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CONFIGURATIONAL VARIANTS OF HYDROXYPHENYLKAINOIDS: THEIR POTENT DEPOLARIZING ACTIVITIES IN THE RAT CENTRAL NERVOUS SYSTEM

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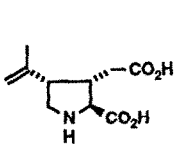
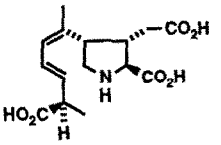
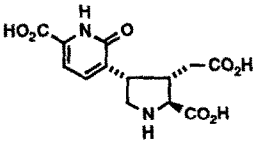
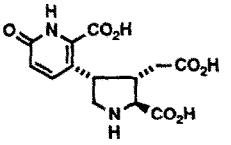
Abstract. Four diastereomers of 3-carboxymethyl-4-(2-hydroxyphenyl)proline were synthesized and their depolarizing activities were examined in the isolated rat spinal cord. The (2S,3S,4S)-isomer was the most potent among them, and the relationship between depolarizing activities and configurational variation was discussed.

Kainoids, such as kainic acid, domoic acid and acromelic acids A and B, have demonstrated potent excitatory actions on mammalian central neurons, therefore, they have been useful probes for analysis of physiological functions of excitatory amino acids.[1,2] In particular, powerful excitatory actions of kainic acid gave rise to the excitotoxic concept that glutamate destroys neurons by excessive activation of excitatory receptors.[3] However, kainate and acromelates did not always demonstrate similar pharmacological and neurological actions in the rat.[2] This may be due to the diversity of functions of kainate receptors. Various agonists seem to provide a promising clue for the explanation of these poorly understood phenomena. Recently we synthesized (2*S*,3*S*,4*S*)-3-carboxymethyl-4-(2-hydroxyphenyl)proline[1c] and revealed its considerably high depolarizing activities in the rat spinal motoneuron.[1c,2a] In order to examine the depolarizing activity from the stereochemical aspect, some stereoisomers of hydroxyphenylkainoid were synthesized and peak amplitudes of depolarizing responses to these compounds were compared at various concentrations in the spinal motoneuron.


kainic acid


domoic acid

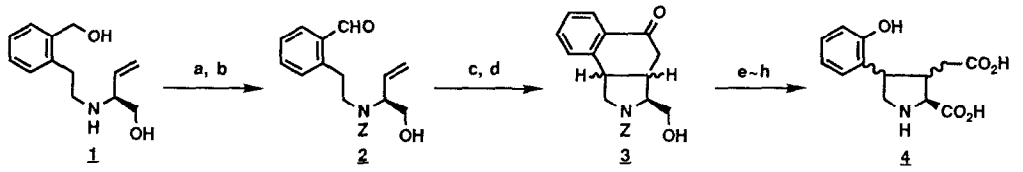

acromelic acid A


acromelic acid B

The secondary amine **1** was protected by a Z(benzyloxycarbonyl) group and benzyl alcohol part was converted to aldehyde (Fig 1). The aldehyde **2** was irradiated by a medium pressure mercury lamp in toluene and smoothly underwent a Diels-Alder reaction via photo-induced enolization of the aldehyde group.[4] A configurational mixture of the diol thus obtained was oxidized with MnO_2 to a mixture of ketone **3** which was separated by Lobar column chromatography into four diastereoisomers. The stereochemistry of each isomer was

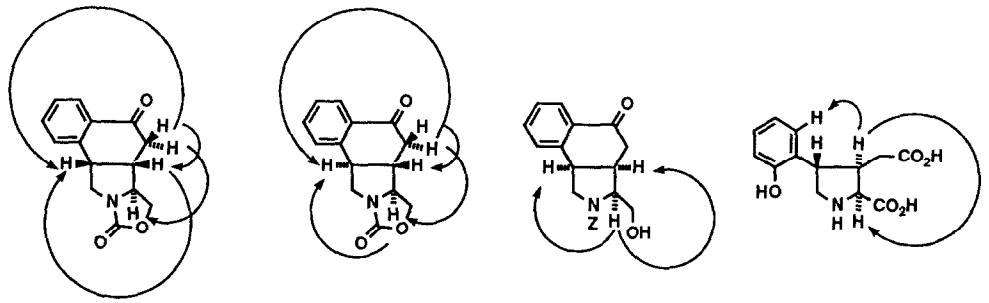
determined mainly by NOE in NMR mesurement as follows. 2,3-*trans*-Isomers were converted to cyclic carbamates by treatment with alkaline solution but 2,3-*cis*-isomers remained unchanged. Though somewhat restricted rotation of the amide bond of **3** made its NMR spectra illegible, the cyclic carbamates, **5** and **6** with fixed conformation by assembling the amide bond in the carbamate ring showed distinct and analyzable spectra. The NOE observed by NOESY method distinguished 2,3-*trans*-3,4-*cis*- and 2,3-*trans*-3,4-*trans*-compounds (Fig 2). The conformation of 2,3-*cis*-3,4-*cis*-compound **7** seemed to be constrained and its NMR spectrum was clear enough to observe NOE. Furthermore the kainoid derived from this compound was converted to 2,3-*trans*-3,4-*cis*-kainoid by epimerization at C2 which coincided with those derived from 2,3-*trans*-3,4-*cis* ketone **5** except their chirality. The configuration of the last ketone was determined as 2,3-*cis*-3,4-*trans* by NOE observation after converting to a kainoid **8**.

Fig 1



a) ZCl, K_2CO_3 b) MnO_2 c) hv / toluene d) MnO_2 e) PDC / DMF then CH_2N_2 f) mCPBA then MeONa / MeOH g) KOH h) TMSOTf, thioanisole / TFA

Fig 2

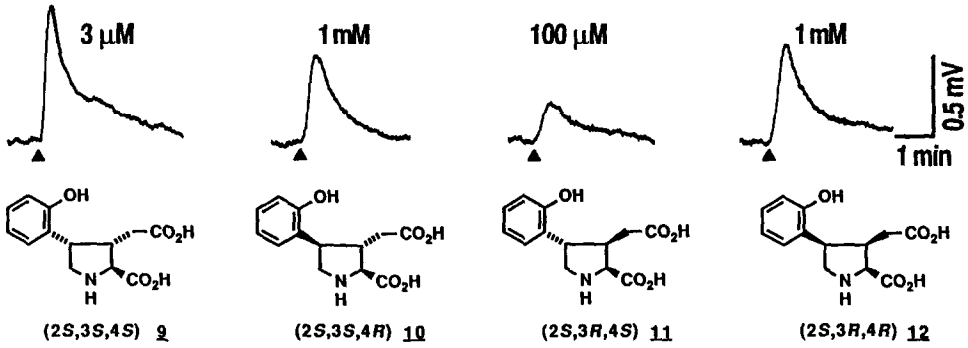


2,3-*trans*-3,4-*cis* 5 **2,3-*trans*-3,4-*trans* 6** **2,3-*cis*-3,4-*cis* 7** **2,3-*cis*-3,4-*trans* 8**

The depolarizing activities of four hydroxyphenylkainoids with different configurations were determined in the isolated newborn rat spinal cord. The methods used for the electrophysiological experiments in the isolated rat spinal cord were essentially similar to those described previously.[5] The potential changes were recorded extracellularly from the L3-L5 ventral root and test samples were added to the bathing solution. Four hydroxyphenylkainoids demonstrated almost similar time courses of actions but their depolarizing activities markedly varied. (2*S*,3*S*,4*S*)-Isomer **9**[6] with the same configuration as that of *L*-kainic acid demonstrated the most potent depolarizing activity among four diastereomers, which was almost comparable to that of acromelic acids A and B.[2a] (2*S*,3*S*,4*R*)-Isomer **10**[6] with the same configuration as that of allokainic acid, which has the common skeleton of kainoids and different configuration

at C4, showed extremely low activity, whereas (2*S*,3*R*,4*S*)-isomer **11**[6] with different configuration at C3 (the configuration of the glutamic acid moiety is different from that of *L*-kainic acid) from that of kainoids and with the same configuration at C4 showed somewhat higher activity than *L*-glutamic acid. This suggests that the stereochemistry of C4-substituents may play a key role for producing high depolarizing activity and sometimes it is more effective than the conformation of the glutamic acid part of kainoids. The depolarizing activities of (2*S*,3*R*,4*R*)-isomer **12**[6], which is configurationally quite different at C3 and C4 from *L*-kainic acid, was considerably low (Fig 3). The relative potencies of hydroxyphenylkainoids were as follows (the numeral in the parenthesis represents an approximate value, *L*-glutamic acid = 1) ; **9** (300) > *L*-kainic acid (150) > **11** (2) > *L*-glutamic acid (1) > **12** (0.5) = **10** (0.5).

Fig 3 Sample records of depolarization in the newborn rat spinal motoneuron



In conclusion, it was revealed that a kainate type receptor could accept phenyl groups at C4 of the kainoids with α-configuration but could not accommodate those with β-configuration and the receptor could flexibly fit to a glutamic acid fragment with various configurations or conformations to a certain extent. Moreover, we have found that the excitatory activities of kainoids depend on the hight of HOMO (highest occupied molecular orbital) energies of the π-electron systems existing in the substituents at C4.[7] The kainate receptor probably has a pocket which fits to and interacts with the π-electron system of the substituent and this interaction is very important to exhibit strong depolarization.

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4 This reaction is essentially the same as one employed in the work of ref. 1(c).

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6 ^{1}H NMR for **9** (500MHz, D_2O, pH 4~5, DSS=0.0 ppm) : δ 2.01 (1H, dd, J=9.8, 16.1 Hz), 2.47 (1H, dd, J=5.4, 16.1 Hz), 3.16 (1H, dddd, J=5.4, 7.5, 7.8, 9.8), 3.81 (1H, dd, J=7.3, 11.7 Hz), 3.86 (1H, dd, J=7.8, 11.7 Hz), 3.93 (1H, ddd, J=7.3, 7.5, 7.8 Hz), 4.16 (1H, d, J=7.8 Hz), 6.94 (1H, ddd, J=1.5, 7.8, 7.8 Hz), 6.96 (1H, dd, J=1.5, 7.8 Hz), 7.10 (1H, dd, J=1.5, 7.8 Hz), 7.27 (1H, ddd, J=1.5, 7.8, 7.8 Hz).
^{1}H NMR for **10** (500MHz, D_2O, pH 5.2, DSS=0.0 ppm) : δ 2.52 (1H, dd, J=7.8, 15.1 Hz), 2.69 (1H, dd, J=4.4, 15.1 Hz), 3.00 (1H, m), 3.6~3.8 (3H, m), 4.07 (1H, d, J=8.8 Hz), 6.95 (1H, d, J=7.8Hz), 6.98 (1H, ddd, J=1.0, 7.0, 7.3 Hz), 7.24~7.28 (2H, m).
^{1}H NMR for **11** (500MHz, D_2O, pH 4.3, DSS=0.0 ppm) : δ 2.45 (1H, dd, J=6.3, 16.6 Hz), 2.57 (1H, dd, J=8.8, 16.6 Hz), 3.28 (1H, dddd, J=6.3, 8.8, 8.8, 9.8 Hz), 3.52 (1H, ddd, J=8.3, 9.8, 10.3 Hz), 3.60 (1H, dd, J=10.3, 11.2 Hz), 3.88 (1H, dd, J=8.3, 10.3 Hz), 4.50 (1H, d, J=8.8 Hz), 6.98 (1H, dd, J=1.0, 7.8 Hz), 7.01 (1H, ddd, J=1.0, 7.2, 7.3 Hz), 7.28 (1H, dd, J=1.0, 7.2 Hz), 7.30 (1H, ddd, J=1.0, 7.3, 7.8 Hz).
^{1}H NMR for **12** (500MHz, D_2O, pH 4.9, DSS=0.0 ppm) : δ 2.24 (1H, dd, J=8.8, 16.5 Hz), 2.28 (1H, dd, J=4.9, 16.5 Hz), 3.60 (1H, dddd, J=4.9, 7.3, 7.5, 8.8 Hz), 3.72 (1H, dd, J=8.3, 11.7Hz), 3.92 (1H, dd, J=10.7, 11.7 Hz), 4.13 (1H, ddd, J=7.5, 8.3, 10.7 Hz), 4.52 (1H, d, J=7.3 Hz), 6.93 (1H, dd, J=1.0, 7.8 Hz), 6.95 (1H, ddd, J=1.0, 7.3, 7.3 Hz), 7.22 (1H, dd, J=1.0, 7.3 Hz), 7.27 (1H, ddd, J=1.0, 7.3, 7.8 Hz).

7 Ohwada, S.; Ohuchi, T; Hashimoto, K.; Konno, K.; Shirahama, H. 59th National Meeting of Chemical Society of Japan, Yokohama, Apr. 1990, Abstr., No 4C518. It will be published in due course.