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BIOACTIVE MARINE METABOLITES VI.[1] STRUCTURE ELUCIDATION OF DISCODERMIN A, AN ANTIMICROBIAL PEPTIDE FROM THE MARINE SPONGE *DISCODERMIA KIIENSIS*

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Abstract: The structure of an antimicrobial peptide discodermin A isolated from the marine sponge *Discodermia kiiensis* has been elucidated as HCO-D-Ala-L-Phe-D-Pro-D-*t*-Leu-L-*t*-Leu-D-Trp-L-Arg-D-Cys(O_3H)-L-Thr-L-MeGln-D-Leu-L-Asn-L-Thr-Sar.

Sponges have attracted the attention of marine natural product chemists, since a number of novel and bioactive metabolites have been isolated from them.[2,3] Although several antineoplastic peptides have been reported from a mollusc[4] and from tunicates,[5-7] no bioactive peptides have been known from sponges. From the marine sponge *Discodermia kiiensis* we have isolated an antimicrobial peptide named discodermin A (**1**),[8] which inhibits *B. subtilis, S. aureus, E. coli*, and *P. aeruginosa* at 1 μg/disk, respectively. Its amino acid composition has been determined as follows; D-Cys(O_3H) (1 mole), L-Asp (1), L-MeGlu (1), L-Thr (2), Sar (1), D-Pro (1), D-Ala (1), L-*t*-Leu (1), D-*t*-Leu (1), D-Leu (1), L-Phe (1), D-Trp (1), and L-Arg (1). In the present paper we describe the structure elucidation of the peptide.

A FAB mass spectrum[9] of discodermin A gave the $M+H^+$ and the $M+Na^+$ ions at *m/z* 1705 and 1727, respectively, which led us to assign a molecular weight of 1704. The IR spectrum (1740 cm^{-1}) and DMSO/DCC oxidation[10] followed by amino acid analysis revealed that one hydroxyl group of Thr was esterified and the other was free. Since potentiometric titration showed the absence of free acids and amines, the molecular weight is calculated to be 1676, to leave a unit of 28 daltons unidentified. Then we paid attention to a ^{13}C NMR signal at δ 163.4 d,[11] which suggested the presence of a formamide moiety. HCl/MeOH (rt, 5h) treatment[12] of discodermin A gave a ninhydrin positive compound, desformyldiscodermin A (**2**), which exhibited a molecular weight of 1676 in FABMS. Edman degradation[13] of **2** established the N-terminal sequence of Ala-Phe-Pro-*t*-Leu-*t*-Leu-Trp.

1: HCO-Ala-Phe-Pro-*t*Leu-*t*Leu-Trp-Arg-Cys(O_3H)-Thr-MeGln-Leu-Asn-Thr-Sar

2: H-Ala-Phe-Pro-*t*Leu-*t*Leu-Trp-Arg-Cys(O_3H)-Thr-MeGln-Leu-Asn-Thr-Sar

3: H-Arg-Cys(O_3H)-Thr-MeGln-Leu-Asn-Thr-Sar

4: H-Thr-MeGln-Leu-Asn-Thr-Sar

5: H-MeGln-Leu-Asn-Thr-Sar

6: HCO-Ala-Phe-Pro-*t*Leu-*t*Leu-Trp-Arg-Cys(O_3H)-Thr-MeGln-Leu-Asn-Thr-Sar-OCH_3

7: HCO-Ala-Phe-Pro-*t*Leu-*t*Leu-Trp-Arg-Cys(O_3H)-Thr-MeGln-Leu-Asn-Thr-Sar-OH

8: H-Arg-Cys(O_3H)-Thr-MeGln-Leu-Asn-Thr-Sar-OCH_3

9: H-Arg-Cys(O_3H)-Thr-MeGln-Leu-Asn-Thr-Sar-OH

BNPS-skatol[14] cleaved the carboxyl side of the Trp residue of discodermin A to give an octapeptide **3** possessing the molecular weight of 949 (FABMS). When subjected to two cycles of the Edman degradation it only gave the sequence Arg-Cys(O_3H). Moreover, only a small amount of PTH-Thr was obtained in the third degradation cycle. The FAB mass spectrum of the product revealed the major peak at *m/z* 643 ascribed to the non-reacted hexapeptide **4** and a minor peak at *m/z* 560 attributable to the open chain pentapeptide **5**, which lacked the Thr residue. This indicated that most of the peptide remained unreacted and a small portion underwent lactone cleavage prior to the third cycle of the Edman degradation. On the other hand, dansylation after the second cycle of the Edman degradation gave Dns-Thr. These results confirmed that this Thr residue was present adjacent to Cys(O_3H) and that its hydroxyl group was lactonized.

Discodermin A was treated with 0.1N KOH/MeOH (rt, 8h) followed by reversed-phase HPLC to yield two compounds; the major methyl ester **6** and the free acid **7**. BNPS-skatol treatment of **6** gave the C-terminal fragment **8** with a molecular weight of 981 (FABMS). Its sequence was deduced to be Arg-Cys(O_3H)-Thr-MeGlx-Leu-Asn by the Edman-dansyl method. The presence of Asn was implied by the fact that discodermin A gave 2,3-diaminopropionic acid upon treatment with bis(trifluoroacetoxyl)iodobenzene[15] followed by amino acid analysis. On the other hand, BNPS-skatol treatment of **7** gave the C-terminal fragment **9** possessing the molecular weight of 967 (FABMS). Hydrazinolysis[16] of **9** yielded Sar, which was thus placed at the C-terminal position of **1** and its carboxyl group was esterified. $LiBH_4$ treatment[17] of discodermin A in MeOH (reflux, 4 h) followed by amino acid analysis revealed the absence of Sar, suggesting that the carboxyl group of the C-terminal Sar was linked in the lactone ring and the carboxyl group of MeGlx was amidated.

The configuration of the two *t*-Leu residues was confirmed as follows. Desformyldiscodermin A was subjected to four cycles of the Edman degradation, and the decapeptide containing a *t*-Leu residue was hydrolyzed by acid. Configuration of the *t*-Leu was determined as L by GCMS analysis of the heptafluorobutyl methyl ester derivative on a Chirasil Val column (Applied Science). Therefore the remaining *t*-Leu had D-configuration.

The results mentioned above unambiguously imply that the structure of discodermin A is HCO-D-Ala-L-Phe-D-Pro-D-*t*-Leu-L-*t*-Leu-D-Trp-L-Arg-D-Cys(O_3H)-L-Thr-L-MeGln-D-Leu-L-Asn-L-Thr-Sar—┘

Discodermin A is a peptide of unusual structural features, since it contains two *t*-Leu[18,19] residues and several D-amino acids. It is interesting to speculate whether this unusual peptide is biosynthesized by the sponge or produced by an associated organism.

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References and Notes

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1: $[\alpha]_D^{22}$ -6.3°, mp 226-228°, ^{1}H NMR (D_2O) δ 0.67 1H m, 0.76 1H m, 0.85 3H d J=8Hz, 0.93 3H d J=7Hz, 0.95 3H d J=7Hz, 1.00 9H s, 1.04 9H s, 1.28 3H d J=7Hz, 1.30 1H m, 1.33 3H d J=7Hz, 1.50 1H m, 1.56 2H m, 1.66 1H m, 2.02 3H m, 2.10 1H m, 2.13 2H m, 2.20 1H m, 2.34 1H m, 2.53 1H dd J=6,14Hz, 2.57 1H dd J=10,14Hz, 2.77 1H dd J=9,14Hz, 2.82 t J=6Hz, 2.89 3H s, 3.07 1H dd J=4,14Hz, 3.24 3H s, 3.34 1H m, 3.38 2H m, 3.60 1H d J=18Hz, 3.68 1H m, 3.68 1H q J=8Hz, 3.77 1H m, 4.03 1H s, 4.06 2H m, 4.23 1H qd J=7,4Hz, 4.49 1H s, 4.54 1H d J=18Hz, 4.59 1H dd J=5,11Hz, 4.64 1H dd J=4,10Hz, 4.95 1H brs, 5.06 1H m, 5.14 1H dd J=6,8Hz, 5.56 1H brq J=7Hz, 6.94 2H m, 7.16 1H t, 7.24 5H m, 7.49 1H d J=8Hz, 7.70 1H d J=8Hz, 7.89 1H s; ^{13}C NMR ($^{12}CD_3OD$) δ 17.9 q, 18.7 q, 20.6 q, 22.9 q, 23.3 q, 25.4 t, 26.1 t, 26.3 d, 27.6 q(3C), 27.7 q(3C), 28.9 t, 30.2 t, 30.6 t, 32.7 q, 32.9 t, 34.8 s, 36.2 s, 37.1 q, 38.5 t, 39.1 t(2C), 42.4 t, 43.2 t, 48.9 t, 48.9 d, 49.1 d, 52.1 t, 52.8 t, 53.2 d, 53.6 d, 54.0 d, 54.3 d, 54.6 d, 56.1 d, 57.8 d, 61.7 d, 62.1 d, 62.5 d, 64.0 d, 68.0 d, 71.3 d, 111.8 s, 112.6 d, 119.7 d, 120.1 d, 122.8 d, 125.1 d, 128.0 d, 129.1 s, 129.7 d(2C), 130.7 d(2C), 138.5 s(2C), 159.1 s, 163.4 d, 170.2 s, 171.7 s, 172.0 s, 172.4 s(2C), 172.9 s(4C), 173.6 s, 173.8 s(2C), 173.9 s, 174.6 s, 175.6 s, 177.5 s.

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