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MOLECULAR CONFORMATION OF DESTRUXIN A

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Abstract: The peptide backbone conformation of destruxin A in $CDCl_3$, as studied by NOESY, closely resembles its crystal conformation which was deduced by a single crystal x-ray analysis.

The destruxins, insecticidal cyclodepsipeptides, were originally isolated from the entomogenous fungus *Metarhizium anisopliae*.[1] Recently, destruxin B was isolated from the plant pathogenic fungus, *Alternaria brassicae*[2] and roseotoxin B is known to be produced by *Trichothecium roseum*.[3] Destruxins have been shown to possess immunodepressant activity in insect model systems, and cytotoxic and cytostatic effects on mouse leukemia cells.[4] Little is known about the mode of action of destruxins, although the available literature suggests that unlike beauvericin, a cationophoric cyclodepsipeptide,[5] destruxins do not have ionophoretic properties.[6] In order to understand the mechanisms involved in the action of these important peptides, it is necessary to have knowledge of their conformational characteristics. In the present report, we compare the conformation determined from the crystal structure of destruxin A with that determined by a NOESY experiment.

Destruxin A (**1**), isolated from the culture broth of *M. anisopliae*,[7] was obtained as crystalline flakes from hexane-benzene at 10°C. Figure 1 shows the perspective drawing of the crystal structure of **1**.[8] The x-ray crystal analysis of roseotoxin B[3] and destruxin B[9] have already been reported. The crystal structure of destruxin A (**1**) is essentially identical to that of destruxin B and roseotoxin (crystal from benzene at room temperature). **1** is cyclo (2(R)-hydroxy-4-pentenoyl-L-prolyl-L-isoleucyl-N-methyl-L-valyl-N-methyl-L-alanyl-β-alanyl). Four α-amino acids have the L(S) configuration while the α-hydroxy-4-pentenoic acid has the D(R) configuration.[10] The ester linkage and four of the five peptide bonds are *trans*, while the N-methyl alanyl-N-methyl valyl peptide bond is *cis*. It has been observed that N-methylated cyclic peptides tend to possess *cis* amides, and destruxin B was speculated to have one *cis* peptide bond on the basis of NMR studies.[11]

Though the peptide backbone of **1** is essentially asymmetric, the overall geometry of the molecule appears to be roughly rectangular. This is commonly observed in the case of cyclic hexapeptides characterized by 4-->1 *cis* chain reversal at one end and a β-turn at the other.[12] Isoleucine and β-alanine are the linking units, and β-alanine assumes the *gauche* conformation about the C(6α)-C(6β) bond. Isoleucine and proline side chains protrude towards the one end of the molecule, which is more hydrophobic, while N-methyl alanine and N-methyl valine, at the other end, are linked by a *cis* peptide bond. This arrangement results in a conformation where the amide nitrogens of isoleucine and β-alanine project towards the interior of the molecule and allow the formation of two transannular 4-->1

type hydrogen bonds. This intramolecular hydrogen bonding results in the formation of two ten membered rings inside the covalent 19-membered macrocylic lactone which leads to a stabilized conformation with two ends of the molecule constrained to β-turns. It was suggested for roseotoxin B that the peptide conformation actually leads to the formation of cross ring bridges which probably are less significant for the rigidity of the backbone.[13] Usually the peptide backbone in cyclo- and depsipeptides is a conformationally flexible structure capable of having nonplanar geometries.[3,12]

The occurrence of well resolved sharp signals in the ^{1}H NMR spectrum of **1** suggests the presence of a stable conformation in solution. Completely N-demethylated analog of destruxin B, protodestruxin, gives a complex pattern of resonances. This suggests the presence of multiple conformers in solution and that N-methylation results in the formation of stable conformation, probably by limiting the number of free amides available for intramolecular hydrogen bonding.[11] The monodemethylated analog of destruxin B, desmethyldestruxin B, gives a well resolved ^{1}H NMR spectrum suggesting little or no role for N-methylation of valine in the conformational stability. Yet, unlike the other common destruxins, desmethyldestruxin B cannot be readily crystallized.

The solution conformation of destruxin A (**1**) in $CDCl_3$ was studied by ^{1}H 2D NOE (NOESY) spectroscopy.[14] Figure 2 shows the contour plot of the NOESY of **1** from 0.5-5.5 ppm.[15] Several COSY correlations were also observed along with the NOESY correlations. The latter (marked by perpendicular lines) could be assigned readily to the specific protons by comparison with the fully assigned ^{1}H NMR spectrum of **1**.[16] The NOE connectivities observed are shown in figure 3. The important correlations were the ones between alanine N-methyl (2.69 ppm) and one of the β-methylene protons of β-alanine (4.01 ppm), and between the α-methine proton of N-methyl valine (4.93 ppm) and the α-methine proton of N-methyl alanine (5.16 ppm). Examination of a molecular model of **1** clearly revealed that these interactions could only be possible if the peptide bond between N-methyl alanine and N-methyl valine was *cisoid*, as opposed to the rest of the peptide bonds in the molecule, and the ester linkage, which were *transoid*. The *cisoid* geometry of this peptide bond results in a conformation in which the N-methyl of the alanine moiety is projected towards the center of the macrocycle which places it in close proximity to one of the β-methylene protons of β-alanine (which is in a *gauche* conformation as has also been reported for destruxin B)[9] and also with the γ1 methylene of isoleucine which has the β-S configuration. The NOESY did show an interaction between the N-methyl of alanine (2.69 ppm) and the protons which appeared as a multiplet centered at 1.28 ppm. Alanine methyl of destruxin A resonates at 1.28 ppm (doublet), partially overlapping the signal from one of the γ1 protons of isoleucine (multiplet centered at 1.25 ppm). An unambiguous assignment for the above NOE correlation could not be made, but from the analysis of the molecular model, it could be safely concluded that the twisting of the peptide bond between N-methyl alanine and N-methyl valine in order to establish spatial proximity between alanine N-methyl and β-alanine β-protons brought the former sterically close to the γ1 protons of isoleucine. This interaction is also favored by the slight β-helical turn of the peptide chain on proline side of the molecule. The conformation is apparently stabilized by the formation of two intramolecular hydrogen bonds between the amide protons of β-alanine and isoleucine with carbonyls of each other as also suggested by the x-ray analysis. A similar situation was suggested for destruxin B and desmethyldestruxin B on the basis of a deuterium exchange rate study by NMR.[11]

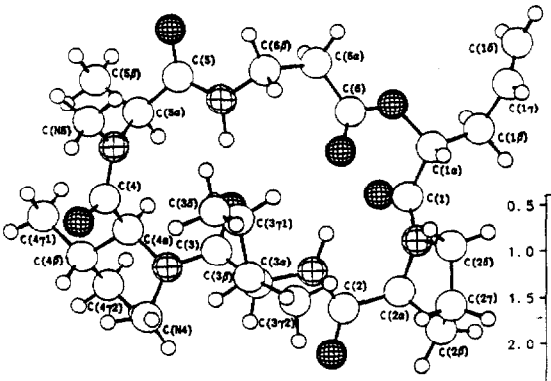

Figure 1. Crystal Structure of Destruxin A (1)

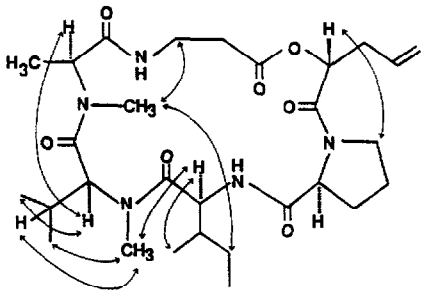

Figure 3. NOESY Interactions of 1

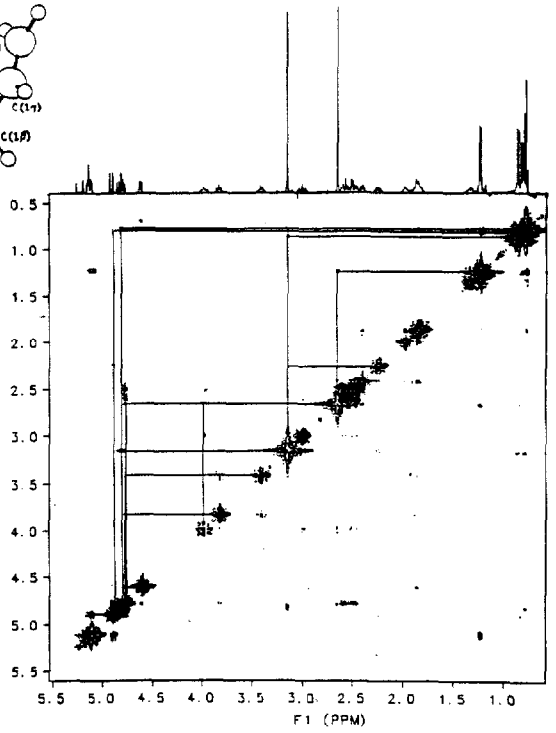

Figure 2. Contour Plot of the NOESY of 1

Another correlation observed in the NOESY was between the α-methine proton of isoleucine and the N-methyl of valine. Orientation of isoleucine side chain with the β-S configuration, as suggested above, indicated the existence of this interaction. Also, the conformationally most favorable orientation of valine side chain showed proximity of the β-methine proton to one of the methyls and to the N-methyl of valine, and these NOESY interactions were indeed present. Such an arrangement brings the other valine methyl in spatial proximity with the α-methine proton of valine, resulting in an interaction which is observed in the NOESY. A slightly puckered conformation of proline nucleus projects the δ-methylene protons (3.46 and 3.87 ppm) close to the α-methine proton of the pentenoic acid moiety as confirmed by the appearance of the relevant correlations. Also the α-methine proton of isoleucine moiety (4.86 ppm) shows a correlation with one of the methyls (~0.83 ppm) which apparently accounts for the interaction between the α-methine proton of isoleucine with its γ2 methyl which is likely if the the γ1 methylene points towards the center of the macrocycle. These observations suggest that the peptide backbone conformation of **1** in solution resembles its crystal conformation. However, a difference seems to occur in the orientation of the N-methyl of alanine, which in solution is apparently tilted more towards the inside of the ring, aided by extra twist of the peptide backbone bringing it close to β-alanine β-proton on the one side and to the γ1 methylene of isoleucine on the other. In the crystal conformation, the N-methyl is pointing away from the center of the macrocycle. These findings may have important relevance to the

mode of action of destruxins which probably interact with specific receptor sites.

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8 Destruxin A, $C_{29}H_{47}O_7N_5.C_6H_6$ (**1**), monoclinic, $P2_1$ with a=9.986(3), b=10.378(2), c=18.549(2) Å, β=104.11(2)°, V=1864 $Å^3$, Z=2, D_c=1.17 gcm^{-3}, μ(Cu Kα)=6.29 cm^{-1}. The intensity data were measured on a four-circle diffractometer using graphite-monochromated Cu Kα radiation (λ=1.5418 Å) in a range of $0° \leq 2\theta \leq 114.0°$. A total of 2686 unique reflections were collected and 2118 reflections with $|Fo| > 3\sigma(|Fo|)$ were considered observed. The structure was solved by direct methods and refined by full-matrix least-squares to R=0.071, Rw=0.082 ($w=1/\sigma^2(|Fo|)$). Archival data have been deposited with the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK. Please give a complete literature citation when ordering.

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15 The NOESY was performed on a Varian XL 400 NMR spectrometer with 155 mM solution of **1** in $CDCl_3$. FIDs were acquired without spinning the sample. PW90=15.6, D1=1.6, MIX=0.2.

16 S. Gupta, D. W. Roberts, and J. A. A. Renwick, *J. Chem. Soc. Perkin Trans. I*, 1989, in press. Destruxin A (**1**), ^1H NMR ($CDCl_3$) (ppm) (Hz): 4.81 (dd, J=9, 7, 1α), 2.63 (m, 1β), 5.77 (m, J=7, 9, 16, 1γ), 5.14 and 5.19 (each bd, J=9 and 16 resp., 1δ), 4.64 (bd, J=6.9, 2α), 1.89 and 2.47 (each m, 2β), 1.89 and 2.02 (each m, 2γ), 3.46 and 3.87 (each m, 2δ), 4.86 (dd, J=6.5, 9.4, 3α), 1.89 (m, 3β), 1.25 and 1.37 (each m, 3γ1), 0.83 (d, J=5.5, 3γ2), 0.82 (t, J=6.5, 3δ), 7.13 (d, J=9.4, 3N<u>H</u>), 4.93 (d, J=10.9, 4α), 2.29 (m, 4β), 0.86 (d, J=6.9, 4γ1), 0.9 (d, J=5.6, 4γ2), 3.19 (s, 4NC<u>H</u>$_3$), 5.16 (q, J=6.6, 5α), 1.28 (d, J=6.6, 5β), 2.69 (s, 5NC<u>H</u>$_3$), 2.59 and 2.63 (each m, 6α), 3.03 and 4.01 (each m, 6β), 8.18 (d, J=9.4, 6N<u>H</u>). C-methyl signal assignments may be interchanged.

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