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L-2-AMINO-4-CHLORO-4-PENTENOIC ACID, A NEW NATURAL AMINO ACID FROM AMANITA PSEUDOPORPHYRIA HONGO*

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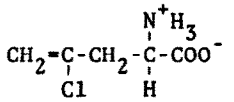
Several interesting amino acids (1) (2) (3) as well as toxic peptides (4) are known from fruit bodies of some species of Amanita. Among others a chlorine-containing amino acid, trans-2-amino-5-chloro-4-hexenoic acid (3), was recently isolated.

We now report another chloro amino acid, L-2-amino-4-chloro-4-pentenoic acid, from a fungus of the same genus, Amanita pseudoporphyria Hongo. This amino acid has not yet been reported as a natural product.



The fruit bodies were collected from natural habitats and stored at -20° for 4 months. They were extracted with 80% ethanol and the extracts treated with Amberlite IR-120 (H^+). Amino acids were eluted with 2N NH_4OH and the eluate was concentrated to a small volume. In the two-dimensional paper chromatograms of the concentrate developed with n-butanol-acetic acid-water and phenol-water, this amino acid locates very near to valine. It gives an yellow-brown coloration with ninhydrin, suggesting that it is an unsaturated amino acid.

After the removal of acidic amino acids with Dowex 1-column (CH_3COO^-), the

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amino acid mixture was repeatedly fractionated and purified by the use of cellulose-columns and t-amyl alcohol (or n-butanol)-methyl ethyl ketone-ammonia (28%)-water (15 : 9 : 4 : 2). Hydrochloric acid was not used throughout the isolation procedure to avoid the possible addition reaction of unsaturated amino acids. Yield of the new amino acid was 150 mg from 3 kg fruit bodies.

Pure crystals decompose at 190°. Elementary analysis, found: C, 40.31; H, 5.36; N, 9.32; Cl, 25.61. $C_5H_8NO_2Cl$ requiers: C, 40.15; H, 5.39; N, 9.36; Cl, 23.70. $[\alpha]_D^{32°}$ = -31° (c=2.4, H_2O), -23° (c=1.2, 3N HCl). NMR, τ 4.5 (s. CH_2=), 5.9 (broad, -CH-), 7 (broad, $-CH_2-$). The MS shows a $^{35}Cl/^{37}Cl$ doublet of the decarboxylated ion.

The chloro amino acid was reduced on the catalytic hydrogenation with uptake of 2 moles of hydrogen, giving norvaline hydrochloride, which was identified with cellulose TLC and IR spectrum. The oxidation of the amino acid with $KMnO_4$ gave aspartic acid. The values of optical rotation suggest that this chloro amino acid belongs to L-series.

DL-2-Amino-4-chloro-4-pentenoic acid was synthesized by the acetamidomalonate alkylation with 2,3-dichloropropene. The crystalline mass of the condensation product was hydrolyzed in alkali under reflux. After the removal of the alkali with Amberlite IR-120 (H^+), dicarboxylic acid formed was refluxed in water to give free amino acid. The natural and synthetic amino acids showed the same Rf-values on cellulose TLC with several different solvents, fragmentation patterns in MS and NMR spectra.

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