(*S*)-*CIS*-2-AMINO-5-CHLORO-4-PENTENOIC ACID FROM THE FUNGUS *AMANITA VERGINEOIDES*

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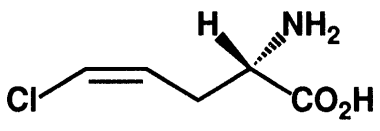
A chlorinated amino acid was isolated from *Amanita vergineoides*. The stereostructure was elucidated as (*S*)-*cis*-2-amino-5-chloro-4-pentenoic acid, on the basis of spectroscopic analysis.

KEY WORDS *Amanita; vergineoides*; Basidiomycetes; chlorine; unsaturated amino acids; (*S*)-*cis*-2-amino-5-chloro-4-pentenoic acid

Mushrooms belong to genus *Amanita* are famous for the Amanita poisoning that is caused by cyclic peptides such as amanitins.[1] Some *Amanita* mushrooms are also known to produce variously unsaturated amino acids, some of which are reported to show toxicities.[2] The white mushroom *Amanita vergineoides* is found occasionally in Japanese forests in early autumn. From this mushroom we isolated cyclopropylalanine and determined its structure.[3] Further survey of this mushroom led to the isolation of a new chlorinated amino acid. In this paper we wish to report the isolation and the structural elucidation of this amino acid.

20 g of *A. vergineoides* was extracted with MeOH, and the MeOH extract was partitioned between EtOAc and water. The water fraction was subjected to ion exchange chromatography using Amberlite IR 120B. The fraction eluted with 1N NH_4OH was fractionated further by chromatography over a cation exchange resin Dowex 50W that was buffered with ammonium-formate, pH 3.00. The amino acid (0.12%) was crystallized from water-EtOH after being dialyzed using Dowex 50W to give colorless needles, mp 186 °C (decomp.), $[\alpha]_D^{20}$ -79.0° (*c* 0.1, H_2O). The FD-MS peaks at *m/z* 153 and 151 [each $(M+2)^+$] and IR absorption at ν 688 cm^{-1} indicated the existence of a chlorine substituent in the amino acid. The presence of a carboxylate was implied by ^{13}C NMR (D_2O) signal at δ 173.6 (s) and by IR absorption at ν 1580 cm^{-1}. Treatment of this amino acid with acidic MeOH (HCl) and subsequent trifluoroacetylation gave a volatile substituent (**2**) which has the molecular formula $C_8H_9NO_3ClF_3$, *m/z* 261.0202 ($C_8H_9NO_3{}^{37}ClF_3$, Δmmu +9), 259.0222 ($C_8H_9NO_3{}^{35}ClF_3$, Δmmu 0). In the ^1H and ^{13}C NMR (D_2O) of the amino acid, signals due to an α–methine



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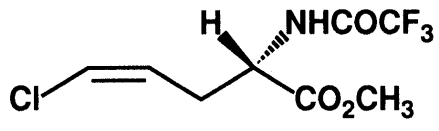

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(δ_H/δ_C 3.90/53.7), a methylene (δ_H/δ_C 2.88/28.2), and a disubstituted double bond (δ_H/δ_C 5.89, 6.42/122.7, 124.6) were observed.

The above three functions were connected in order as listed above, a methine – a methylene – a double bond. A remaining chlorine atom, thus, should be substituted at the end of a double bond whose geometry is indicated as *cis* with the coupling constant, J=6.6 Hz. The IR absorption at ν 740 cm^{-1} supported the *cis*- assignment. CD sign of the amino acid (**1**), $[\theta]_{207}$ +4100 denotes (*S*)-configuration of this amino acid.[4] Thus the stereostructure of the amino acid was elucidated as (*S*)-*cis*-2-Amino-5-chloro-4-pentenoic acid (**1**).

Unsaturated amino acids such as allylglycine and propargylglycine are known to cause convulsions in rats due to the inhibition of glutamate decarboxylase. The isolated amino acid (**1**) may be expected to show a similar activity.

Table 1. ^1H and ^{13}C NMR Spectral Data for the Amino Acid (**1**)

No.	δ_H	δ_C
1		173.6 (s)
2	3.90 t, J=5.7	53.7 (d)
3	2.88 dd, J=5.7, 6.6	28.2 (t)
4	5.89 dt, J=6.6, 6.6	122.7 (d)
5	6.42 d, J=6.6	124.6 (d)

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