CYCLOPEPTIDE FROM THE SEEDS OF ANNONA GLABRA

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Abstract: A new cyclopeptide glabrin A has been isolated from the seeds of Annona glabra. Its structure was elucidated by 2D-NMR and FAB-MS.

The fruits of Annona glabra Linn. (Annonaceae) is edible in Yunnan province . Recently from the plant we have obtained a new cyclopeptide which was first found in the family of Annonaceae, named glabrin A. In this communication we report its structure.

The dried seeds (3kg) of A. glabra were degreased by petroleum ether and percolated by 95% EtOH at room temperature. Then the 95% EtOH extract was extracted by $CHCl_3$. The $CHCl_3$ extract was separated by silical gel column chromatography to furnish glabrin A (1, 0.023%).

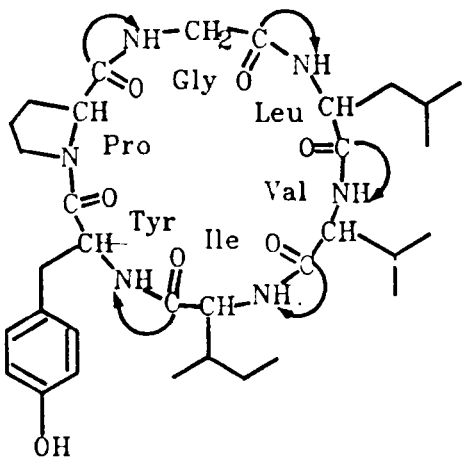

Fig.1 The sequence is shown as arrows for glabrin A by COLOC spectra.

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Glabrin A (1), white needles ($CHCl_3$–MeOH), mp 300–303℃ , $[\alpha]_D^{28}$–195.86℃ (c, 0.845 ,MeOH), is negative to ninhydrin reaction but positive after hydrolysis with 12N HCl, and showed a peak due to $(M+1)^+$ at m / z 643 in the pos. FAB–MS. IR spectrum exhibited intense N–H and C = O absorptions at 3280, 1635 cm^{-1}. The ^{13}C NMR spectrum showed the presence of six amide carbons (174.62, 173.17, 172.63, 172.23, 171.26, 170.34) and six α–carbons (64.87, 61.58, 56.79, 55.55, 54.64, 42.90) of six amino acids. The ^1H NMR spectrum showed the presence of five amide protons of five amino acids (10.23, d, 2.4; 9.91, dd, 2.4, 9.6; 9.17, d, 8.8; 9.04, d,5.6; 8.40, d, 9.6) and seven α–protons of six amino acids (5.19, m; 5.09, dd, 9.6, 16.2 (3.96, m); 4.98, t, 9.9; 4.71, m; 4.47, t, 9.9; 3.96, m). These facts indicated that (1) is a cyclohexapeptide[1].

Using COSY, ^{13}C–^1H COSY, and COLOC spectra these amino acids were determined to be glycine (Gly), valine (Val), leucine (Leu), isoleucine (Ile), tyrosine (Tyr), and proline (Pro) units. Then the sequence of amino acids was disclosed by COLOC experiments as summarized in Fig.[2], i.e. the sequence was

–N–Pro–Gly–Leu–Val–Ile–Tyr–CO–

To further corroborate the peptide to be a cyclopeptide, pos. FAB–MS was pursued. The peptide gave $(M+1)^+$ at m / z 643 which proved the molecular weight was agreeable with that of the sequence above after cycling and several useful fragment ion at m / z 480[–CO–Ile–Val–Leu–Gly–Pro–N–], 367[–CO–Val–Leu–Gly–Pro–N–], 268 [–CO–Leu–Gly–Pro–N–], 431[–CO–Gly–Pro–Tyr–Ile–NH– or –CO–Leu–Gly–Pro–Tyr–NH–], 403[431–CO], 339[367–CO], 303[–CO–Tyr–Ile–NH–CO–], 277[303–CO], 240[–CO–Ile–Val–NH–CO– or –CO–Val–Leu–NH–CO–], and 213[240–CO] which showed that the sequence was identical with the sequence above.

Therefore , the structure of (1) was elucidated as follows:

cyclo–(prolyl–glycyl–leucyl–valyl–isoleucyl–tyrosyl)

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