

A NOVEL NEUROEXCITATORY AMINO ACID FROM
CLITOCYBE ACROMELALGA
A POSSIBLE INTERMEDIATE IN THE BIOGENESIS OF
ACROMELIC ACID A †

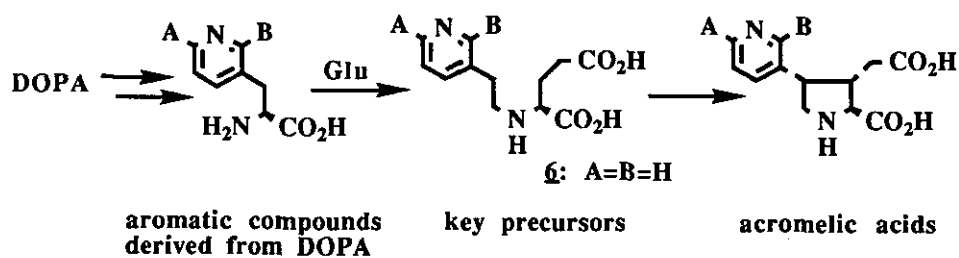
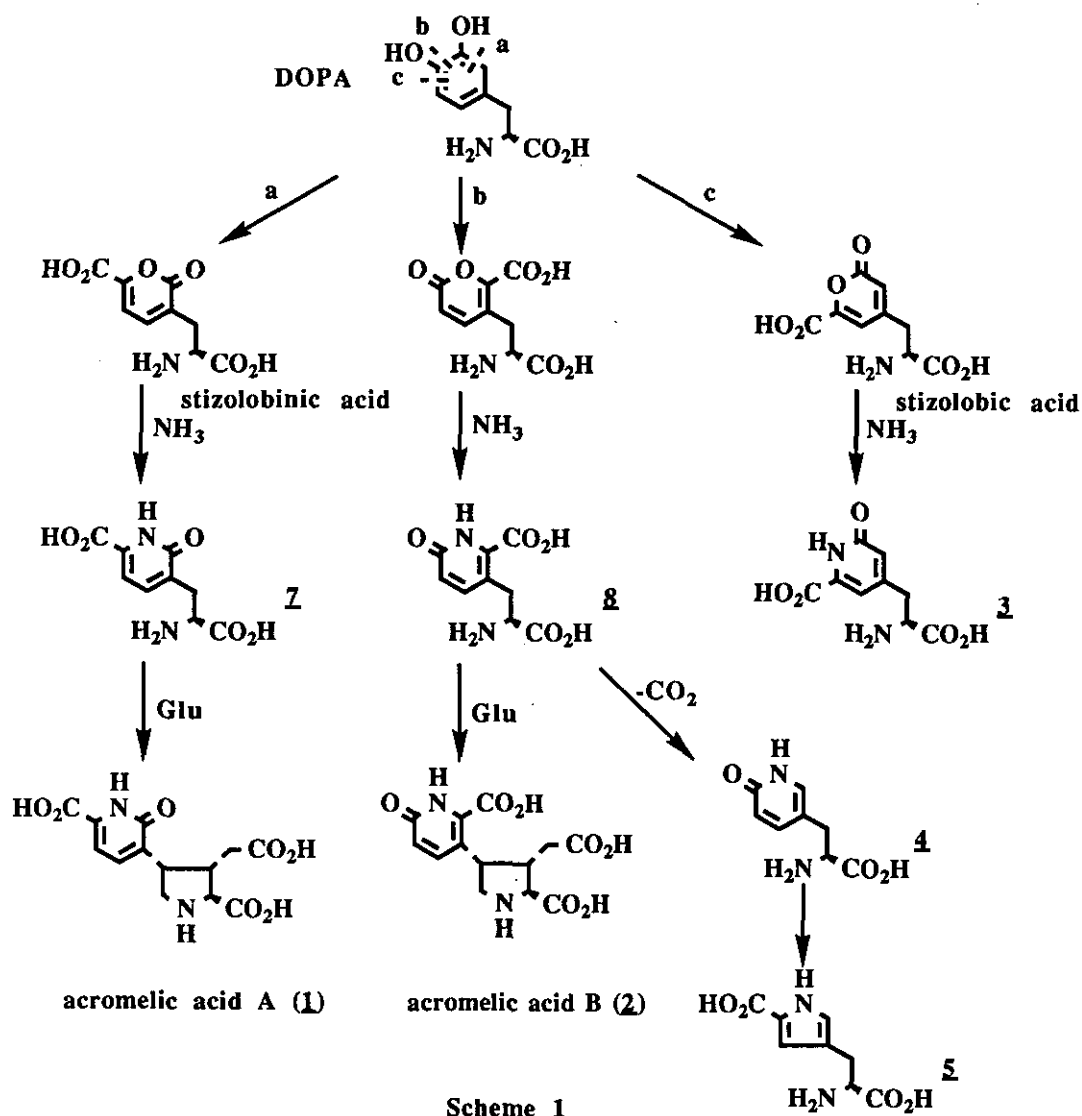
Kimiaki Yamano, Katsuhiro Konno, and Haruhisa Shirahama*

Department of Chemistry, Faculty of Science, Hokkaido University,
Sapporo 060, Japan

Abstract- A new neuroexcitatory amino acid, *L*-3-(6-carboxy-2-oxo-3-pyridyl)alanine (**7**), was isolated from the poisonous mushroom Clitocybe acromelalga and its structure was confirmed by chemical conversion. Its occurrence in this fungus supports the previously proposed biogenesis of acromelic acid A.

Acromelic acids A (**1**) and B (**2**), the potent neuroexcitatory amino acids, were recently found as toxic principles in the poisonous mushroom, Clitocybe acromelalga (Japanese name: Dokusasako). ¹ They have attracted considerable interest in the field of neurobiology because of their physiological and pharmacological activities. ² During further investigation to seek other toxins, we isolated several amino acids such as stizolobic acid, *L*-3-(6-carboxy-2-oxo-4-pyridyl)alanine (**3**), ³ *L*-3-(2-oxo-5-pyridyl)alanine (**4**), ⁴ *L*-3-(2-carboxy-4-pyrrolyl)alanine (**5**) ^{4, 5} and *L*-*N*-[2-(3-pyridyl)ethyl]glutamic acid (**6**). ⁶ They may be important in understanding the biogenesis of acromelic acids. We proposed that acromelic acids were biosynthetically derived by condensation of a DOPA derivative and glutamic acid (Scheme 1, 2). ^{1, 6} Furthermore, we isolated recently a new amino acid, *L*-3-(6-carboxy-2-oxo-3-pyridyl)alanine (**7**). We would like to describe the isolation and

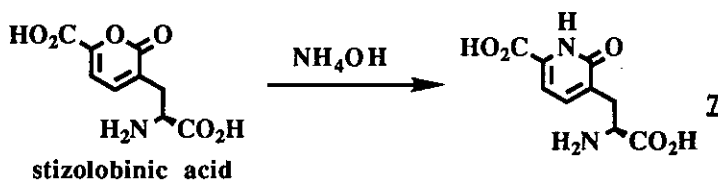
† Dedicated to Professor Edward C. Taylor on the occasion on his 70th birthday.



structural determination of this amino acid (**7**) which gives further support to the proposed biogenetic pathway (Scheme 1).

Fractionation of the water extracts was carried out by monitoring the lethal effect in mice. The new amino acid (**7**) was separated from a poisonous fraction containing acromelic acids by means of paper electrophoresis.

This new amino acid (**7**), $[\alpha]_D^{25} -5.8^\circ$ (c 0.13, H_2O), $C_9H_{10}N_2O_5$, showed a weak brown coloration with ninhydrin. 1H -Nmr spectrum (400 MHz) of **7** in D_2O indicated the presence of two aromatic protons (δ 7.03, 1H, d, $J=7.1$; 7.69, 1H, d, $J=7.1$) and an alanine side chain (δ 3.04, 1H, dd, $J=8.1, 14.6$; 3.22, 1H, dd, $J=5.3, 14.6$; 4.07, 1H, dd, $J=5.3, 8.1$). The uv spectrum exhibited a maximum at around 241 and 315 nm which were very similar to those of acromelic acids.¹ Furthermore, the coupling constants and chemical shifts of the two aromatic protons in the 1H -nmr spectrum suggested a 6-carboxy-3-substituted pyridone moiety. These spectroscopic data and biogenetic considerations (Scheme 1) implied structure (**7**) for the newly isolated amino acid, and confirmation of this structure was achieved by the conversion from stizolobinic acid (Scheme 3). The nmr spectral data, Rf value (R_f 0.55, $nBuOH/HCO_2H/H_2O=6/1/2$) on cellulose tlc and $[\alpha]_D$ value (-4.9° , c 0.12, H_2O) of synthetic **7** were identical with those of natural one.



Scheme 3

This amino acid (**7**) exhibited weakly depolarizing activity in the preparation of new born rat spinal cord. The occurrence of **7** in *C. acromelalgae* supports our biogenic pathway of acromelic acid A from DOPA. Isolation of the compound (**8**) and the compounds corresponding to acromelic acids derived from intermediates such as **3**, **5** and **6** is expected. Further studies on the isolation of new compounds is in progress.

ACKNOWLEDGEMENT

We are grateful to Drs. H. Shinozaki and M. Ishida (Tokyo Metropolitan Institute for Medical Science) for the biological test. We thank Dr. Y. Ohfuné (Suntory Institute for Bioorganic Research) for providing *DL*-stizolobinic acid.

REFERENCES

1. K. Konno, K. Hashimoto, Y. Ohfuné, H. Shirahama, and T. Matsumoto, J. Am. Chem. Soc., 1988, **110**, 4807.
2. M. Ishida and H. Shinozaki, British J. Pharmacol., 1991, **104**, 873; H. Shinozaki and M. Ishida, Pacific J. Pharmacol., 1991, 293; and references cited therein.
3. K. Yamano, K. Hashimoto, and H. Shirahama, Heterocycles, 1992, **34**, 445.
4. K. Yamano and H. Shirahama, Tetrahedron, 1992, **48**, 1457.
5. K. Yamano, K. Konno, and H. Shirahama, Chem. Lett., 1991, 1451.
6. K. Yamano and H. Shirahama, Chem. Lett., in press.

Received, 18th November, 1992