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STRUCTURE AND SYNTHESIS OF FR900490, A NEW IMMUNOMODULATING PEPTIDE ISOLATED FROM A FUNGUS

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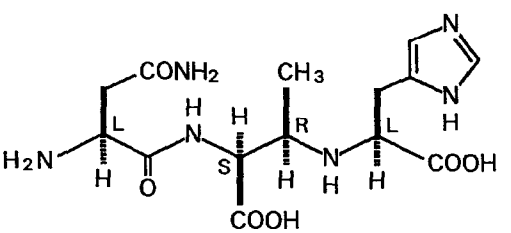
Summary : The structure of FR900490 ($C_{14}H_{22}N_6O_6$) has been shown to be 1 on the basis of its physical and chemical properties and confirmed by a synthesis.

FR 900490 (1) is a peptidyl compound recently isolated from Discosia sp. F-11809 as a new immunomodulator.[1] Herein we report the structural elucidation and synthesis of this natural product.

FR 900490 was isolated as a colorless powder: $C_{14}H_{22}N_6O_6$(SIMS, m/z 371 (M^++1). Anal. Calcd for $C_{14}H_{22}N_6O_6 \cdot 2H_2O$: C, 41.38; H, 6.45 ; N, 20.68. Found: C, 40.55; H, 6.19; N, 20.48); $[\alpha]_D$ +8.5° (c 0.7, H_2O); pKa 2.3, 5.7, 7.1, 8.9; positive ninhydrin test. The ^{13}C NMR spectrum (D_2O) of 1 showed, in the sp^3-carbon region, seven signals attributable to four methines (δ 51.0 (d),



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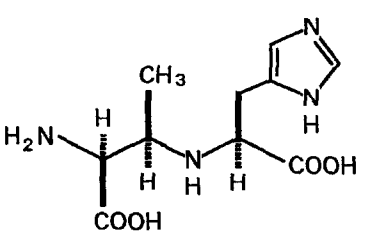

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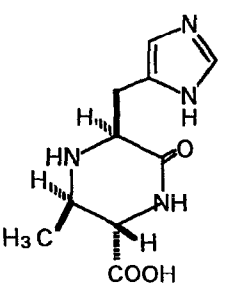

3

53.6 (d), 56.1 (d), 60.2 (d)), two methylenes (δ 28.3 (t), 36.7 (t)), and one methyl (δ 12.5 (q)). In the sp^2-carbon region, seven signals were observed assignable to four carbonyls (δ 171.1 (s), 174.2 (s), 174.3 (s), 174.3 (s)) and three aromatic carbons (δ 117.5 (d), 131.7 (s), 136.4 (d)). A spin decoupling experiment of the ^1H NMR spectrum (Table 1) of 1 revealed the ^1H-^1H relationships and the presence of an imidazole as shown in Figure 1, leading to partial structures A, B, and C, which are consistent with the ^{13}C NMR data described above.

Table 1. ^1H NMR (270MHz, D_2O) data of FR900490 (1)

Proton	Chemical shift (δ)	Multiplicity	J(Hz)
a	8.01	s	
b	7.17	s	
c	4.44	d	5.0
d	4.24	dd	4.9, 7.9
e	3.99	dd	5.3, 8.2
f	3.55	dq	5.0, 6.6
g	3.30	dd	5.3,15.0
h	3.16	dd	8.2,15.0
i	2.96	dd	4.9,16.5
j	2.83	dd	7.9,16.5
k	1.16	d	6.6

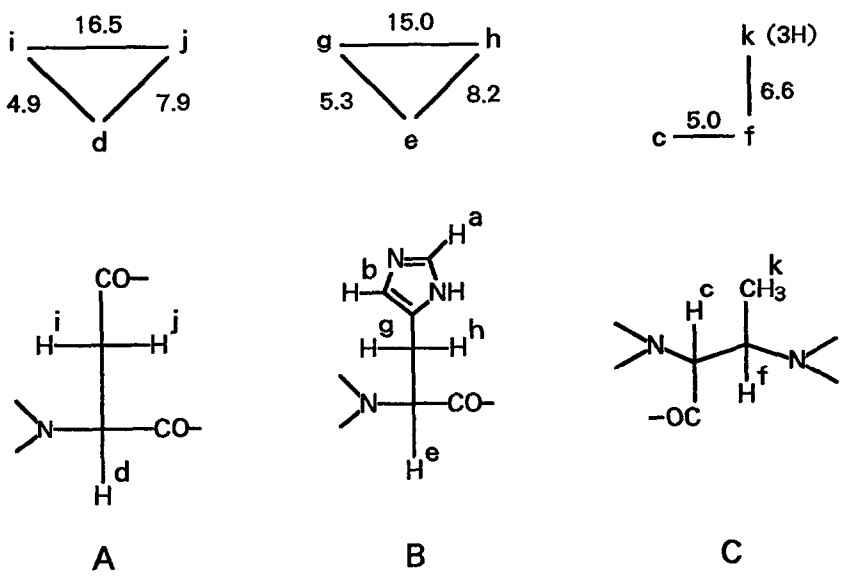

Figure 1. Partial structures A-C and the ^1H-^1H relationships (H^a-H^k).

The partial units A and B were confirmed by the fact that hydrolysis of 1 with 6N HCl (110°C, 24h) gave L-Asp and L-His (molar ratio, 1:0.86) which were identified by amino acid analysis and CD measurement. For determination of the N-terminal amino acid in 1, the Edman degradation[2] was applied to 1 in combination with MS analysis. The PTH-amino acid obtained in the first step was determined to be PTH-Asn which was along with PTH-Asp as a minor product (EIMS: PTH-Asn, m/z 249 (M^+); PTH-Asp, m/z 250 (M^+); ratio, 3:1). The latter amino acid seemed to be derived from the former by the acid treatment in the Edman degradation. These data led to the conclusion that the N-terminus of 1 is L-Asn.

The residue obtained after removal of the PTH-amino acids was purified by chromatography of CM cellulose (0.05M pyridine-AcOH,pH 4.85), to give amino acid 2 (SIMS, m/z 257 (M^++1))[3] and its cyclized product 3 (SIMS, m/z 239 (M^++1)).[4] The latter compound was also obtained together with L-Asp by acid hydrolysis of 1 (6N HCl, 100°C, 5h). An extensive NMR study on 3 (Figure 2) revealed that 5-H and 6-H are in 1,2-diaxial(J=9.7Hz), and 3-H and 5-H are in 1,3-diaxial(NOE), leading, on the basis of the S-configuration of the His residue, to the conclusion that the configurations at C-5 and C-6 are R and S, respectively. Hence the full structure of the natural product was deduced to be 1 including its absolute configuration.

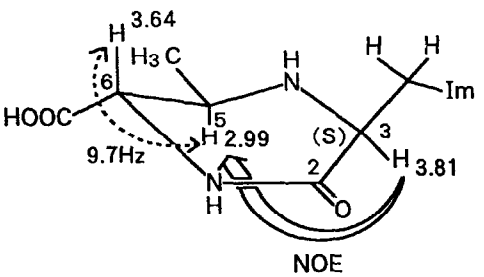

Figure 2. The stereochemistry and NMR data of 3

A final confirmation for the structure 1 was obtained by a synthesis starting from L-His and L-Asp. Methyl (2S,3S)-1-(p-toluenesulfonyl)-3-methyl-2-aziridinecarboxylate (4), prepared from L-Thr according to the method reported in the literature,[5] was allowed to react with L-His (3 equiv) in the presence of 1N NaOH (5 equiv) in MeOH (r.t., 30 min) to produce a mixture of 5 and 6, which, without purification, was directly treated with Na in liq. NH_3 at -78°C and the products were separated by chromatography on CM cellulose eluting with a pyridine-AcOH buffer (pH 4.5) to provide the compound (mp 235-6°C, 44%) identical with 2 and its isomer 7 (27%).[6]

Coupling reaction of 2 with Z-L-Asn(Mbh)OSu (Mbh=p-methoxybenzhydryl) (Et_3N/dioxane-H_2O) and removal of the protecting group in the product 8 (mp 183°C, 100%)[6] by treatment with 30% HBr in AcOH, followed by purification by chromatography on Dowex 50W x 8 eluting with 1% NH_4OH to afford the desired product 1 (mp 185°C, $[\alpha]_D$ +9.1° (c 0.5, H_2O), 73%), which was identical in all

respects with the natural product. FR900490 was thus established to have the structure 1.

FR900490 has an ability to restore the depression of bone marrow cell maturation by immunosuppressive factors in tumor-bearing mouse serum.[1]

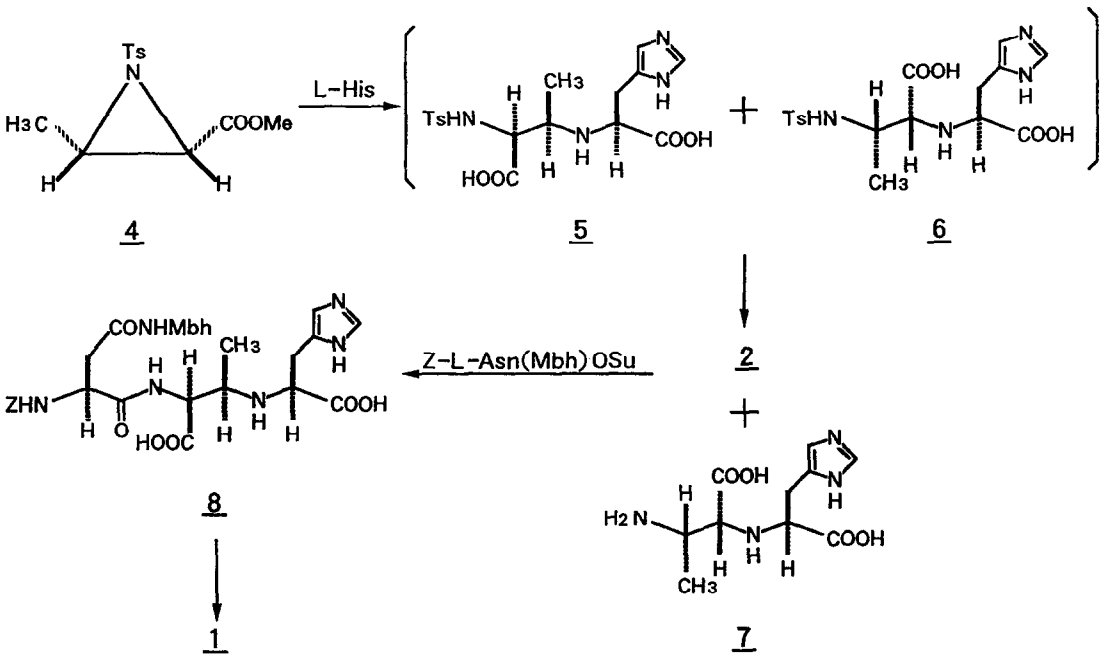

Figure 3. Synthesis of FR900490

Reference and Notes

1. T. Shibata, O. Nakayama, N. Okuhara, Y. Tsurumi, H. Terano, and M. Kohsaka, J. Antibiot, submitted for publicaton.
2. P. Edman and A. Henshen, "Protein Sequence Determination", Ed, by J.M. Needleman, Springer-Verlag, Berlin-Heiderberg-New York, 1975, p.232.
3. ^{1}H NMR (D_2O) data of 2: δ 8.4 (brs,1H), 7.3(brs,1H), 3.77(t,J=6.6Hz,1H), 3.74(d,J=4.8Hz,1H) 3.3(m,1H), 3.16(d,J=6.6Hz,2H), 1.20(d,J=6.6Hz,3H).
4. ^{1}H NMR (D_2O) data of 3: δ 8.62 (brs,1H), 7.17(brs,1H), 3.81(dd,J=7.7,4.5Hz), 3.64(d,J=9.7Hz,1H), 3.24(dd,J=15.5,4.5Hz,1H), 3.04(dd,J=15.5,7.7Hz,1H), 2.99(dq,9.7,6.3Hz,1H), 1.20(d,J=6.3Hz,3H).
5. K. Nakajima, F. Takai, T. Tanaka, and K. Okawa, Bull.Chem.Soc.Jpn., 51, 1577 (1978).
6. The selected physical data of the synthetic intermediates. 2: $[\alpha]_D$ -26.2° (c 0.22, H_2O): the ^{1}H NMR spectrum was identical with that of the compound derived from the natural product.[3] 7: ^{1}H NMR(D_2O-NaOD) δ 7.61(s,1H), 6.88(s,1H), 3.33(dd,J=7, 5Hz,1H), 3.0-2.6(4H,m), 0.97(d,J=7Hz,3H). 8: ^{1}H NMR($CDCl_3$) δ 7.48(s,1H), 6.82(s,1H), 5.09(s,2H), 4.56(m,1H), 3.66(s,3H), 3.64(s,3H), 3.53(m,1H), 3.05(m,1H), 2.88(dd,J=15,3Hz,1H), 2.71(dd,J=15, 6.5Hz,1H), 0.87(d,J=6.5Hz,3H).

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