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A New Amino Acid, L-3-(2-Carboxy-4-pyrrolyl)-alanine from the Poisonous Mushroom *Clytocybe acromelalga*

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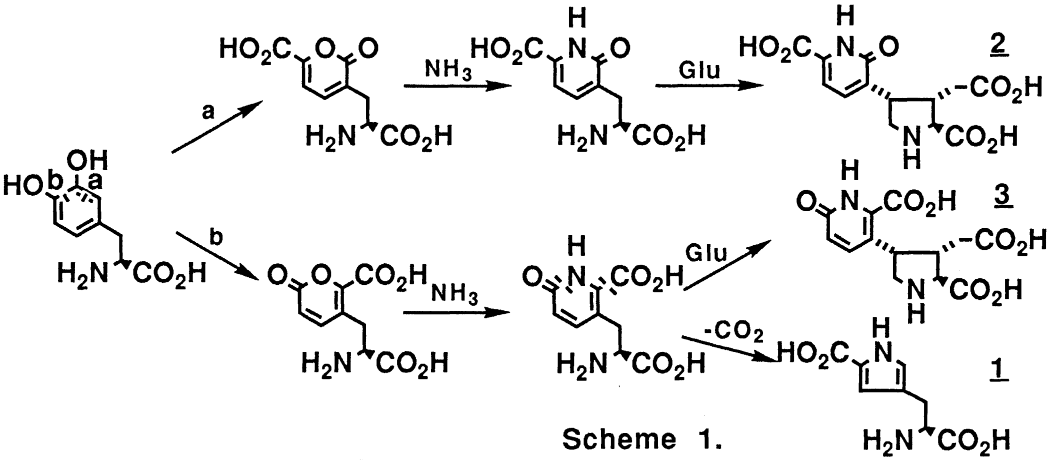
A new amino acid, L-3-(2-carboxy-4-pyrrolyl)-alanine was isolated from *Clytocybe acromelalga* . Its structure was determined by spectral data and a synthesis.

The title mushroom is found only in Japan. The accidental ingestion of this mushroom causes a violent pain and a marked reddish edema in the hand and foot after several days which continues for about a month. We have made efforts to isolate these characteristic toxins from the fungus and found several principles. They are clitidine, 1) clitioneine, 2) 4-amino quinolinic acid 3) and acromelic acid A (2) and B (3).4) Farther and continuous investigation led to the isolation of a new amino acid 1. In this paper, we wish to describe the determination of structure and the synthesis of 1.

The water extracts of fruit bodies were diluted by acetone to give precipitates which were dialysed against water. Dialysate was fractionated by chromatograpy and paper erectrophoresis monitoring the lethal effect in mice and 1 was isolated from a poisonous fraction.The weakly acidic property of 1 was obvious from its behavior on ion-exchange column chromatography and paper electrophoresis. ^{1}H-NMR and ^{13}C-NMR spectrum of 1 in D_2O indicated the presence of two aromatic protons, four carbons of aromatic ring, an alanine side chain and a carboxyl group on the aromatic ring.5) These observation and biogenetic consideration implied stucture 1 for the newly isolated amino acid (Scheme 1).



Scheme 1.

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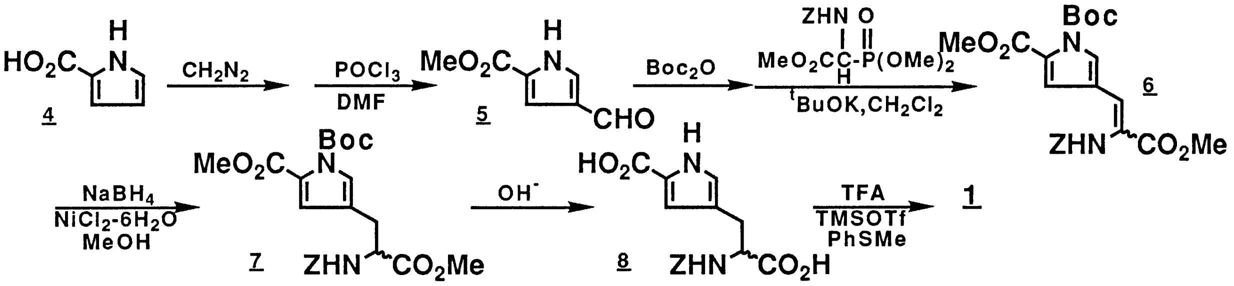
Due to a small amount of the sample the structure 1 was confirmed by synthesis (Scheme2). Pyrrole-2-carboxylic acid (4) was treated with diazomethane followed by Vilsmeier reagent to afford an aldehyde 5 which was converted to an αβ-unsaturated ester 6 with Horner-Emmons reagent 7) after protection of the imino group. Hydrogenation of 6 was carried out with NaBH$_4$ assisted by NiCl$_2$-6H$_2$O.8) Removal of protective groups furnished a racemic amino acid which underwent optical resolution employing TLC with chiral plate (Rf=0.56 and 0.65 MeOH/H$_2$O/MeCN=1/1/4).9) CD spectra showed (+) Cotton effect for the faster moving compound and (-) for the other. Since L-amino acid exhibits usually (+) Cotton effect in CD spectrum,10) the compound with Rf 0.65 should be L-isomer. The NMR and CD spectra, HPLC retention time and TLC (chiral plate) Rf of the synthetic L-amino acid were completely coincident with those of the natural product.



Scheme 2.

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5) Spectral data for 1: mp 200-202 °C (dec); UV (H$_2$O) λ_{max} 234 (log ε 3.76), 256 nm (4.06); ^{1}H-NMR (250 MHz, D$_2$O) δ 2.89 (1H, dd, J=5.1, 14.5 Hz), 2.98 (1H, dd, J=7.2, 14.5 Hz), 3.78 (1H, dd, J=5.1, 7.2 Hz), 6.48 (1H, brs), 6.80 (1H, brs); ^{13}C-NMR (67.5 MHz, D$_2$O) δ 28.6 (t), 56.4 (d), 115.9(d), 118.3 (s), 121.6 (s), 123.7 (d), 163.8 (s), 176.5 (s); HR-FABMS found: m/z 199.0710 [M+H]$^+$, calcd for C$_8$H$_{11}$O$_4$N$_2$: 199.0800.

6) Since this type of pyridonecarboxylic acid undergoes easily decarboxylation (unpublished observation), the biogenesis shown in Scheme1 is most probable. The chemical shifts of aromatic protons and carbons of pyrrole, furan and thiophene are quite different individually. The presence of pyrrole nucleus was suggested by NMR data.

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9) Macherey-Nagel Chiralplate 811-056. The instruction manual of M. Nagel Company describes that L-isomer moves always faster than D-isomer on this plate.

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