Determination of the Turnover Number during Inactivation of MAO by 1. MAO (8 mM) containing various concentrations of 1 in 50 mM potassium phosphate, pH 7.2, buffer (300-μL total volume) was incubated at 25 °C in closed tubes in the dark. Aliquots (20 μL) were periodically removed for up to 17 days and assayed for enzyme activity. After 17 days, the control enzyme retained 66% of its original activity. Inactivator/enzyme ratios of 0, 0.5, 1, 1.5, 2, 2.5, 3, and 5 were used.

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Registry No. 1, 27067-03-4; 2, 935-42-2; MAO, 9001-66-5; 1-phenylcyclopropanecarboxaldehyde, 6120-95-2.

A Dipeptide Derived from Kainic and L-Glutamic Acids: A Selective Antagonist of Amino Acid Induced Neuroexcitation with Anticonvulsant Properties

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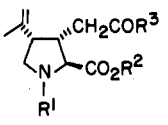
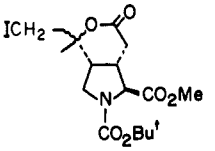
The dipeptide *N*-[[[2′*S*-(2′α,3′β,4′β)]-2′-carboxy-4′-(1″-methylethenyl)-3′-pyrrolidinyl)acetyl]-L-glutamic acid (**6**) has been synthesized by a route that involves the selective protection of the α-carboxyl function of kainic acid. This dipeptide inhibits the stimulation of Na^+ fluxes induced in brain slices by the neuroexcitant *N*-methyl-D-aspartic acid. Administered intracerebroventricularly, it is also effective in protecting mice from picrotoxin-induced convulsions with an ED_{50} of 0.17 μmol.

The finding that γ-dipeptides of the neuroexcitants D- and L-glutamic acids with α-amino carboxylic acids are capable of inhibiting the effects of neuroexcitatory amino acids,[1-3] as well as acting as anticonvulsants,[4] led us to question whether the analogous derivatives of the excitatory dicarboxylic amino acid kainic acid (**1**), namely γ-kainyl derivatives of type **2**, would show similar or modified activities.[5] We are now reporting the synthesis and the pharmacological properties of a member of this class of compounds, γ-kainyl-L-glutamic acid (**6**).





8

1 $R^1=R^2=H$, $R^3=OH$
2 $R^1=R^2=H$, $R^3=NHCH(R)CO_2H$
3 $R^1=CO_2Bu^t$, $R^2=H$, $R^3=OH$
4 $R^1=CO_2Bu^t$, $R^2=Me$, $R^3=OH$
5 $R^1=CO_2Bu^t$, $R^2=Me$, $R^3=NHCH(CH_2CH_2CO_2Me)CO_2Me$ (L)
6 $R^1=R^2=H$, $R^3=NHCH(CH_2CH_2CO_2H)CO_2H$ (L)
7 $R^1=R^2=H$, $R^3=NHCH_2CO_2H$

Chemistry. The preparation of γ-dipeptides (**2**) derived from kainic acid (**1**) required the development of a method for the exclusive protection of the α-carboxyl group of kainic acid (**1**). This was achieved by the transient incorporation of the γ-carboxyl group into a δ-lactone ring formed by iodolactonization involving the isopropenyl side chain. The synthetic sequence leading to the dipeptide γ-kainyl-L-glutamic acid (**6**) through the intermediate monoester **4** started with the iodolactonization of the *N*-*tert*-butyloxy derivative (**3**)[6] followed by esterification with diazomethane. The iodolactone **8** was obtained as a mixture of two diastereoisomers.[6] Reduction of this mixture with zinc in aqueous acetic acid regenerated both the isopropenyl side chain and the free γ-carboxyl group. The monoester **4** thus obtained was treated with dimethyl glutamate and *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to give the fully protected dipeptide **5**, which was hydrolyzed and acidolyzed to the desired γ-kainyl-L-glutamic acid (**6**).

Table I. Percent Response to Agonists in the Presence of Antagonists in a $^{22}Na^+$ Efflux Assay[a]

	agonist			
antagonist	30 μM NMDA	0.1 mM KA	0.5 mM L-Glu	0.1 mM Quis
1 mM **6**	16 ± 9**[b]	84 ± 5	90 ± 31	69 ± 11*[b]
1 mM γ-DGG	5 ± 2*[b]	47 ± 14*[b]	71 ± 11*[b]	100 ± 4

[a] For the method by which the stimulation of $^{22}Na^+$ efflux from preloaded rat striatum slices and its inhibition by antagonists were measured see ref 8 and 9. One hundred percent response is the increase in $^{22}Na^+$ efflux rate induced by the given concentration of the agonist in the absence of any antagonist. The data represent the mean response plus or minus standard deviation from at least triplicate experiments. [b] The statistical significance of the difference from control as calculated by variance analysis: *, $p < 0.01$; **, $p < 0.001$.

Biological Results and Discussion. The dipeptide **6** was tested[7] for its ability to block the increase in the permeability of the neuronal membrane to sodium ions produced in rat striatum slices by excitatory amino acids.[8] The excitants used were *N*-methyl-D-aspartic acid (NMDA), kainic acid (KA), L-glutamic acid (L-Glu), and quisqualic acid (Quis), representing four classes of recep-

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tors.[8] The data obtained for the antagonist activity of γ-kainyl-L-glutamic acid (6) and for the dipeptide γ-D-glutamylglycine (γ-DGG) as a standard antagonist[1,8] are shown in Table I. The results clearly indicate that 6 at 1 mM concentration is a selective NMDA antagonist.

It is of interest to note that 6, a dipeptide composed of two excitatory amino acids, acts as an antagonist of amino acid induced excitation. The fact that it is active mainly at NMDA sites and almost not at all at KA and L-Glu sites is in agreement with the results of previous studies that showed that derivatization of either kainic[6,9] or L-glutamic[1,2] acid through the γ-carboxyl group may lead to compounds exhibiting antagonist properties with receptor specifities different from those of the agonists from which they are produced.

Several NMDA antagonists, including the recently reported γ-kainylglycine (7),[5] have been found to possess anticonvulsant properties.[4,5,10] The dipeptide γ-kainyl-L-glutamic acid (6) is an anticonvulsant capable of protecting mice from picrotoxin-induced seizures (see the Experimental Section) with an ED_{50} value of 0.17 μmol. The corresponding value for γ-DGG is 0.1 μmol.

Experimental Section

Melting points were determined on a Buchi apparatus and are uncorrected. Mass spectra were obtained with a Varian MAT-731 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Proton NMR spectra were recorded at 270 MHz on a Brucker WH-270 instrument. Tetrahydrofuran was dried by distillation from $LiAlH_4$. Organic solutions were dried over anhydrous $MgSO_4$.

1,1-Dimethylethyl Methyl [2*S*-(2α,3β,4β)]-3-(Carboxymethyl)-4-(1-methylethenyl)-1,2-pyrrolidinedicarboxylate (4). To a solution of a mixture of the isomers of the iodolactone 8, prepared by iodolactonization of 470 mg (1.5 mmol) of the *tert*-butyloxy derivative 3,[6] followed by treatment with diazomethane,[6] in 12 mL of acetic acid was added 1.2 mL of water and 1 g of zinc dust. The mixture was stirred during 1 h and filtered. The filtrate was evaporated to leave a residue that was taken up in ethyl acetate, washed consecutively with saturated NH_4Cl solution and with water, dried, and evaporated to give 408 mg (83% from 3) of the monoester 4: mp 127–128 °C (colorless prisms from hexane–ethyl acetate); $[\alpha]^{22}_D$ −26.9° (c 1, $CHCl_3$); ^1H NMR ($CDCl_3$ + D_2O) δ 1.41 (s) and 1.47 (s) (ratio 3:2, *t*-Bu), 2.30 (dd, *J* = 8.6 and 17 Hz, one of CH_2CO), 2.40 (dd, *J* = 5.6 and 17 Hz, one of CH_2CO), 2.75–2.9 and 2.9–3.1 (H_3 and H_4), 3.4–3.5 (one of H_5), 3.55–3.8 [consists of a m centered at 3.68, one of H_5, and 3.75 (s), CO_2Me], 4.08 (d, *J* = 3.8 Hz) and 4.22 (d, *J* = 2.4 Hz) (ratio 3:2, H_2), 4.72 (s, olefinic H), 4.94 (s, olefinic H); high-resolution mass spectrum $C_{12}H_{17}NO_6$ [(M − C_4H_8)$^+$] calcd *m/e* 271.1055, found *m/e* 271.1031.

***N*-[[[2′*S*-(2′α,3′β,4′β)]-2′-Carboxy-4′-(1″-methylethenyl)-3′-pyrrolidinyl]acetyl]-L-glutamic Acid (6).** To a stirred solution of 327 mg (1 mmol) of the monoester 4, 233 mg (1.1 mmol) of dimethyl L-glutamate hydrochloride, and 115 mg (1.1 mmol) of *N*-hydroxysuccinimide in 5 mL of dry tetrahydrofuran was added, at 0 °C, 0.16 mL (1.15 mmol) of triethylamine followed by 227 mg (1.1 mmol) of dicyclohexylcarbodiimide. The mixture was stirred for 1 h at 0 °C and at ambient temperature overnight. A mixture of 15 mL of ethyl acetate, 0.1 mL of acetic acid, and 0.5 mL of 1 N HCl was added, and after an additional stirring during 2 h the precipitate was filtered off and the solution was washed consecutively with 1 N HCl, 5% $NaHCO_3$, and saturated NaCl solution, dried, and evaporated. The residue was chromatographed over silica gel, using 3:1 (v/v) hexane–ethyl acetate as solvent, to give 315 mg of an oil consisting of the fully protected dipeptide 5; high-resolution mass spectrum for $C_{23}H_{38}N_2O_9$ (M^+) calcd *m/e* 484.2420, found *m/e* 484.2416. To a solution of 5 in 7 mL of methanol was added 1.95 mL of 1 NaOH. After 2 days, water was added and the solution was acidified with 1 N HCl, at 0 °C, to pH 2 and extracted with ethyl acetate. The organic solution was dried and evaporated to leave a residue that was treated with 98% formic acid during 5 h. Evaporation followed by trituration of the residue with ethyl acetate and recrystallization from water afforded 138 mg (40% from 4) of the dipeptide 6 as colorless needles: mp 244 °C dec; $[\alpha]^{22}_D$ −15.4° (*c* 0.5, H_2O); ^1H NMR (D_2O)[11] δ 1.78 (s, CH_3), 1.94–2.12 (one of H_3), 2.12–2.26 (one of H_3), 2.37 (dd, *J* = 7.5 and 15 Hz, one of CH_2CON), 2.46 (d, *J* = 6.5 Hz, part of dd of one of CH_2CON), 2.51 (t, *J* = 7 Hz, 2 H_4), 2.98–3.1 ($H_{4'}$), 3.1–3.21 ($H_{3'}$), 3.46 (t, *J* = 11.5 Hz, one of $H_{5'}$), 3.67 (dd, *J* = 6.5 and 11.5 Hz, one of $H_{5'}$), 4.15 (d, *J* = 3.6 Hz, $H_{2'}$), 4.40 (dd, *J* = 6 and 8.3 Hz, H_2), 5.07 (olefinic H).[12] Anal. ($C_{15}H_{22}N_2O_7$) C, H, N.

Measurement of Anticonvulsant Potency with Picrotoxin-Injected Mice. CDl mice of either sex, weight 20–30 g, were used. Intracerebroventricular (icv) injections were performed without anaesthesia and directed into the left or right ventricle, 1 mm posterior and 1 mm lateral to the bregma, at a depth of 2.6 mm, as dictated by the length of the needle of a type 702 Hamilton microsyringe. The compound tested for anticonvulsant activity was dissolved in phosphate-buffered saline, pH 7.4. Mice were injected with 5 μL of vehicle (saline) or test compound solution. After 10 min, picrotoxin was injected intravenously in the tail vein at 3 mg/kg. Volumes of 0.1–0.2 mL of picrotoxin in phosphate-buffered saline were administered, using a Tuberculin syringe and a 27-g needle. The animals were observed during 30 min following the picrotoxin injection. The incidence and timing of stage 5 seizures[13] in response to picrotoxin were recorded. Groups of a minimum of eight animals were used. The ability of the tested compound to protect mice from picrotoxin-induced convulsions is expressed in terms of the median effective dose (ED_{50}) and is calculated according to ref 14.

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Registry No. 1, 487-79-6; 3, 75466-86-3; 4, 98394-36-6; 5, 98394-37-7; 6, 98394-38-8; 8 (isomer 1), 83220-93-3; 8 (isomer 2), 83289-39-8; dimethyl L-glutamate hydrochloride, 23150-65-4.

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