Methanolysis Products of Disorazole A_1

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Received September 26, 2005

Two new disorazole analogues were synthesized by acid-promoted methanolysis of disorazole A_1 (**1**). Structural elucidation of both products (**2** and **3**), through 1D and 2D NMR experiments, verified that each resulted from epoxide cleavage. With antiproliferative activities in susceptible cell lines comparable to that of disorazole A_1, these methanolysis products indicate that the C-9−C-10 epoxide is not an essential structural component for biological activity.

The disorazoles comprise a family of structurally complex polyketide natural products originally isolated from the fermentation broth of *Sorangium cellulosum*, strain So ce12, by researchers at the Gesellschaft für Biotechnologische Forschung mbH.[1] Multidisciplinary interest in the disorazoles is highlighted by the elucidation of mode of action,[2] identification of biosynthetic genes,[3] and synthetic studies[4] that include the total synthesis of disorazole C_1.[4e] Disorazole A_1 (**1**), the major component of the fermentation, displays potent cytotoxicity with pM IC_{50} values against several human cancer cell lines.[2] Its antimitotic activity stems from irreversible tubulin binding at the vinblastine binding site, resulting in inhibition of tubulin polymerization as well as microtubule destabilization.[2b,4d] The disorazoles thus join a growing list of tubulin binding natural products that include the pharmaceutically important *Vinca* alkaloids, taxanes, and epothilones.

Surprisingly little information has been published to aid in establishing a disorazole SAR. Although the structures of 29 different naturally occurring disorazoles have been disclosed, cytotoxicity data for only those analogues containing a C-9−C-10 epoxide have been reported.[2,5] The inherent reactivity of such epoxides, especially divinyl species as found in **1**, has raised concerns of instability and potential pharmacological liabilities. As a result of these concerns, the total synthesis of a C-9−C-10 cyclopropyl disorazole analogue is currently being pursued by Hoffmann and co-workers.[4d]

A more direct method to evaluate the biological role of this epoxide involves examining simple, semisynthetic derivatives of the natural product itself; consequently, we performed solvolysis studies on **1** to establish an initial disorazole SAR. Methanolic solutions of **1** were treated with strong protic acids such as methanesulfonic acid and trifluoroacetic acid to yield a major methanolysis product (**2**) as well as multiple compounds resulting from dehydration as determined by LCMS. The use of a weaker acid, dichloroacetic acid, reduced the formation of these dehydration products; however, a previously unobserved methanol adduct (**3**) was generated in equal amounts to **2** (Scheme 1).

The molecular formula of **2** was assigned as $C_{44}H_{58}N_2O_{11}$ on the basis of HRMS and NMR data, and complete structural elucidation required analysis of COSY, HSQC, HMBC, and selective TOCSY spectra. Stereochemistry as drawn in Scheme 1 reflects data reported by Höfle and was not determined during the course of this work.[6] Comparison of the ^{1}H NMR spectra of **1** and **2** highlighted two noteworthy differences. First, two methoxy singlets (δ 3.36 and 3.15) were observed in the ^{1}H NMR spectrum of **2**, not one as is the case for **1**. Second, resonances corresponding to H-9 and H-10 of **2**, assigned on the basis of COSY correlation beginning at the H-5 doublet (δ 6.13, J = 11.8 Hz), are shifted downfield relative to those of **1** by approximately 0.2 and 0.6 ppm, respectively, suggesting fragmentation of the epoxide.[7] The regiochemistry of the methanolysis of the epoxide was determined on the basis of HMBC correlation from H-9 to C-22. Olefin geometries were assigned using ^{1}H−^{1}H coupling constants (Table 1), except for three olefins with unresolved signals, C-11−C-12, C-17−C-18, and C-17′−C-18′. The configuration of the C-11−C-12 double bond was assigned as *Z* on the basis of selective TOCSY data of H-10 indicating a correlation with H-11 at 5.44 ppm (dd, J = 11.2, 7.1). The differences in the ^{13}C NMR resonances of C-19 and C-19′ for **1** and **2** are approximately 0.1 ppm each, suggesting no olefin isomerization at C-17−C-18 or C-17′−C-18′.[7]

Structural elucidation of **3**, $C_{44}H_{58}N_2O_{11}$ on the basis of HRMS data, also required analysis of COSY, HSQC, and HMBC spectra. Initial inspection of the ^{1}H NMR for **3** suggested that this compound also resulted from methanolysis of the C-9−C-10 epoxide because of the presence of two methoxy singlets (δ 3.25 and 3.24) and because H-10 had shifted downfield by 0.9 ppm relative to H-10 of **1**. Furthermore, an upfield shift of the H-7 signal of **3** by approximately 2 ppm relative to that of **1** indicated a disruption of the C-5−C-8 diene system.[8] An HMBC correlation from H-7 to C-22 verified that the epoxide had fragmented by a 1,4-addition to C-7. Connectivity was established by a COSY spectrum beginning with the resonance at 6.38 ppm (dd, J = 11.9, 1.1 Hz, H-5), and olefin geometries were assigned on the basis of ^{1}H−^{1}H coupling constants (Table 1), except for two olefins with unresolved signals, C-17−C-18 and C-17′−C-18′. Comparison of ^{13}C NMR data for C-19 and C-19′ of **3** and **1** resulted in the assignment of both olefins, C-17−C-18 and C-17′−C-18′, as *E*-isomers.[8]

Both **2** and **3** are potent inhibitors of cell proliferation, as indicated in Table 2. In the susceptible cell lines MCF-7, A549, and CCRF-CEM, **2** is equipotent to disorazole A_1 but is 3- to 5-fold less active than **1** in multidrug-resistant cell lines NCI/ADR and CCRF-CEM/PTX that overexpress P-glycoprotein. Although **3** retains substantial potency, it is clearly less active than **1** especially in the resistant lines.[9]

In conclusion, acid-promoted methanolysis of disorazole A_1 generated two new disorazole analogues, **2** and **3**, each resulting from the fragmentation of the C-9−C-10 epoxide. Cell viability assays verified that biological activity is not dependent on the presence of this epoxide. Given these results, semisynthesis may provide access to new disorazole analogues that retain potent cytotoxicity yet possess more favorable pharmacological profiles including reduced instability. Interestingly neither of these methanolysis products corresponded to the methanol adducts, disorazole D_4 and D_5, originally isolated from *S. cellulosum*.[1]

Experimental Section

General Experimental Procedures. Disorazole A_1 was isolated from the fermentation broth of *S. cellulosum*, strain So ce12, essentially

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10.1021/np050367n CCC: $33.50
Published on Web 12/14/2005

Scheme 1. Methanolysis of Disorazole A_1 (**1**)

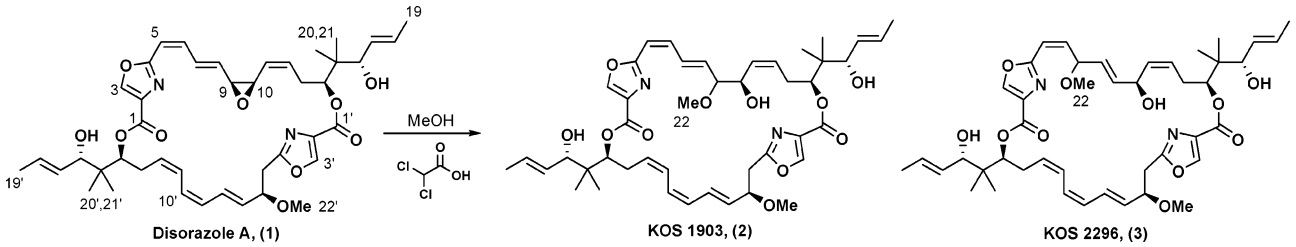

Table 1. NMR Data for **2** in $CDCl_3$ and **3** in Acetone-d_6

	KOS 1903 (**2**)		KOS 2296 (**3**)	
position	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1		161.1[k]		161.6[v]
2		133.4		135.2[w]
3	7.99(s)	143.2	8.48 (s)	145.2[x]
4		161.5		161.1
5	6.13 (d, 11.8)		6.38 (dd, 11.9, 1.1)	115.9
6	6.57 (t, 11.8)[a]		6.03 (dd, 12.0, 8.3)	142.2
7	7.12 (dd, 15.1, 11.7)		5.13 (dd, 7.8, 7.2)	78.6
8	5.84 (dd, 15.3, 6.8)		5.61 (dd, 16.0, 6.2)[c]	134.1[y]
9	3.79−3.82 (m)[e]		5.82 (ddd, 15.6, 4.6, 0.8)	136.7
10	4.55 (dd, 7.2, 2.8)	69.4	4.96 (dd, 8.0, 4.8)	67.6
11	5.44 (dd, 11.2, 7.1)[b]	128.9[l]	5.19−5.28 (m)[p]	134.4
12	5.40−5.66 (m)[f]	130.3	5.38 (ddd, 10.7, 7.8, 7.1)	128.2
13	2.63 (m)	28.6	2.45−2.70 (m)[q]	28.8[z]
	2.42 (dd, 15.4, 5.1)			
14	5.18 (dd, 10.0, 2.4)	78.5	5.19−5.28 (m)[p]	78.3[aa]
15		41.6[m]		42.6[bb]
16	3.79−3.82 (m)[e]	76.0[d]	3.92 (d, 6.5)[r]	76.8[d]
17	5.40−5.66 (m)[f]	128.9[l]	5.49−5.68 (m)[s]	132.1[d,y]
18	5.40−5.66 (m)[f]	129.2[d,l]	5.49−5.68 (m)[s]	132.1[d,y]
19	1.64 (d, 5.6)[g]	17.8[n]	1.64−1.67 (m)[t]	18.0[d]
20	0.93 (s)[h]	18.3°	0.99 (s)[u]	19.2[d,cc]
21	0.91 (s)[h]	18.5°	0.96 (s)[u]	19.2[d,cc]
22-OMe	3.36 (s)	56.7	3.24 (s)	56.0
1′		161.3[k]		161.5[v]
2′		132.9		134.4[w]
3′	8.10 (s)	144.1	8.47 (s)	145.5[x]
4′		162.2		163.0
5′	3.00 (dd, 15.1, 6.5)	34.9	3.15 (dd, 15.1, 4.1)	34.1
	2.90 (dd, 15.1, 6.5)		2.96 (dd, 15.1, 9.2)	
6′	3.97 (app dd, 14.1, 6.7)	78.8	4.11 (ddd, 8.9, 8.7, 4.2)	80.5
7′	5.40−5.66 (m)[f]	132.9	5.49−5.68 (m)[s]	130.4[y]
8′	6.52 (dd, 15.1, 11.4)[a]	128.4[d]	6.57 (dd, 15.1, 11.5)	130.2
9′	5.94 (t, 11)	128.4[d]	5.87 (dt, 11.1)	129.1
10′	6.24 (t, 11.1)	125.2	6.25 (t, 11.4)	126.2
11′	6.41 (t, 11.1)[a]	126.3	6.41 (t, 11.1)	126.4
12′	5.40−5.66 (m)[f]	129.3[d,l]	5.49−5.68 (m)[s]	128.2[y]
13′	2.75 (m)	27.8	2.45−2.70 (m)[q]	29.1[z]
	2.29 (d br)			
14′	5.30 (dd, 11.2, 2.4)	77.7	5.19−5.28 (m)[p]	78.7[aa]
15′		41.5[m]		42.8[bb]
16′	3.79−3.82 (m)[e]	76.0[d]	3.86 (d, 6.5)[r]	76.8[d]
17′	5.40−5.66 (m)[f]	129.3[d,l]	5.49−5.68 (m)[s]	127.9[y]
18′	5.40−5.66 (m)[f]	129.5[l]	5.49−5.68 (m)[s]	127.0[y]
19′	1.64 (d, 5.6)[g]	17.8[n]	1.64−1.67 (m)[t]	18.0[d]
20′	0.88 (s)[h]	19.0[o]	0.94 (s)[u]	19.5[cc]
21′	0.86 (s)[h]	19.3[o]	0.91 (s)[u]	19.7[cc]
22′-OMe	3.15 (s)	56.4	3.25 (s)	56.2

[a] Due to signal overlap in $CDCl_3$; shift and *J* values measured in acetone-d_6. [b,c] Shift and *J* values determined by selective TOCSY (4.55 and 5.13 ppm, respectively). [d] Double intensity. [e−z,aa−cc] Interchangeable resonances.

as previously reported.[2a] Optical rotations were measured using a Perkin-Elmer 241 polarimeter; UV spectra were acquired by diode-array-detected HPLC; NMR spectra were obtained using a Bruker DRX 400 spectrometer. ^{1}H NMR spectra were collected at 400 MHz, and signals are reported in parts per million (δ ppm) relative to residual solvent peak ($CHCl_3$ = 7.26 ppm; CD_3COCD_2H = 2.05 ppm). ^{13}C NMR spectra were collected at 100 MHz, and signals are reported in parts per million (δ ppm) relative to residual solvent peak ($CDCl_3$ = 77.0 ppm; CD_3-$COCD_3$ = 29.9 ppm). HRMS were obtained by flow injection analysis on a Mariner TOF spectrometer (Applied Biosystems, Foster City, CA) equipped with a turbo-ionspray source in positive mode (spray tip potential, 5000 V; spray chamber temperature, 400 °C; nozzle potential, 115 V). The resolution of the measured mass was >8000 full width/half-maximum. Analytical HPLC was performed using a model L-7100 pump, a model L-7200 autosampler equipped with a 40 μL loop (Hitachi Instruments, Inc., Fremont, CA), and a model Spectra 100 UV detector (Thermo Electron Corporation, Boston, MA). The column was a 5 μm 4.6 mm × 250 mm Luna C18 (2) (Phenomenex, Torrance, CA). Analyses were run at 1.25 mL/min with a 55:45 mixture of acetonitrile and water while monitoring at 270 nm. Preparative HPLC was performed using a Hitachi Prep-800 preparative chromatography system equipped with a Knauer spectrophotometer K-2501 UV detector and a

Table 2. Inhibition of Cellular Proliferation by Disorazole A_1 (**1**), **2**, and **3**

		compound, IC_{50} (nM)		
cell line	tumor origin	**1**	**2**	**3**
MCF-7	breast	0.25	0.24	0.62
NCI/ADR	breast, MDR	0.52	2.8	28
A549	lung	0.31	0.25	0.73
CCRF-CEM	T-cell leukemia	0.070	0.11	0.33
CCRF-CEM/PTX	T-cell leukemia, MDR	0.066	0.24	1.3

2 mL injection loop. A 7.5 cm Varian Load & Lock preparative HPLC column was packed to a bed height of 28 cm with Lichroprep RP-18, 15−25 μm (EM Science, NJ). The system was operated at a flow rate of 125 mL/min with a monitoring wavelength of 270 nm.

Methanolysis of Disorazole A_1 (1). To a stirred solution of disorazole A_1 (502 mg, 0.661 mmol) in MeOH (125 mL) in a 0 °C water−ice bath was added dichloroacetic acid (313 μL). The reaction was placed in a 4 °C refrigerator and incubated at this temperature for 17 h, then at ambient temperature for 30 min. After quenching with phosphate buffer (0.05 M, pH 8), the reaction solution was concentrated by 50% by rotary evaporation. The concentrate was partitioned between EtOAc and phosphate buffer (0.05 M, pH 8). The layers were separated, and the aqueous phase was extracted with EtOAc (3×). The solvent was removed, and the crude residue was purified by preparative HPLC to produce a 1:1 mixture of **2**:**3** (169 mg, 0.214 mmol, 32%). This mixture was rechromatographed to provide both **2** (40 mg, 0.051 mmol, 8%) and **3** (25 mg, 0.032 mmol, 5%) in pure form and the balance of the material in mixed fractions.

KOS 1903 (2): off-white solid; $[\alpha]_D$ −63.3 (*c* 1.52, MeOH); UV (DAD, MeCN−H_2O, 55:45) λ_{max} 271 nm; ^1H and ^{13}C NMR, see Table 1; MS *m*/*z* $[M + Na]^+$ calcd for $C_{44}H_{58}N_2O_{11}Na$ 813.3933, obsd 813.3929.

KOS 2296 (3): off-white solid; $[\alpha]_D$ +33.1 (*c* 0.72, MeOH); UV (DAD, MeCN−H_2O, 55:45) λ_{max} 263, 271 (sh) nm; ^1H and ^{13}C NMR, see Table 1; MS *m*/*z* $[M + Na]^+$ calcd for $C_{44}H_{58}N_2O_{11}Na$ 813.3933, obsd 813.3941.

Biological Assay. MCF-7 and A549 cells from American Type Culture Collection (ATCC, Manassus, VA) and NCI/ADR cells from the National Cancer Institute (NCI, Bethesda, MD) were cultured in RPMI 1640 media with 10% fetal bovine serum (Mediatech, Inc., Herndon, VA). CCRF-CEM and CCRF-CEM/PTX cell lines from Memorial Sloan-Kettering Institute for Cancer Research (New York, NY) were cultured in RMPI 1640 media with 5% fetal bovine serum. All human cancer cell lines were maintained in air atmosphere with 5% CO_2 at 37 °C. Cells were seeded in duplicate in 96-well microtiter plates at approximately 5000 cells per well, then incubated in 5% CO_2 for 24 h. Serial dilutions (10-fold) of compounds were added to the wells, and the mixtures were incubated for 72 h. Cell viability was determined using the CellTiter-Glo luminescent cell viability assay (Promega US, Madison, WI). IC_{50} is defined as the concentration of compound required to inhibit cell growth by 50%.

Acknowledgment. We thank Dr. J. Carney, C. Tran, and N. Viswanathan for mass spectral analyses and for helpful discussions. We also thank Professor J. Ellman and H. Peltier at the University of California, Berkeley, for assistance with optical rotations.

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(7) Selected NMR data for disorazole A_1 (**1**) in $CDCl_3$: ^1H NMR (400 MHz) δ 3.55 (dd, J = 9.7, 4.3 Hz, H-9), 3.99 (dd, J = 9.7, 4.2 Hz, H-10); ^{13}C NMR (100 MHz) δ 17.9 (double intensity, C-19 and C-19′).
(8) NMR data for **3** were compared with reported data for disorazole A_1 in acetone-d_6, ref 1.
(9) Using the Kosan standard operating procedure to determine cell viability (see Experimental Section), we were unable to reproduce the previously reported single digit pM IC_{50} values for **1**, ref 2.

NP050367N