A Piperidine Amino Acid, 2,4,5-Piperidinetricarboxylic Acid from *Clitocybe acromelalga*

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A new piperidine amino acid, 2,4,5-piperidinetricarboxylic acid (**11**) was isolated from the poisonous mushroom, *Clitocybe acromelalga.* The structure determination and its biogenetic potential are discussed.

Introduction

Clitocybe acromelalga (Japanese name: Dokusasako), found only in Japan, is a famous toadstool which has a unique biological activity. The accidental ingestion of this fungus causes violent pain, a marked reddish edema on the hands and feet after several days, and the pain continues for about a month (Miura, 1936). These physiologically characteristic properties prompted us to study the toxic constituents of this mushroom. Since it was difficult to reproduce the symptoms in experimental animals' based on the lethal effect in mice, acromelic acids A and B were isolated as the toxic principles (Konno *et al.,* 1988). Acromelic acids are extraordinary potent neuroexcitatory amino acids and have attracted significant interest both pharmacologically and physiologically. Not only the complete study of these acids (Ishida and Shinozaki, 1991; Shinozaki and Ishida, 1991) but also investigation of other toxins are thus expected. Further separation of the water extracts of this mushroom had led to the isolation of ten new compounds possessing structures **1–10** (Fig. 1) (Hirayama *et al.,* 1989; Konno *et al.,* 1982; Konno *et al.,* 1984; Yamano and Shirahama, 1994; Yamano *et al.,* 1991; Yamano and Shirahama, 1992; Yamano *et al.,* 1992; Yamano and Shirahama, 1993; Yamano *et al.,* 1993; Yamano and Shirahama, 1993; Yamano and Shirahama, 1994). The amino acids **6–8** exhibited a weakly depolarizing activity in the preparation of a newborn rat spinal cord. The new pyridine nucleoside **1** and nucleotide **2** also showed a lethal effect in mice after intraperitoneal injection.

Further and continuous investigation resulted in the isolation of a new amino acid **11** which had a piperidine skeleton in contrast to kainoids which have a pyrrolidine ring. In this report, we describe the isolation and structural determination of 2,4,5-piperidinetricarboxylic acid (**11**) along with its biogenetic potential.

Results and Discussion

The water extracts were diluted with acetone to give a copious residue which was dialyzed against water. The dialyzate was further fractionated by chromatography and paper electrophoresis. Each step of the fractionation was monitored by assaying the lethal effect in mice. A novel amino acid **11** was separated from the poisonous fraction.

This newly isolated amino acid **11** did not show a yellow coloration but a violet coloration upon application of ninhydrin. The compound **11** is not likely a proline derivative. Its acidic property was obvious from the behavior during ion-exchange column chromatography and paper electrophoresis. In the FAB-MS spectrum, ion peaks at *m/z* 218 $[M+H]^+$ and 240 $[M+Na]^+$, suggesting the molecular formula $C_8H_{11}NO_6$, were observed. NMR spectra of **11** in D_2O exhibited signals due to two methylene and three methine groups (Table I). Particularly, the signal at δ 4.02 in the ^1H NMR spectrum seemed to be assigned to the α proton of the amino acid. Moreover, the measurement of

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Table I. NMR data of 2,4,5-piperidinetricarboxylic acid (**11**).

	2	3-α	3-β	4	5	6-α	6-β
H	4.02 dd (5.0, 7.8)	2.39 ddd (5.0, 6.8, 14.7)	2.32 ddd (4.4, 7.8, 14.7)	2.99 dt (4.4, 6.8)	3.11 dt (3.9, 6.8)	3.45 dd (3.9, 13.2)	3.57 dd (6.8, 13.2)
C	67.7 d	27.2 t		41.4 d	42.4 d	43.1 t	

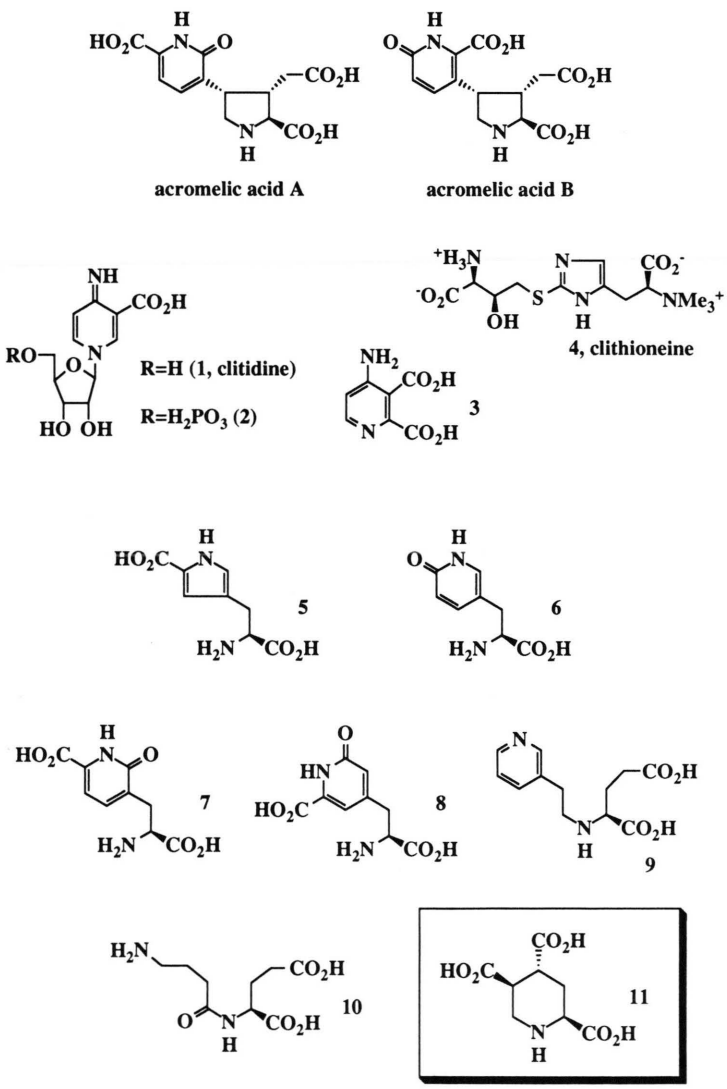
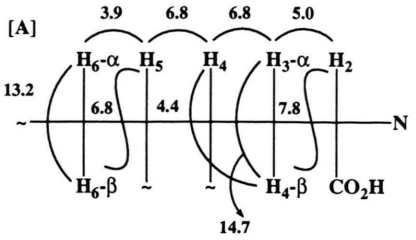


Fig. 1. The compounds isolated from *Clitocybe acromelalga* so far.



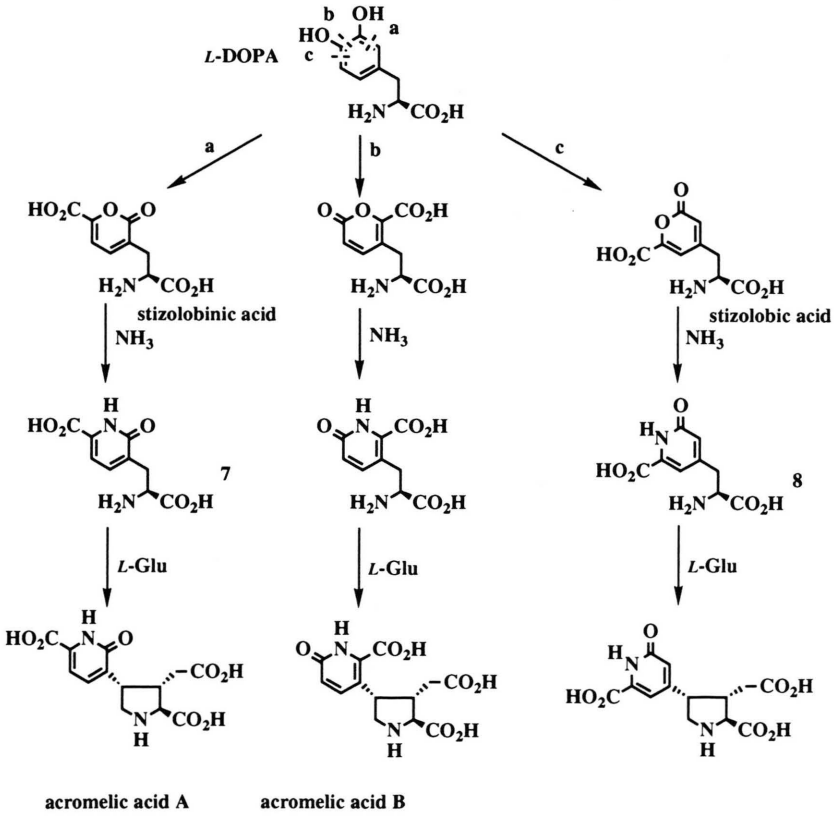
Coupling constants in Hz are given in figure.

Fig. 2. The partial structures A and B deduced from $^1H-^1H$ COSY spectrum and ^1H and ^{13}C NMR spectra, respectively.

the $^1H-^1H$ COSY spectrum suggested the partial structure A (Fig. 2). Although carbon signals of three carboxyl groups were not detectable because of their weak intensity in the ^{13}C NMR spectrum, the chemical shifts in the NMR spectra (^1H and ^{13}C) and the acidic property implied the piperidinetricarboxylic acid structure B (Fig. 2). The relative configuration of **11** was established by the splitting patterns in the ^1H NMR spectrum and an NOE experiment (Fig. 3).

We proposed the biogenetic pathway of acromelic acids, such as Schemes 1 and 2, in previous papers (Konno *et al.*, 1988). In this route, the left-half moieties of the acromelic acids were derived from 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) *via* stizolobinic acid, stizolobic acid, *etc.* Aromatic compounds derived from DOPA coupled with L-glutamic acid (L-Glu) with concomitant decarboxylation and deamination, subsequently generated a 3–4 bond to produce the pyrrolidine derivatives, that is, the acromelic acids.

We thought that **11** might also be derived from Asp and L-Glu *via* a biosynthetic pathway similar to that for acromelic acids (Scheme 3). Kainoids



Scheme 1. Biogenesis of acromelic acids – cleavage of L-DOPA and its recyclization gives the pyridone moieties of acromelic acids.

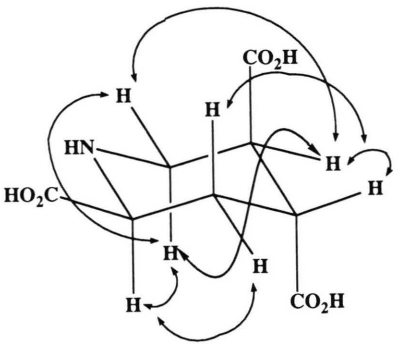


Fig. 3. Nuclear Overhauser effects observed between protons indicated by double-headed arrows.

are formed by the bond formation so as to give a pyrrolidine ring. In the generation of **11,** however, the final bond formation seems to occur between the two α-carbons to produce the piperidine ring. This hypothesis suggests, therefore, that **11** also has a 2-(*S*) configuration based on L-Glu. Furthermore, there may be the possibility of isolating the kainoid **12.** Indeed, the isolation of **12** was recently reported (Fushiya *et al.,* 1993).

Experimental

General procedure

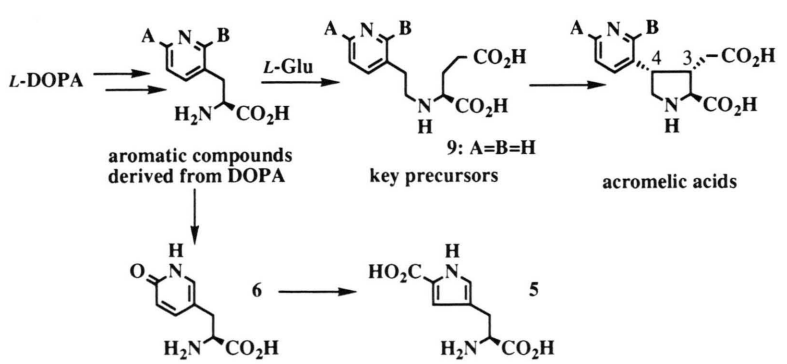
The MS spectrum was obtained using a JEOL model JMS-DX 300 spectrometer. The ^1H NMR spectrum was recorded in D_2O using a JEOL model JNM-FX 400 (400 MHz). Chemical shifts were obtained as δ values in ppm relative to HDO (4.8 ppm) in D_2O. The ^{13}C NMR spectrum was measured in D_2O using a JEOL model JNM-FX 400 (100 MHz), and dioxane (67.4 ppm) was employed as an internal standard.

Mushroom material

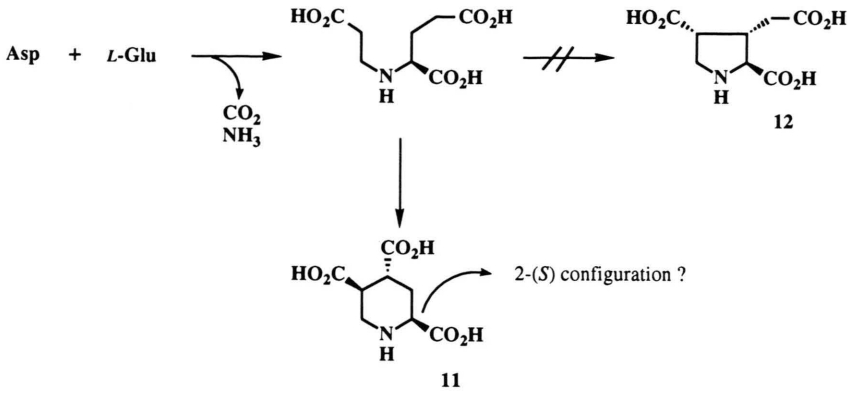
Fruiting bodies of *Clitocybe acromelalga* were collected in the autumn in Nagaokashi, Niigata-ken, Japan. They were frozen upon collection, and stored at −20 °C.

Isolation of **11**

Frozen fruit bodies (6 kg) were extracted with H_2O (7 l×3) at 4 °C overnight. The combined extracts were concentrated *in vacuo* to about 1 l. To this turbid solution was added acetone (2.5 l) and the mixture was allowed to stand at 4 °C overnight. The supernatant was decanted and the lower muddy layer was then evaporated. The copious residue was dialyzed against H_2O (3 l×4) at 4 °C overnight. The combined dialyzate was evaporated and the residue (338 g) was applied to a column of charcoal (300 g, packed in H_2O). The column was eluted stepwise with several concentrations of aq. EtOH (H_2O, 2.5, 5, 10 and 30% aq. EtOH, each 10 l). The 2.5–5% aq. EtOH fraction was collected and the solvent was removed *in vacuo* (residue: 42 g). The residue (6 g×7) was chromatographed on a column of weakly basic ion-exchange resin (Amberlite IR-45, HCO_2^- form) using H_2O–HCO_2H (H_2O, 5, 10 and 20% aq. HCO_2H, each 6 l) as a solvent. The eluate with 10–20% aq. HCO_2H was concentrated *in vacuo* and a portion of the resultant paste (3.7 g) was subjected to paper electrophoresis (46×20 cm, pH 4.6, 600 V, 1.5 h). An area of moving length, +3–9 cm was cut off and the strips extracted with H_2O. The solvent was then removed. The residue was placed on cellulose TLC plates and developed using a mixed solvent (MeOH–Py–H_2O, 15:1:5). A new amino acid **11** was isolated from the band



Scheme 2. Biogenesis of acromelic acids – the condensation of various pyridylalanines and L-Glu gives acromelic acids.



Scheme 3. A probable biogenesis of 2,4,5-piperidinetricarboxylic acid (**11**).

at R_f 0.52 (2.0 mg). **11:** NMR data are shown in the text; HR-FAB MS found: *m/z* 218.0679 $[M+H]^+$, calcd for $C_8H_{12}NO_6$: 218.0765 and 240.0504 $[M+Na]^+$, calcd for $C_8H_{11}NO_6Na$: 240.0484.

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