*, 651–652.
- (14) Srivastava, R. P.; Proksch, P. *Entomol. Gener.* **1991**, *15*, 265–274.

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