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Isolation of *L-N*-[2-(3-Pyridyl)ethyl]-glutamic Acid from the Poisonous Mushroom *Clitocybe acromelalga*. A Possible Intermediate in the Biogenesis of Acromelic Acids

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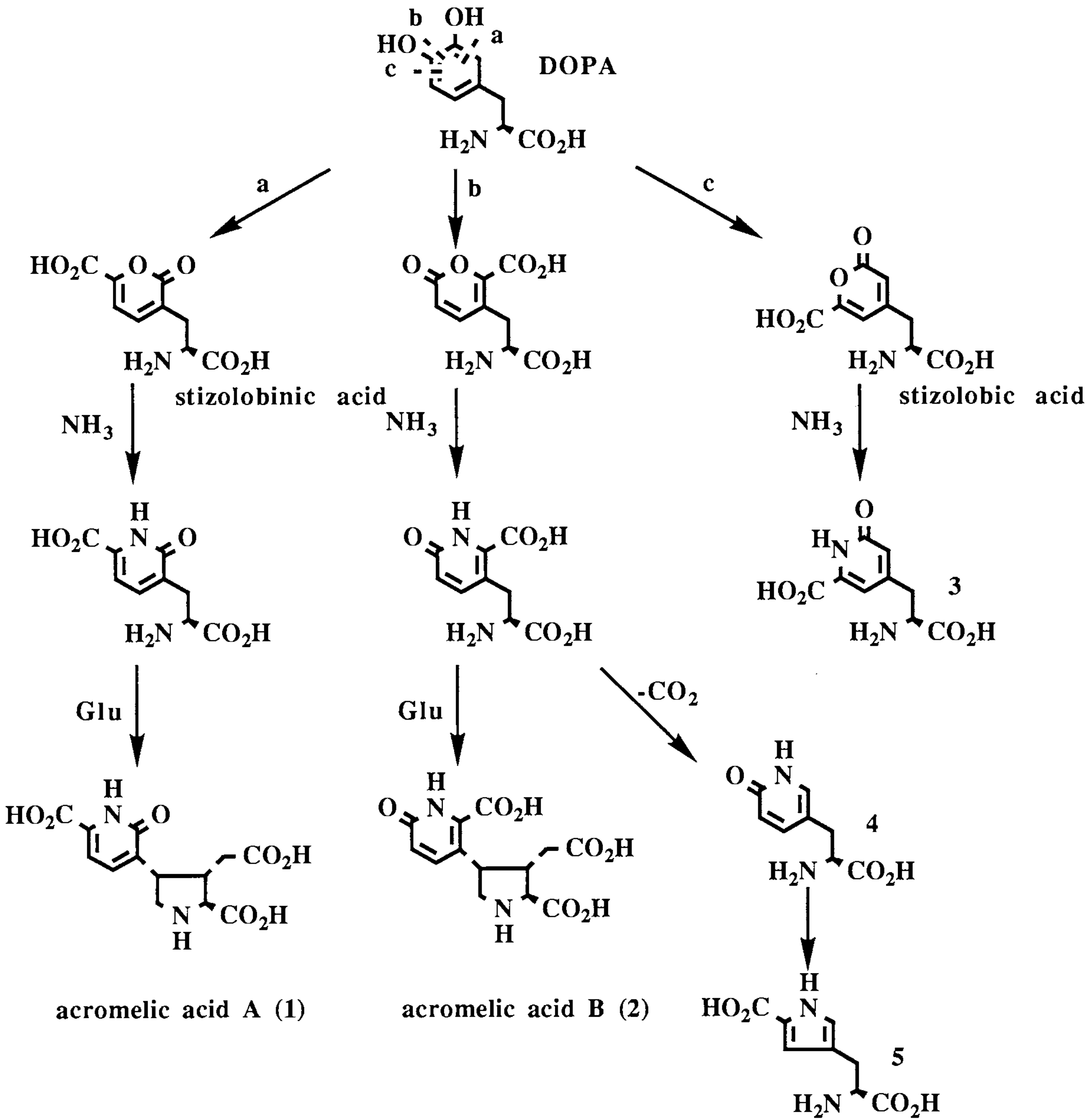
A new amino acid, *L-N*-[2-(3-pyridyl)ethyl]-glutamic acid, was isolated from the poisonous mushroom *Clitocybe acromelalga* and the structure confirmed by synthesis. Its occurrence in this fungus supports the previously proposed biogenesis of acromelic acids.

The extraordinarily potent neuroexcitatory amino acids, acromelic acids A (**1**) and B (**2**), were recently found as toxic principles in the poisonous mushroom, *Clitocybe acromelalga* (Japanese name: Dokusasako). 1) Because of their physiological and pharmacological activities, they have attracted considerable interest in neurobiology studies. 2) During further separation of the extracts of this mushroom 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) and *L*-3-(2-carboxy-4-pyrrolyl)alanine (**5**), 4, 5) which may be important in understanding the biogenesis of acromelic acids, and in our original proposal for the biogenesis of acromelic acids, we proposed that stizolobic acid was derived from DOPA. 1, 6) In this biogenetic Scheme 1, DOPA is cleaved in the catechol ring and subsequently recyclized to pyrone derivatives, stizolobic acid and a similar compound, which combines with glutamic acid to give acromelic acids. 7) Recently, the biosynthesis of the related neuroexcitant domoic acid by a marine diatom was reported. 8) It was shown that domoic acid is assembled by condensation of geranyl pyrophosphate and glutamic acid, and is very similar to our proposed pathway for the acromelic acids. In this report we would like to describe the isolation and structural determination of *L-N*-[2-(3-pyridyl)ethyl]-glutamic acid (**6**) which appears to be biosynthetically derived by condensation of a DOPA derivative and glutamic acid. The isolation of **6** will give further support to our biogenetic scheme as well as the pathway proposed for domoic acid in microalgae.

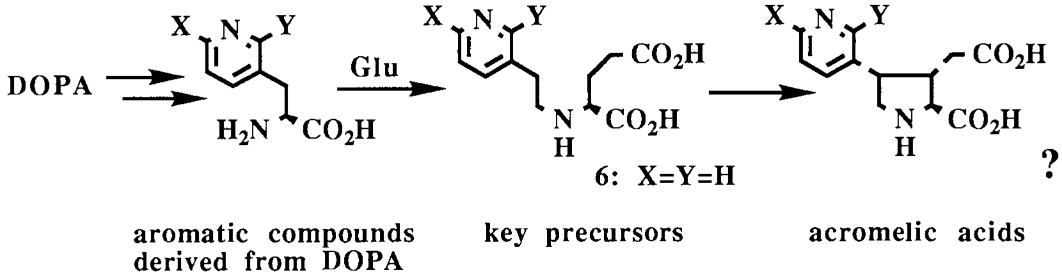
Fractionation of the extracts was carried out by monitoring the lethal effect in mice. From a poisonous fraction containing an acromelic acid, a new amino acid **6** was separated by means of paper electrophoresis and HPLC.

This new amino acid **6**, obtained as an amorphous powder, mp 176-180°C (decomp), $[\alpha]_D^{25}$ +129° (H_2O; *c* 0.09), showed a violet coloration with ninhydrin and exhibited a protonated ion at m/z 253 (FABMS; $[M+H]^+$), corresponding to the molecular formula $C_{12}H_{17}N_2O_4$. ^{1}H-NMR spectrum of **6** in D_2O indicated the presence of four aromatic protons [δ 7.72, 1H, dd, J=6.6, 7.8 Hz; 8.13, 1H, brd, J=7.8 Hz; 8.66, 2H, m], a glutamic acid moiety [δ 2.20, 2H, m; 2.52, 2H, brt, J=6.8 Hz; 3.77, 1H, t, J=5.9 Hz] and signals due to two methylene groups [δ 3.28, 2H, t, J=7.8 Hz; 3.49, 2H, t, J=7.8 Hz]. The UV spectrum exhibited a maximum at around 260 nm, corresponding to absorption of a substituted pyridine ring. Furthermore, the coupling constants and chemical shifts of the four aromatic protons in the ^{1}H-NMR spectrum suggested a 3-

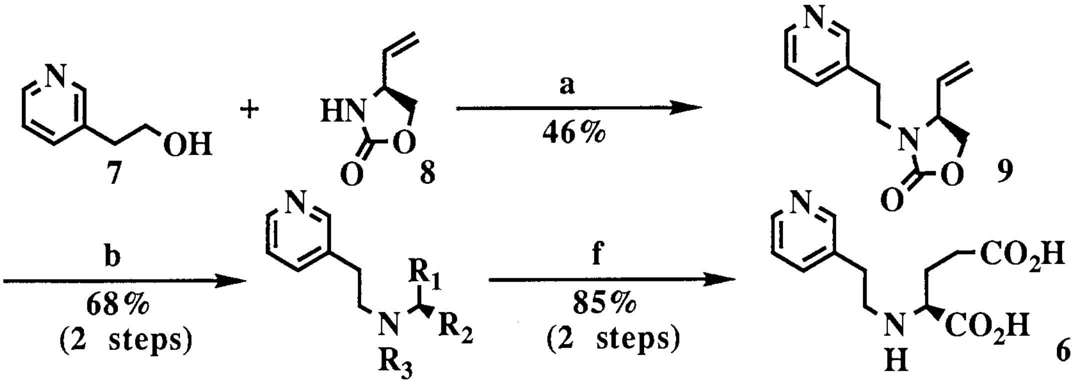
substituted pyridine moiety. These chemical data and biogenetic considerations (Schemes 1 and 2) implied structure **6** for the newly isolated amino acid, and confirmation of this structure was achieved by synthesis (Scheme 3).



Scheme 1.



Scheme 2.



c **10** R_1= CH=CH_2, R_2= CH_2OH, R_3= Boc
11 R_1= CH=$CHCO_2Me$, R_2= CH_2OH, R_3= Boc (42%, 2 steps)
d **12** R_1= $CH_2CH_2CO_2Me$, R_2= CH_2OH, R_3= Boc (99%)
e **13** R_1= $CH_2CH_2CO_2Me$, R_2= CO_2Me, R_3= Boc (91%, 3 steps)

a) Tf_2O, KH/CH_2Cl_2 b) (1) 3 M KOH/MeOH, (2) Boc_2O c) (1) O_3, (2) Ph_3P=$CHCO_2Me$ d) $NaBH_4$, $NiCl_2$-$6H_2O$ e) (1) Dess-Martin reagent, (2) $NaClO_2$, (3) CH_2N_2 f) (1) 1 M KOH/MeOH, (2) TFA (1 M=1mol dm^{-3})

Scheme 3.

Since many attempts to N-alkyl glutamic acid or its derivatives by various pyridylethyl moieties were by no means successful, vinyloxazolidone **8** [9)] was used as a source of glutamic acid moiety. Coupling of the triflate of 2-(3-pyridyl)ethanol **7** with vinyloxazolidone **8** was carried out with KH in CH_2Cl_2. The adduct **9** [10)] was converted to **10** [10)] through hydrolysis and N-protection. The compound **10** was oxidized by ozone and the resulting aldehyde was treated with Wittig reagent to afford an αβ-unsaturated ester **11**.[10)] Hydrogenation of the double bond of **11** was carried out with $NaBH_4$ assisted by $NiCl_2 \cdot 6H_2O$. The primary alcohol of saturated ester **12** [10)] was stepwise oxidized with Dess-Martin reagent and $NaClO_2$ into an acid whose ester **13** [10)] was deprotected to furnish the product **6**. Final purification of the amino acid product was performed by paper electrophoresis. The NMR spectral data, Rf value (Rf 0.31, ^{n}BuOH/HCO_2H/H_2O=6/1/2) on TLC and $[\alpha]_D$ value (+126°, H_2O; *c* 0.09) of the synthetic **6** were identical with those of the natural product.

The probable biosynthesis of acromelic acids (Scheme 2) would invoke *N*-pyridylethyl-glutamic acids corresponding to **6** as precursors of various acromelic acids A-E. However, the acromelic acids derived from intermediates such as **3**, **5**, and **6** have not been isolated yet, and the possibility exists that they are merely shunt products in the acromelic acid biosynthetic pathway.

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References

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10) The spectral data of **9-13** are as follows. **9:** $[\alpha]_D^{30}$ -2.6° ($CHCl_3$, *c* 0.55); IR ν_{max} (neat) cm^{-1}: 3680-3040, 1745, 1657, 1640, 1592, 1573, 1475, 1415, 1370, 1260-1210, 1188, 1158, 1082, 1025, 940, 805, 767, and 717; ^1H-NMR ($CDCl_3$): δ 2.89 (2H, m), 3.30 (1H, dt, J=7.0 and 14 Hz), 3.59 (1H, dt, J=7.0 and 14 Hz), 3.92 (1H, t, J=8.0 Hz), 4.05 (1H, q, J=8.0 Hz), 4.38 (1H, t, J=8.0 Hz), 5.29 (1H, brd, J=16.5 Hz), 5.35 (1H, brd, J=9.0 Hz), 5.57 (1H, brdd, J=9.0 and 16.5 Hz), 7.26 (1H, m), 7.58 (1H, brd, J=7.8 Hz), 8.47 (1H, brs), and 8.50 (1H, brd, J=4.5 Hz); EI-MS m/z (rel. int.): 218 $[M]^+$ (34), 191 (2), 174 (1), 149 (1), 126 (71), 106 (14), 92 (7), 78 (5), 65 (8), 55 (100), 41 (18), and 39 (18); HR-MS found: m/z 218.1037 $[M]^+$, calcd for $C_{12}H_{14}N_2O_2$: 218.1056. **10:** $[\alpha]_D^{26}$ +26.4° ($CHCl_3$, *c* 0.28); IR ν_{max} (neat) cm^{-1}: 3600-3020, 1720-1625, 1580, 1484, 1453, 1445, 1425, 1368, 1250, 1160, 1029, 928, 896, 857, 808, 781 and, 720; ^1H-NMR ($CDCl_3$): δ 1.53 (9H, s), 2.87 (2H, t, J=7.0 Hz), 3.37 (2H, t, J=7.0 Hz), 3.79 (2H, d, J=6.0 Hz), 4.35 (1H, m), 5.19 (1H, brd, J=17 Hz), 5.24 (1H, brd, J=11 Hz), 5.87 (1H, m), 7.23 (1H, dd, J=4.5 and 7.0 Hz), 7.53 (1H, brd, J=7.0 Hz), and 8.46 (2H, m); EI-MS m/z (rel. int.): 292 $[M]^+$ (1), 261 (12), 219 (8), 205 (10), 161 (70), 149 (5), 144 (5), 106 (13), 100 (39), 93 (40), 68 (7), 57 (100) and 41 (28); HR-MS found: m/z 292.1796 $[M]^+$, calcd for $C_{16}H_{24}N_2O_3$: 292.1787. **11:** $[\alpha]_D^{27}$ +3.6° ($CHCl_3$, *c* 0.78); IR ν_{max} (neat) cm^{-1}: 3600-3040, 1725, 1688, 1665, 1480, 1451, 1425, 1407, 1365, 1310, 1274, 1248, 1159, 1042, 1028, 980, 860, 904, 778, and 718; ^1H-NMR ($CDCl_3$): δ 1.51 (9H, s), 2.87 (2H, t, J=7.0 Hz), 3.38 (2H, t, J=7.0 Hz), 3.74 (3H, s), 3.87 (2H, d, J=6.0 Hz), 4.47 (1H, m), 5.89 (1H, d, J=16 Hz), 6.94 (1H, dd, J=5.0 and 16 Hz), 7.24 (1H, dd, J=4.5 and 7.0 Hz), 7.52 (1H, m), and 8.47 (2H, m); EI-MS m/z (rel. int.): 350 $[M]^+$ (1), 320 (7), 277 (6), 263 (9), 219 (50), 158 (45), 106 (16), 93 (54), 69 (6), 57 (100), and 41(25); HR-MS found: m/z 350.1841 $[M]^+$, calcd for $C_{18}H_{26}N_2O_5$: 350.1841. **12:** $[\alpha]_D^{25}$ -4.3° ($CHCl_3$, *c* 0.92); IR ν_{max} (neat) cm^{-1}: 3660-3040, 1750-1730, 1690, 1678, 1665, 1596, 1580, 1582, 1414, 1367, 1252, 1160, 885, 864, 805, 780, and 720; ^1H-NMR ($CDCl_3$): δ 1.50 (9H, s), 1.92 (2H, m), 2.37 (2H, m), 2.87 (2H, t, J=7.0 Hz), 3.33 (2H, t, J=7.0 Hz), 3.68 (3H, s), 3.70(2H, m), 3.85 (1H, m), 7.24 (1H, dd, J=4.5 and 7.0 Hz), 7.53 (1H, m), and 8.46 (2H, m); EI-MS m/z (rel. int.): 352 $[M]^+$ (1), 321 (4), 295 (1), 279 (6), 265 (7), 221 (38), 204 (13), 189 (5), 165 (15), 160 (42), 149 (7), 128 (21), 106 (22), 99 (6), 93 (47), 71 (7), 57 (100), and 41 (24); HR-MS found: m/z 352.2017 $[M]^+$, calcd for $C_{18}H_{28}N_2O_5$: 352.1999. **13:** $[\alpha]_D^{28}$ -4.4° ($CHCl_3$, *c* 0.90); IR ν_{max} (neat) cm^{-1}: 3690-3060, 1738, 1700, 1687, 1490-1338, 1368, 1299, 1250, 1213, 1155, 1023, 760, and 718; ^1H-NMR ($CDCl_3$): δ 1.45 (9H, s), 2.00-2.46 (4H, m), 2.91 (2H, m), 3.22 (1H, m), 3.40-3.80 (2H, m), 3.70 (3H, s), 3.75 (3H, s), 7.23 (1H, dd, J=5.0 and 7.0 Hz), 7.57 (1H, brd, J=7.0 Hz), and 8.47 (2H, m); EI-MS m/z (rel. int.): 380 $[M]^+$ (9), 293 (5), 288 (8), 279 (7), 265 (2), 249 (6), 221 (17), 188 (88), 165 (7), 156 (18), 128 (24), 106 (21), 93 (39), 68 (9), 57 (100), and 41 (25); HR-MS found: m/z 380.1949 $[M]^+$, calcd for $C_{19}H_{28}N_2O_6$: 380.1948.

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