Dictyosphaeric Acids A and B: New Decalactones from an Undescribed *Penicillium* sp. Obtained from the Alga *Dictyosphaeria versluyii*†

Tim S. Bugni,‡ Jeffrey E. Janso,§ R. Thomas Williamson,§ Xidong Feng,§ Valerie S. Bernan,§ Michael Greenstein,§ Guy T. Carter,§ William M. Maiese,§ and Chris M. Ireland*,‡

Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112, and Wyeth Research, Pearl River, New York 10965

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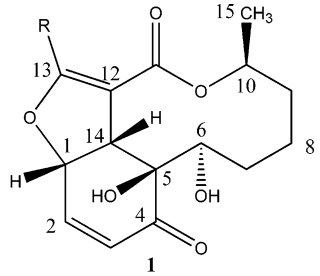
Fungal isolate F01V25 was obtained from the alga *Dictyosphaeria versluyii* collected near Dravuni, Fiji, in 2001 and represented a previously undescribed *Penicillium* sp. Fermentation of isolate F01V25 resulted in the production of two new polyketides, dictyosphaeric acids A and B, along with the known anthraquinone carviolin. The relative stereochemistry of dictyosphaeric acids A and B was determined using the *J*-based configuration analysis method in conjunction with ROE and NOE correlations.

In our continuing efforts to discover potential pharmaceutical leads, our research group has investigated fungi from Fijian marine substrates such as ascidians,[1] sponges,[2] and algae. Most recently, a previously undescribed *Penicillium* sp. (isolate F01V25) was obtained from the green alga *Dictyosphaeria versluyii*. Fermentation extracts of F01V25 showed selective activity against Gram-positive bacteria. Although the most active metabolites were not identified, two new polyketide decalactones, dictyosphaeric acids A (**1**) and B (**2**), were isolated along with the known anthraquinone carviolin (**3**).[3] Analyses of NMR data showed that **1** and **2** were related to colletofragerone A2 (**4**), which was isolated from the fungus *Colletotrichum fragariae*.[4] The latest stereostructures for colletofragerones A1 and A2 (**4**) were subsequently reported in a review.[5] Although decalactones are common among fungal metabolites, the colletofragerones are the only other compounds that have the same carbon skeleton as the dictyosphaeric acids. The relative stereochemistry was determined using the *J*-based configuration method[6] in conjunction with molecular modeling and analysis of NOE and ROE correlations. Long-range coupling constants were measured using the G-BIRD$_R$-HSQMBC.[7] Dictyosphaeric acid A (**1**) showed weak antibacterial activity toward Gram-positive bacteria, while dictyosphaeric acid B (**2**) showed no antibacterial activity.

A lyophilized culture was extracted with MeOH, and the extract was dried and partitioned between aqueous MeOH and hexanes followed by partitioning between aqueous MeOH and chloroform. The chloroform-soluble material was separated using LH-20 and reversed-phase HPLC to yield dictyosphaeric acids A (**1**) and B (**2**) and carviolin (**3**).

Dictyosphaeric acid A (**1**) was isolated as an amorphous yellow solid. The molecular formula, $C_{22}H_{24}O_8$, was determined by high-resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. Initial inspection of the ^{1}H and ^{13}C NMR data indicated that compound **1** contained 10 olefinic carbons, three oxygenated methines, one oxygenated quaternary sp^3 carbon, and one ketone. HSQC, HMBC, COSY, and ROESY NMR data were utilized for the structural elucidation of dictyosphaeric acid A (**1**).



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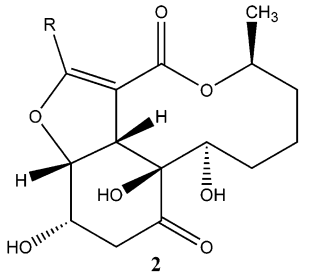

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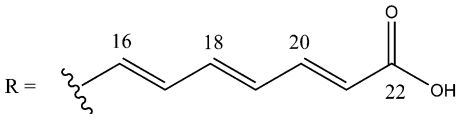

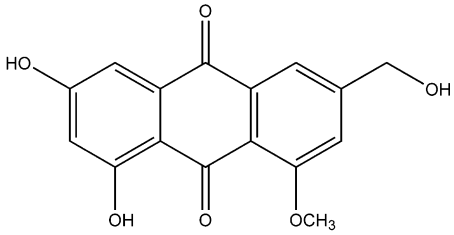

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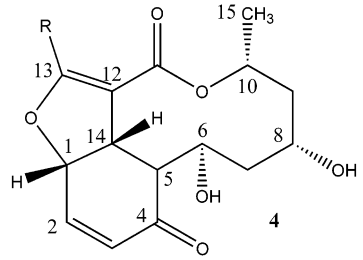

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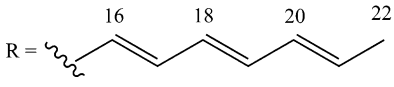

† Dedicated to the late Dr. D. John Faulkner and the late Dr. Paul J. Scheuer for their pioneering work on bioactive marine natural products.

* To whom correspondence should be addressed. Phone: (801) 581 8305. Fax: (801) 585 6208. E-mail: cireland@pharm.utah.edu.

‡ University of Utah.

§ Wyeth Research.

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Table 1. NMR Data for Dictyosphaeric Acids A (**1**) and B (**2**)

	dictyosphaeric acid A (**1**)						dictyosphaeric acid B (**2**)		
position	δ ^{13}C	δ ^{1}H	mult., *J* Hz	HMBC	COSY	ROESY	δ ^{13}C	δ ^{1}H	mult., *J* Hz
1	77.9	5.50	ddd, 8.7, 3.3, 0.8	2, 3, 12, 13	2, 3, 14	2, 14	84.0	4.88	dd, 8.9, 3.3
2	141.2	6.58	dd, 10.5, 3.3	1, 4, 14	1, 3	1, 3	68.2	4.49	ddd, 6.6, 3.3, 1.1
3	129.1	6.09	dd, 10.5, 0.8	1	1, 2	2	44.7	2.87	dd, 18.3, 6.6
								2.93	d, 18.3
4	202.8						212.9		
5	78.0						82.0		
6	74.1	3.80	d, 8.9	5, 7, 8	7α	8b, 9b	70.8	3.68	d, 8.5
7α	29.4	1.45	m	6, 8, 9	6, 7*β*	7*β*	30.6	1.90	m
7*β*		2.01	m	8, 9	7α	7α, 14			
8a	23.0	1.61	m	7	8b		21.1	1.57	br m
8b		1.90	m	10	8a	6		1.90	m
9a	34.8	1.61	m	8, 10	9b, 10	10, 15	30.6	1.49	br m
9b		1.85	m	7, 8	9a	6, 10, 15		2.04	dt, 13.1, 1.9
10	75.3	4.84	m, 6.2		9a, 15	9a, 9b, 15	73.5	5.08	m
11	165.6						164.8		
12	106.0						105.3		
13	161.6						164.9		
14	52.2	4.23	d, 8.7	1, 2, 4, 11, 12, 13	1	1, 7*β*	53.0	3.93	d, 8.9
15	20.6	1.32	d, 6.2	9, 10	10	9a, 9b, 10	18.8	1.30	d, 6.6
16	121.4	6.59	m	13, 18	17		121.9	7.04	d, 15.6
17	136.2	6.83	dd, 15.5, 11.0	13, 19, 18	16, 18	19	137.8	6.86	dd, 15.4, 11.0
18	139.2	6.63	m	16, 20	17, 19	20	139.2	6.64	dd, 14.9, 11.0
19	135.5	6.53	dd, 14.8, 10.9	17, 18, 20	18, 20	17, 21	136.0	6.54	dd, 14.9, 11.0
20	144.5	7.27	dd, 15.5, 10.9	18, 19, 22, 21	19, 21	18	144.4	7.28	dd, 15.2, 11.2
21	124.0	5.93	d, 15.5	19, 22	20	19	124.4	5.93	d, 15.4
22	169.6						169.6		

The starting point for the elucidation was the all-*trans*-heptatrienoic acid side chain. Analysis of the coupling constants (see Table 1) supported the all-*trans* arrangement. However, there was enough overlap that analysis of GHMBC data was necessary for an unambiguous assignment of the side chain resonances. The downfield shift of H-20 (δ 7.27) indicated that it was β to a carbonyl group. HMBC correlations between H-20 and a signal at δ 169.6 (C-22) supported this assignment. The chemical shift of C-22 (δ 169.6) indicated that the carbonyl was most likely an acid since unsaturated esters are typically closer to 165 ppm.[8]

Using the heptatriene chain for substructure queries led to the related structures of the colletofragerones.[4,5] By comparison of the NMR data to that of colletofragerone A2 (**4**), the remaining signals of the dictyosphaeric acids could be assigned, and the structure for compound **1** was proposed. Although the structures were related, there were several key differences that hindered the elucidation of dictyosphaeric acid A (**1**). The main difference was the position of one hydroxyl and the stereochemistry.

The proposed structure shown was consistent with all NMR data, but no HMBC correlations were observed for H-10 (due in part to the broad nature of the signal), and the possibility existed that C-10 could be linked via an ether linkage to either C-6 or C-5. Support for the decalactone system came from analysis of ^{13}C chemical shifts and a ROESY spectrum. The ^{13}C shift of C-11 (δ 165.6) suggested that the carbon was most likely an ester rather than the acid that would be present if C-10 formed an ether bridge with either C-6 or C-5. Additionally, a ROESY spectrum was collected and carefully analyzed to see if correlations across the putative decalactone system were observed. This analysis was performed in conjunction with molecular modeling of the possible cyclic ethers and esters as well as the different stereoisomers. As shown in Figure 1, ROESY correlations were observed that confirmed the decalactone structure and helped establish the relative stereochemistry of dictyosphaeric acid A (**1**). A key ROESY correlation that supported the decalactone system was

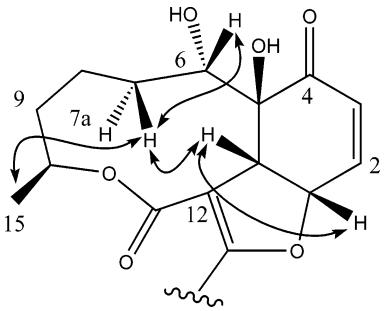

Figure 1. Key NOE/ROE correlations supporting the relative stereochemistry of dictyosphaeric acid A (**1**).

observed between H-14 and H-7*β*. The results from the molecular modeling also indicated that the dihedral angle between H-10 and C-11 is approaching 90°, suggesting that the $^{3}J_{C,H}$ would be very small. These results help rationalize the lack of an HMBC correlation between H-10 and C-11 as well as provide additional support that dictyosphaeric acid A (**1**) had different C-10 stereochemistry as compared to colletofragerone A2 (**4**).

The relative stereochemistry as shown for dictyosphaeric acid A (**1**) in Figure 1 was established using an extension of the *J*-based configuration analysis method[6] in conjunction with analyses of observed ROE and NOE correlations. Representations of the two possible conformations, depending on whether H-14 and H-1 were *cis* or *trans*, are shown in Figure 2 and were based on molecular modeling (Macromodel Ver. 5.1.12). Analysis of the long-range heteronuclear couplings in addition to ^{1}H−^{1}H scalar couplings and NOE studies indicated that H-14 and H-1 were in a *cis* orientation. As shown in Figure 2, all coupling constants were consistent with the *cis* configuration, while there were two conflicts (indicated by italics) for the *trans* configuration. A series of DPFGSE 1D NOE experiments using six mixing times (100−750 ms) was performed. Antiphase scalar coupling artifacts were observed only at the shortest mixing time, indicating that through-space interactions are the major contributors to the signal observed, providing further support for the proposed model. Additionally, the

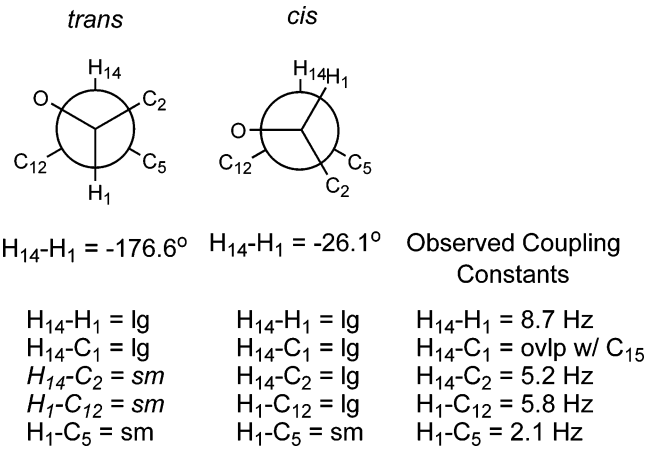

Figure 2. Newman projections and coupling constant analysis for $H_{14}-H_1$ *cis* and *trans* conformations of compound **1**.

observed coupling constant (8.7 Hz) between H-14 and H-1 was consistent with a similar system found in cryptocaryone, the structure of which was confirmed by X-ray crystallographic analysis.[9]

The stereochemistry at position C-5 was indicated in part by the $^3J_{C,H}$ value (4.0 Hz) observed between C-4 and H-14. Molecular modeling of each of the two possible configurations at position C-5 indicated that when C-5 and H-14 presented an *anti* conformation, the dihedral angle between C-4 and H-14 was near 60°, which would result in a small coupling constant. However, in the model where C-5 and H-14 presented a *syn* conformation, the dihedral between C-4 and H-14 was about 7°, which would result in a larger coupling constant and be consistent with the observed value. Additional support for the configuration at position C-5 came from observed NOE/ROE correlations (see Figure 1). The NOE buildups for H-14 to H-7β and H-14 to H-1 were similar, suggesting that both H-7β and H-1 are a similar distance to H-14. This observation strongly supported the assigned stereochemistry at position C-5 and agreed well with the distance predicted in the molecular modeling. For the C-5 stereochemistry shown in Figure 2, the distance (2.1 Å) between H-14 and H-7β was nearly identical to the distance between H-14 and H-1. However, for the opposite configuration at position C-5, the distance between H-14 and H-7β was 3.7 Å and would be inconsistent with the similar buildup curves observed in the DPFGSE 1D NOE experiments. Overall, the observed NOE and ROE correlations in conjunction with the *J*-based configuration analyses clearly distinguish the stereochemistry shown. An attempt was made to prepare a Mosher ester at position 6, but only decomposition of starting material was observed.

Dictyosphaeric acid B (**2**) was isolated as an amorphous yellow solid. The molecular formula, $C_{22}H_{26}O_9$, was established by FTICRMS and supported an addition of H_2O as compared to **1**. The ^{1}H olefinic region was simplified and indicated that dictyosphaeric acid B (**2**) had one less olefin than dictyosphaeric acid A (**1**). The COSY spectrum showed that H-1 was not coupled to an olefinic methine as in **1**, but was coupled to an oxygenated methine and confirmed the hydration. The H-2 oxygenated methine was additionally coupled to a diastereotopic methylene (H-3a and H-3b). The NMR data (Table 1) clearly indicated the relationship between dictyosphaeric acids A (**1**) and B (**2**). The assigned relative stereochemistry of the C-2 hydroxyl was based on the $^1H-^1H$ vicinal coupling value between H-1 and H-2. Both diastereomers were modeled, and the dihedral angle was measured. When H-1 and H-2 shared a *cis*-relationship, the dihedral angle was −48°, which would result in a small coupling constant, consistent with the experimentally measured coupling constant (3.3 Hz). However, when H-1 and H-2 presented a *trans*-relationship, the dihedral angle was 150°, which would result in a large coupling constant.

Dictyosphaeric acid A (**1**) showed antibacterial activity toward Gram-positive bacteria and when tested at 50 μg/well yielded 11 and 12 mm hazy zones of inhibition against methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant *S. aureus*, respectively. Additionally, dictyosphaeric acid A (**1**) yielded a 7 mm zone against vancomycin-resistant *Enterococcus faecium* and a very hazy 7 mm zone against *Candida albicans* at 50 μg/well. Dictyosphaeric acid B (**2**) and carviolin (**3**) showed no antimicrobial activity at 50 and 200 μg, respectively.

Dictyosphaeric acids A (**1**) and B (**2**) add to a structurally rare class of decalactones, and to date only two metabolites have been reported containing the same carbon skeleton, colletofragarones A1 and A2 (**4**).[4] Colletofragarones A1 and A2 (**4**) differ from dictyosphaeric acids A (**1**) and B (**2**) in that they do not contain the carboxylic acid and also display a different hydroxylation pattern. Although members of the Penicillia are ubiquitous fungi, isolate F01V25 represents an undescribed species. The isolation of such unique metabolites from a new *Penicillium* sp. provides additional support that unique species from the marine environment yield new metabolites. Currently, a formal taxonomic description of isolate F01V25 is in progress.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C spectra were obtained in 1:1 $CDCl_3/CD_3OD$ at 500 and 125 MHz, respectively. Proton shifts are reported in parts per million relative to the reference solvent signals of CD_3OD at δ 3.30 for ^{1}H and δ 49.0 for ^{13}C. High-resolution mass spectra (HRMS) were obtained using an APEXII FTICR mass spectrometer equipped with an actively shielded 7.1 T superconducting magnet.

Biological Material. Strain F01V25 was obtained from a macerated sample of the alga *Dictyosphaeria versluyii* collected near Dravuni, Fiji, in January 2001. Strain F01V25 has been deposited in the Wyeth Culture Collection in Pearl River, NY. Strain F01V25 was identified as belonging to the genus *Penicillium*, but represents a previously undescribed species. An article providing a taxonomical description of F01V25 (tentatively *Penicillium dravuni*) will follow. The identification was based on morphology and sequence analysis of the ITS regions (GenBank accession AY494856).

Dictyosphaeric acid A (1): $[\alpha]_D^{25}$ +126° (*c* 0.210, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.74), 336 (4.09); IR ν_{max} 3528 (br), 3017, 2978, 2939, 1720−1640 (br), 1631, 1600, 1361 cm^{-1}; ^{1}H, ^{13}C, and HMBC NMR data, Table 1; FTICRMS *m/z* 417.15449 ([M + H]$^+$ calcd for $C_{22}H_{25}O_8$, 417.15440).

Dictyosphaeric acid B (2): $[\alpha]_D^{25}$ +76° (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.81), 280 (s, 3.82), 346 (4.13); IR ν_{max} 3532 (br), 3016, 2941, 1711, 1690, 1631, 1559, 1368 cm^{-1}; ^{1}H, ^{13}C, and HMBC NMR data, Table 1; FTICRMS *m/z* 435.16516 ([M + H]$^+$ calcd for $C_{22}H_{27}O_9$, 435.16496).

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Supporting Information Available: Experimental procedures for fermentation, extraction, and isolation are provided free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bugni, T. S.; Abbanat, D.; Bernan, V. S.; Maiese, W. M.; Greenstein, M.; VanWagoner, R. M.; Ireland, C. M. *J. Org. Chem*. **2000**, *65*, 7195−7200.
(2) Smith, C. J.; Abbanat, D.; Bernan, V. S.; Maiese, W. M.; Greenstein, M.; Jompa, J.; Tahir, A.; Ireland, C. M. *J. Nat. Prod*. **2000**, *63*, 142−145. Bugni, T. S.; Bernan, V. S.; Greenstein, M.; Janso, J. E.; Maiese, W. M.; Mayne, C. L.; Ireland, C. M. *J. Org. Chem*. **2003**, *68*, 2014−2017.
(3) Eilbert, F.; Anke, H.; Sterner, O. *J. Antibiot.* **2000**, *53*, 1123−1129.
(4) Inoue, M.; Takenaka, H.; Tsurushima, T.; Miyagawa, H.; Ueno, T. *Tetrahedron Lett*. **1996**, *37*, 5731−5734.
(5) Garcia-Pajon, C. M.; Collado, I. G. *Nat. Prod. Rep*. **2003**, *20*, 426−431.
(6) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem*. **1999**, *64*, 866−876. Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc.* **1999**, *121*, 870−871. Williamson, R. T.; Boulanger, A.; Vulpanovici, A.; Roberts, M. A.; Gerwick, W. H. *J. Org. Chem*. **2002**, *67*, 7927−7936.
(7) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Kover, K. E. *Magn. Reson. Chem*. **2000**, *38*, 265−273. Marquez, B. L.; Gerwick, W. H.; Williamson, R. T. *Magn. Reson. Chem*. **2001**, *39*, 499−530.
(8) Pretsch, E.; Buhlmann, P.; Affolter, C. *Structure determination of organic compounds: Tables of spectral data*, 3rd ed.; Springer-Verlag: New York, 2000.
(9) Dumontet, V.; Gaspard, C.; Van Hung, N.; Fahy, J.; Tchertanov, L.; Sevenet, T.; Gueritte, F. *Tetrahedron* **2001**, *57*, 6189−6196.

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