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STRUCTURAL ELUCIDATION OF TALOPEPTIN (MK-I), A NOVEL METALLO PROTEINASE INHIBITOR PRODUCED BY STREPTOMYCES MOZUNENSIS MK-23

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Summary: The structure of talopeptin (MK-I), a novel metallo proteinase inhibitor produced by *Streptomyces mozunensis* MK-23 was elucidated to be 6-deoxy-α-L-talopyranosyloxyphospho-L-leucyl-L-tryptophan.

As reported in the previous papers [1,2], a novel proteinase inhibitor talopeptin (MK-I), which shows a specific inhibitory activity against metallo proteinases, was isolated from the culture filtrate of *Streptomyces mozunensis* MK-23. It was also reported that talopeptin is composed of 6-deoxyaldohexose, phosphoric acid, leucine and tryptophan. In this paper, structural elucidation of talopeptin is reported.

Partial hydrolysis with 0.5N HCl (70°C, 1 hr), talopeptin gave 6-deoxyhexose, phosphoric acid and a peptide, which were separated from each other by column chromatographies.

The peptide component was separated by carbon chromatography: mp 133°(dec.); $[\alpha]_D^{22}$ 9° (c 1.0, H_2O). ^{1}H-NMR[90MHz, δ value (ppm) in 0.5N NaOD]: 0.75 and 0.77 [each 3H, d, J=6Hz, $(C\underline{H}_3)_2CH$(in Leu)], 0.9∿1.5[3H, m, $(CH_3)_2C\underline{H}$-$C\underline{H}_2$(in Leu)], 3.1 and 3.4[2H, J_{AB}=15Hz, J_{AX}=8Hz, J_{BX}=5Hz, $C\underline{H}_2$(in Trp)], 3.2[1H, dd, J=6Hz and 7Hz, α-C$\underline{H}$(in Leu)], 4.55[1H, dd, J_{AX}=8Hz, J_{BX}=5Hz, α-C$\underline{H}$(in Trp)], and 7.0∿7.8[5H, m, indolyl protons(in Trp)]; ^{13}C-NMR[25MHz, δ value (ppm) in D_2O down field from external TMS]: 178.6 and 177.4[C=O], 136.2, 127.7, 124.1, 121.8, 119.2, 118.8, 110.9 and 110.5[indolyl carbons(in Trp)], 55.7 and 53.5[α-carbons(in Leu or Trp, respectively)], 43.3[β-carbon(in Leu)], 27.7[β-carbon(in Trp)], 24.1[γ-carbon(in Leu)], 22.6 and 22.1[δ,δ'-carbons(in Leu)]. This peptide was identified as L-

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Leucyl-L-tryptophan in comparison with the authentic sample.

The sugar component was successively purified by carbon, Dowex 50(H^+), and IRA-402 (bicarbonate) column chromatographies: $[\alpha]_D^{20}$ -20° (c 0.5, H_2O); HPLC: Fig. 1; *Rf* 0.75[cellulose, TLC, AcOEt:pyridine:AcOH:H_2O (5:5:1:3), diphenylamine-aniline]. ^{1}H-NMR: [90MHz, δ value (ppm) in D_2O)]: ~1.2(total 3H, three or more doublets, J=6.5Hz, 6 position of isomers), 3.7~4.5(total 4H), 4.9(0.4H, d, J~1Hz, anomeric proton), 5.4(0.55H, two doublets, J~1Hz, anomeric proton), and 5.5(0.05H, d, J~1Hz, anomeric proton). ^{1}H-NMR data suggest that more than three isomers exist in aqueous solution.

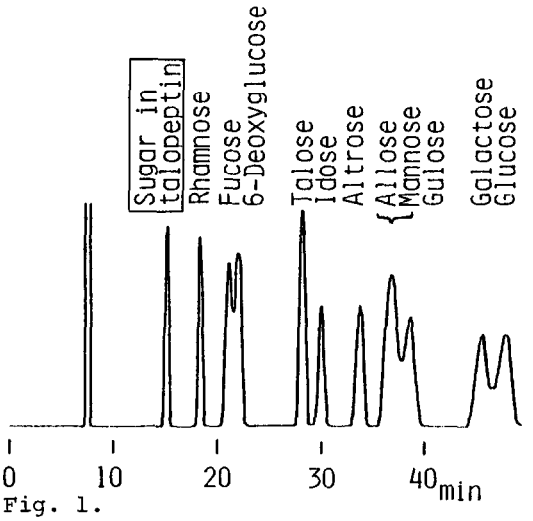


Fig. 1.
HPLC of the sugar in talopeptin and related monosaccharides.
Column: Hitachi #3013-N (8φx500mm),
Eluent: 70% CH_3CN (pH 7)
Flow rate: 2ml/min,
Temp.: 50°C, Detection: RI

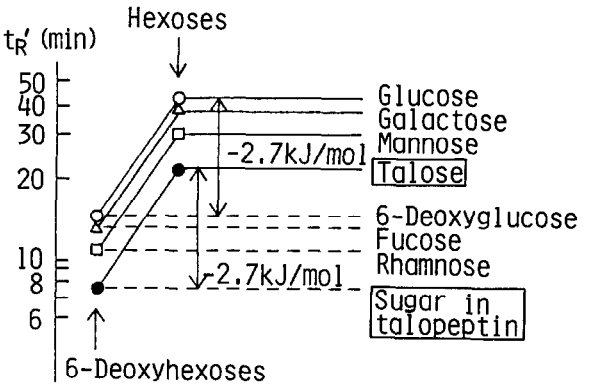
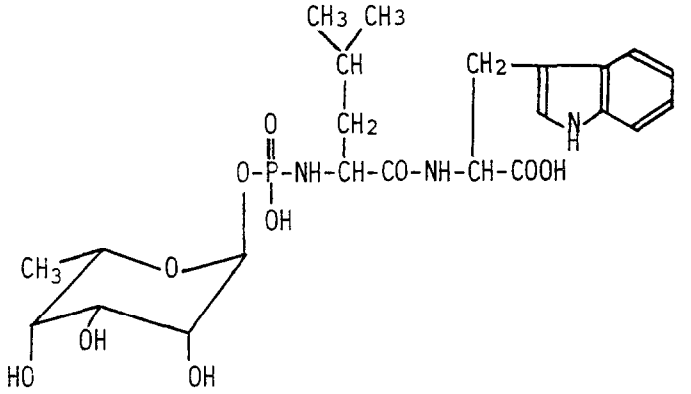


Fig. 2.
Correlation of the sugar in talopeptin to be 6-deoxytalose on the basis of the quantitative structure-mobility relationship of monosaccharides in HPLC (normal phase partition chromatography).
HPLC conditions: same as in Fig. 1.

Structure of the sugar was studied on the basis of the quantitative structure-mobility relationship in HPLC of monosaccharides. As reported in the previous paper [3], 6-deoxyaldohexoses (such as 6-deoxyglucose, fucose, or rhamnose) are eluted faster than the corresponding aldohexoses (such as glucose, galactose, or mannose, respectively) and the difference in free energy value between them is constant (-2.7 kJ/mole in 70% CH_3CN at 50°C) in each case. The regularity is applied to the structural determination of the sugar. As shown in Fig. 2, an aldohexose which is eluted slower than the 6-deoxyaldohexose with free energy difference, Δ(ΔG°), in 2.7 kJ/mole is talose. Thus, the sugar in talopeptin is assumed to be 6-deoxytalose. The 6-deoxytalose is assigned to be L-isomer on the

basis of its optical rotation, lit. $[\alpha]_D^{21}$ -21° (c 1.0, H_2O). The sugar in talopeptin was identified as 6-deoxy-L-talose which was synthesized according to the methods described by Collins et al. [4] and Brimacombe et al. [5].



The Structure of Talopeptin

The sequence of talopeptin was studied by means of ^{1}H-NMR and ^{13}C-NMR spectroscopy. ^{1}H-NMR spectrum of talopeptin and assignment of signals are shown in Fig. 3, and ^{13}C-NMR spectrum of talopeptin in Fig. 4.

As shown in Fig. 3, protons at 1 position of 6-deoxytalose and α position of leucine are coupled with phosphorus ($J_{P-O-C-H}$=8Hz,

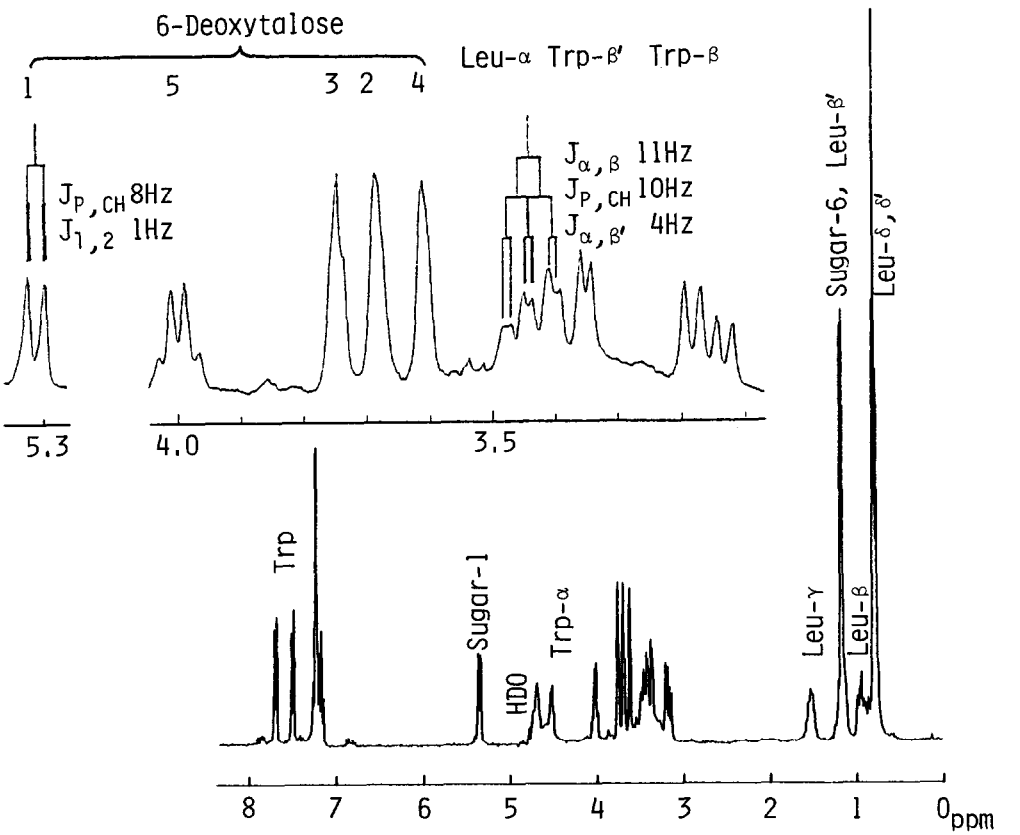


Fig. 3. ^{1}H-NMR spectrum of talopeptin (300MHz, in D_2O).

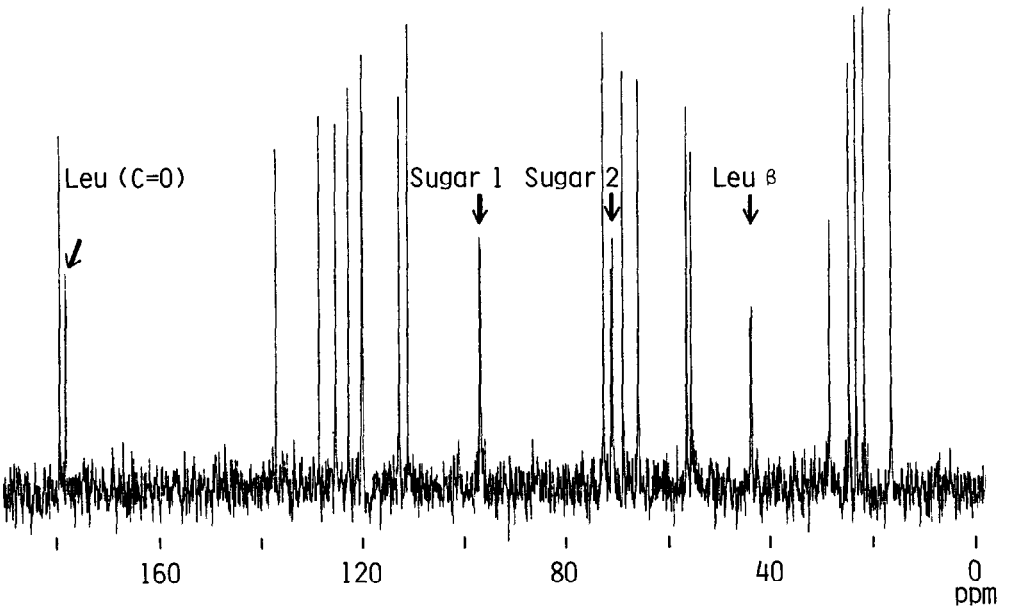


Fig. 4. Proton decoupled ^{13}C-NMR spectrum of talopeptin (25MHz, in D_2O)

$J_{P-N-C-H}$=10Hz). Also as shown in Fig. 4, carbons at 1 and 2 positions of 6-deoxytalose, β position of leucine, and carbonyl carbon of leucine are coupled with phosphorus (J_{P-O-C_1}=5.4Hz, $J_{P-O-C_1-C_2}$=7.9Hz, $J_{P-N-C_\alpha-C_\beta}$=6.1Hz, and $J_{P-N-C_\alpha-C_{carbonyl}}$=3.0Hz, respectively). These results suggest that phosphoric acid bonds to the hydroxyl group at 1 position of 6-deoxytalose as ester and to the amino group of leucine as phosphoramide. Thus the sequence of talopeptin is confirmed to be 6-deoxy-L-talopyranosyloxyphospho-L-leucyl-L-tryptophan.

Finally, the configuration of 6-deoxy-L-talosyl residue was studied. Proton chemical shifts and coupling constants indicate that the configuration of 6-deoxy-L-talosyl residue is α-pyranose form. ^{13}C-NMR supports the above discussion on the basis of the chemical shift at 1 position and the coupling constant ($J_{^{13}C,^1H}$=172Hz).

Thus the structure of talopeptin is concluded to be 6-deoxy-α-L-talopyranosyloxyphospho-L-leucyl-L-tryptophan.

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