SYNTHESES AND EXCITATORY ACTIVITIES OF SOME AROMATIC KAINOIDS, ACROMELIC ACID ANALOGS†

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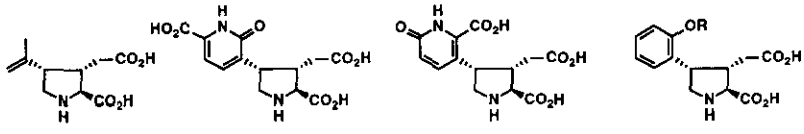
Abstract- The hydroxyiodophenyl, iodoanisyl and carboxyphenyl kainoids, (**9**, **10**, and **13**) were synthesized from the hydroxyphenyl compound (**6**). These new kainoids showed weak depolarizing activities in the preparation of a new-born rat spinal motoneuron.

Kainoid is a group of nonproteinogenic amino acids possessing a 4-substituted 3-carboxymethylproline part as a common structure with some of them showing potent depolarization in neurocells.[1] They act on the excitatory amino acid receptor. Their depolarizing activity significantly depends on the structure and the chemical property of the C4-substituent.[2] We have currently studied the excitatory kainoids, acromelic acids[3] and their analogs,[4] and two kinds of new analogs have been synthesized and their biological activity estimated.

The first ones were the iodophenyl analogs whose radioactive form would be useful to study the biological action mechanism. The phenol (**6**)[4] was treated with chloramine T and NaI in DMF to afford the iodo compound (**7**) in 53% yield, which was substituted exclusively at the *para*-position relative to the hydroxyl group. Deprotection of **7** was carried out by treatment with aqueous KOH in MeOH followed by TMSOTf and thioanisole in TFA[5] at 0°C to furnish iodide (**9**). Methylation of **7** with diazomethane followed by deprotection through the same treatment as above provided iodide (**10**).



† Dedicated to Prof. Rolf Huisgen (University of Munich) in celebration of his 75th birthday.
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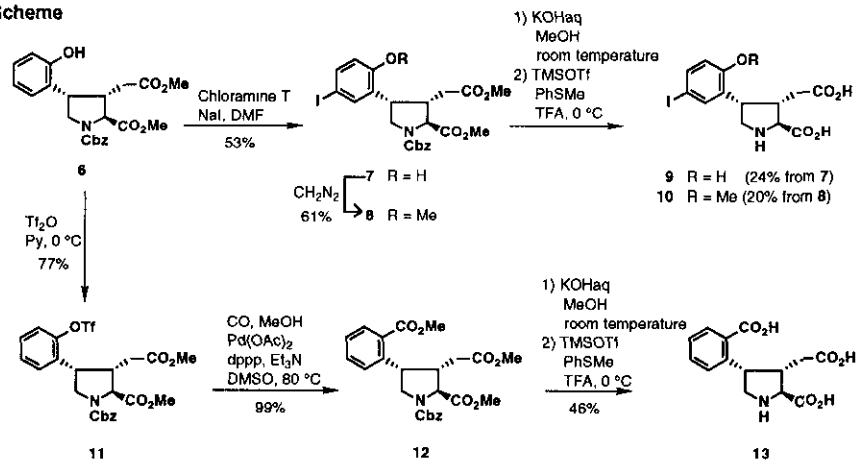
Second, the *ortho*-carboxyphenyl kainoid was synthesized since the potently active acromelic acid B was the *ortho*-carboxypyridone kainoid, and then the biological action of *ortho*-carboxyphenyl analog was expected to reveal the role of the *ortho*-carboxyl group. The phenol (**6**) was converted to triflate (**11**) by treatment with Tf_2O and pyridine in CH_2Cl_2 at 0°C. Carbonylation[6] of **11** was performed in the presence of $Pd(OAc)_2$ as a catalyst with dppp (1,3-bis(diphenylphosphino)propane), CO, MeOH and Et_3N in DMSO at 80°C to give the methyl ester (**12**) in 99% yield. Through hydrolysis followed by removal of the benzyloxycarbonyl group, the ester (**12**) afforded a new acromelic acid analog (**13**) in 46% yield.

Scheme



Excitatory activity of the newly synthesized kainoids was estimated by the strength of depolarization measured in the preparation of the new-born rat spinal motoneuron. The relative potencies of the natural and newly synthesized kainoids are as follows; **5>2>4>3>1>10>9>13**=L-Glu. The iodinated methoxy- and hydroxyphenyl kainoids (**10**, **9**) had significantly reduced activities compared to the original compounds (**4** and **5**). Low activity of the iodine compounds (**9**) and (**10**) would be due to the steric effect of a bulky iodine group which probably prevents fitting of the substrate in a receptor, or the electronic effect of iodine which reduces interaction with the receptor. The carboxyphenyl kainoid (**13**) also has considerably reduced activity compared to the original compounds, acromelic acid B (**3**) and hydroxyphenyl and anisyl kainoids (**4**) and (**5**) respectively. This could be caused by a conformational change in the C4-substituent and/or an unfavorable electronic structure of the phenyl group to display potent activity.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Ir spectra were recorded on a JASCO IR-S infrared spectrophotometer. ^{1}H Nmr spectra were recorded on a Hitachi R-90Hs (90 MHz), a R-250Hs (250 MHz), a JEOL model JMN-FX-400 (400 MHz), and a FX-500 (500 MHz) spectrometers. All chemical shifts are reported as δ values in parts per million relative to the peak of tetramethylsilane as an internal standard set at 0 ppm, if the solvent was $CDCl_3$, or the HDO peak set at 4.80 ppm, if the solvent was D_2O. The data are reported as follows: chemical shift, number of proton, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broadened), and coupling constants. Low (EI and FAB) and high (EI and FAB) resolution mass spectra were determined on a JEOL model JMS-DX 303 and a JMS-HX 110 spectrometers.

Methyl (2*S*,3*S*,4*S*)-1-benzyloxycarbonyl-4-(2-hydroxy-5-iodophenyl)-2-methoxycarbonyl-3-pyrrolidineacetate (7)

To a solution of **6** (42 mg, 98.3 mmol) in DMF (1 ml) at room temperature was added NaI (18 mg, 0.118 mmol), and the solution was stirred for 1.5 h at room temperature. The reaction mixture was acidified to pH 2 with 1M aqueous HCl. The mixture was poured into ether (5 ml) and washed with water (2 x 3 ml), 5% aqueous $Na_2S_2O_3$, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to give iodide **7** (29 mg, 53%). : ^{1}H Nmr (250 MHz, $CDCl_3$), δ 7.52 (1H, d, J=8.5 Hz), 7.2-7.4 (6H, m), 6.60 (1H, d, J=8.5 Hz), 6.02 (0.5x1H, brs), 5.82 (0.5x1H, brs), 5.22 (1H, d, J=12.5 Hz), 5.23 (0.5x1H, d, J=12.5 Hz), 5.17 (0.5x1H, d, J=12.5 Hz), 5.10 (0.5x1H, d, J=12.5 Hz), 4.19 (0.5x1H, d, J=4.8 Hz), 4.15 (0.5x1H, d, J=4.8 Hz), 3.7-4.0 (3H, m), 3.75 (0.5x3H, s), 3.61 (0.5x3H, s), 3.59 (3H, s), 3.20 (1H, m), 2.18 (1H, dd, J=8.0, 16.2 Hz), 2.15 (1H, dd, J=8.0, 16.2 Hz), 2.06 (1H, d, J=5.2, 16.2 Hz), 2.05 (1H, d, J=5.2, 16.2 Hz).

Methyl (2*S*,3*S*,4*S*)-1-benzyloxycarbonyl-2-methoxycarbonyl-4-(5-iodo-2-methoxyphenyl)-3-pyrrolidineacetate (8)

To a solution of **7** (56 mg, 0.131 mmol) in MeOH (1 ml) was added an ethereal solution of diazomethane until the solution turned yellow. The solution was concentrated in vacuo and the residue was purified by column chromatography on silica gel to give methyl ether (**8**) (45 mg, 61%): $[\alpha]_D^{21}$ –28.8° (*c* 0.90, $CHCl_3$); ir (neat), 3000, 2935, 2880, 2835, 1735, 1702, 1587, 1483, 1410, 1350, 1280, 1240, 1200, 1158, 1123, 1080, 1016, 810, 750, 690 cm^{-1}; ^{1}H nmr (250 MHz, $CDCl_3$), δ 7.52 (1H, d, J=8.5 Hz), 7.1-7.4 (6H, m), 6 60 (1H, d, J=8.5 Hz), 5.25 (0.5x1H, d, J=12.2 Hz), 5.23 (0.5x1H, d, J=12.2 Hz), 5.13 (0.5x1H, d, J=12.2 Hz), 5.07

(0.5x1H, d, J=12.2 Hz), 4.19 (0.5x1H, d, J=4.9 Hz), 4.15 (0.5x1H, d, J=4.9 Hz), 3.7-4.0 (3H, m), 3.79 (0.5x3H, s), 3.72 (0.5x3H, s), 3.71 (0.5x3H, s), 3.60 (0.5x3H, s), 3.57 (3H, s), 3.20 (1H, m), 2.19 (0.5x1H, dd, J=7.9, 16.5 Hz), 2.16 (0.5x1H, dd, J=7 9, 16.5 Hz), 2.03 (0.5x1H, d, J=5.5, 16.5 Hz), 2.02 (0.5x1H, d, J=5.5, 16.5 Hz); EI-HRms calcd for $C_{24}H_{26}NO_7I$ (M^+) 567.0754, found 567.0775.

(2*S*,3*S*,4*S*)-2-Carboxy-4-(2-hydroxy-5-iodophenyl)-3-pyrrolidineacetic acid (9)

To a solution of iodide (**7**) (29 mg, 52.4 mmol) in MeOH (0.5 ml) at room temperature was added 2M aqueous KOH. After 1 h, the solution was acidified to pH 2 with 1M aqueous HCl. The mixture was extracted with EtOAc (3 x 3 ml) and the combined extracts were washed with brine (5 ml), dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was directly used for the next reaction.

The residue was dissolved in trifluoroacetic acid (0.5 ml) under argon. The solution was cooled to 0 °C and thioanisol (10.1 mg, 81.5 mmol) and TMSOTf (15.1, 81.5 mmol) were added at 0 °C. After 1 h, the mixture was concentrated in vacuo. The residue was subjected to a column of Dowex 50W-X8 (H^+ form, 1 x 5 cm). The column was eluted first with water and then 5% aqueous NH_4OH and the latter eluate was concentrated to give an ammonium salt of amino acid (**9**). The water solution of ammonium salt was passed through a column of Amberlite IRC-50 (H^+ form, 1 x 5 cm) to give amino acid (**9**) (5 mg, 24%): ^1H Nmr (250 MHz, D_2O), δ 7.43 (1H, dd, J=ca. 1.0, 8.5 Hz), 7.28 (1H, d, J=ca. 1.0 Hz), 6.61 (1H, d, J=8.5 Hz), 4.02 (1H, d, J=7.9 Hz), 3.6-3.8 (3H, m), 3.06 (1H, m), 2.44 (1H, dd, J=5.2, 16.5 Hz), 1.98 (1H, dd, J=9.8, 16.5 Hz).

(2*S*,3*S*,4*S*)-2-Carboxy-4-(5-iodo-2-methoxyphenyl)-3-pyrrolidineacetic acid (10)

Experimental procedure was the same as described for compound (**9**). Iodide (**8**) (45 mg, 79 4 mmol) was treated with this procedure to give amino acid (**10**) (6.4 mg, 20%): Ir (nujol), 3600-2200, 1730, 1650-1530, 1315, 1250, 1210, 1175, 1130, 1020, 910, 880, 820, 790, 720 cm^{-1}; ^1H Nmr (250 MHz, D_2O), δ 7.54 (1H, dd, J=1.8, 8.5 Hz), 7.29 (1H, d, J=1.8 Hz), 6.71 (1H, d, J=8.5 Hz), 3.93 (1H, d, J=6.7 Hz), 3.71 (3H, s), 3.5-3.8 (3H, m), 3.06 (1H, m), 2.28 (1H, dd, J=5.5, 16.5 Hz), 1.84 (1H, dd, J=9.8, 16.5 Hz); FAB-HRms calcd for $C_{14}H_{17}NO_5I$ ($(M+H)^+$) 406.0152, found 406.0173.

Methyl (2*S*,3*S*,4*S*)-4-(2-trifluoromethanesulfonyloxyphenyl)-1,2-dimethoxycarbonyl-3-pyrrolidineacetate (11)

To a solution of **6** (200 mg, 0.468 mmol) in CH_2Cl_2 (4.0 ml) were added dropwise pyridine (0.140 ml, 1.68 mmol) and Tf_2O (0.100 ml, 0.562 mmol) at 0 °C under argon. After 1 h, the mixture was poured into water (5 ml), and extracted with ether (3 x 5 ml). The combined extracts were washed with saturated aqueous solution of $CuSO_4$ (10 ml) and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by

column chromatography on silica gel to give triflate (**11**) (202 mg, 77%): $[\alpha]_D^{24}$–34.6° (*c* 1.90, $CHCl_3$); ir (neat), 2960, 2900, 1750, 1715, 1495, 1420, 1355, 1250, 1215, 1140, 1090, 1080, 1010, 900, 885, 780, 740, 700 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$), δ 7.2-7.4 (9H, m), 5.26 (0.5x1H, d, J=12.0 Hz), 5.22 (0.5x1H, d, J=12.0 Hz), 5.17 (0.5x1H, d, J=12.0 Hz), 5.07 (0.5x1H, d, J=12.0 Hz), 4.30 (0.5x1H, d, J=4.5 Hz), 4.27 (0.5x1H, d, J=4.5 Hz), 3.8-4.1 (3H, m), 3.80 (0.5x3H, s), 3.64 (0.5x3H, s), 3.57 (3H, s), 3.20 (1H, m), 2.29 (0.5x1H, dd, J=8.0, 16.5 Hz), 2.25 (0.5x1H, dd, J=8.0, 16.5 Hz), 2.04 (1H, dd, J=6.5, 16.5 Hz); EI-HRms calcd for $C_{24}H_{24}NO_9F_3S$ (M^+) 559.1124, found 559.1078.

Methyl (2*S*,3*S*,4*S*)-1,2-dimethoxycarbonyl-4-(2-methoxycarbonylphenyl)-3-pyrrolidineacetate (12)

To a solution of triflate (**11**) (180 mg, 0.322 mmol), MeOH (0.72 ml), Et_3N (0.10 ml, 0.708 mmol), and 1,3-bis(diphenylphosphino)propane (dppp, 39.8 mg, 96.6 mmol) in DMSO (3.6 ml) was added $Pd(OAc)_2$ (21.7 mg, 96.6 mmol) at room temperature. The mixture was thoroughly evacuated under liquid air temperature and allowed to stand for 1.5 h at 80°C. The mixture was poured into ether (10 ml), washed with water (2 x 5 ml) and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to give ester (**12**) (149 mg, 99%): $[\alpha]_D^{25}$–44.2° (*c* 1.00, $CHCl_3$); ir (neat), 3040, 2960, 2920, 1750, 1720, 1420, 1360, 1260, 1210, 1130, 1090, 1010, 970, 760, 700 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$), δ 7.88 (0.5x1H, d, J=6.5 Hz), 7.85 (0.5x1H, d, J=6.5 Hz), 7.2-7.5 (8H, m), 5.26 (0.5x1H, d, J=12.0 Hz), 5.22 (0.5x1H, d, J=12.0 Hz), 5.16 (0.5x1H, d, J=12.0 Hz), 5.06 (0.5x1H, d, J=12.0 Hz), 4.61 (1H, m), 4.27 (0.5x1H, d, J=4.8 Hz), 4.24 (0.5x1H, d, J=4.8 Hz), 3.8-4.0 (2H, m), 3.86 (3H, s), 3.82 (0.5x3H, s), 3.64 (0.5x3H, s), 3.53 (3H, s), 3.23 (1H, m), 2.1-2.2 (2H, m); EI-HRms calcd for $C_{25}H_{27}NO_8$ (M^+) 469.1737, found 469.1721.

(2*S*,3*S*,4*S*)-2-Carboxy-4-(2-carboxyphenyl)-3-pyrrolidineacetic acid (13)

Experimental procedure was the same as described for compound (**9**). Ester (**12**) (35 mg, 74.6 mmol) was treated with this procedure to give amino acid (**13**) (10 mg, 46%): $[\alpha]_D^{25}$–92.0° (*c* 0.26, H_2O); ir (nujol), 3600-2200, 1730, 1710, 1690, 1650-1520, 1250, 1090, 1050, 765, 720 cm^{-1}; ^1H nmr (250 MHz, D_2O), δ 7.72 (1H, dd, J=1.5, 7.8 Hz), 7.46 (1H, ddd, J=1.5, 7.3, 7.8 Hz), 7.35 (1H, ddd, J=ca. 1.0, 7.3, 7.8 Hz), 7.26 (1H, dd, J=ca. 1.0, 7.8 Hz), 4.43 (1H, ddd, J=7.3, 7.8, 11.2 Hz), 4.03 (1H, d, J=4.4 Hz), 3.76 (1H, dd, J=7.8, 11.7 Hz), 3.72 (1H, dd, J=11.2, 11.7 Hz), 3.26 (1H, dddd, J=4.4, 6.4, 7.3, 8.3 Hz), 2.35 (1H, dd, J=6.4, 16.6 Hz), 2.20 (1H, dd, J=8.3, 16.6 Hz); FAB-HRms calcd for $C_{14}H_{15}NO_6$ ($(M+H)^+$) 294.0978, found 294.0950

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