SYNTHESIS AND NEUROEXCITATORY ACTIVITY OF SOME ACROMELIC ACID ANALOGS

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Abstract- The pyridine derivatives (**6~9**), analogs of acromelic acid A (**1**) which were potent excitatory amino acids, were synthesized. In the crayfish neuromuscular junction, depolarizing activity of these derivatives was comparable to those of acromelic acid A and domoic acid. At the isolated rat spinal motoneurons, compounds (**6**), (**7**) and (**8**) were found to be powerful excitants as potent as kainic acid, while **9** was inactive.

Excitatory amino acids are putative neurotransmitters at majority of excitatory synapses in vertebrates and invertebrates. According to recent investigations excitatory amino acids appear to play key roles for crucial physiological functions including memory and learning, and development of some neurological disorders, such as epilepsy and Huntington's chorea.[1] At present, the receptors of excitatory amino acids are divided into at least five subtypes in the mammalian central nervous system; NMDA (*N*-methyl-*D*-aspartate), kainate, AMPA ((*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate), *L*-AP4 (*L*-2-amino-4-phosphonobutylate) and metabotropic receptors.[2,3] Much effort

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This paper is dedicated to Professor Emeritus Masatomo Hamana on the occasion of his 75th birthday.

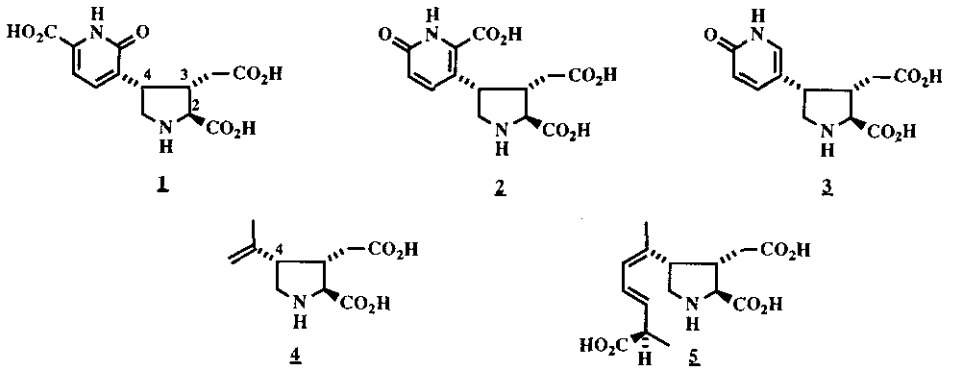

Figure 1

has been made to develop a variety of glutamate agonists and antagonists for each receptor subtype.[4,5]

We isolated acromelic acids A (**1**) and B (**2**) from a poisonous mushroom as toxic principle.[6] Recently acromelic acid C (**3**) was also obtained from the same fungus.[7] Of these, **1** and **2** have proved to be potent excitatory amino acid: these amino acids exhibit marked depolarizing activity in the crayfish opener muscle fibers,[8] in the isolated rat spinal motoneurons[9] and in the frog spinal cord.[10] In particular, **1** shows much greater depolarizing activity than that of kainic acid (**4**) and domoic acid (**5**), regardless of vertebrates or invertebrates. Furthermore, **1** specifically inhibits the binding of [^{3}H]kainic acid at the frog spinal cord, which is approximately 100 times more potent than **4**.[10] Interestingly, systemic administration of **1** to rat induces behavioral and pathological effects which are quite distinct from those induced by systemic administration of kainic acid;[11] specific lesions of interneurons in the lower spinal cord with little or no damage to the hippocampal neurons where kainate preferentially affects. Accordingly, acromelic acids have been expected to be useful tool for elucidating the function of excitatory amino acid receptors, in particular kainate and AMPA receptors which have recently been cloned.[12]

The remarkable neurophysiological properties of acromelic acids prompted us to synthesize their analogs, which might lead to more potent glutamate agonists and/or ant-

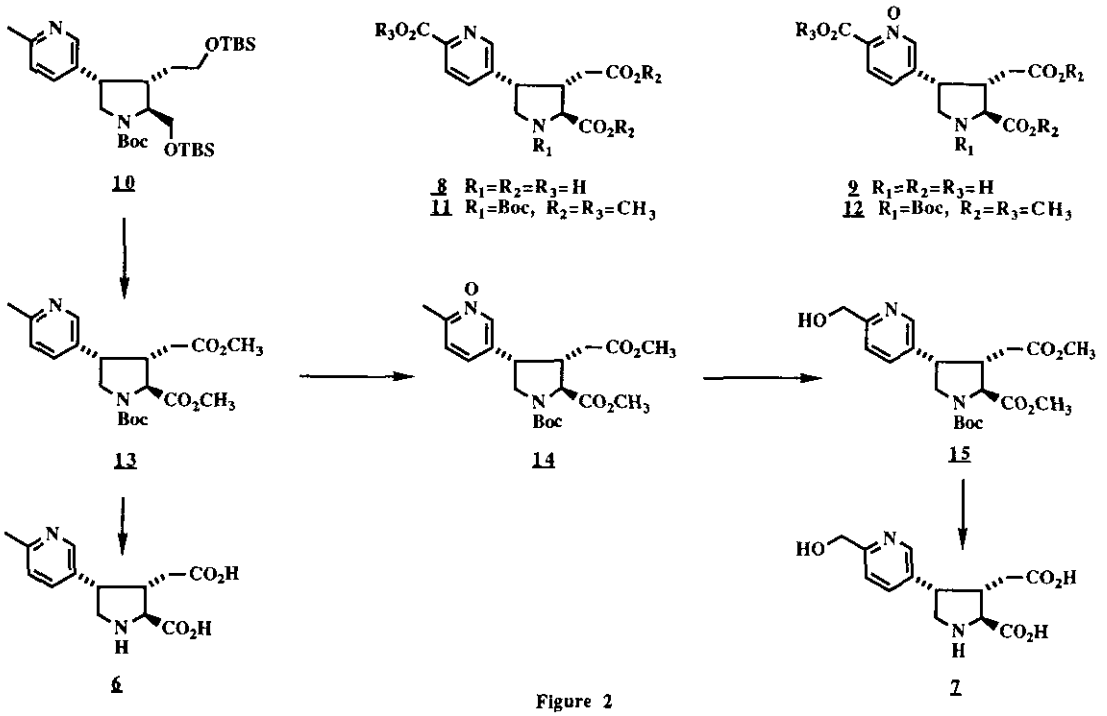

Figure 2

agonists.[13] Moreover, structure-activity relationships of these analogs would provide further insight into the mode of action for the excitatory amino acids. We report here the synthesis and neuroexcitatory activity of acromelic acid analogs (**6~9**).[14]

All the analogs were easily accessible from compounds (**10~12**), the intermediates for the synthesis of **1**. Removal of silyl groups of methylpyridine (**10**)[6] by p-TsOH/MeOH, subsequent oxidation of the resultant diol with PDC/DMF, followed by esterification with CH_2N_2 gave dimethyl ester (**13**) in 41% overall yield. The oxidation of the methyl group of **13** was achieved by the newly developed procedure of pyridine *N*-oxide chemistry.[15] Thus, mCPBA oxidation of the diester **13** gave the corresponding *N*-oxide (**14**) in 56% yield, which upon treatment with TFAA/DMF, produced the desired hydroxymethylpyridine (**15**) in 52% yield. Deprotection [(1) KOH (2) TFA] of **13** and **15** finally afforded the pyridine derivatives (**6**) and (**7**), respectively. Similarly, deprotection of **11** and **12** gave the amino acids (**8**) and (**9**), respectively.

The depolarizing activity of the synthetic compounds (**6**~**9**) was examined in both invertebrates and vertebrates preparations because kainoids are not so active in invertebrates, whereas they are extremely potent excitants in the mammalian central nervous system. The estimated order of potency is as follows,

at the crayfish neuromuscular junction: 1=2=7=8>5=6=9>4

in the isolated newborn rat spinal cord: 1>2=5>4=6=7=8>>9

At the crayfish neuromuscular junction, compounds (**7**) and (**8**) were as potent as the parent compound (**1**), while compounds (**6**) and (**9**) were less potent than **1** but still comparable to that of domoic acid (**5**). At this preparation, **1** was about 100 times more potent than kainic acid (**4**) and 10 times more than **5**.[8] On the other hand, the rank order of potency in the isolated rat spinal cord was different from that in the crayfish preparation: the compounds (**6**), (**7**) and (**8**) were powerful excitants as potent as **4** but compound (**9**) was inactive. The previous studies have shown that the presence of (1) unsaturated C-C or C-O bonds in C4 substituents on pyrrolidine ring of kainic acid derivatives and (2) cis-stereochemistry between C3 and C4 substituents was essential for potent excitatory activity of the kainoids.[3] This holds for the results presented here except for the case of *N*-oxide (**9**) in rat spinal cord.

Noteworthy is that the activity of the *N*-oxide (**9**) is weaker than that of the pyridine (**8**) in the both preparations, in spite of the fact that only slight difference in their structures. This indicates that electronic state of C4 substituent greatly affects depolarizing activity, in other words, interaction of this site is crucial for binding to the receptor. Furthermore, the hydroxymethyl or carboxyl functionality at C-6 of pyridine ring enhances the activity in crayfish, whereas it does not have any role in the rat spinal cord: **7** and **8** are much more potent than **6** in the crayfish, but all of them exhibit the same activity in the rat spinal cord. The difference on the rank order of potency between the two preparations may reflect the role of these oxygen functionality on the pyridine side chain. Further studies along this line are now in progress.

EXPERIMENTAL

The spectroscopic measurements were carried out with the following instruments: ^{1}Hnmr, Hitachi R 90-S (90MHz), JEOL JNM-FX 400 (400MHz); ir, JASCO IR-S; hrms, JEOL JMS-DX 300; uv, Hitachi 200-10; optical rotations, JASCO DIP-360; CD, JASCO J-40. TMS in $CDCl_3$ and DSS in D_2O were employed as internal standard in ^{1}Hnmr.

Methyl (2S,3S,4S)-*N*-(*tert*-butyloxycarbonyl)-3-methoxycarbonylmethyl-4-(2-methyl-5-pyridyl)-2-pyrrolidinecarboxylate (13).

To a solution of methylpyridine **10** (1.62 g, 2.87 mmol) in MeOH (16 ml) was added p-TsOH (1.1 g, 5.78 mmol) and the mixture was allowed to stand at room temperature for 30 min. The mixture was poured into aq. Na_2CO_3 and extracted with AcOEt. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (45 g, 2-8% MeOH-$CHCl_3$) to give diol (843 mg, 88%) as syrup. $[\alpha]_D$ -57.0° (c 0.58, $CHCl_3$), hrms: m/z 337.2146 $(M+H)^+$ calcd for $C_{18}H_{24}N_2O_4$ 337.2127, ir (neat): 3440, 1685, 1610, 1415, 1374, 1170, 1136, 758 cm^{-1}, nmr (90 MHz, $CDCl_3$): δ 1.48 (9H, s), 2.52 (3H, s), 7.10 (1H, d, J=8.1), 7.30 (1H, d, J=8.1), 8.25 (1H, s).

A mixture of the diol (444 mg, 1.32 mmol) and PDC (6 g, 16 mmol) in DMF (22 ml) was stirred at 40°C overnight under argon. To the reaction mixture an ether solution of CH_2N_2 was added and the mixture was poured into aq. Na_2CO_3 and extracted with AcOEt. The combined extracts were washed twice with brine, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (13 g, 1% MeOH-$CHCl_3$) to give diester (**13**) (238 mg, 46%) as syrup. $[\alpha]_D$ -2.0° (c 0.35, $CHCl_3$), hrms: m/z 392.1938 (M^+) calcd for $C_{20}H_{28}N_2O_6$ 392.1946, uv λ_{max} (EtOH): 267 nm (log ε 3.56), ir (neat): 1760, 1713, 1605, 1445, 1410, 1375, 1264, 1210, 1180, 1170, 1135, 760 cm^{-1}, nmr (90 MHz, $CDCl_3$): δ 1.45 (9H, s), 2.00 (1H, dd, J=8.1, 17.1), 2.30 (1H, dd, J=7.3, 17.1), 2.54 (3H, s), 2.99 (1H, q, J=6.4), 3.63 (3H, s), 3.78 (3H, s), 3.99 (1H, d, J=6.4), 7.10 (1H, d, J=8.1), 7.30 (1H, d, J=8.1), 8.24 (1H, s).

Methyl (2S,3S,4S)-*N*-(*tert*-butoxycarbonyl)-3-methoxycarbonylmethyl-4-(2-methyl-5-pyridyl)-2-pyrrolidinecarboxylate *N*-oxide (14).

To a stirred solution of methylpyridine **13** (238 mg, 0.61 mmol) in CH_2Cl_2 (4 ml) was added mCPBA (252 mg, 1.46 mmol) and the mixture was stirred at room temperature overnight. To the reaction mixture an ether solution of CH_2N_2 was added and the solvent was evaporated. The residue was purified by silica gel chromatography (6 g, 0-1% MeOH-$CHCl_3$) to give *N*-oxide (**14**) as syrup. $[\alpha]_D$ +2.1° (c 0.58, $CHCl_3$), hrms: m/z 408.1902 (M^+) calcd for $C_{20}H_{28}N_2O_7$ 408.1852, uv λ_{max} (EtOH): 265 nm (log ε 3.82), ir (neat): 1756, 1710, 1410, 1377, 1210, 1175, 1140, 1010, 760 cm^{-1}, nmr (90 MHz, $CDCl_3$): δ 1.37 (9H, s), 1.96 (1H, dd, J=7.7, 16.9), 2.30 (1H, dd, J=6.6, 16.9), 2.43 (3H, s), 2.7-3.0 (1H, m), 3.58 (3H, s), 3.71 (3H, s), 3.92 (1H, d, J=9.2), 6.82 (1H, d, J=8.1), 7.16 (1H, d, J=8.1), 8.00 (1H, s).

Methyl (2S,3S,4S)-*N*-(*tert*-butoxycarbonyl)-3-methoxycarbonylmethyl-4-(2-hydroxymethyl-5-pyridyl)-2-pyrrolidinecarboxylate (15).

To a stirred solution of *N*-oxide (**14**) (140 mg, 0.034 mmol) in DMF (1.5 ml) was added TFAA (0.24 ml, 0.17 mmol) and the mixture was stirred at room temperature overnight under argon. The reaction was quenched by aq. Na_2CO_3, then the mixture was poured into water and extracted with AcOEt. The combined extracts were washed twice with brine, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (4.5 g, 1% MeOH-$CHCl_3$) to give hydroxymethylpyridine (**15**) (73 mg, 52%) as syrup. $[\alpha]_D$ +3.2° (c 1.00, $CHCl_3$), hrms: m/z 408.1878 calcd for $C_{20}H_{28}N_2O_7$ 408.1896, uv λ_{max} (EtOH): 265 nm (log ε 3.73), ir (neat): 3520, 1760, 1713, 1610, 1450, 1412, 1380, 1265, 1214, 1185, 1172, 1140, 764 cm^{-1}, nmr (90 MHz, $CDCl_3$): δ 1.38 (9H, s), 1.99 (1H, dd, J=8.4, 16.9), 2.30 (1H, dd, J=5.1, 16.9), 2.6-3.1 (2H, m), 3.55 (3H, s), 3.71 (3H, s), 3.99 (1H, d, J=5.1), 4.67 (2H, s), 7.15 (1H, d, J=8.4), 7.34 (1H, d, J=8.4), 8.23 (1H, s).

(2S,3S,4S)-3-Carboxymethyl-4-(2-methyl-5-pyridyl)-2-pyrrolidinecarboxylic acid (6).

To a solution of diester (**13**) (70 mg, 0.18 mmol) in MeOH (2.2 ml) was added 1N-KOH (1.1 ml, 1.1 mmol) and the mixture was allowed to stand overnight at room temperature. After evaporation of the solvent, the residue was dissolved in TFA (2 ml) and the solution was left for 30 min at room temperature. The solvent was evaporated

and the residue was purified by ion-exchange chromatography (Amberlite IR-120B, H^+, 2x9 cm, 5% NH_4OH then Amberlite IRC-50, H^+, 2x9 cm, water) to give amino acid (**6**) (30 mg, 65%) as amorphous powder. $[\alpha]_D$ +21.8° (c 1.00, H_2O), uv λ_{max} (H_2O): 266 nm (log ε 3.78), fdms: m/z 265 $(M+H)^+$, nmr (400 MHz, D_2O): δ 1.93 (1H, dd, J=9.5, 16.1), 2.30 (1H, dd, J=5.9, 16.1), 2.60 (3H, s), 3.13 (1H, dd, J=5.9, 9.5), 3.64 (1H, m), 3.87 (2H, m), 3.98 (1H, d, J=5.9), 7.60 (1H, d, J=8.1), 8.04 (1H, dd, J=1.5, 8.1), 8.33 (1H, d, J=1.5).

(2S,3S,4S)-3-Carboxymethyl-4-(2-hydroxymethyl-5-pyridyl)-2-pyrrolidinecarboxylic acid (7).

The amino acid (**7**) (36 mg, 77% yield, amorphous powder) was obtained from **15** (73 mg, 0.18 mmol) by the same manner as described for **6** from **13**. $[\alpha]_D$ +13.0° (c 0.90, H_2O), uv λ_{max} (H_2O): 266 nm (log ε 3.65), fdms m/z 281 $(M+H)^+$, nmr (400 MHz, D_2O): δ.2.08 (1H, dd, J=9.2, 16.5), 2.45 (1H, dd, J=5.5, 16.5), 3.23 (1H, dt, J=9.2, 5.5), 3.75 (1H, m), 3.97 (2H, m), 4.08 (1H, d, J=5.5), 4.86 (2H, s), 7.71 (1H, d, J=8.0), 8.06 (1H, dd, J=1.5, 8.1), 8.46 (1H, D, J=8.1).

(2S,3S,4S)-3-Carboxymethyl-4-(2-carboxy-5-pyridyl)-2-pyrrolidinecarboxylic acid (8).

The amino acid (**8**) (39 mg, 75% yield, amorphous powder) was obtained from **11**[3] (92 mg, 0.21 mmol) by the same manner as described for **6** from **13**. $[\alpha]_D$ +19.3° (c 0.70, H_2O), uv λ_{max} (H_2O): 270 nm (log ε 3.75), fdms: m/z 295 $(M+H)^+$, nmr (400 MHz, D_2O): δ 2.18(1H, dd, J=9.2, 16.5), 2.53 (1H, dd, J=6.1, 16.5), 3.33 (1H, dddd, J=5.4, 6.1, 8.5, 9.2), 3.82(1H, t, J=11.6), 4.02 (1H, dd, J=8.0, 11.6), 4.10 (1H, ddd, J=8.0, 8.5, 11.6), 4.14 (1H, d, J=5.5), 8.28 (1H, d, J=8.1), 8.37 (1H, d, J=8.1), 8.63 (1H, s).

(2S,3S,4S)-3-Carboxymethyl-4-(2-carboxy-5-pyridyl)-2-pyrrolidinecarboxylic acid *N*-oxide (9).

The amino acid (**9**) (30 mg, 77% yield, amorphous powder) was obtained from **12** (67 mg, 0.15 mmol) by the same manner as described for **6** from **13**. $[\alpha]_D$ +7.9° (c 0.95, H_2O), uv λ_{max} (H_2O): 262 nm (log ε 3.99), fdms: m/z 311 $(M+H)^+$, nmr (500MHz, D_2O): δ 2.08 (1H, dd, J=8.3, 16.1), 2.39 (1H, dd, J=6.4, 16.1), 3.20 (1H, ddt, J=5.9, 6.4, 8.3), 3.71 (1H, dd, J=9.3, 11.7), 3.88 (1H, ddd, J=7.8, 8.3, 9.3), 3.96 (1H, dd, J=7.8, 11.7),

4.08 (1H, d, J=5.9), 7.53 (1H, d, J=8.3), 7.61 (1H, dd, J=1.5, 8.3), 8.16 (1H, d, J=1.5).

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