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New Amino Acids from the Poisonous Mushroom *Clitocybe acromelalga*

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Key Word Index- *Clitocybe acromelalga*; amino acid; *L*-3-(2-carboxy-4-pyrrolyl)-alanine; *L*-3-(2-oxo-5-pyridyl)-alanine; stizolobic acid

Abstract- *New amino acids, L-3-(2-carboxy-4-pyrrolyl)-alanine (1) and L-3-(2-oxo-5-pyridyl)-alanine (2), were isolated from Clitocybe acromelalga and their structures were deduced by spectral data and biogenesis and confirmed by syntheses. Stizolobic acid (5) was also found in this fungus.*

Clitocybe acromelalga (Japanese name ; dokusasako) is a poisonous mushroom found only in Japan. The accidental ingestion of this mushroom causes a violent pain and a marked reddish edema in hand and foot after several days and it continues for about a month. These characteristic physiological properties prompted us to study the toxin of the fungus. We have already isolated several principles so far. They are clitidine,[1] clitioneine,[2] 4-amino quinolinic acid[3] and acromelic acids A (**3**) and B (**4**).[4] Further and continuous investigation led to the isolation of new amino acids **1**,[5] **2** and stizolobic acid (**5**).[6] In this paper, we wish to describe the isolation of **1**, **2** and **5**, the determination of structures and syntheses of **1** and **2** which are biosynthetically close to acromelic acids A and B, probably.[4, 7]

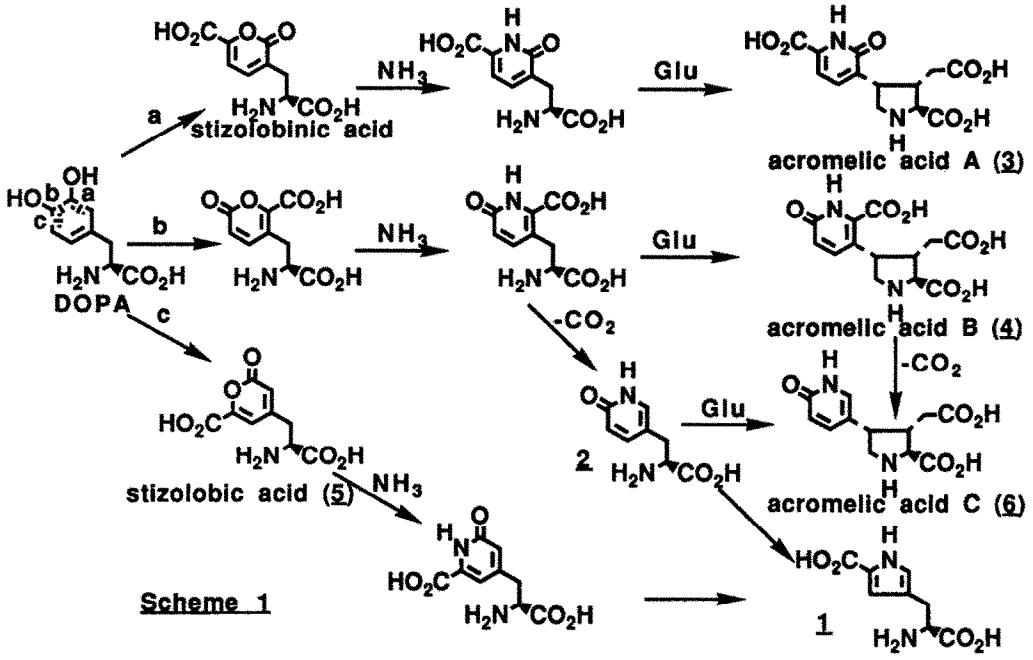
The water extracts of frozen fruit bodies were diluted by acetone to give precipitates which were dialysed against water. Dialyzate was fractionated by chromatography and paper electrophoresis monitoring the lethal effect in mice. Amino acids, **1** and **2**, were isolated from a poisonous fraction. A fraction showed depolarizing activity in the preparation of new born rat spinal cord. The active compound of this fraction was found to be stizolobic acid (**5**) whose uniqe activity on neurons of vertebrate and invertebrate was recently reported.[8]

The weakly acidic property of **1** was obvious from its behavior on ion-exchange column chromatography and paper electrophoresis. The molecular formula, $C_8H_{10}O_4N_2$, was deduced from the $[M+H]^+$ peak of HR-FAB mass spectrum. ^{1}H-NMR and ^{13}C-NMR spectra of **1** in D_2O indicated the presence of two aromatic protons [δ 6.48, 1H, brs and 6.80, 1H, brs], four carbons of aromatic ring [δ 115.9 (d), 118.3 (s), 121.6 (s) and 123.7 (d)], an alanine side chain [δ 2.89, 1H, dd, J=5.1, 14.5; 2.98, 1H, dd, J=7.2, 14.5; 3.78, 1H, dd, J=5.1, 7.2. δ 28.6 (t), 56.4 (d) and 176.5 (s)] and a carboxyl group on the aromatic ring [δ 163.8 (s)]. The chemical shifts of two singlet peaks due to aromatic protons and those of four signals due to aromatic carbons

implied a 2,4-disubstituted pyrrole structure. Pyrrole and furan exhibit individually signals of their protons and carbons at quite different chemical shifts. The UV spectrum also supported above implication.

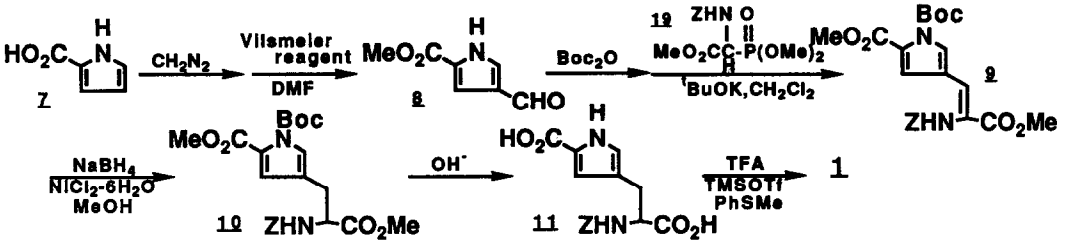
On the other hand, ^{1}H-NMR spectrum of **2** in D_2O showed also the presence of alanine side chain [δ 2.68, 1H, dd, J=7.0, 14.5; 2.80, 1H, dd, J=4.9, 14.5; 3.41, 1H, dd, J=4.9, 7.0] and three aromatic proton signals [δ 6.39, 1H, d, J=8.5; 7.36, 1H, brd, J=8.5; 7.64, 1H, brs]. The UV spectra of **2** exhibited two maxima around at 225 and 300 nm which were very similar to those of 5-methyl-2-pyridone. Furthermore, the coupling constants and chemical shifts of three aromatic protons in the ^{1}H-NMR spectrum suggested 5-substituted-2-pyridone structure.

These observation and biogenetic consideration implied structures **1** and **2** for the newly isolated amino acids (Scheme 1). The biogenesis was previously figured for acromelic acids and formation of **1** and **2** could be involved in this scheme. Due to a small amount of the sample the structures **1** and **2** were confirmed by syntheses.



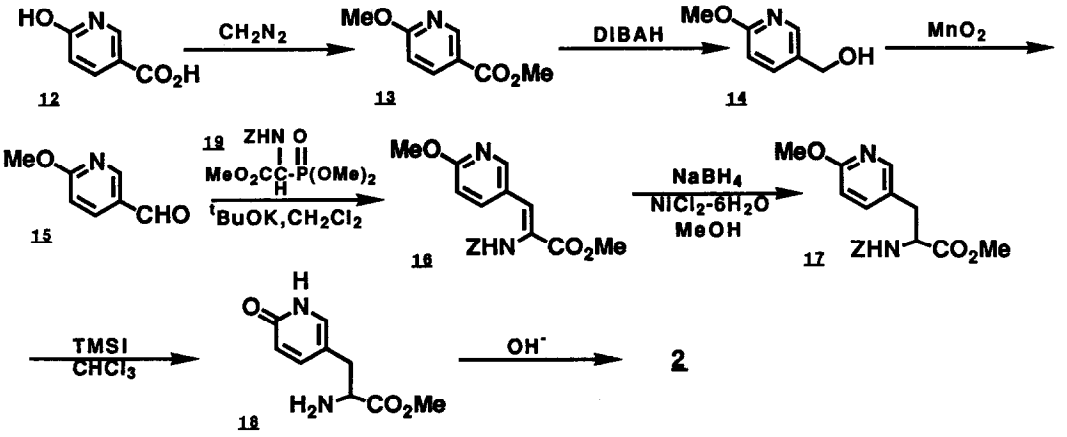
Scheme 1

The synthesis of **1** was performed starting from pyrrole-2-carboxylic acid **7** whose methyl ester was treated with Vilsmeier reagent to afford an aldehyde **8**. The aldehyde **8** was converted to an αβ-unsaturated ester **9** with Horner-Emmons reagent **10**[9] after protection of the imino group. Hydrogenation of the double bond of **9** was carried out with $NaBH_4$ assisted by $NiCl_2$-$6H_2O$.[10] Removal of protective groups[11] furnished racemic amino acid **1** (Scheme 2).



Scheme 2

Next, the synthesis of **2** was achieved starting from 6-hydroxy nicotinic acid. Reduction of ester **13** with DIBAH followed by oxidation with manganese dioxide gave an aldehyde **15** which was converted to **17** in the similar way to those employed in the synthesis of **1**. Removal of protective groups afforded a racemic amino acid **2** (Scheme 3).



Scheme 3

Synthetic **1** and **2** underwent optical resolution employing TLC with chiral plate (**1**: Rf=0.56 and 0.65, **2**: Rf=0.42 and 0.35 MeOH/H_2O/MeCN=1/1/4).[12] CD spectra of both **1** and **2** showed (+) Cotton effect for the faster moving compounds and (-) for the slower moving ones. Since L-amino acid exhibits usually (+) Cotton effect in CD spectrum,[13] the compounds with Rf 0.65 for **1** and Rf 0.42 for **2** should be L-isomer. The NMR and CD spectra, HPLC retention time and TLC (chiral plate) Rf value of the synthetic L-amino acids were completely coincident with those of natural products.

In the biogenesis of acromelic acids, the fission of DOPA at an outside and an inside of diol (a and b respectively) was assumed so far (Scheme 1). In this study the fission at another outside of diol (c) was also concerned. Acromelic acid C[14] is probably derived from **2** via similar route to the biogenesis of the acromelic acids A and B. But we found that acromelic acid B underwent decarboxylation under one year preservation in a refrigerator. The pyrrolyl alanine **1** may be derived from pyridone **2** or the pyridone related to stizolobic acid. Thus, it can be assumed that all compounds of acromelic acid family obtained from this mushroom are biosynthetically derived from DOPA. The biological activities of **1** and **2** are now under investigation.

EXPERIMENTAL

The ^{1}H-NMR (250MHz) was recorded in D_2O. The ^{13}C-NMR (67.5MHz) was measured in D_2O using dioxane as an internal standard. Paper electrophoresis was performed at pH 4.6 (pyridine/AcOH/H_2O=3/3/994), 600V, for 1.5h. Cellulose TLC was carried out using the following solvents; (A) MeOH/pyridine/H_2O (15/1/5), (B) ^{n}BuOH/HCO_2H/H_2O (6/1/2), and visualized by a UV lamp or ninhydrin.

Fruiting bodies of the mushroom were collected at Nagaoka city, Niigataken, Japan, frozen upon collection and stored at -20°C.

Isolation of 1, 2 and 5. Frozen fruit bodies (6.0kg) were extracted with H_2O(3× 7L) at 4°C overnight. The combined extracts were concentrated *in vacuo* to about 1L. To this turbid solution was added acetone (2.5L) and the mixture was allowed to stand at 4°C overnight. The supernatant was decanted and then the lower muddy layer was evaporated. The residue was dialyzed against H_2O (4× 3L) at 4°C overnight. The combined dialyzate was evaporated and the residue (338g) was applied on a column of charcoal (300g, packed in H_2O). The column was eluted stepwise with several concentration of EtOH (H_2O, 2.5, 5, 10 and 30% aq.EtOH, each 10L). The 2.5-5% aq.EtOH fraction were collected and the solvent was removed *in vacuo* (42g). The residue (7× 6g) 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 6L) as a solvent. The eluate with 10-20% aq.HCO_2H was concd *in vacuo* and the resultant paste (3.7g) was subjected to paper electrophoresis (46× 20cm, pH 4.6, 600V, 1.5h). The area of 0~+9cm was cut out and the strips extracted with H_2O and the solvent was removed. The residue was placed on cellulose TLC (20× 20cm) and developed with solvent system (A). The band at Rf 0.19 which absorbed UV light and the fluorescent band at Rf 0.67 were extracted with H_2O respectively. In these procedure stizolobic acid (Rf 0.19, 2mg, **5**) and **2** (Rf 0.67, 0.8mg) were separated. The amino acid **1** was separated from Rf 0.25-0.44 fraction by HPLC (Shodex Sugar SC-1821: H_2O, 52°C, Rt 7-8min and Lichrosorb NH_2: H_2O, Rt 5min). The crude **1** was purified by cellulose TLC with solvent system (B) (Rf 0.26, 1mg). **1**: mp 200-202 (decompose); UV λ_{max} (H_2O) nm (log ε): 234 (3.76) and 256 (4.06); CD λ_{ext} (H_2O) nm (Δε): 215 (+67.2); IR ν_{max}(nujol) cm^{-1}: 3620-2400 and 1740-1620; ^{1}H-NMR (D_2O): 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) and 6.80 (1H, brs); ^{13}C-NMR (D_2O): δ 28.6 (t), 56.4 (d), 115.9 (d), 118.3 (s), 121.6 (s), 123.7 (d), 163.8 (s) and 176.5 (s); HR-FABMS found: m/z 199.0710 $[M+H]^+$, calcd for $C_8H_{11}N_2O_4$: 199.0719. **2**: UV λ_{max} (H_2O) nm (log ε): 225 (3.01) and 300 (2.48); CD λ_{ext} (H_2O) nm (Δε): 225 (+49.6); IR ν_{max}(nujol) cm^{-1}: 3640-2400, 1720-1480, 1400 and 840; ^{1}H-NMR (D_2O): δ 2.68 (1H, dd, J=7.0, 14.5 Hz), 2.80 (1H, dd, J=4.9, 14.5 Hz), 3.41 (1H, dd, J=4.9, 7.0), 6.39 (1H, d, J=8.5 Hz), 7.36 (1H, brd, J=8.5 Hz) and 7.64 (1H, brs); HR-FABMS found: m/z 183.0789 $[M+H]^+$, calcd for $C_8H_{11}N_2O_3$: 183.0770.

2-Carboxymethyl-4-formyl-pyrrole (8). To a solution of pyrrole-2-carboxylic acid **7** (1g) in MeOH (50mL) was added an etherial solution of diazomethane until the yellow color was developed, and then the solvent was removed to afford methyl ester (1.12g, 99%) as a white powder. To a solution of methyl ester (1.1g, 8.8mmol) in DMF (20mL) was added Vilsmeier reagent (1.2eq) at room temperature, and the reaction mixture was stirred overnight. Saturated aq.$NaHCO_3$ was added to the mixture which was extracted with ether (3× 100mL). The combined extracts were dried over anhydrous sodium sulfate and evaporated to yield aldehyde **8** (860mg, 64%) with 5-formyl isomer (21%). The isomers were readily separable by silica gel column chromatography (100g, $CHCl_3$). More polar isomer was the desired one. **8**: IR ν_{max}(neat) cm^{-1}: 3410,

1750-1650, 1560, 1443, 1415, 1360, 1250, 1212 and 758; ^{1}H-NMR ($CDCl_3$): δ 3.90 (3H, s), 7.32 (1H, brs), 7,60 (1H, brs) and 9.85 (1H, s); EI-MS m/z (rel. int.): 153 $[M]^+$ (80), 122 (48), 120 (100), 94 (8), 66 (17), 60 (3), 53 (3) and 39 (14); HR-MS found: m/z 153.0438 $[M]^+$, calcd for $C_7H_7NO_3$: 153.0426.

Methyl (Z)-2-benzyloxycarbonylamino-3-(N-t-butyloxycarbonyl-2-methoxycarbonyl-4-pyrrolyl)-propenoate (9). To a solution of aldehyde **8** (500mg, 3.3mmol) in MeCN (30mL) was added DMAP (480mg, 3.9mmol, 1.2eq) and Boc_2O (1.05g, 4.8mmol, 1.5eq), and the mixture was stirred at room temperature for 2.5h. The reaction mixture was poured into aq.NH_4Cl and extracted with ether (3× 100mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated to give a N-protected form of aldehyde **8** (818mg, 99%) as a colorless oil. To a stirred suspension of ^{t}BuOK (40mg, 0.36mmol, 1.2eq) in CH_2Cl_2 (4mL) under argon at -20°C was added a solution of Horner-Emmons reagent **19** (120mg, 0.36mmol, 1.2eq) in CH_2Cl_2 (2mL). After 5min, a solution of N-protected form of aldehyde **8** (100mg, 0.4mmol) in CH_2Cl_2 (2mL) was added to the mixture which was stirred at 0°C for 2h. The reaction mixture was poured into H_2O and extracted with AcOEt (3× 100mL). The combined extracts were washed with H_2O, dried over anhydrous sodium sulfate and evaporated. The residue was purified by silica gel column chromatography (30g, ether) to afford adduct **9** (105mg, 81%) as a single isomer. **9**: IR ν_{max}(neat) cm^{-1}: 3560-3080, 1780-1660, 1648, 1480, 1435, 1396, 1368, 1320, 1223, 1150, 1060, 910, 848 and 780-720; ^{1}H-NMR ($CDCl_3$): δ 1.56 (9H, s), 3.76 (3H, s), 3.84 (3H, s), 5.18 (2H, s), 6.28 (1H, brs), 7.01 (1H, s), 7.19 (1H, brs), 7.32 (5H, brs) and 7.52 (1H, brs); EI-MS m/z (rel. int.): 458 $[M]^+$ (3), 385 (3), 372 (1), 358 (21), 250 (28), 223 (25), 218 (12), 196 (7), 191 (23), 163 (7), 146 (17), 131 (14), 108 (19), 91 (100), 79 (17), 65 (8), 57 (84) and 41 (71); HR-MS found: m/z 458.1687 $[M]^+$, calcd for $C_{23}H_{26}N_2O_8$: 458.1689.

Methyl 2-benzyloxycarbonylamino-3-(N-t-butyloxycarbonyl-2-methoxycarbonyl-4-pyrrolyl)-propionate (10). To the mixture of adduct **9** (70mg, 0.15mmol) and $NiCl_2$-$6H_2O$ (37mg, 0.15mmol, 1.0eq) in MeOH (2mL) was added the suspension of $NaBH_4$ (58mg, 1.5mmol, 10eq) in MeOH (2mL). The mixture was stirred at room temperature for 20min. Water was added to the reaction mixture which was extracted with AcOEt (3× 100mL). The combined extracts were dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed by silica gel column (30g, ether) to afford reduction product **10** (63mg, 90%) as a colorless oil. **10**: IR ν_{max}($CHCl_3$) cm^{-1}: 3520, 3420, 1790-1660, 1588, 1499, 1440, 1400, 1372, 1328, 1240-1190, 1153, 1090-1030, 953, 905 and 848; ^{1}H-NMR ($CDCl_3$): δ 1.56 (9H, s), 2.92 (2H, d, J=7.1 Hz), 3.75 (3H, s), 3.83 (3H, s), 4.58 (1H, t, J=7.1 Hz), 5.12 (2H, s), 5.30 (1H, brs), 6.59 (1H, brs), 7.10 (1H, brs) and 7.36 (5H, s); EI-MS m/z (rel. int.): 460 $[M]^+$ (1), 387 (4), 360 (1), 329 (1), 309 (2), 269 (3), 228 (10), 209 (60), 193 (3), 153 (5), 138 (100), 106 (37), 91 (69), 79 (6), 65 (6), 57 (69) and 41 (22); HR-MS found: m/z 460.1857 $[M]^+$, calcd for $C_{23}H_{28}N_2O_8$: 460.1846.

Racemic 3-(2-Carboxy-4-pyrrolyl)-alanine (1). To a stirred solution of **10** (50mg, 0.1mmol) in MeOH (2mL) was added 1N KOH (0.5mL, 0.5mmol, 5eq), and the mixture was allowed to stand at room temperature overnight. The mixture was acidified to pH 2 with 1N HCl and extracted with AcOEt (3× 50mL). The combined extracts were evaporated. The residue was stirred again in 1N NaOH at room temperature for 3h. The reaction mixture was acidified to pH 2 with 1N HCl and extracted with AcOEt (3× 50mL). The combined extracts were dried over anhydrous sodium sulfate and evaporated to afford **11** (36mg, 87%). **11**: IR ν_{max} ($CHCl_3$) cm^{-1}: 3520-2400 and 1750-1620; ^{1}H-NMR (CD_3OD): δ 2.93 (1H, dd, J=8.6, 15.7 Hz), 3.12 (1H, dd, J=6.4, 15.7), 4.44 (1H, dd, J=6.4, 8.6), 5.12 (1H, ABd, J=11.4), 5.20 (1H, ABd, J=11.4), 6.86 (1H, brs), 6.94 (1H, brs) and 7.40 (5H, s); HR-FABMS found: m/z 333.1101 $[M+H]^+$, calcd for $C_{16}H_{17}N_2O_6$: 333.1087.

To a solution of **11** (20mg, 0.06mmol) in TFA was added thioanisole (1drop) and TMSOTf (1drop) at 0°C, and the mixture was stirred for 45min. The solvent was removed *in vacuo*, and H_2O was added to the residue. The mixture was washed with AcOEt and H_2O layer was evaporated. The residue was subjected to a column of Amberlite IR-45 (HCO_2^- form) and eluted with 20% aq.HCO_2H to give **1** (9mg, 75%). **1**: mp 200-202 (decompose); UV λ_{max} (H_2O) nm (log ε): 234 (3.76) and 256 (4.06); IR ν_{max}(nujol) cm^{-1}: 3620-2400 and 1740-1620; ^{1}H-NMR (D_2O): 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) and 6.80 (1H, brs); ^{13}C-NMR (D_2O): δ 28.6 (t), 56.4 (d), 115.9 (d), 118.3 (s), 121.6 (s), 123.7 (d), 163.8 (s) and 176.5 (s); HR-FABMS found: m/z 199.0710 $[M+H]^+$, calcd for $C_8H_{11}N_2O_4$: 199.0719.

5-Carboxymethyl-2-methoxy-pyridine (13). To a solution of 6-hydroxy nicotinic acid **12** (3g) in MeOH (100mL) was added an etherial solution of diazomethane until the yellow color was developed, and then the solvent was removed. The residue was separated by silica gel column chromatography (50g, ether) to afford **13** (1.02g, 34%) as a white powder. **13**: IR ν_{max}($CHCl_3$) cm^{-1}: 3340, 1715, 1595, 1260, 1115, 1010 and 790; ^{1}H-NMR ($CDCl_3$): δ 3.96 (3H, s), 4.00 (3H, s), 6.76 (1H, d, J=8.5 Hz), 8.15 (1H, dd, J=1.5, 8.5) and 8.83 (1H, d, J=1.5); Anal. calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. found: C, 57.59; H, 5.60; N, 8.09.

5-Hydroxymethyl-2-methoxy-pyridine (14). To a solution of **13** (1.02g, 6.1 mmol) in CH_2Cl_2 (20ml) was added 0.93M DIBAH-hexane (14.5mL, 13.5mmol, 2.2eq) at -20°C under argon and the mixture was stirred. To the reaction mixture was added MeOH (3mL) after 10min and then H_2O was added at room temperature. The mixture was filterd through Celite. The filtrate was washed with brine, dried over anhydrous sodium sulfate and evaporated to afford alcohol **14** (708.4mg, 83%) as a colorless oil. **14**: IR ν_{max} (neat) cm^{-1}: 3320, 1605, 1570, 1487, 1385, 1283, 1255, 1210, 1120, 1015 and 830; ^{1}H-NMR ($CDCl_3$): δ 3.90 (3H, s), 4.58 (2H, s), 6.71 (1H, d, J=8.5 Hz), 7.58 (1H, dd, J=2.0, 8.5) and 8.03 (1H, brs); EI-MS m/z (rel. int.): 139 $[M]^+$ (71), 138 (100), 122 (20), 109 (39), 95 (11), 78 (17), 53 (17) and 42 (19); HR-MS found: m/z 139.0611 $[M]^+$, calcd for $C_7H_9NO_2$: 139.0633.

5-Formyl-2-methoxy-pyridine (15). To a solution of alcohol **14** (708.4mg, 5.1mmol) in $CHCl_3$ (14mL) was added MnO_2 (5g), and the mixture was stirred at toom temperature. After 5min, the mixture was filterd through Celite, and filtrate was evaporated to yield aldehyde **15** (690mg, 99%) as an yellow oil. **15**: IR ν_{max} (neat) cm^{-1}: 3360, 2720, 2560, 1705, 1660, 1585, 1350, 1205, 1115, 1000, 835 and 753; ^{1}H-NMR ($CDCl_3$): δ 6.84 (1H, d, J=8.5 Hz), 8.07 (1H, dd, J=1.5, 8.5 Hz), 8.64 (1H, d, J=1.5 Hz) and 9.95 (1H, s); Anal. calcd for $C_7H_7NO_2$: C, 61.31; H, 5.15; N, 10.21. found: C, 61.17; H, 5.12; N, 10.09.

Methyl (Z)-2-benzyloxycarbonylamino-3-(2-methoxy-5-pyridyl)-propenoate (16). To a stirred suspension of ^{t}BuOK (884mg, 7.9mmol, 1.5eq) in CH_2Cl_2 (50mL) under argon at -20°C was added a solution of Horner-Emmons reagent **19** (2.6g, 7.9mmol, 1.5eq) in CH_2Cl_2 (10mL). After 5 min, a solution of aldehyde **15** (730mg, 5.3mmol) in CH_2Cl_2 (10mL) was added to the mixture which was stirred at -20°C for 2h and cotinuously at 0°C for 2h. The reaction mixture was poured into H_2O and extracted with AcOEt (3× 100mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was purified by silica gel column chromatography (50g, ether) to afford adduct **16** (1.4g, 78%) as a single isomer. **16**: IR ν_{max} ($CHCl_3$) cm^{-1}: 3600-3080, 1750-1655, 1635, 1590, 1485, 1360, 1290-1200, 1122, 1050, 1015 and 755; ^{1}H-NMR ($CDCl_3$): δ 3.90 (3H, s), 3.95 (3H, s), 5.10 (2H, s), 6.65 (1H, d, J=8.5 Hz), 7.2-7.4 (6H, m), 7.76 (1H, brd) and 8.28 (1H, brs); EI-MS m/z (rel. int.): 342 $[M]^+$ (1), 298 (2), 234

(3), 221 (2), 207 (9), 175 (9), 148 (16), 119 (7), 91 (100), 79 (9), 65 (14), 59 (8) and 42 (9); HR-MS found: m/z 342.1240 $[M]^+$, calcd for $C_{18}H_{18}N_2O_5$: 342.1216.

Methyl 2-benzyloxycarbonylamino-3-(2-methoxy-5-pyridyl)-propionate (17). To the mixture of adduct **16** (300mg, 0.9mmol) and $NiCl_2$-$6H_2O$ (210mg, 0.9mmol, 1.0eq) in MeOH (8mL) was added the suspension of $NaBH_4$ (330mg, 8.7mmol, 9.7eq) in MeOH (5ml). The mixture was stirred at room temperature for 5min. Water was added to the reaction mixture which was extracted with ether (3× 100mL). The combined extracts were dried over anhydrous sodium sulfate and evaporated. The residue was purified by silica gel column chromatography (50g, ether) to afford reduction product **17** (290mg, 96%) as a colorless oil. **17**: IR ν_{max} (neat) cm^{-1}: 3560-3190, 1765-1650, 1603, 1490, 1436, 1390, 1285, 1250, 1208, 1050, 1022, 831 and 750; ^{1}H-NMR ($CDCl_3$): δ 2.98 (1H, dd, J=5.5, 14.0 Hz), 3.09 (1H, dd, J=5.5, 14.0 Hz), 3.70 (3H, s) 3.95 (3H, s), 4.62 (1H, t, J= 5.5 Hz), 5.10 (2H, s), 5.36 (1H, brs), 6.65 (1H, d, J=6.5 Hz), 7.2-7.4 (6H, m) and 7.95 (1H, brs); EI-MS m/z (rel. int.): 344 $[M]^+$ (1), 313 (1), 285 (1), 241 (2), 221 (7), 209 (4), 193 (68), 177 (7), 149 (9), 122 (100), 108 (15), 91 (82), 79(7), 65 (13) and 42 (2); HR-MS found: m/z 344.1375 $[M]^+$, calcd for $C_{18}H_{20}N_2O_5$: 344.1372.

Racemic 3-(2-Oxo-5-pyridyl)-alanine (2) To a stirred solution of **17** (324mg, 0.9mmol) in $CHCl_3$ (10mL) was added TMSI (422mg, 2.1mmol, 2.3eq) under reflux, and the mixture was stirred for 40min. The mixture was poured into H_2O and washed with AcOEt (3× 100mL). Removal of the solvent afforded pyridone **18** (283mg, 91%). **18**: IR ν_{max} (nujol) cm^{-1}: 3600-3280, 1760-1710, 1650, 1605, 1410, 1350, 1295, 1210 and 860-800; ^{1}H-NMR (D_2O): δ 2.68 (1H, dd, J=7.0, 14.0Hz), 2.78 (1H, dd, J=7.0, 14.0Hz), 3.62 (3H, s), 3.68 (1H, t, J=7.0Hz), 6.49 (1H, d, J=9.5Hz), 7.27 (1H, s) and 7.48 (1H, d, J=9.5Hz); EI-MS m/z (rel. int.): 196 $[M]^+$ (1), 151 (1), 137 (13), 120 (6), 109 (100), 102 (3), 91 (13), 88 (17), 80 (9), 53 (9) and 43 (4); HR-MS found: m/z 196.0834 $[M]^+$ calcd for $C_9H_{12}N_2O_3$: 196.0848.

To a solution of pyridone **18** (40mg, 0.2mmol) in MeOH (2mL) was added 1N KOH (0.5mL, 0.5mmol, 2.5eq), and the mixture was allowed to stand at room temperarure overnight. The solvent was removed, and the residue was applied to cellulose TLC (20× 20cm, 4sheets) and developed with a solvent system of ^{n}BuOH/HCO_2H/H_2O (6/1/2) to afford racemic **2** (31.6mg, 85%). **2**: UV λ_{max} (H_2O) nm (log ε): 225 (3.01) and 300 (2.48); IR ν_{max}(nujol) cm^{-1}: 3640-2400, 1720-1480, 1400 and 840; ^{1}H-NMR (D_2O): δ 2.68 (1H, dd, J=7.0, 14.5 Hz), 2.80 (1H, dd, J=4.9, 14.5 Hz), 3.41 (1H, dd, J=4.9, 7.0), 6.39 (1H, d, J=8.5 Hz), 7.36 (1H, brd, J=8.5 Hz) and 7.64 (1H, brs); HR-FABMS found: m/z 183.0789 $[M+H]^+$, calcd for $C_8H_{11}N_2O_3$: 183.0770.

Optical resolutions of 1 and 2. Racemic **1** was placed on chiral plate (10× 20cm) and developed with a solvent system of MeOH/H_2O/MeCN (1/1/4). The bands at Rf 0.56 and 0.65 were separately gathered and extracted with H_2O, and the extracts were evaporated respectively. The compound at Rf 0.65 exhibited (+) Cotton effect [λext (H_2O) 215 (Δε +62.4) nm] in CD spectrum (L-isomer) and the compound at Rf 0.56 exhibited (-) effect [λext (H_2O) 215 (Δε -72.0) nm] (D-isomer). Similarly, racemic **2** also underwent optical resolution. The compound at Rf 0.42 exhibited (+) Cotton effect [λ_{ext} (H_2O) 225 (Δε +41.0) nm] in CD spectrum (L-isomer) and the compound at Rf 0.35 exhibited (-) effect [λ_{ext} (H_2O) 225 (Δε -66.2) nm] (D-isomer).

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