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THE STRUCTURE OF NEOPEPTINS, INHIBITORS OF FUNGAL CELL WALL BIOSYNTHESIS

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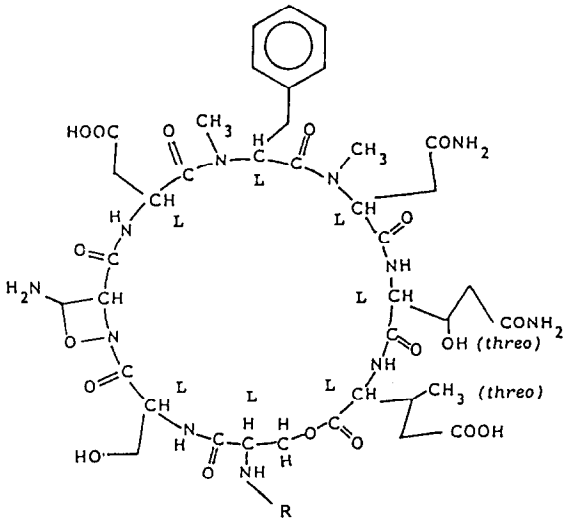
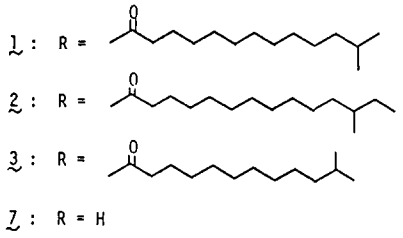
Summary: The structures of neopeptins A (1), B (2), and C (3) were elucidated on the basis of chemical and spectroscopic evidences.

Neopeptins are antifungal antibiotics isolated from a culture of Streptomyces sp.[1] They inhibit growth of several plant pathogenic fungi with swelling of mycelia. They inhibit in vitro proteoheteroglycan and β-1,3-glucan synthetases from Saccharomyces cerevisiae.

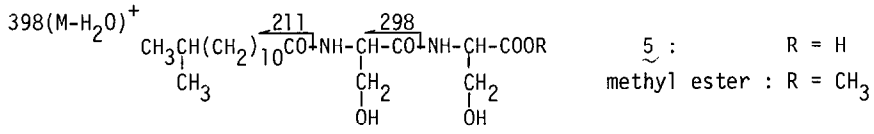
Neopeptin A (1), $C_{53}H_{81}N_{11}O_{19}$ [m.p. > 200°C (dec), $[\alpha]_D^{20}$ -9.2° (*c* 0.48, MeOH), m/z 1176 $(M+1)^+$, m/z 1198 $(M+Na)^+$, m/z 1214 $(M+K)^+$, (SIMS)], neopeptin B (2), $C_{54}H_{83}N_{11}O_{19}$ [m.p. > 200°C (dec), $[\alpha]_D^{20}$ -28.5° (*c* 0.12, MeOH), m/z 1190 $(M+1)^+$, m/z 1212 $(M+Na)^+$, m/z 1228 $(M+K)^+$, (SIMS)], neopeptin C (3),[2] $C_{52}H_{79}N_{11}O_{19}$ [m.p.190-205°C (dec), $[\alpha]_D^{27}$ -3.7° (*c* 0.29, MeOH), m/z 1162 $(M+1)^+$, m/z 1184 $(M+Na)^+$, (SIMS)] are amphoteric compounds, which have a basic group (pKa' 9.5) and two acidic groups (pKa' 5.9-4.0).





Acid hydrolysis of 1 gave seven amino acids and a fatty acid. Six amino acids were identified using authentic samples, namely 2 moles of L-serine and each one mole of L-aspartic acid, L-N-methylaspartic acid,[3] L-threo-β-methylglutamic acid,[4] L-threo-β-hydroxyglutamic acid,[5] L-N-

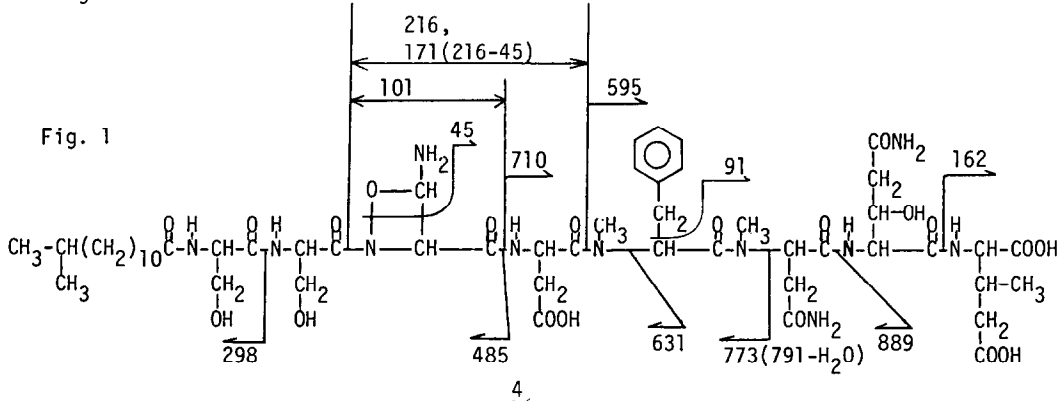
methylphenylalanine.[6] The fatty acid was esterified with diazomethane and analyzed by GC/MS [m/z 242 M^+, m/z 211 $(M-OCH_3)^+$, m/z 199 $(M-C_3H_7)^+$]. The methyl ester of authentic 12-methyltridecanoic acid[7] gave an essentially identical mass spectrum. Acid hydrolysis of 2 and 3 gave identical amino acids with those from 1, 12-methyltetradecanoic acid[7] [m/z 256 M^+, m/z 227 $(M-C_2H_5)^+$, m/z 225 $(M-OCH_3)^+$, m/z 199 $(M-C_4H_9)^+$, as methyl ester] and 11-methyldodecanoic acid [m/z 228 M^+, m/z 197 $(M-OCH_3)^+$, m/z 185 $(M-C_3H_7)^+$, as methyl ester] respectively. Subtraction of the sum ($C_{50}H_{77}N_9O_{17}$) of seven amino acids and a fatty acid linked by seven amide bonds (ν_{MaX}^{KBr} 1650, 1530 cm^{-1}) and one lactone bond (ν_{MaX}^{KBr} 1740 cm^{-1}) plus two primary amides (δ 6.80, 6.88, 7.25, 7.40 ppm in DMSO-d_6, 400 MHz) from the molecular formula of 1 ($C_{53}H_{81}N_{11}O_{19}$) gives a formula ($C_3H_4N_2O_2$), an amino acid which accounts the basicity of 1. The presence of a lactone group was proved by obtaining an open chain acid (4) [m/z 1194 $(M+1)^+$, m/z 1216 $(M+Na)^+$, (SIMS)] by treatment of 1 with 0.035 M NaOH. The C-terminal amino acid of 4 was identified as β-methylglutamic acid by carboxypeptidase[8] treatment. Selective acid hydrolysis (0.25 M AcOH, reflux for 24 hrs) of 4 gave aspartic acid, acyl peptide (5) and tetrapeptide (6). The structure of 5 was deduced from GC/MS analysis of the corresponding methyl ester as illustrated bellow.



5 : R = H
methyl ester : R = CH_3

Amino acid analysis of 6 shows N-methylaspartic acid, N-methylphenylalanine, threo-β-methylglutamic acid, and threo-β-hydroxyglutamic acid. From the ^1H NMR data (δ 6.80, 6.89, 7.30, 7.42 ppm in DMSO-d_6, 400 MHz), 6 has two primary amides and only possible positions are the γ-carboxyl group of threo-β-hydroxyglutamic acid and β-carboxyl group of N-methylaspartic acid, because carboxypeptidase treatment of 6 gave β-methylglutamic acid but not β-methylglutamine. Three successive Edman degradations revealed the sequence, N-MePhe-N-MeAsn-HyGln-MeGlu. The PTH amino acids were determined by GC/MS analysis[9] (m/z 296 M^+, PTH-N-MePhe; m/z 263 M^+, PTH-N-MeAsn; m/z 279 M^+, PTH-HyGln). In addition, 4 was analyzed by SIMS. Assignment[10] of main fragment ions is shown in Fig. 1.

Fig. 1



4

The position of the lactone was determined as follows. Chromic acid oxidation of 1 in acetic acid-pyridine followed by acid hydrolysis resulted in the recovery of one mole of serine but no threo-β-hydroxyglutamic acid. Borohydride reduction of 1 in water followed by acid

hydrolysis resulted in disappearance of β-methylglutamic acid. Therefore, in consideration of pKa' value of 1, the lactone position was concluded as between one of the β-hydroxyl groups of serine and the α-carboxyl group of β-methylglutamic acid. Treatment of 1 with polymyxin acylase[11] gave deacylneopeptin (7). participation of acylated serine hydroxyl in the lactone formation was proved as follows. By Edman degradation of 1, PTH amino acids were detected by HPLC (1st step, not detected; 2nd step, Ser; 3rd step, unknown peak). In contrast, Edman degradation of deacyl open chain acid (8), which was obtained by treatment of 4 with polymyxin acylase, clarified the sequence (1st step, Ser; 2nd step, Ser; 3rd step, unknown peak).

Finally, an intact labile basic amino acid could not be isolated but the structure was deduced to be 3-amino-2-oxazetidine-4-carboxylic acid (Aoc) from the following chemical and spectral evidences. Hydrogenation of 1 over Pt at 4 atm, followed by acid hydrolysis gave a small amount of diaminopropionic acid in addition to the seven amino acids. SIMS spectrum (Fig. 1) of 4 indicated fragment ion yielded by a loss of formamide (-45 amu). ^{13}C NMR data (β-carbon atom of Aoc, δ 71.4 ppm, J_{CH} = 150 Hz, in DMSO-d_6, 100 MHz) are consistent with a four membered ring.[12] The apparent basicity of 1 and negative ferric chloride test exclude an oxime possibility and the presence of a doublet attributable to proton of Aoc excludes an epoxide possibility. Protons of amino acids of 1 are observed as a pair of signals. Mesurement of ^1H NMR at different temperature (55°C, 23°C, 5°C, -10°C, -35°C in MeOH-d_4, 400 MHz) gave a stepwise sift in the ratio of a pair of signals (ca. 1:1 to 3:1). Dreiding model examination indicated that the two stable conformations regarding cis and trans of the amide bond between serine and Aoc are possible[13] (Fig. 2).

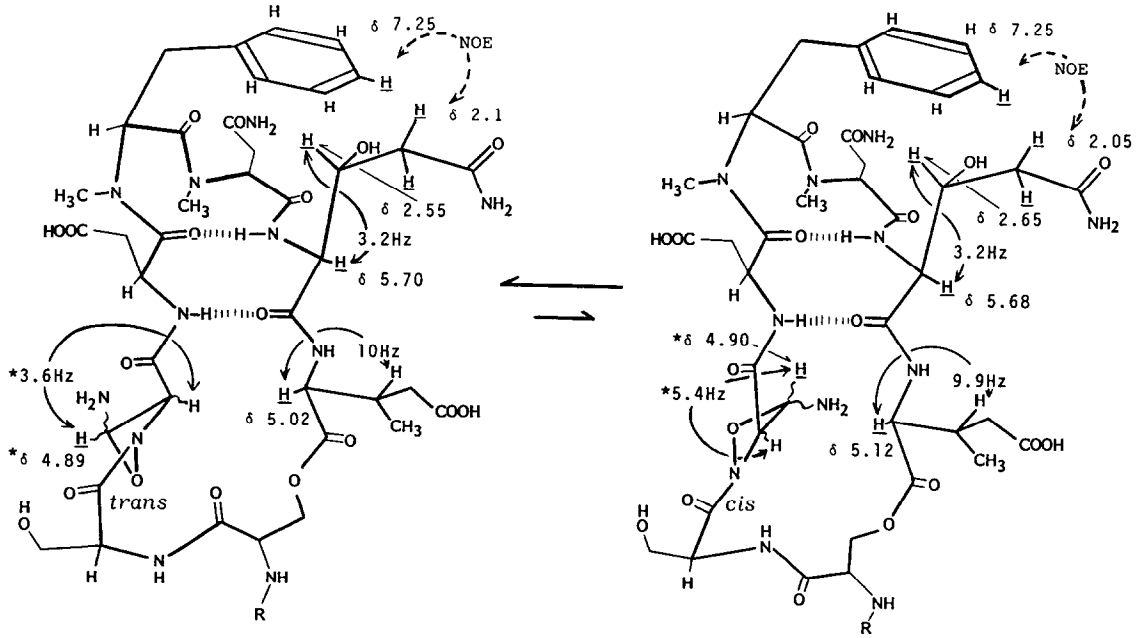


Fig. 2 Two stable conformations of neopeptins; ^1H NMR was measured at 23°C, asterisks show measurment at 55°C.

^{1}H NMR (2D NMR COSY[14], in MeOH-d_4, 400 MHz) shows a pair of two α protons at δ 5.02 (d, J = 10 Hz, α proton of major conformer, MeGlu), 5.12 (d, J = 9.9 Hz, α proton of minor conformer, MeGlu) and 5.68 (d, J = 3.2 Hz, α proton of minor conformer coupled with β proton at δ 2.65, HyGln), 5.70 (d, J = 3.2 Hz, α proton of major conformer coupled with β proton at δ 2.55, HyGln). Therefore, another pair of doublet, which could not be observed owing to overlapping of solvent peak at 23°C, should be assigned to β protons of Aoc [δ 4.89 (d, J = 3.6 Hz, coupled with α proton at δ 4.58, major conformer), 4.90 (d, J = 5.4 Hz, coupled with α proton at δ 4.5, minor conformer), 55°C, WEFT, in MeOH-d_4, 400 MHz]. 2D NMR NOESY[15] (in MeOH-d_4, 400 MHz) shows obviously NOE between δ 7.25 (aromatic protons of major and minor conformers, N-MePhe) and δ 2.05 (γ protons of minor conformer coupled with β proton at δ 2.65, HyGln), δ 2.1 (γ proton of major conformer coupled with β proton at δ 2.55, HyGln). Figure 2 illustrates the remarkable sielding of β protons of HyGln, NOE between aromatic protons of N-MePhe and γ protons of HyGln, and two intramolecular hydrogen bonds in two stable conformations. Though stereochemistry of Aoc remains to be solved, coupling constants between α and β protons of the four membered ring suggest trans relationship.[16]

Because treatment of 2 and 3 with polymyxin acylase gave 7, 2 and 3 differ only at the fatty acid side chain.

From the data described above, we propose the structures 1, 2, and 3, for neopeptin A, neopeptin B, and neopeptin C respectively. The structures show some close resemblance to lipopeptins reported previously from our laboratory.[7]

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