Dendroamides, New Cyclic Hexapeptides from a Blue-Green Alga. Multidrug-Resistance Reversing Activity of Dendroamide A

Junichi Ogino,[†] Richard E. Moore,*[,†] Gregory M. L. Patterson,[†] and Charles D. Smith[‡]

Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, and Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

Received January 9, 1996[⊗]

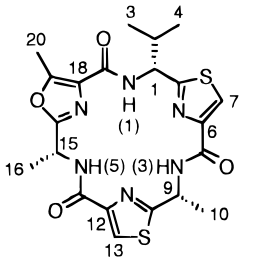
Dendroamide A (**1**), one of three new bistratamide-type cyclic hexapeptides from the terrestrial blue-green alga (cyanobacterium) *Stigonema dendroideum* Fremy, exhibits multidrug-resistance reversing activity. The gross structures of the three compounds, dendroamides A−C, were determined by NMR and mass spectral analyses. Their absolute stereochemistries were determined by Marfey and chiral GC/MS analyses of derivatives formed from acid hydrolysis of the intact and ozonized peptides.

Tumor cells that survive initial chemotherapy often increase their resistance not only to the original drug, but also to other unrelated drugs. The phenomenon, termed multidrug resistance (MDR),[1] results in the ultimate failure of chemotherapy. As a consequence, there is a growing interest to develop agents that circumvent or overcome MDR. Recently, we have discovered that the cryptophycins, a novel class of strongly cytotoxic peptolides from certain *Nostoc* spp., are very effective against drug-resistant and multidrug-resistant tumors.[2,3] We have also identified several noncytotoxic to weakly cytotoxic natural products from blue-green algae that reverse P-glycoprotein-mediated MDR and restore the effectiveness of common antitumor drugs.[4−6]

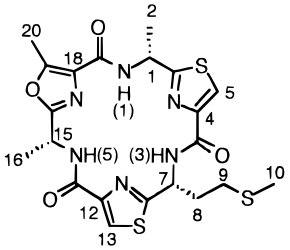
In screening additional extracts of blue-green algae for anti-MDR activity, we have found that the lipophilic extract of *Stigonema dendroideum* Fremy (Family Stigonemataceae) (UH strain IA-45-3) reverses MDR in a P-glycoprotein-overexpressing subline of a human breast carcinoma cell line (MCF-7/ADR). Using a bioassay-guided fractionation, we have isolated three new bistratamide-type cyclic hexapeptides, dendroamides A−C (**1**−**3**), from IA-45-3. The dendroamides are characterized by the presence of two thiazole (Tzl) and one methyloxazole (mOzl) amino acid units. Cyclic peptides containing thiazole (Tzl) and methyloxazoline (mOzn) residues were first isolated in 1980 from a marine tunicate.[7] Since then, a large number of these cyclic peptides have been reported from other marine organisms[8−18] and more recently from terrestrial blue-green algae (cyanobacteria).[19−21] In this paper, we report the structures of the three new peptides and the anti-MDR activity of dendroamide A (**1**).

Results and Discussion

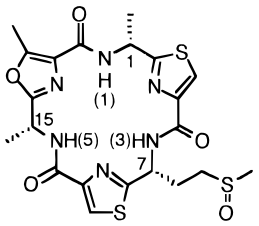
Isolation and Gross Structure Determination. *S. dendroideum* IA-45-3 was isolated from a Maui rock sample and mass cultured in the laboratory as previously described.[22] The lipophilic extract (1:1 CH_2Cl_2/2-propanol) of the freeze-dried blue-green alga was fractionated by reversed-phase column chromatography and HPLC to give **1**, **2**, and **3** in yields of 0.58, 0.15, and 0.17%, respectively, based on the dry weight of the alga.



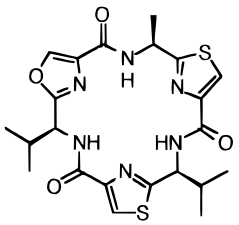
1
dendroamide A



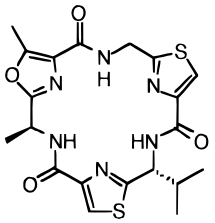
2
dendroamide B



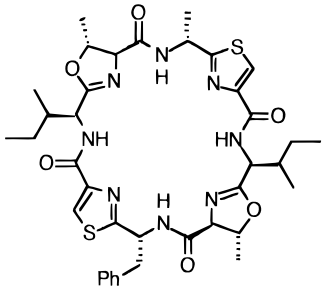
3
dendroamide C



4
bistratamide C



5
nostocyclamide



6
patellamide D

Dendroamide A (**1**) has the molecular formula $C_{21}H_{24}N_6O_4S_2$ based on a high-resolution mass measurement of the molecular ion. The electron impact mass spectrum (EIMS) also shows a large M − 43 fragment ion (loss of C_3H_7) and some important frag-

† University of Hawaii.
‡ Fox Chase Cancer Center.
⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1996.

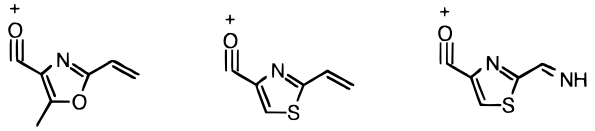
S0163-3864(96)00178-4 CCC: $12.00

Table 1. NMR Data for Dendroamide A (1) (Cyclo-D-Val-Tzl-D-Ala-Tzl-D-Ala-mOzl)

C or N position	δ (^{13}C)	δ (^{1}H) (mult, J_{HH}, Hz)	^{1}H−^{1}H COSY	HMBC[a]
N1		8.44 (br d; 8.2)	1	21, 1, 5
1	56.1	5.31 (dd; 8.2, 4.8)	N1H, 2	21, 2, 3, 4, 5
2	35.6	2.28 (d of septet; 4.8, 6.9)	1, 3, 4	1, 3, 4, 5
3	18.3	1.02 (d; 6.9)	2	1, 2, 4
4	18.5	0.95 (d; 6.9)	2	1, 2, 3
5	168.9			
6	149.2			
7	124.0	8.11 (s)		5, 6, 8[b]
8	159.7			
N3		8.52 (br d; 8.2)	9	8, 9, 11
9	47.3	5.69 (dq; 8.2, 6.8)	N3H, 10	8, 10, 11
10	25.1	1.68 (d; 6.8)	9	9, 11
11	171.7			
12	149.2			
13	123.8	8.09 (s)		11, 12, 14[b]
14	160.0			
N5		8.61 (br d; 6.6)	15	14, 15, 17
15	44.6	5.17 (br quintet; 6.6)	N5H, 16	14, 16, 17
16	21.1	1.65 (d; 6.6)	15	15, 17
17	162.1			
18	154.0			
19	128.8			
20	11.7	2.63 (s)		8, 19, 21
21	160.7			

[a] Carbon correlations. [b] Determined by selective INEPT experiment.

ment ions consistent with thiazole- and methyloxazole-containing residues.



Intense absorptions in the IR spectrum at 3396 cm^{-1} (NH stretching vibration of secondary amide), 1668 cm^{-1} (amide-I band), and 1538 cm^{-1} (amide-II band) and its lipophilic nature indicated that **1** is a cyclic peptide.

The ^{1}H- and ^{13}C-NMR spectral data for **1**, including COSY and HMBC correlations, are shown in Table 1. The chemical shifts are in good agreement with values reported for the two thiazole amino acid units (Val-Tzl and Ala-Tzl) in bistratamide C (**4**)[15] and the methyloxazole amino acid unit (Ala-mOzl) in nostocyclamide (**5**).[20] ^{1}H−^{13}C correlations from HMBC and selective INEPT experiments, viz. H9−C8, H15−C14, and H1−C21, have allowed us to sequence the three amino acid units into a total gross structure.

Dendroamide B (**2**) possesses the formula $C_{21}H_{24}N_6O_4S_3$ from high-resolution mass spectrometry. Comparison of the ^{1}H and ^{13}C-NMR data for **1** and **2** indicated that **2** also contains Ala-Tzl and Ala-mOzl units. The chemical shifts and patterns for the remaining signals suggested that the third unit ($C_8H_{10}N_2OS_2$) is Met-Tzl where C-1 of a 1-amido-3-(methylthio)propyl group is attached to C-2 of a thiazole-4-carboxamide. In support of a CH_2CH_2SMe side chain in the Met-Tzl unit, the EIMS spectrum showed relatively large M − 48 (loss of MeSH) and M − 74 (loss of CH_2=CHSMe) fragment ions. HMBC correlations for H7 − C6, H15 − C14, and H1 − C21 permitted us to sequence the three units into a total gross structure.

Dendroamide C (**3**) has the formula $C_{21}H_{24}N_6O_5S_3$. The ^{1}H- and ^{13}C-NMR data for **3** show that Ala-Tzl and Ala-mOzl units are present. In addition to the amide bands, the IR spectrum of **3** displays a strong absorption at 1046 cm^{-1} (S=O stretching vibration of sulfoxide). The presence of the sulfoxide group is supported by the EIMS which shows an intense M − 64 fragment ion for loss of CH_3SOH from the molecular ion. All these data have strongly suggested the existence of a unit originating from methionine sulfoxide, viz. a Met(O)-Tzl unit. Again, H7−C6, H15−C14, and H1−C21 correlations in the HMBC spectrum established the sequence of the three units and the gross structure for **3**.

Absolute Stereochemistry. To determine the absolute configuration of the methyloxazole amino acid unit (Ala-mOzl), each dendroamide was subjected to vigorous acid hydrolysis and the acid hydrolysate analyzed by the Marfey method.[23] Only the oxazole ring was cleaved by direct acid hydrolysis to generate D-alanine from all three dendroamides.[24−26] The configuration of C-15 in **1**−**3** was therefore *R*.

To determine the absolute configuration of the thiazole amino acids, the peptides were first ozonized and then subjected to vigorous acid hydrolysis. Ozonolysis resulted in cleavage of both the oxazole and thiazole rings. After acid hydrolysis, D-alanine and D-valine were detected in the hydrolysate of ozonized **1** and D-alanine and D-methionine sulfone were found in the acid hydrolysates of ozonized **2** and **3** by Marfey and GC/MS[27] analyses. The D-methionine sulfone had obviously been generated from the D-methionine residue in **2** and D-methionine sulfoxide residue in **3** during the ozonolysis. All of the chiral carbons in the dendroamides were therefore *R*.

Since most of the ^{1}H and ^{13}C NMR signals for the Met(O)-Tzl unit in **3** are doubled, we have concluded that the dendroamide C described here is actually a mixture of *R* and *S* sulfoxides. Separation of the two diastereomers, however, was not achieved, and no studies were undertaken to determine whether **3** is a natural product or an artifact.

Biological Activity. Cells that express the efflux pump P-glycoprotein are resistant to most currently used anticancer drugs because of impaired intracellular accumulation of the drugs. Reduced cytotoxicity and lower intracellular accumulation of anticancer drugs can be at least partially reversed by compounds which interact with the drug transporter, e.g., verapamil. During the fractionation of IA-45-1, samples containing anti-MDR compounds were identified by their ability to chemosensitize P-glycoprotein-overexpressing breast carcinoma (MCF-7/ADR) cells to daunomycin and/or actinomycin D and to promote the accumulation of [^{3}H]-vinblastine in these cells. As indicated in Figure 1, dendroamide A was more potent than verapamil in its ability to increase the accumulation of [^{3}H]vinblastine in MCF-7/ADR cells, reaching ~750% of control at 5 μM and maximal effect at 20 μM. Neither dendroamide A nor verapamil increased the accumulation of [^{3}H]vinblastine in MCF-7 cells (data not shown), indicating that their effects are due to inhibition of drug transport by P-glycoprotein.

MCF-7/ADR cells were then treated with combinations of daunomycin or actinomycin D and dendroamide A, and cell survival was assessed. As indicated in

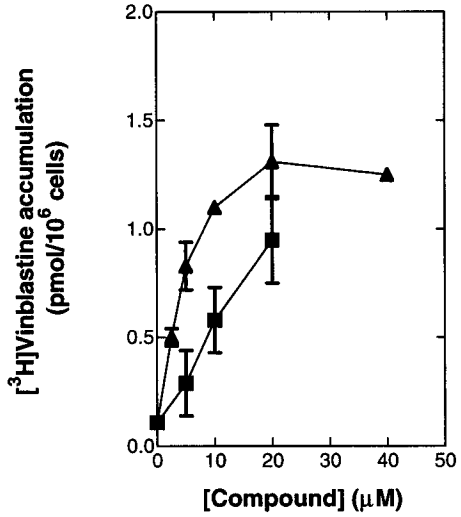


Figure 1. Effects of dendroamide A on [3H]vinblastine accumulation by MCF-7/ADR cells. Cells were incubated with the indicated concentrations of dendroamide A (▲) or verapamil (■) for 30 min. [^{3}H]Vinblastine was then added, and its intracellular accumulation after 60 min was determined. Values represent the mean ± SD accumulation of [^{3}H]vinblastine in triplicate samples in one of three similar experiments.

Figure 2A, these cells were killed much more effectively by combinations of daunomycin or actinomycin D and dendroamide A than with identical doses of the drugs alone. Dose−response curves for this activity were very similar to those for intracellular drug accumulation, demonstrating activity at submicromolar concentrations. In contrast, dendroamide A did not alter the cytotoxicity of cisplatin, which is not a substrate for P-glycoprotein. Dendroamide A concentrations of 0.6 μM or greater increased the sensitivity of MCF-7/ADR cells to vinblastine (Figure 2B) to that of parental MCF-7 cells, indicating that this compound is able to completely overcome P-glycoprotein-mediated MDR in this model system.

The reduction of photoaffinity labeling of P-glycoprotein[28] by [^{3}H]azidopine is strongly indicative of direct binding of a modulating agent to P-glycoprotein. Increasing concentrations of verapamil or dendroamide A caused progressive decreases in the binding of [^{3}H]-azidopine to P-glycoprotein in membranes from MCF-7/ADR cells, such that photolabeling of P-glycoprotein was reduced by 50% by 0.5 and 10 μM dendroamide A and verapamil, respectively (data not shown).

MCF-7/VP cells were used to test the effects of dendroamide A on MRP-mediated drug resistance. In comparison with MCF-7 cells, MCF-7/VP cells were 15-fold resistant to vincristine. As indicated in Figure 3, treatment of MCF-7/VP cells with either 20 μM verapamil or 5−10 μM dendroamide A increased the cytotoxicity of vincristine, such that resistance was fully reversed by 10 μM dendroamide A. Similarly, dendroamide A was able to sensitize MCF-7/VP cells to etoposide and doxorubicin but did not modulate responses to cisplatin (data not shown).

The abilities of dendroamide A to (1) selectively potentiate the cytotoxicity of P-glycoprotein-transported drugs, (2) increase the intracellular accumulation of vinblastine, and (3) compete with [^{3}H]azidopine for binding to P-glycoprotein are characteristic of MDR-reversing agents which act as antagonists for drug transport by P-glycoprotein. Interestingly, this compound also fully reverses resistance due to overexpressions of MRP. While numerous compounds with anti-MDR activity have been identified in the past 10 years, most of these agents fall into a small number of families of structurally similar chemicals. Dendroamide A is an example of an MDR-reversing agent belonging to a class of modified cyclic peptides characterized by the presence of three thiazole (Tzl), thiazoline (Tzn), oxazole (Ozl), and/or oxazoline (Ozn) amino acid units. Patellamide D (**6**),[11,12] a related cyclic peptide possessing four of these amino acid units, has been reported to reverse MDR in a human leukemia cell line.[6]

Dendroamides B and C were essentially inactive in the anti-MDR assays described above.

Experimental Section

Spectral Analysis. NMR spectra were determined on an 11.75-T (GN-OMEGA) instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{1}H chemical shifts are referenced to residual $CHDCl_2$ (5.32 ppm) in CD_2Cl_2. ^{13}C chemical shifts are referenced to the solvent (CD_2Cl_2, 52.8 ppm). All ^{1}H and ^{13}C assignments are based on detailed COSY,[29] HMQC, and HMBC analyses where one-bond ^{1}H−^{13}C connectivities have been determined by HMQC,[30] and two- and three-bond (also four-bond) ^{1}H−^{13}C connectivities have been determined by HMBC.[31] Some of the important ^{1}H−^{13}C correlations, viz. the ones deciding the sequence of the peptide, were supported by selective INEPT experiments.[32] Mass spectra, including high-resolution mass measurements, were determined in the EI mode on a VG-70SE instrument. UV spectra, IR spectra, and optical rotations were measured on a Hewlett-Packard 8452A diode array spectrophotometer, a Perkin-Elmer 1600 FTIR, and a JASCO DIP-370 digital polarimeter, respectively.

Isolation and Cultivation of Alga. An epilithic alga, *S. dendroideum* Fremy, designated UH strain IA-45-3, was isolated from a rock sample collected at Wainapanapa, Maui (20° 47′ 34″ N, 156° 00′ 28″ W) in June 1989 and purified by repeated subculture on solidified media.

The cyanophyte was cultured in an autoclaved 20L glass carboy containing an inorganic medium (modified BG-11) adjusted to pH 7.0 with MOPS. Cultures were continuously illuminated at an incident intensity at 80−100 μmol photons m^{-2} s^{-1} (photosynthetically active radiation) from banks of cool-white fluorescent tubes and vigorously aerated at a rate of 5 L/min with a mixture of 0.5% CO_2 in air at a temperature of 24 ± 1 °C.

After 28 days, the alga was harvested by filtration onto Whatman No. 4 paper. The yield of lyophilized cells was 0.64 g/L.

Isolation of Dendroamides. The freeze-dried alga (12.9 g) was extracted twice with 2 L of CH_2Cl_2/2-propanol (50/50) for 12 and 5 h, respectively. The extracts were combined and concentrated *in vacuo* to give a dark green solid that was partitioned between water and CH_2Cl_2. The lipophilic portion was applied to an ODS-coated silica column (55 g, 20 × 3 cm) and subjected to flash chromatography with CH_3CN/H_2O (35/65, 200 mL), CH_3CN/H_2O (65/35, 400 mL), CH_3CN/H_2O (90/10, 400 mL), CH_3CN (800 mL), and CH_2Cl_2 (800 mL). The fraction that was eluted with CH_3CN/H_2O

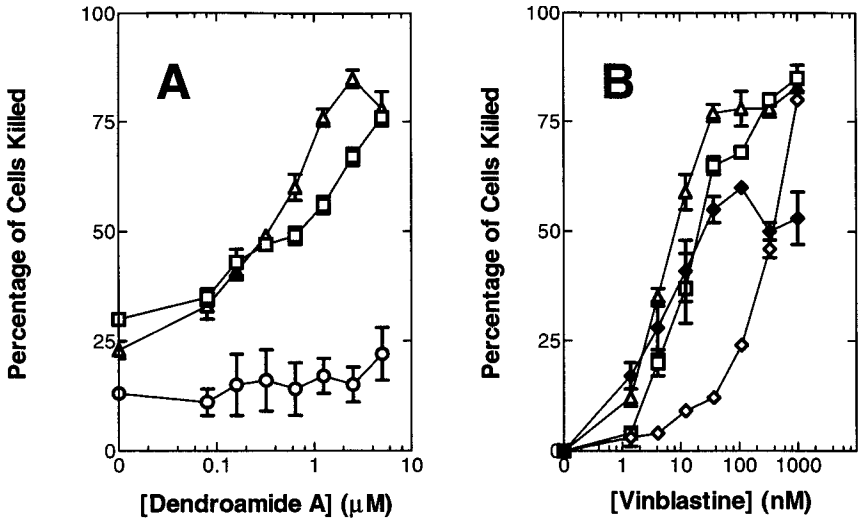


Figure 2. Chemosensitization of MCF-7/ADR cells by dendroamide A. (A) MCF-7/ADR cells were treated with 15 μM daunomycin (□), 200 nM actinomycin D (△), or 4 μM cisplatin (○) in the presence of the indicated concentrations of dendroamide A. (B) MCF-7 cells (◆) or MCF-7/ADR cells treated with 1% EtOH (◇), 0.6 μM dendroamide A (□), or 2.5 μM dendroamide A (△) were incubated with the indicated concentrations of vinblastine. Cell survival was assayed after 48 h of treatment using the SRB binding assay and is expressed as the percentage of cells killed. Values represent the mean ± SD for triplicate samples in one of two similar experiments.

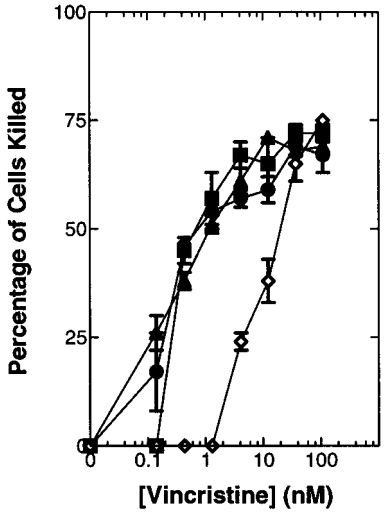


Figure 3. Chemosensitization of MCF-7/VP cells by dendroamide A. MCF-7/VP cells were treated with the indicated concentrations of vincristine in the presence of ethanol (◇), 20 μM verapamil (■), 5 μM dendroamide A (●), or 10 μM dendroamide A (▲). Cell survival was assayed after 48 h of treatment using the SRB binding assay and is expressed as the percentage of cells killed. Values represent the mean ± SD for triplicate samples in one of three similar experiments.

(65/35) showed excellent anti-MDR activity. Subjection of this fraction to reversed-phase HPLC (Econosil C8, 10 μm, 25 cm × 10 mm, UV detection at 254 nm, flow rate 3.0 mL/min) using CH_3CN/H_2O (62/38) as the eluant gave the three cyclic hexapeptides (**1**, t_R 12.2 min, 74.6 mg; **2**, t_R 11.0 min, 19.4 mg; **3**, t_R 5.2 min, 22.1 mg).

Dendroamide A (1): $[\alpha]_D$ +40.5° (*c* 3.50, CH_2Cl_2); UV λ_{max} (MeOH) 224 nm (ϵ 28 000); IR (neat) ν_{max} 3396, 1668, 1538 cm^{-1}; EIMS *m/z* (rel intensity) 488 (7.6), 445 (100), 139 (30), 138 (37), 136 (26); high-resolution EIMS *m/z* 488.1342 ($C_{21}H_{24}N_6O_4S_2$, −4.2 mmu error), 445.0690 ($C_{18}H_{17}N_6O_4S_2$, +6.3 mmu error); ^{1}H- and ^{13}C-NMR see Table 1.

Dendroamide B (2): $[\alpha]_D$ +44.5° (*c* 1.53, CH_2Cl_2); UV λ_{max} (MeOH) 224 nm (ϵ 31 400); IR (neat) ν_{max} 3396, 1668, 1538 cm^{-1}; EIMS *m/z* (rel intensity) 520 (30), 472 (24), 446 (100), 139 (56), 138 (65), 136 (23); high-resolution EIMS *m/z* 520.1021 ($C_{21}H_{24}N_6O_4S_3$, ±0 mmu error), 472.0934 ($C_{20}H_{20}N_6O_4S_2$, +5.3 mmu error); ^{1}H- and ^{13}C-NMR see Table 2.

Table 2. NMR Data for Dendroamide B (2) (Cyclo-D-Ala-Tzl-D-Met-Tzl-D-Ala-mOzl)

C or N position	δ (^{13}C)	δ (^{1}H) (mult, J_{HH}, Hz)	^{1}H−^{1}H COSY	HMBC[a]
N1		8.61 (br d; 6.7)	1	21, 3
1	47.9	5.42 (quintet; 6.7)	N1H, 2	21, 2, 3
2	25.1	1.68 (d; 6.7)	1	1, 3
3	172.0			
4	148.9			
5	124.6	8.14 (s)		3, 4, 6[b]
6	159.8			
N3		8.49 (br d; 8.7)	7	6, 11
7	50.2	5.78 (dt; 8.7, 6.7)	N3H, 8	6, 8, 9, 11
8	38.0	2.26 (m)	7, 9a, 9b	7, 9, 11
9	30.0	2.49 for 9a (ddd; −13.3, 8.7, 6.9)	8, 9b	7, 8, 10
		2.67 for 9b (ddd; −13.3, 8.7, 6.0)	8, 9a	7, 8, 10
10	15.4	2.11 (s)		
11	169.4			
12	149.8			
13	123.7	8.10 (s)		11, 12, 14[b]
14	159.9			
N5		8.61 (br d; 6.7)	15	14, 15, 17
15	44.4	5.23 (quintet; 6.7)	N5H, 16	14, 16, 17
16	21.0	1.65 (d; 6.7)	15	15, 17
17	162.1			
18	154.2			
19	128.8			
20	11.7	2.67 (s)		18, 19, 21
21	160.6			

[a]Carbon correlations. [b]Determined by selective INEPT experiment.

Dendroamide C (3): $[\alpha]_D$ +26.0° (c 3.75, CH_2Cl_2); UV λ_{max} (MeOH) 224 nm (ϵ 30 900); IR (neat) ν_{max} 3395, 1667, 1538, 1046 cm^{-1}; EIMS *m/z* (rel intensity) 536 (3.0), 472 (100), 139 (25), 138 (29), 136 (11); high resolution EIMS *m/z* 536.1009 ($C_{21}H_{24}N_6O_5S_3$, −3.9 mmu error), 472.0956 ($C_{20}H_{20}N_6O_4S_2$, +3.1 mmu error); ^{1}H and ^{13}C-NMR see Table 3.

Direct Acid Hydrolysis. Each dendroamide (1.0 mg) in 0.3 mL of 6 N HCl was heated in a reaction vial

Table 3. NMR Data for Dendroamide C (3) (Cyclo-D-Ala-Tzl-D-Met(O)-Tzl-D-Ala-mOzl)

C or N position	δ (^{13}C)	δ (^{1}H) (mult, J_{HH}, Hz)	^{1}H−^{1}H COSY	HMBC[a]
N1		8.61 (br d; 6.7)	1	21, 3
1	47.9	5.43 (quintet; 6.7)	N1H, 2	21, 2, 3
2	25.1	1.68 (d; 6.7)	1	1, 3
3	172.1			
4	148.5			
5	124.9	8.15 (s)	3, 4, 6	
6	160.1			
N3		8.54 (m)	7, 7′	6, 11
7	50.3	5.81 (dt; 5.1, 8.1)	N3H, 8a, 8b	6, 8, 9, 11
7′[b]	50.0	5.81 (dt; 5.1, 8.1)	N3H, 8′a, 8′b	6, 8′, 9′, 11
8	31.7	2.35 for 8a (m)	7, 8b, 9a, 9b	7, 9, 11
		2.51 for 8b (m)	7, 8a, 9a, 9b	7, 9, 11
8′[b]	32.4	2.35 for 8′a (m)	7′, 8′b, 9′a, 9′b	7′, 9′, 11
		2.51 for 8′b (m)	7′, 8′a, 9′a, 9′b	7′, 9′, 11
9	50.0	2.80 for 9a (ddd; −13.2, 10.2, 5.6)	8a, 8b, 9b	7, 8, 10
		2.84 for 9b (ddd; −13.2, 10.4, 5.6)	8a, 8b, 9a	7, 8, 10
9′[b]	50.6	2.72 for 9′a (ddd; −13.0, 10.4, 4.9)	8′a, 8′b, 9′b	7′, 8′, 10′
		2.90 for 9′b (ddd; −13.0, 10.6, 5.8)	8′a, 8′b, 9′a	7′, 8′, 10′
10	38.9	2.53 (s)		9
10′[b]	39.2	2.54 (s)		9′
11[b]	168.9			
12	149.8			
13	124.1	8.13 (s)		11, 12, 14
14	159.7			
N5		8.60 (br d; 6.9)	15	14, 17
15	44.4	5.24 (quintet; 6.9)	N5H, 16	14, 16, 17
16	21.0	1.65 (d; 6.9)	15	15, 17
17	162.0			
18	154.2			
19	128.7			
20	11.7	2.65 (s)		18, 19, 21
21	160.6			

[a] Carbon correlations. [b] Signals doubled due to presence of *R* and *S* sulfoxides.

at 110 °C for 16 h. The solvent was removed under vacuum, and the dried acid hydrolysate was derivatized and subjected to Marfey analysis.

Ozonolysis and Acid Hydrolysis. A stream of O_3 was bubbled through a solution of each dendroamide (1.0 mg) in 10 mL of CH_2Cl_2 at 25 °C for 10 min. The solvent was removed under a stream of N_2, and the residue was dissolved in 0.3 mL of 6 N HCl and heated at 110 °C for 24 h. After removal of the solvent, the residue was derivatized and subjected to Marfey and chiral GC/MS analyses.

Marfey Analysis. Dried acid hydrolysate described above (0.3−1.0 mg) in 100 μL of water was treated successively with 1 mg of sodium (5-fluoro-2,4-dinitrophenyl)-L-alaninamide (FDAA, Marfey reagent) in 200 μL of acetone followed by 40 μL of 1 N sodium bicarbonate solution, and the mixture was heated at 45 °C for 1 h. After the mixture was cooled to room temperature, 20 μL of 2 N HCl was added, and the resulting solution was analyzed by reversed-phase HPLC on a 250 × 4.6 mm C18 column (ALTEX ultrasphere 5 μm) using a linear gradient of 10−50% MeCN in 0.05 M Et_3N/phosphate buffer at pH 3 over 45 min (isocratic conditions thereafter) at a flow rate of 1 mL/min. Standards were prepared using the same procedure by treating each authentic amino acid. The comparison of the retention times and coinjection experiments of the FDAA-derivatized direct hydrolysates, ozonized hydrolysates, and similarly derivatized authentic amino acids established that all amino acid residues in **1**−**3** have the D-configuration.

Chiral GC/MS Analysis. A mixture of the dried hydrolysate described above in 0.3 mL of 2-propanol and 0.05 mL of acetyl chloride was heated at 100 °C for 45 min. The excess reagent was then evaporated at 115 °C under a stream of nitrogen, and the residue, which contained the isopropyl esters of the various amino acids, was treated with 0.5 mL of 1:1 $(CF_3CF_2CO)_2O$/CH_2Cl_2 at 100 °C for 15 min. After the residue was cooled to room temperature, the excess reagent was evaporated with a stream of dry nitrogen, and the resulting mixture of isopropyl esters of *N*-pentafluoropropyl amino acids was dissolved in 0.5 mL of CH_2Cl_2 for GCMS analysis on a 25 m × 0.25 mm Chirasil-Val column (Alltech). The following conditions were used for the GC: a 12 psi head pressure (flow rate estimated to be about 0.6 mL/s) and a column temperature held at 35 °C for 4 min after injection of the sample, then increased from 35 to 100 °C at 2.0°/min, and finally increased from 100 to 195 °C at 6.0 °C/min and held for 12.0 min. The same procedure was repeated for standard samples of authentic amino acid. By comparison of the retention times and mass spectra of the derivatized hydrolysates and authentic amino acids, D-Ala and D-Val were detected in the hydrolysate of ozonized **1**, whereas D-Ala and D-Met-sulfone were detected in the hydrolysate of ozonized **2** and **3**. This result strongly confirmed the result from Marfey analysis that the methionine residue in **2** and the methionine sulfoxide residue in **3** were both oxidized during the ozonolysis to the methionine sulfone.

Evaluation of MDR Reversal. MCF-7 breast carcinoma cells and MCF-7/ADR cells, a MDR subline which overexpresses P-glycoprotein,[33] were obtained from the Division of Cancer Treatment of the National Cancer Institute. MCF-7/VP cells, which express multidrug resistance-related protein (MRP) but not P-glycoprotein,[34] were provided by Drs. Schneider and Cowan. The effects of drugs on cell growth were characterized using the sulforhodamine binding assay[35] as previously described.[36,37] Briefly, MCF-7 or MCF-7/ADR cells were treated with increasing doses of dendroamide A in the presence of minimally toxic doses of cisplatin, daunomycin, or actinomycin D. In experiments with MCF-7/VP cells, cultures were treated with 0, 5, or 10 μM dendroamide A in the presence of varying concentrations of vinblastine, doxorubicin, etoposide, or cisplatin. In both types of experiments, the percentage of cells killed by the combination of agents was determined 48 h later. The effect of dendroamide A on intracellular drug accumulation was determined[36,37] using 20 nM [^{3}H]vinblastine sulfate (10−15 Ci/mmol from Amersham Corp.). For photolabeling studies, samples containing approximately 25 μg of membrane protein from MCF-7/ADR cells, 0.75 μM [^{3}H]azidopine (~1 μCi, from Amersham Corp.) and dendroamide A were incubated at room temperature in the dark for 30 min as previously described.[37] Samples were then exposed to 200 000 μJ of UV light and subjected to sodium dodecylsulfate−polyacrylamide gel electrophoresis on 7.5% gels. Radiolabeled proteins were detected by fluorography using Amplify and Hyperfilm-MP (both from Amersham). Verapamil was used as a positive control in all of the above experiments.

Acknowledgment. Studies on the isolation and structure determination of the dendroamides were supported by Grant No. CA12623 (R.E.M.) from the National Cancer Institute, Department of Health and Human Services. Research on the MDR-reversing activity of **1** was supported by Grant No. CA64983 (C.D.S.). One of us (J.O.) thanks the Toray Research Center, Otsu, Shiga, Japan, for supporting a study leave at the University of Hawaii in 1994−1995.

References and Notes

(1) Endicott, J. A.; Ling, V. *Annu. Rev. Biochem.* **1989**, *58*, 137−171.
(2) Golakoti, T.; Ohtani, I.; Patterson, G. M. L.; Moore, R. E.; Corbett, T. H.; Valeriote, F. A.; Demchik, L. *J. Am. Chem. Soc.* **1994**, *116*, 4729−4737.
(3) Moore, R. E.; Patterson, G. M. L.; Mynderse, J. S.; Barchi, J., Jr.; Norton, T.; Furusawa, E.; Furusawa, S. *Pure Appl. Chem.* **1986**, *58*, 263−271.
(4) Princep, M. R.; Caplan, F. R.; Moore, R. E.; Patterson, G. M. L.; Smith, C. D. *J. Am. Chem. Soc.* **1992**, *114*, 385−386.
(5) Stratmann K.; Burgoyne D. L.; Moore R. E.; Patterson G. M. L.; Smith C. D. *J. Org. Chem.* **1994,** *59*, 7219−7226.
(6) Williams A. B.; Jacobs R. S. *Cancer Lett.* **1993**, *71*, 97−102.
(7) Ireland, C. M.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688−5691.
(8) Wasylyk, J. M.; Biskupiak, J. E.; Costello, C. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 4445−4449.
(9) Sesin, D. F., Gaskell, S. J.; Ireland, C. M. *Bull. Soc. Chim. Belg.* **1986**, *95*, 853−867.
(10) Ireland, C. M.; Durso, A. R., Jr.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807−1811.
(11) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Watters, D. J.; *J. Med. Chem.* **1989**, *32*, 1349−1354.
(12) Schmitz, F. J.; Ksebati, M. B.; Chang, J. S.; Wang, J. L.; Hossain, M. B.; van der Helm, D.; Engel, M. H.; Serban, A.; Silfer, J. A. *J. Org. Chem.* **1989**, *54*, 3463−3472.
(13) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1354−1359.
(14) Hawkins, C. J.; Lavin, M. F.; Marshall, K. A.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1990**, *33*, 1634−1638.
(15) Foster, M. P.; Concepcion, G. P.; Caraan, G. B.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 6671−6675
(16) McDonald, L. A.; Ireland, C. M. *J. Nat. Prod.* **1992**, *55*, 376−379.
(17) McDonald, L. A.; Foster, M. P.; Phillips, D. R.; Ireland, C. M.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1992**, *57*, 4616−4624.
(18) Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; van den Brenk, A. L.; Watters, D. J. *Tetrahedron* **1992**, *48*, 341−348.
(19) Princep, M. R.; Moore, R. E.; Levin, I. A.; Patterson, G. M. L. *J. Nat. Prod.* **1992**, *55*, 140−142.
(20) Todorova, A. K.; Jüttner, F.; Linden, A.; Plüss, T.; von Philipsborn, W. *J. Org. Chem.* **1995**, *60*, 7891−7895.
(21) Admi, V.; Carmeli, S.; Afek, U. *J. Nat. Prod.* **1996**, *59*, 396−399.
(22) Patterson G. M. L.; Baldwin, C. L.; Bolis, C. M.; Caplan, F. R.; Karuso, H.; Larsen, L. K.; Levin, I. A.; Moore, R. E.; Nelson, C. S.; Tschappat, K. D.; Tuang, G. D.; Furusawa, E.; Furusawa, S.; Norton, T. R.; Raybourne, R. B. *J. Phycol.* **1991**, *27*, 530−536.
(23) Marfey, P. *Carlsberg Res. Commun.* **1984,** *49*, 591−596.
(24) Turchi, I. J.; Dewer, M. J. S. *Chem. Rev.* **1975**, *75*, 389−437.
(25) Fusetani, N.; Sugawara, T.; Matsunaga, S. *J. Am. Chem. Soc.* **1991**, *113*, 7811−7812.
(26) Carmeli, S.; Paik, S.; Moore, R. E.; Patterson, G. M. L.; Yoshida, W. Y. *Tetrahedron Lett.* **1993**, *34*, 6681−6684.
(27) Frank, H.; Nicholson, G. J.; Bayer, E. *J. Chromatogr. Sci.* **1977**, *15*, 174−176.
(28) Beck, W. T.; Qian, X.-D. *Biochem. Pharm.* **1992**, *43*, 89−93.
(29) (a) Piantini, U.; Solensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800−6801. (b) Muller, N.; Ernst, R. R.; Wuthrich, K. *J. Am. Chem. Soc.* **1986**, *106*, 6482−6492.
(30) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565−569.
(31) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(32) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314−318.
(33) Fairchild, C. R.; Ivy, S. P.; Kao-Shan, C.-S.; Whang-Peng, J.; Rosen, N.; Israel, M. A.; Melera, P. W.; Cowan, K. H.; Goldsmith, M. E. *Cancer Res.* **1987**, *47*, 5141−5148.
(34) Schneider, E.; Horton, J. K.; Yang, C.-H.; Nakagawa, M.; Cown, K. H. *Cancer Res.* **1994**, *54*, 152−158.
(35) Skehan, P.; Stoneng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.
(36) Smith, C. D.; Prinsep, M. R.; Caplan, F. R.; Moore, R. E.; Patterson, G. M. L. *Oncol. Res.* **1994**, *6*, 211−218.
(37) Smith, C. D.; Zilfou, J. T.; Stratmann, K.; Patterson, G. M. L.; Moore, R. E. *Mol. Pharm.* **1995**, *47*, 241−247.

NP960178S