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STRUCTURES OF ANTI-ACE AND -RENIN PEPTIDES FROM LYCII RADICIS CORTEX

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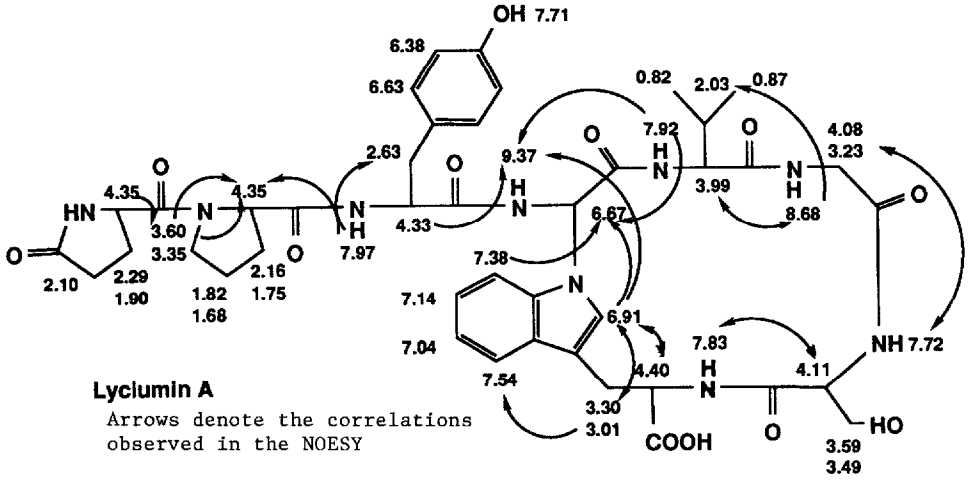
Summary: Two novel octapeptides, lyciumins A and B, have been isolated from Lycii Radicis Cortex as anti-ACE and -renin substances and their structures have been established.

An oriental crude drug, Lycii Radicis Cortex, the root barks of Lycium chinense Mill. has been used as an antifebrile, a tonic and a hypotensive drug. As regards constituents, lyciumamide and kukoamine A were known. Now we have obtained two novel cyclic octapeptides, named lyciumins A (1, 0.007 %) and B (2, 0.01 %), inhibiting renin and ACE activities, from the above crude drug, and have elucidated their structures.

Lyciumin A (1), an amorphous powder, $[\alpha]_D$ +10.1°, ninhydrin reaction (-), showed a peak due to $[M-H]^-$ at m/z 872 in the neg. FAB-MS. UV maxima absorptions at 273, 281 and 291 nm suggested the presence of an indole skeleton i.e. as Trp in the molecule. Acid hydrolysis of 1 revealed 1 to be consisting of each one mole of Glu, Ser, Gly, Pro, Var and Tyr. On DNP reaction 1 afforded one DNP-lized derivative (neg. FAB-MS: $[M-H]^-$ at m/z 1061) of 1. While, 1 on benzylation gave a two-moles benzylized derivative (neg. FAB-MS: $[M-H]^-$ at m/z 1052). Next, respective proton signals on the ^{1}H-NMR spectrum (DMSO-d_6) in each peptide constructing 1 were assigned by ^{1}H-^{1}H and ^{1}H-^{13}C 2D COSY spectra as shown in the formula. However, signals at δ 6.67 (1H, d, J=7.8 Hz) and 9.37 (1H, d, J=7.8 Hz, NH) coupling each other could not be interpreted. Moreover, the indole NH also could not be found. Subsequently, NOESY spectrum disclosed the sequence of amino acids in 1, e.g. NOE was observed between an amide proton (NH) and a proton attached to the α-carbon of the neighbouring amino acid. Particularly, it should be noted that a proton signal at δ 9.37 possessed NOE's against



Lyciumin A

Arrows denote the correlations observed in the NOESY

those of the H-2 (δ 6.91) of Trp indole ring part, the NH (δ 7.92) of Val and the proton (δ 4.33) of Cα of Tyr. Moreover, an unidentified proton signal at δ 6.67, coupled with the above mentioned NH at δ 9.37, also has NOE's against those of the NH of Val and the H-2 and H-7 (δ 7.38) of the indole ring. Therefore, it was suggested that 1 has an internal bonding between the indole N^1 and the CH (δ 6.67) in the other amino acid component. Namely, an unidentified -NH-CH- sequence was revealed to be belonging in a part of an additional Gly, thus all proton signals could be unambiguously assined and the molecular formula derived from a $[M-H]^-$ peak matched to this structure. The ^{13}C-NMR data for 1 also did not be inconsistent with the structure. The chemical shift of the CH appeared at δ 61.4, coupled with the proton signal at δ 6.67 in the COSY, could be reasonably accounted for when the indole N^1 linked to the above CH. Meanwhile, terminal Glu was concluded to be exist as a pyro-form from the accumulated evidence, e.g. chemical reaction, FAB-MS and NMR. Consequently, the structure of 1 could be represented as (glycyl^4 Cα, tryptophan^8 indole N^1)-cyclo-pyroglutaminyl-prolyl-tyrosyl-glycyl-valyl-glycyl-seryl-tryptophan.

Lysiumin B (2), a white powder, $[\alpha]_D$ -3.5°, showed a peak due to $[M-H]^-$ at m/z 895 in the neg. FAB-MS. The structure of 2 was also determined in a similar manner as 1, namely it was found to be replaced by Trp in 2 instead of Tyr in 1.

These plant-origin peptides are unique examples and they are novel in terms of cyclic one with a linkage between the indole N^1 of Trp and the Cα of Gly. Furthermore, these compounds were shown to possess inhibiting activities of renin and ACE activities, i.e. inhibitions of renin activities: lyciumin A, 19.4 % (40 μg/ml); lyciumin B, 32 % (40 μg/ml), inhibitions of ACE activities, lyciumin A, 90.9 % (100 μg/R.M.); lyciumin B, 79.0 % (100 μg/R.M.).

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