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Discokiolides. Cytotoxic Cyclic Depsipeptides from the Marine Sponge *Discodermia kiiensis*

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Cytotoxic cyclic depsipeptides discokiolides A-D were isolated from the marine sponge *Discodermia kiiensis* as methyl esters. The structures were mainly determined by NMR techniques. Discokiolides are the unique depsipeptides containing oxazole ring.

Exploratory studies of bioactive metabolites from marine sponges have been fruitful,[1] and have yet been continued with great interests. Several metabolites have been found from the genus *Discodermia*.[2-6] Here we describe the isolation and characterization of new cyclic depsipeptides, discokiolide-A (**1a**), -B (**2a**), -C (**3a**), and -D (**4a**) from *D. kiiensis* as methyl esters.

The methanol extract of the sponge (160 g, after extraction) was chromatographed on an ODS column (YMCgel ODS 105-125 μm, 150 g) into three fractions (H_2O, MeOH, and CH_2Cl_2 eluates). From the CH_2Cl_2 eluate (0.7 g), 24-methylenecholest-4-en-3-one, cholest-4-en-3,6-dione, and 24-methylenecholest-4-en-3,6-dione were identified. The MeOH eluate (2.9 g) was fractionated by centrifugal partition chromatography (CPC; Hex:MeOH:EtOAc:H_2O = 4:4:1:1, eluted with the upper layer) and the retained material (1.85 g) was subjected again to CPC (CH_2Cl_2:MeOH:H_2O = 10:7:3, eluted with the upper layer). The eluted material (1.67 g) was then applied to a Develosil ODS column (φ20 × 250 mm, 10-20 μm, MeOH:H_2O = 8:2). Three (1.0 g) of five fractions were purified by Asahipak ODP (φ21 × 300 mm, MeOH:H_2O = 8:2) followed by Asahipak GS-310 (φ20 × 500 mm, MeOH), and an acid component (463 mg, single peak) was obtained. It showed a potent proliferation effect *in vitro* (Table 1), while its ester (methylation by diazomethane) had no activity.

Table 1. Proliferation effect *in vitro* (IC_{50} μg/ml)

	P388	P388/ADM	B16-BL6	Lewis	Lu-99	HT-29	CCD-19Lu
Acid	2.6	7.2	1.6	1.2	0.7	1.2	0.5
Ester	>10	>10	>10	>10	>10	>10	>10

Careful examination by RP-HPLC revealed that the ester was not a single compound but a mixture of four components. Thus, the ester mixture was subjected to RP-HPLC (Develosil ODS, 10-20 μm, φ20 × 250 mm, MeOH:H_2O = 8:2) and gave amorphous methyl esters of discokiolide-A (**1b**, 56 mg), -B (**2b**, 136 mg), -C (**3b**, 15 mg), and -D (**4b**, 14 mg) (Table 2).

Structural examination was first done on discokiolide-B methyl ester **2b**, the most abundant component. IR spectrum indicated the presence of NH, ester, and amide groups. High-resolution liquid SIMS (HR-LSIMS) in a matrix of *m*-nitrobenzylalcohol gave $(M+H)^+$ ion at *m/z* 1055.5447, which agreed with the molecular formula $C_{55}H_{75}N_8O_{13}$ (Δ = − 0.2 μmu). Extensive NMR analysis of DQF-COSY, HOHAHA (t_m = 40 ms) and HMQC

Table 2. Physical Properties of Methyl Esters of Discokiolide A-D

	-A (**1b**)	-B (**2b**)	-C (**3b**)	-D (**4b**)
$[\alpha]_D$ (25°C, MeOH)(*c*)	−52.3°(1.0)	−43.5°(1.0)	−28.7°(0.6)	−49.5°(0.6)
UV_{max} (MeOH) nm (ε)	250 (19,600)	251 (19,500)	252 (18,200)	258 (15,900)
IR ($CHCl_3$, cm^{-1})	3404, 1738 1683, 1633	3404, 1736 1682, 1631	3404, 1738 1681, 1633	3404, 1736 1685, 1632
LSIMS (*m/z*) $(M+H)^+$	1041	1055	1025	1055
a	635	649	649	649
b (a - NMeIle 1)	-----	522	-----	522
c	396	410	410	410
d (c - NMeIle 1)	269	283	283	283
$(deH\text{-}discokiic\ acid)^+$	266	266	266	266
e (NMeIle 1-Asn)	240	240	240	240
e - CO (or e - CHMe)	212	212	212	212
$Ph\text{-}CH{=}CH\text{-}CH^+Me$	131	131	131	-----
$Ph\text{-}CH{=}O^+Me$	121	121	(91)	121
$(Ile)^+$	113	113	113	113
$(Asp)^+$ - CO	100	100	100	100

(J = 140 Hz) spectra led to the assignment of all the proton and carbon signals. HOHAHA data revealed the presence of ten spin coupled connectivity networks, and analysis of COSY and HMQC spectra indicated the following partial structures: two NH-CH-CH_2, two CH-CH(CH_3)-CH_2-CH_3, CH=CH-CH-CH_3, NH-CH-CH, CH-CH_2-CH_2-CH_2, CH-CH-CH_3, and two aromatic rings. Connection of the coupled fragments obtained above with the other groups and the non-protonated atoms were determined by using ROESY (300 ms) and HMBC (τ = 60 ms) experiments. Five of the six peptide bonds in the cyclic backbone region were easily elucidated by long-range CH correlation found in the carbonyl region of the HMBC spectrum (Table 3) With the peptide bond of Asn-Pro part, however, we could not discriminate α- and β-peptide bonds using the CH correlation. This descrimination was accomplished by analysing ROESY spectrum. We observed strong NOE peaks between α CH of Asn and δ CH_2 of Pro, implying that α-peptide bond of the Asn-Pro part is preferred. ROESY analysis also gave useful information on the peptide bonds of two NMeIle residues. Strong NOE peak found between each α CH protons of the residues demonstrates *cis* geometry about the NMeIle 1–NMeIle 2 amide bond. In the other part of the molecule, arrangement of the fragments, non-protonated atoms, and the other groups except for the oxazole ring moiety were easily completed in a similar manner, because all the non-protonated carbons had at least one long range CH correlation. The presence of the oxazole ring was determined on the basis of the one bond CH coupling constant ($^1J_{CH}$ = 209 Hz) at the C-5 position in the discokiic acid moiety, because this is the only structure which includes the CH constituent with $^1J_{CH}$ = 209 Hz. Using the same procedure for structure elucidation of the remaining esters **1b**, **3b**, and **4b**, led to the following results: i) NMeIle 1 of **2b** is replaced by NMeVal in **1b**, ii) the methoxyl group of **2b** is substituted by a hydrogen atom in **3b**, and iii) the compound **4b** was the double bond isomer of **2b** ($\Delta^8 \rightarrow \Delta^7$).

The structures deduced for the discokiolides were also supported by HR-LSIMS. The prominent ion peaks of the high mass range are as shown in Fig. 1. Fragment a (*m/z* 649.3598; Δ = 0.0 μmu) is derived from $(M+H)^+$ by β-elimination of the discokiic acid moiety, followed by cleavage between NMeIle 2 and Asn. Fragment c (*m/z* 410.2290; Δ = + 0.2 μmu) agreed with a ion formed by cleavage of the discokiic acid moiety from fragment a. Loss of NMeIle 2 from fragment a and c gives fragment b (*m/z* 522.2604; Δ = + 0.2 μmu) and d (*m/z* 283.1295; Δ = + 0.2 μmu), respectively. For fragment e ($C_{11}H_{18}N_3O_3$, *m/z* 240.1346; − 0.1 μmu), the dipeptide

Table 3. NMR data for **2b** ($CDCl_3$, 400 MHz)

Residue	Carbons	^{13}C (ppm)	^{1}H ppm (mult., J in Hz)	Long-range ^{1}H-^{13}C correlations	
				intra	inter
Phe	1	168.3			
	2	55.4	5.26 (dd, 10.2, 4:1)	C1, C4, C3	C1 (Pro)
	3	83.5	4.70 (d, 4.1)	C1, C4, C5, 9,	C3-MeO
	3-MeO		58.0	3.25 (s)	C3
	4	137.3			
	5, 9	127.8	7.01 (m)	C7, C3	
	6, 8	128.7	7.02 (m)	C4	
	7	128.8	6.98 (m)	C5, 9	
	NH		6.80 (d, 10.2)	C2	C1 (Pro)
Pro	1	172.2			
	2	60.7	4.32 (dd, 8.6, 3.5)	C1, C3, C4, C5	C1 (Asn)
	3	31.2	2.12 (m)	C1, C2, C4, C5	
			1.89 (m)	C1, C4, C5	
	4	25.3	2.01 (m)	C2, C3, C5	
			1.92 (m)	C2, C3	
	5	48.7	3.78 (m)	C3, C4	
			3.69 (m)		
Asn	1	169.8			
	2	47.2	5.34 (ddd, 10.0, 9.6, 5.4)	C4, C1, C3	C1 (NMeIle-2)
	3	39.5	2.85 (dd, 15.3, 9.6)	C4, C1, C2	
			2.38 (dd, 15.3, 5.4)	C4, C1, C2	
	4	172.6			
	NH_2		5.90, 5.59 (br s)		
	NH		7.58 (d, 10.0)	C2	C1 (NMeIle-2)
NMeIle-2	1	169.2			
	2	65.5	4.44 (d, 10.8)	C1, C3, C4, C5, C(NMe)	C1 (NMeIle-1)
	3	34.8	2.10 (qddd, 6.5, 10.8, 9.8, 3.0)		
	4(Me)	16.3	0.86 (d, 6.5)	C2, C3, C5	
	5	25.9	1.34 (qdd, 7.0, 12.6, 3.0)	C3, C4, C6	
			0.85 (qdd, 7.0, 12.6, 9.8)		
	6(Me)	12.6	0.91 (t, 7.0)	C3, C5	
	NMe	30.0	2.94 (s)	C2	C1 (NMeIle-1)
NMeIle-1	1	170.8			
	2	57.3	5.18 (d, 10.8)	C1, C3, C4, C5, C(NMe)	C4 (Asp)
	3	35.1	2.20 (qddd, 6.5, 10.8, 9.8, 2.7)		
	4(Me)	15.0	0.86 (d, 6.5)	C2, C3, C5	
	5	27.3	1.39 (qdd, 7.1, 12.3, 2.7)	C2, C3, C4, C6	
			0.83 (qdd, 7.1, 12.3, 9.8)		
	6(Me)	13.0	0.94 (t, 7.1)	C3, C5	
	NMe	30.6	3.12 (s)	C2	C4 (Asp)
Asp	1	172.1			
	1-OMe	53.1	3.67 (s)	C1	
	2	52.1	4.53 (ddd, 6.7, 10.9, 1.9)	C4, C1, C3	C1 (disco. acid)
	3	35.5	3.32 (dd, 17.0, 10.9)	C4, C1, C2	
			2.38 (dd, 17.0, 1.9)	C4, C1, C2	
	4	174.9			
	NH		7.37 (d, 6.7)	C1, C2	C1 (disco. acid)
Discokiic acid	1	174.2			
	2	43.8	2.93 (qd, 7.0, 10.8)	C1, C4, C3, C2-Me	
	2-Me	14.5	0.84 (d, 7.0)	C1, C2, C3	
	3	70.9	5.87 (d, 10.8)	C1, C5, C4, C2, C2-Me	C1 (Phe)
	4	136.3			
	5	138.8	7.66 (s)	C6, C4	
	6	168.2			
	7	38.3	3.75 (qdd, 7.0, 7.9, 0.9)	C6, C9, C8, C7-Me	
	7-Me	19.4	1.46 (d, 7.0)	C6, C8, C7	
	8	129.5	6.26 (dd, 15.7, 7.9)	C6, C10, C9, C7, C7-Me	
	9	132.4	6.52 (dd, 15.7, 0.9)	C6, C10, C8, C11, 15, C7	
	10	137.3			
	11, 15	127.2	7.39 (m)	C9, C13	
	12, 14	129.6	7.34 (m)	C10	
	13	128.8	7.26 (m)	C11, 15	

ion (NMeIle 2–Asn)$^+$ is more feasible than the ion of the discokiic acid part ($C_{16}H_{18}NO$, *m/z* 240, $\Delta = -4.1$ μmu). Fragment d of **2b** (*m/z* 283) shifted to *m/z* 269 in **1b**, whereas fragment e (*m/z* 240) appeared at the

Hydrolysis of **2b** into the original carboxylic acid by alkali, or isolation of the discokiic acid moiety by hydrolysis using NaOMe gave only intractable materials (many spots on TLC plate). Further research on the regeneration of original acids from the respective esters and on the absolute configurations of three asymmetric centers as well as the respective amino acids are in progress.

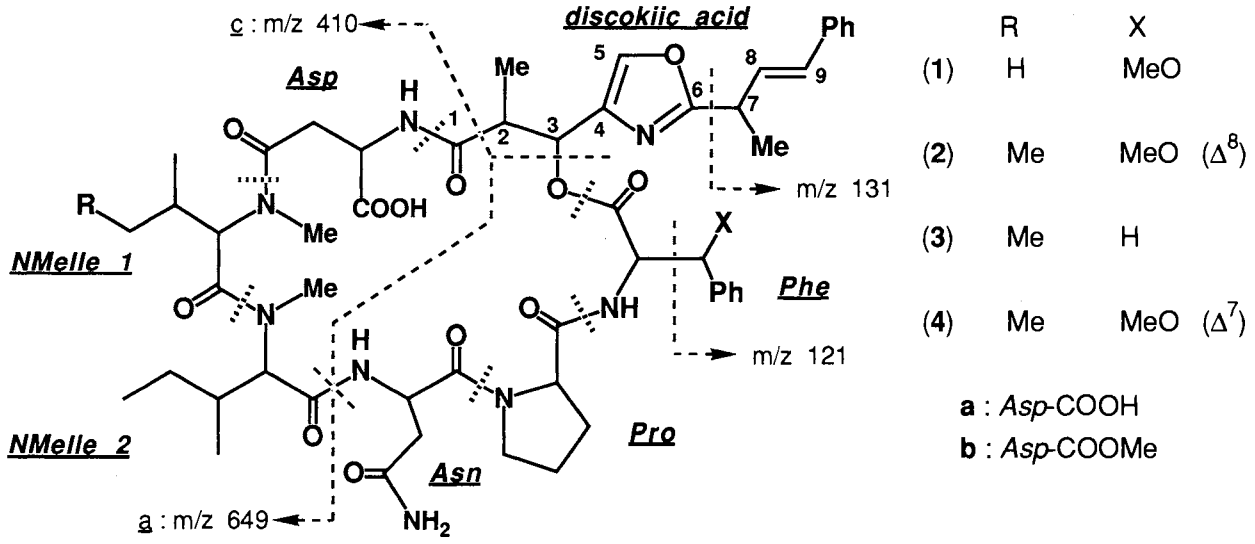

Fig. 1. Discokiolides A-D.

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