NOVEL NEUROEXCITATORY AMINO ACID FROM CLITOCYBE ACROMELALGA

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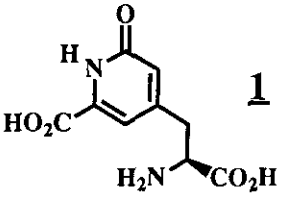
Abstract-Novel neuroexcitatory amino acid, *L*-3-(6-carboxy-2-oxo-4-pyridyl)alanine (1), was isolated from the poisonous mushroom, Clitocybe acromelalga, and its structure was deduced by spectral data and biogenesis and confirmed by the chemical conversion.

The accidental ingestion of Japanese toadstool, Clitocybe acromelalga, causes a violent pain and a remarkable reddish edema in hand and foot, and it continues for a month. We were interested in this unique physiologically characteristic properties and have studied on the toxic constituent of this fungus. We have already isolated several principles so far: clitidine,[1] clitioneine,[2] 4-aminoquinolinic acid[3] and acromelic acids A (2) and B (3).[4] And new amino acids, *L*-3-(2-carboxy-4-pyrrolyl)alanine (4)[5,6] and *L*-3-(2-oxo-5-pyridyl)alanine (5),[6] were also found recently. Further investigation led to the isolation of the novel neuroexcitatory amino acid (1) with stizolobic acid (6).[7,8] These amino acids were probably biosynthetically derived from DOPA.[4,9] This report deals with the isolation and the structure determination of 1 through the chemical conversion of stizolobic acid into 1.



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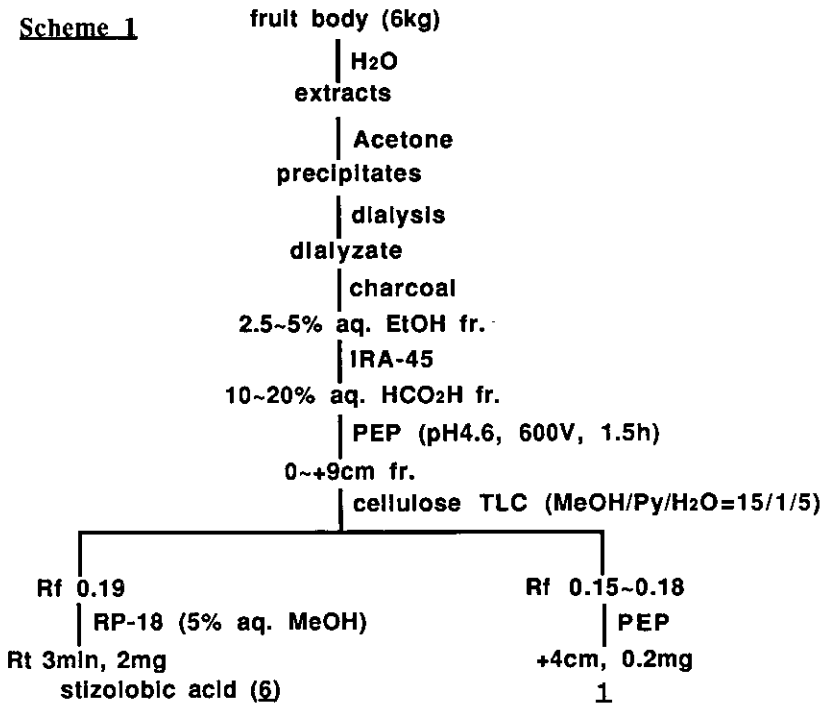
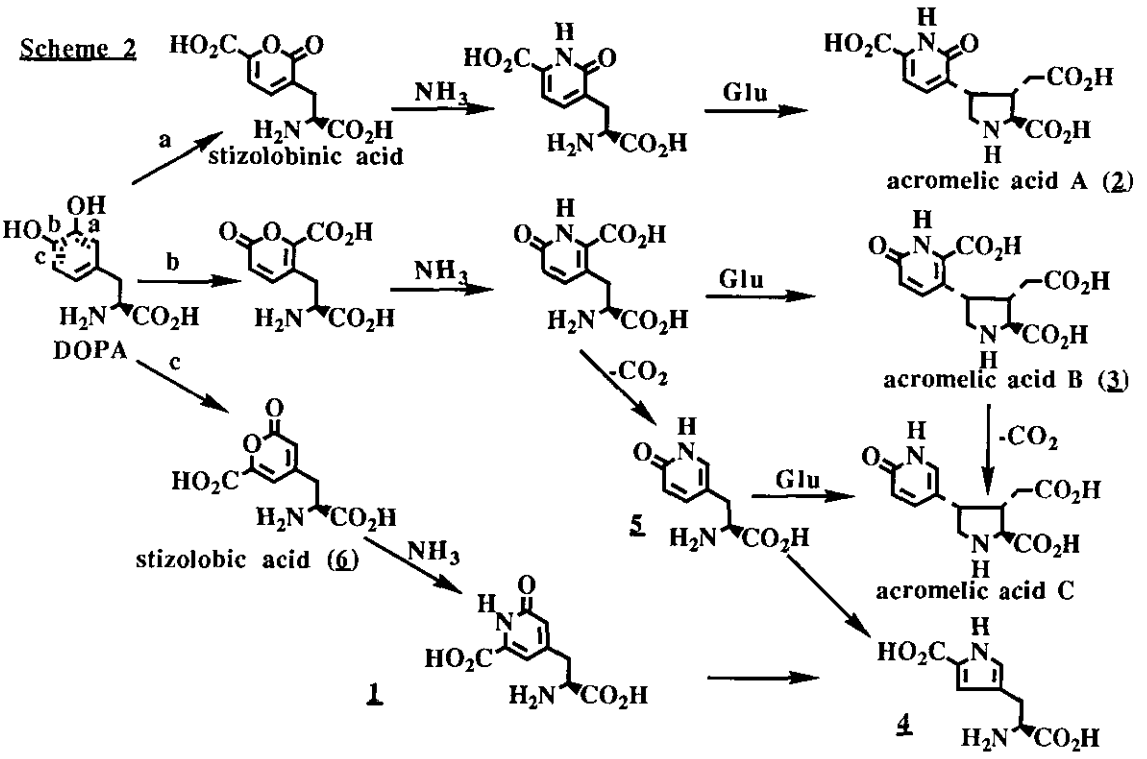
The water extracts of frozen fruit bodies were diluted by acetone to give copious precipitates which were dialyzed against water. Dialyzate was fractionated by chromatography and paper electrophoresis (PEP) monitoring the lethal effect in mice. New amino acid (1) and stizolobic acid were isolated from a poisonous fraction (Scheme 1).

Scheme 1

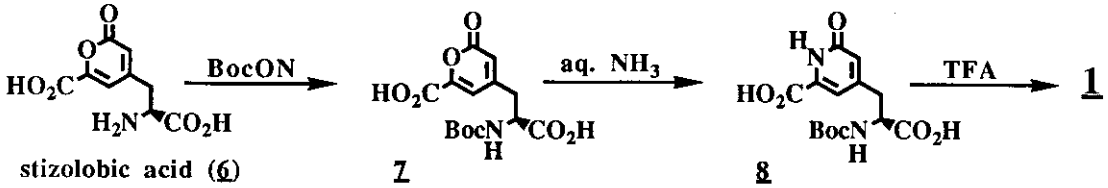


The acid (1), $[\alpha]_D^{22}$ -131° (H_2O; *c* 0.05), $C_9H_{10}N_2O_5$, showed a brown coloration with ninhydrin. The weakly acidic property of 1 was obvious from its behavior on ion-exchange column chromatography and paper electrophoresis. ^{1}H-Nmr spectra of 1 in D_2O indicated the presence of the two aromatic protons [δ 6.60, 1H, d, J=1.4; 6.95, 1H, d, J=1.4] and an alanine side chain [δ 3.01, 1H, dd, J=8.6, 14.6; 3.21, 1H, dd, J=5.4, 14.6; 3.98, 1H, dd, J=5.4, 8.6]. The uv spectra of 1 showed two maxima around at 230 and 310 nm which were very similar to those of acromelic acids.[4] Furthermore, the coupling constants and chemical shifts of two aromatic protons in the ^{1}H-nmr spectrum suggested 6-carboxy-4-substituted pyridone structure. The structure of 1 was implied from these observation and biogenetic consideration (Scheme 2).

Biosynthetical formation of stizolobic acid from DOPA was previously shown employing seedlings of beans[9] and it was proposed by Musso for the explanation of the biosynthesis of flyagaric coloring matters.[10] This route suited to the biogenesis of 1 and was reasonably assembled into the previously proposed biogenetic scheme of acromelic acids A (2) and B (3)[1] and 4.[5]



The confirmation of the structure (1) was carried out by a synthesis. The amino group of stizolobic acid was protected by *tert*-butoxycarbonyl group. *N*-Boc-stizolobic acid (7) was treated with aqueous ammonia to afford pyridone (8). Removal of protective group furnished 1 (88% for three steps, Scheme 3). Purification was performed by paper electrophoresis. The nmr spectra, $[\alpha]_D$ and Rf value on tlc of synthetic 1 were completely coincident with those of the natural product. As the amount of the isolated compound was very little, the bioactivity was tested with the synthetic one. The amino acid (1) exhibited depolarizing activity in the preparation of new born rat spinal cord.



stizolobic acid (6) 7 8

Scheme 3

ACKNOWLEDGMENT

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