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BIOACTIVE MARINE METABOLITES VII.[1] STRUCTURES OF DISCODERMINS B, C, AND D, ANTIMICROBIAL PEPTIDES FROM THE MARINE SPONGE *DISCODERMIA KIIENSIS*

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Abstract: Isolation and structure elucidation of three peptide antibiotics, discodermins B, C, and D, from the marine sponge, *Discodermia kiiensis*, and the revised structure of discodermin A are described.

Several bioactive lipophilic peptides have been isolated from marine tunicates and a sea hare in the 1980s.[2] We have recently reported the isolation[3] and structure elucidation[1] of an antimicrobial tetradecapeptide, discodermin A (**1**), from the marine sponge *Discodermia kiiensis* HOSHINO. Subsequently, we have isolated three more peptides related to discodermin A, which we have named discodermins B, C, and D. In this paper, we describe the isolation and structure elucidation of these antibacterial peptides as well as the revised structure of discodermin A.

The antimicrobial fractions obtained by medium pressure column chromatography on SiO_2[3] were fractionated by medium pressure column chromatography on Chromedia LRP-1 (Whatman, USA) with 64% aq. MeOH to obtain three active fractions. Fraction 1 (yield, 87 mg from 1 kg of wet animal) and 3 (yield, 900 mg) were essentially pure, but fraction 2 was shown to be a mixture of two compounds; fraction 1 was named discodermin D (**4**)[4] and fraction 3 was named discodermin A. Fraction 2 was further purified by reversed-phase HPLC on a YMC A-324 column (YMC-Shimakyu, Japan, 1 x 30 cm) with 66% aq. MeOH, which yielded 46 mg of discodermin C (**3**)[4] and 19 mg of discodermin B (**2**)[4].

Discodermins A-D are indistinguishable on TLC on silica gel, but can be separated by reversed-phase HPLC, suggesting that they differ from each other only in the structure of the non-polar groups. FAB mass spectra showed the molelular weights of both discodermins B and C to be 1690 and discodermin D to be 1676. Amino acid analyses, GCMS analyses of the acid hydrolysate as the *N,O*-heptafluorobutyl methyl ester on a Chirasil Val III column (Applied Science, USA), and digestion with L- and D-amino acid oxidase demonstrated that, instead of D- and L-*t*-Leu residues in discodermin A, discodermin B contains D-Val and L-*t*-Leu residues, while discodermin C contains L-Val and D-*t*-Leu residues, and discodermin D contains D- and L-Val residues. Chiralities of all other amino acid residues in **2-4** except for $Cys(O_3H)$ and MeGlu were deduced to be identical with those of **1** by the above procedures. All four peptides have $Cys(O_3H)$ and MeGlu with the same chiralities, as will be shown below.

HCO-D-Ala-L-Phe-L-Pro-X-D-Trp-L-Arg-D-Cys(O_3H)-L-Thr-L-MeGln-D-Leu-L-Asn-L-Thr-Sar

1: X = D-*t*-Leu-L-*t*-Leu
2: X = D-Val-L-*t*-Leu
3: X = D-*t*-Leu-L-Val
4: X = D-Val-L-Val

L-Arg-D-Cys(O_3H)-L-Thr-L-MeGln-D-Leu-L-Asn-L-Thr-Sar

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Treatment of **2-4** with 10% HCl/MeOH (rt, 4h)[5] followed by two Edman degradation cycles indicated the N-terminal sequences of Ala-Phe to be identical for **2-4**. Though FAB mass spectra gave no useful sequential ions, EI mass spectra provided valuable fragment ions. EI mass spectrum of discodermin A gave peaks at *m/z* 344, 457, and 569 corresponding to the sequence of Y-*t*-Leu-*t*-Leu (Y = HCO-Ala-Phe-Pro). On the other hand, EI mass spectrum of discodermin B exhibited fragments at *m/z* 344, 443, and 555 (Y-Val-*t*-Leu), discodermin C at *m/z* 344, 457, 555 (Y-*t*-Leu-Val), and discodermin D at *m/z* 344, 443, 541 (Y-Val-Val). These results imply the sequences of the five residues from the N-terminal in **2-4**. Chiralities of the two Val residues in **4** were determined as in the case of discodermin A.[1] BNPS-skatol treatment[6] of **1-4** gave the C-terminal octapeptides **5** indistinguishable by TLC, HPLC, FABMS, and 400 MHz ^{1}H NMR. This indicates that the Trp residue is placed at the 6th position from the N-terminal and the structures of the eight residues from the C-terminal are identical in the four peptides; the chiralities of Cys(O_3H) and MeGlu in **1-4** are concluded to be identical among them because the other six amino acid residues possess the same chiralities and the all octapeptides were indistinguishable. Thus the structures of the discodermins B-D are determined as designated in **2-4**.

Contrary to the previous assignment of the chirality of the Pro residue in discodermin A,[3] Pro residues in **2-4** were shown to possess the L-configuration by GC analysis on a chiral column. This led us to reinvestigate the chirality of the Pro residue in **1** by chiral GC analysis, which demonstrated that the Pro residue in discodermin A is also of the L-configuration. Accordingly the structure of **1** is revised as depicted above.

Discodermins B, C, and D all showed *in vitro* antimicrobial activity against *Mortierella ramannianus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis*, and *Mycobacterium smegmatis*. They also inhibited the development of starfish (*Asterina pectinifera*) embryo; discodermin A at 5 μg/mL, C at 5 μg/mL, and D at 50 μg/mL.

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References and Notes

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4. **2**: mp. 217-219°, $[\alpha]_D^{23}$ -3.2° (c 1.0, MeOH), λ_{max} (MeOH) 290.1 (ϵ 4600), 281.9 (5390), 275.0 (5030) nm. **3**: mp. 222-224°, $[\alpha]_D^{23}$ -6.6° (c 1.0, MeOH), λ_{max} (MeOH) 290.1 (ϵ 4950), 281.9 (5810), 275.0 (5450) nm. **4**: mp. 215-219°, $[\alpha]_D^{23}$ -4.7° (c 1.0, MeOH), λ_{max} (MeOH) 290.1 (ϵ 4380), 281.9 (5150), 275.0 (4800) nm.
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