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Spiro[4.5] and Spiro[4.6] Carboxylic Acids: Cyclic Analogues of Valproic Acid. Synthesis and Anticonvulsant Evaluation¹

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Spiro[4.5]decane-2-carboxylic acid (12a), spiro[4.5]decane-2,2-dicarboxylic acid (11a), spiro[4.6]undecane-2-carboxylic acid (12b), spiro[4.6]undecane-2,2-dicarboxylic acid (11b), and spiro[4.6]undecane-2-acetic acid (13) were synthesized by an improved method and evaluated for anticonvulsant activity. These analogues were synthesized to evaluate the role of the carboxylic acid group as an essential substituent in valproic acid (di-n-propylacetic acid, 1). Carbocyclic spiranes are known to resist metabolic alteration so that any activity elicited by these compounds would be due to the carboxylic acid function and not to any metabolic change. Spiro[4.6]undecane-2-carboxylic acid (12b) was the most active analogue tested and the pentylenetetrazol and picrotoxin evaluations of 12b compared favorably to 1. However, 12b failed to provide adequate protection against maximal electroshock seizures, bicuculline, or strychnine in mice. Possible reasons for these results are discussed.

Valproic acid (di-*n*-propylacetic acid, 1) was introduced into the United States in 1978 as an anticonvulsant specifically for the treatment of absence (petit mal) seizures.²



Metabolic alteration of 1 is a significant disadvantage, however.³ A recent report⁴ detailed the synthesis and anticonvulsant evaluation of rigid homologues of 1. This study showed that (\pm) -(E)-2,3-diethylcyclopropanecarboxylic acid (2) and dicyclopropylacetic acid (3) were as active as 1 against pentylenetetrazol-induced seizures in mice. We herein report our studies on spiro carboxylic acid analogues of 1 as potential anticonvulsants.



Chemistry. The target compounds were synthesized according to the procedure shown in Scheme I. This synthesis has been detailed in part previously.⁵ Starting with the appropriate ketone (cyclohexanone or cycloheptanone, 4), ethyl cyanoacetate was reacted under Cope conditions⁶ and the product, the cycloalkylidenecyanoacetic acid ester 5, was then reacted with potassium cyanide in a hydroalcoholic solution. After the mixture stood at room temperature for 48 h, the solvents were evaporated, and the residue was treated with hydrochloric acid, refluxed overnight, and upon cooling, the 1-carboxy 1-acetic acid derivative 6 precipitated in nearly pure form.

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Scheme I^a



^a Reaction conditions: $A = AcOH, NH_aOAc; B = KCN$, HCl; C = EtOH, H₂SO₄; D = LAH or NaAlH₂-(O(CH₂)₂OCH₃)₂; E = HBr, H₂SO₄, or SOCl₂; F = CH₂(CO₂Et)₂, NaOEt; G = KOH, EtOH, HCl; H = Δ ; I = $SOCl_2$; CH_2N_2 , Et_3N ; $C_6H_sCO_2Ag$, Et_3N ; NaOH, HCl. Series a = cyclohexanone; series b = cycloheptanone.

Esterification under standard conditions⁸ produced the diethyl ester 7. When 7 was reduced with lithium alu-

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Table I. Phase I Anticonvulsant Testing

	dose,	MI	ESª	scMet	b	Tox ^c			
compd	mg/kg	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	comment	
12a	300	$1/1^{d}$	$0/1^{d}$	$1/1 (4/4)^g$	0/1 ^d	3/4 (1/4)	0/2		
	600	0/0	0/0	1/1	0/1	4/4	0/1	2/4 died in respiratory depression; $2/4$ anesthetized	
1 2b	300	$1/1^{d}$	0/1	1/1 (2/4)	0/1	1/4 (0/4)	0/2		
	600	0/0	0/0	1/1	1/1	4/4	1/1	death same as in 12a	
11a	300	0/1	0/1	0/1	0/1	0/4	0/2		
	600	0/1	0/1	1/1 (4/4)	0/1	0/4 (0/4)	2/2		
11b	300	0/1	$0/1^e$	1/1 (3/4)	0/1	0/4	0/2		
	600	$0/1^{d}$	$0/0^d$	1/1(4/4)	$0'/0^{d}$	3/4 (3/4)	$2/2^{e}$		
13	300	$1/1^{d}$	$0/1^{d}$	1/1(1/4)	$0'/1^{d}$	3/3(2/4)	0/2		
	600	1/1	0'/1	1/1	1/1	4/4	0'/2	4/4 anesthetized	
1 2b (Na) [/]	300	0'/1	0'/1	0/1	0/1	0/4	0'/2	,	
	600	0/1	0/1	1/1	0/0	4/4	2/2		

^a Maximal electroshock test (number of animals protected/number of animals tested). ^bSubcutaneous pentylenetetrazol test. ^cToxicity (number of animals exhibiting toxicity/number of animals tested). ^dToxic at 0.5 h. ^eToxic at 4 h. ^fDisodium salt of 12b. ^gData in parentheses indicates the results of a second trial.

minum hydride under conditions previously reported,⁹ the glycol 8 was produced in fair yields. It was found that the inorganic precipitate formed during the workup adsorbed a considerable quantity of 8. Continuous extraction of the dried inorganic residue with ethyl acetate-acetone (3:1) improved the vields dramatically. Several trials were attempted on the reduction of the diacid 6 with lithium aluminum hydride,¹⁰ but due to their poor solubility in organic solvents, the results were unpredictable. Reduction was also performed with sodium dihydrobis(2-methoxyethoxy)aluminate (Vitride (Hexcel)). The latter reducing agent required a shorter reaction period (2 h vs. 24-48 h) and the workup was less tedious. On bromination of the glycol 8 with fuming hydrobromic acid and sulfuric acid,⁹ an unstable product formed, which had to be stored at -15°C under reduced pressure and reacted within 4 h after isolation. This crude dibromide 9 could, however, be condensed with diethyl malonate to produce the desired 2,2-dicarboethoxy spiranes 10 in good yields. Saponification of the diester in alcoholic potassium hydroxide, followed by acidification of the dipotassium salt, produced the diacid 11. The diacids were readily decarboxylated to 12 by heating above their melting point and distilling the residue under reduced pressure. The overall yield from cyclohexanone (series a) was 20%, while with cycloheptanone (series b) was 18%. Halogenation of the glycol 8 with a 0.1 molar excess of thionyl chloride in methylene chloride produced a stable dichloride, which, after washing with fresh methylene chloride and evaporation, could be used without further purification in the subsequent steps.¹¹ This modification resulted in an increased overall yield of 25% and 23%, respectively. Spiro[4.6]undecane-2-acetic acid (13) was prepared by the Arndt-Eistert synthesis¹² of 12b. This reaction was preferred over the earlier work⁵ involving the Emmons modification¹³ of the Wittig reaction on the respective spiro ketone.

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 Table II. Phase II Quantification of Antipentylenetetrazol

 Activity and Neurotoxicity

compd	scMet ED ₅₀ , ^a mg/kg	TD ₅₀ , mg/kg	PI ^b
12b	83 (60-105)	193 (176-226)	2.3
11b	194 (168-258)	275 (225-326)	1.4
valproic acid ^e	149 (123-177)	426 (369-450)	2.9

^aMeasured at time of peak effect, 0.5 h. Values in parentheses are 95% confidence intervals determined by probit analysis. ^bProtective index (TD_{50}/ED_{50}). ^cValues from ref 16.

 Table III. Phase V Quantification of Antipicrotoxin Activity

 and Neurotoxicity

compd	scPic ED ₅₀ , ^a mg/kg	${ m TD}_{50}$, mg/kg	PI
12b	167 (137-195)	193 (176-226)	1.2
valproic acid ^b	387 (341-444)	426 (369-450)	1.1
			0 - 0

^aSubcutaneous picrotoxin (3.2 mg/kg). ^bValues from ref 16.

Biological Results and Discussion

Preliminary pharmacological testing of compounds 11a,b, 12a,b, and 13 were performed by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological and Communicative Disorders and Stroke. Phase I analysis in mice is shown in Table I. The three tests were maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (scMet), and neurologic toxicity (Tox). All compounds, including the disodium salt of 12b, displayed little activity against seizures induced by MES. The latter compound was submitted for testing due to the high lipophilicity exhibited by 12a and 12b.14 This compound, however, was slightly soluble in water and thus did not provide the anticipated distribution.¹⁴ Protection against pentylenetetrazol-induced seizures was shown for 11b, 12a, and 12b at 300 mg/kg at 0.5 h and by 11a at 600 mg/kg. The low order of toxicity of 11a, 11b, and 12b at 300 mg/kg at 0.5 h prompted further evaluation. Phase II results on the quantification against scMet for 11b and 12b are shown in Table II. These data were provided by the Antiepileptic Drug Development (ADD) Program. Valproic acid is shown for comparison. The ED_{50} for 12b is lower than for valproic acid with a protective index comparable to the latter: thus 12b was evaluated in phase V quantification against bicuculline, picrotoxin, pentylenetetrazol, and strychnine. Data for picrotoxin are shown in Table III. Valproic acid is shown for comparison. As with scMet, 12b had a lower ED_{50} and a comparable protective index in the picrotoxin test. Bicuculline and strychnine data were disappointing, as no protection was shown in doses up to

⁽¹⁴⁾ Zalucky, T. B., unpublished data.

	· · · · · · · · · · · · · · · · · · ·		av onset, ^b min (no. of mice protected/no. of mice tested)				
pretreatment	pretreatment time, min	dose, mg/kg, sc	clonic seizure ^c	tonic seizure ^d	death		
PEG 30%	15	· · · · · · · · · · · · · · · · · · ·	$7 \pm 2 (0/8)$	$11 \pm 4 \ (0/8)$	$13 \pm 4 \ (0/8)$		
PEG 30%	30		$6 \pm 2 (0/4)$	$9 \pm 2 (0/4)$	$9 \pm 2 (0/4)$		
valproic acid	15	360	$9 \pm 2 (0/3)$	11 (2/3)	$15 \pm 2 (1/3)$		
aproio acia	15	400	$15 \pm 8 (1/4)$	$17 \pm 11 \ (2/4)$	$18 \pm 11 (2/4)$		
11b	30	300	$9 \pm 4 (0/4)$	$12 \pm 3 (1/4)$	$13 \pm 5 (1/4)$		
129	30	200	$8 \pm 4 (0/4)$	$11 \pm 3 (0/4)$	$12 \pm 3 (0/4)$		
12h	15	150	$7 \pm 2 (0/4)$	$10 \pm 3 (0/4)$	$10 \pm 2 (0/4)$		
12b	30	150	$8 \pm 3 (0/4)$	$13 \pm 6 (0/4)$	$14 \pm 6 \ (0/4)$		

Table IV. Anticonvulsant Testing against Bicuculline-Induced Seizures^a

^aBicuculline (3.75 mg/kg) administered subcutaneously. ^bValues are mean ±SD. ^cIndicates at least one 5-s episode of clonic spasms. ^d Indicates a period of hindlimb flexion followed by hindlimb extension.

Table	v.	Anticonvulsant	Testing	against	Picr	otoxin-	Induced	Seizures ^a
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pretreatment	pretreatment	dose,	av onset	no. of mice
	time,	mg/kg,	(min) ^b of	protected/no. of
	min	sc	clonic seizure ^c	mice tested
PEG 30% valproic acid 12b	15 15 15	400 180	13 ± 1 16 ± 4 21 ± 4	0/4 1/4 0/4

^aPicrotoxin (4.0 mg/kg) administered subcutaneously. ^bValues are mean ±SD. ^cIndicates at least one 5-s episode of clonic spasms.

400 mg/kg. The subcutaneous bicuculline and subcutaneous picrotoxin tests were repeated in the laboratory with 11b, 12a, 12b, and valproic acid. Results are shown in Tables IV and V. Bicuculline was administered in a dose of 3.75 mg/kg whereas the picrotoxin dose was 4.0 mg/kg. It should be noted that both bicuculline and picrotoxin dosages differed from the ADD Program protocol.^{15,17} Under the conditions used in this laboratory, 11b protected one out of four mice from bicuculline-induced tonic seizures and death. Neither 12a nor 12b demonstrated any activity when evaluated against bicuculline, and 12b also failed to provide protection against picrotoxin-induced convulsions. Valproic acid did provide some protection at both the 360 and 400 mg/kg doses against bicuculline-induced seizures and at the 400 mg/kg dose against seizures induced by picrotoxin. The ED_{50} values for valproic acid protection against bicuculline and picrotoxin induced seizures in mice were previously reported to be 360 and 387 mg/kg, respectively.¹⁶ The convulsant doses used to determine the ED_{50} values were somewhat lower than those used in this laboratory, however: 2.7 mg/kg for bicuculline and 3.2 mg/kg for picrotoxin. The effectiveness of 12b against bicuculline and picrotoxin would have added much to the mechanism of action of valproic acid as these convulsants are considered antagonists of γ -aminobutyric acid (GABA),^{19,20} a major inhibitory neurotransmitter in the brain.²¹ Valproic acid has been shown to increase GABA levels in the brain in animals.²² Whether it acts reversibly is questioned.²³

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Recently,²⁴ the 2-ene and 4-ene forms of valproic acid were found in the brain of rats infused with valproic acid. These metabolites were found to be 50 times as potent as the parent drug against pentylenenetetrazol-induced seizures. Since 12b is not significantly metabolized,¹⁴ its mode of action must be due to the intact carboxylic acid moiety. It was shown that valproic acid bears a steric resemblance to GABA.²⁵ The resemblance of 12b to valproic acid is likewise apparent. The rigid orientation of the spiro rings in 12b, however, allow little torsional vibration within the molecule. This limited orientation may be used as a biologic probe in determining the active conformation of valproic acid. More importantly, the absence of a biotransformation mechanism for the carbocyclic spiranes would provide information on the reversible nature of valproic acid. The kinetics of 12b will be reported subsequently.

The mechanism of action of 12b in protecting the animals from the convulsant action of pentylenetetrazol was investigated. A theory has been proposed that anticonvulsants may act on voltage-dependent channels regulating neurotransmitter release.²⁶ Thus 11b, 12a, 12b, and Thus 11b, 12a, 12b, and valproic acid were submitted for testing.²⁷ None of the compounds displayed any inhibitory or stimulatory effects within the range 10^{-8} to 10^{-4} M.²⁹

In summary, 12b, the most active of a series of spiro carboxylic acids is effective against pentylenetetrazol-induced seizures. This seizure protection would indicate effectiveness against absence (petit mal) seizures.²⁹ In comparing 12b to 13, it can be stated that increasing the distance of the carboxylic acid group from the ring by a

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⁽¹⁵⁾ The ADD Program used 2.70 and 3.2 mg/kg for the bicuculline and picrotoxin evaluations, respectively.

⁽²⁴⁾ Pollack, G. M.; Shen, D. D. Proceedings of the 15th Epilepsy International Symposium, Washington, DC, Sept 26-30, 1983; American Epilepsy Society and Epilepsy Foundation of America, 1983; p 456 (abstract).

methylene group decreases pentylenetetrazol inhibition and increases toxicity. A general structure-activity correlation would have to await the synthesis and evaluation of a considerable number of spiro carboxylic acid analogues.

Experimental Section

Chemistry. Melting points were obtained with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Observed boiling points were also uncorrected. IR spectra were obtained on a Perkin-Elmer 1330 infrared spectrophotometer either as KBr pellets or neat. ¹H NMR spectra were determined on a 60-MHz Varian EM-360A spectrometer with Me₄Si as the internal reference. Drying of organic extracts was performed by storage overnight over sodium sulfate. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY. Where analyses are indicated only by the symbols of the elements, analytical results for the elements were within 0.4% of the theoretical values.

Ethyl α -Cycloheptylidene- α -cyanoacetate (5b). The general procedure of Cope and co-workers⁶ was followed with use 1.2 mol of 4b, 1.0 mol of ethyl cyanoacetate, 7.7 g NH₄OAc, and 12 g of HOAc in 150 mL of benzene. After refluxing for 8 h, the mixture was cooled to room temperature, and washed with H₂O (100 mL) and saturated NaCl (100 mL), and the combined washings were extracted with 150 mL of Et₂O. The combined organic layers were dried (Na₂SO₄), concentrated under reduced pressure, and distilled: bp 108–110 °C (0.45 mm) (lit.³⁰ bp 160 °C (12 mm)); yield, 177 g (85%), as a light yellow viscous oil; NMR (CDCl₃) δ 1.3 (t, 3 H, CH₃, J = 10.5 Hz), 1.6 (br s, 8 H, C₃-C₆ protons on ring), 2.8 (m, 4 H, C₂, C₇ protons on ring), 4.2 (q, 2 H, CH₂, J = 10.5 Hz); IR (neat) 2210, 1720 cm⁻¹. Anal. (C₁₂H₁₇NO₂) C, H, N.

1-Carboxycycloheptane-1-acetic Acid (6b). The general procedure of Vogel⁷ was followed with use of 0.4 mol of 5b in 300 mL of EtOH and 0.8 mol of KCN in 200 mL of H₂O. After mixing of the two solutions, the clear yellow mixture was stoppered and allowed to stand at room temperature for 2 days. After the solvents were removed under reduced pressure, the residue was reacted with 500 mL of HCl, added over 1 h with stirring. The suspension was refluxed for 16 h, and on cooling to room temperature, the acid 6b separated. It was filtered, washed thoroughly with cold water (500 mL), and dried. All of the washings and the remaining HCl were combined, boiled to half its volume, and refrigerated overnight. The crude acid thus obtained was separated and washed as before and the total recrystallized from H₂O: mp 160-162 °C (lit.³⁰ mp 159 °C); yield, 66 g (82%), as white needles; NMR (Me₂CO- d_6) δ 1.5 (br s, 8 H, C₃-C₆ protons on ring), 2.0 (br s, 4 H, C₂, \tilde{C}_7 protons on ring), 2.5 (s, 2 H, CH₂CO₂), 8.5 (br, 2 H, CO₂H); IR (KBr) 3000, 1710 cm⁻¹.

Ethyl 1-(Ethoxycarbonyl)cycloheptane-1-acetate (7b). The ester was prepared with use of 6b (104 g, 0.52 mol), 650 mL of absolute EtOH, 325 mL of benzene, and 45 g of H₂SO₄. After stirring and refluxing for 24–36 h, the mixture was cooled to room temperature and washed with H₂O (3×200 mL) and saturated KHCO₃ (2×200 mL). The aqueous extract was extracted with Et₂O (2×200 mL) and the Et₂O separated and washed with KHCO₃ (2×100 mL). The combined organic layers were dried (Na₂SO₄), concentrated under reduced pressure, and distilled, bp 113–114 °C (0.7 mm), as a clear mobile oil: yield, 110.5 g (83%); NMR (CDCl₃) δ 1.2 (t, 6 H, 2 CH₃, J = 7.2 Hz), 1.5 (br s, 8 H, C₃-C₆ protons on ring), 2.0 (br m, 4 H, C₂, C₇ protons on ring), 2.6 (s, 2 H, CH₂CO₂), 4.13 (m, (two overlapping quartets), 4 H, 2 CH₂, J = 7.2 Hz); IR (neat) 1720 cm⁻¹. Anal. (C₁₄H₂₄O₄) C, H.

1-(Hydroxymethyl)-1-(β -hydroxyethyl)cycloheptane (8b). Method A. To LAH, 45 g (1.19 mol) in 1 L of dry Et₂O, was added 7b (71 g, 0.28 mol) in dry THF (300 mL) over 90 min with vigorous stirring. After the addition, the mixture was refluxed for 24-48 h, cooled, and decomposed with H₂O, and after filtration of the inorganic residue, the organic solvents were dried (Na₂SO₄). The residue was dried at 80 °C overnight, triturated into a fine powder, and transferred to a Soxhlet apparatus where it was extracted continuously with a mixture of EtOAc-Me₂CO (3:1) for 24 h. The solvent mixture was combined with the organic layer, concentrated under reduced pressure, and distilled: bp 125–129 °C (0.25 mm); yield, 37.5 g (59%) of a viscous, pale yellow oil that solidified on standing: mp 56–58 °C (MeOH) as clear waxy crystals; NMR (CDCl₃) δ 1.4 (br s, 14 H, C₂–C₇ protons on ring + CH₂ (triplet massed)), 3.3 (s, 2 H, CH₂), 3.7 (t, 2 H, CH₂, J = 10.5 Hz), 4.4 (s, 2 H, OH (exchanged with D₂O)); IR (neat) 3290 cm⁻¹. Anal. (C₁₀H₂₀O₂) C, H.

Method B. To a vigorously stirred solution of sodium bis(2methoxyethoxy)aluminate (Vitride, 70% solution in toluene), 124 mL (0.44 mol), in 300 mL of toluene was added 7b (51.2 g, 0.2 mol) over 20 min. The mixture was refluxed for 2 h and after cooling was slowly added to 900 mL of 6 N HCl, while the mixture was stirred constantly and the temperature maintained below 50 °C with external cooling. After all the salts had dissolved, the mixture was separated and the organic layer washed with H₂O (3×100 mL) and dried (Na₂SO₄). The aqueous washings were extracted first with toluene (100 mL) and separated and then with Et₂O (100 mL) and combined with the organic layer. Concentration, under reduced pressure, and distillation of the residue produced 8b (bp 134-139.5 °C (0.35 mm)), yield 19 g (56%).

1-(Chloromethyl)-1-(β -chloroethyl)cycloheptane (9b, R = Cl). A solution of 8b (23.8 g, 0.14 mol) in CH₂Cl₂ (150 mL) was added to a stirred solution of SOCl₂ (24 mL, 0.32 mol) in CH₂Cl₂ (300 mL). The mixture was refluxed overnight. The solvent and excess SOCl₂ were removed under reduced pressure, and fresh CH₂Cl₂ (3 × 200 mL) was added and the mixture evaporated. IR spectra showed no absorption between 3000 and 3500 cm⁻¹. Yield: 24.6 g (84%). An analytical sample was prepared, bp 116–118 °C (0.45 mm), as a clear liquid that darkens on standing. It must be stored at -15 °C under reduced pressure until use. Anal. (C₁₀H₁₈Cl₂) C, H, Cl.

1-(Bromomethyl)-1-(β -bromoethyl)cycloheptane (9b, R = Br). To 48.8 g (0.29 mol) of 8b in 600 mL of HBr (48%) was added 300 mL of H₂SO₄ with stirring and cooling to maintain a temperature not exceeding 15 °C. After the addition, the mixture was heated on a steam bath for 24 h. After cooling to room temperature, the solid residue was separated by vacuum filtration and thoroughly extracted with Me₂CO (350 mL) and Et₂O (500 mL). The acid layer was poured into a beaker containing ice and extracted with Et_2O (3 × 500 mL). The Et_2O was then washed successively with H_2O (2 × 200 mL), saturated KHCO₃ (3 × 100 mL), and saturated NaCl (3×100 mL), combined with the Me₂CO-Et₂O extract, and dried (Na₂SO₄). The remaining undissolved solid was treated with H2O (500 mL) and excess solid KHCO₃ and the mixture allowed to stand at room temperature overnight. The mixture was separated by vacuum filtration, the aqueous layer extracted with Et₂O (200 mL), and the solid macerated with Me₂CO (200 mL) and Et₂O (250 mL). The organic solvents were combined and concentrated under reduced pressure to yield a mobile, dark brown liquid which did not distill. It gave a single spot on TLC (CHCl₃-MeOH, 2:1), R_f 0.79. It was stored at -15 °C under reduced pressure and used within 4 h.

2,2-Dicarboethoxyspiro[4.6]undecane (10b). To 200 mL of absolute EtOH was added 7.8 g (0.34 mol) of Na with stirring. Gentle warming shortens the reaction time. Diethyl malonate (27.0 g, 0.17 mol) was added at once and the mixture was refluxed for 15 min and the crude halide 9b (0.15 mol) was added as rapidly as possible and the mixture refluxed for 24 h. The suspension was cooled to room temperature, acidified with $10\%\,$ HCl (125 mL), diluted with H_2O (100 mL), and extracted with Et_2O (3 × 300 mL). The combined Et_2O extracts were washed with H_2O (100 mL) and saturated NaCl (100 mL) and dried (Na₂SO₄). Evaporation of the solvent under reduced pressure and distillation at 9.5 mm produced two fractions: fraction 1, boiling point below 150 °C; fraction 2, boiling point above 150 °C. Fraction 2 was redistilled and produced 10b: bp 116-124 °C (0.35 mm); yield, 21.6 g (48%), as a clear liquid; NMR (CDCl₃) δ 1.2 (t, 6 H, 2 CH₃, J = 7.2 Hz), 1.5 (br s, 14 H, protons on seven-membered ring + CH2 on C4), 2.1 (s, 2 H, CH2 on C1), 2.2 (t, 2 H, CH2 on C3 (overlaps with singlet at 2.1), 4.2 (q, 4 H, 2 CH₂, J = 7.2 Hz); IR (neat) 1730 cm⁻¹. Anal. (C₁₇H₂₈O₄) C, H.

Spiro[4.6]undecane-2,2-dicarboxylic Acid (11b). A solution of KOH (25.2 g, 0.45 mol) in EtOH (280 mL) was heated to reflux and 10b (25.2 g, 0.085 mol) added with stirring while a gentle reflux was maintained. The mixture was heated an additional 2 h, and

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on cooling, the dipotassium salt precipitated. The precipitate was collected by vacuum filtration, washed with absolute EtOH, and dried. The salt was dissolved in H₂O, decolorized with C, heated to boiling, and filtered into an ice-cold stirred 10% HCl solution. After filtration, the filtrate was refrigerated overnight to yield an additional quantity of crude 11b. The EtOH filtrate and washings provided an additional 2 g of crude 11b after workup. The crude acid was recrystallized from MeOH to provide 11b, 15.9 g (78%); mp 181–181.5 °C dec (gas evolution), as white needles: NMR (Me₂CO-d₆) δ 1.5 (br s, 14 H, protons on sevenmembered ring + CH₂ on C₄), 2.1 (m, 4 H, CH₂ on C₁ and C₃ overlap), 7.6 (br, 2 H, CO₂H); IR (KBr) 3300–3020, 1680 cm⁻¹.

Spiro[4.6] undecane-2-carboxylic Acid (12b). The dicarboxylic acid 11b (10 g, 0.04 mol) was gradually heated in an oil bath until the temperature reached 200 °C, where it was maintained for 1 h. The residue was distilled to yield 12b: bp 105-110 °C (0.35 mm), yield, 8.2 g (85%) as a clear oil; NMR (CDCl₃) δ 1.4 (s, 14 H, protons on seven-membered ring + CH₂ on C₄), 1.8 (m, 4 H, CH₂ on C₁ and C₃), 2.86 (quintet, 1 H, CHCO₂), 11.31 (s, 1 H, CO₂H); IR (neat) 3480-2990, 1695 cm⁻¹. Anal. (C₁₂H₂₀O₂) C, H.

Spiro[4.6]undecane-2-acetic Acid (13). A solution of SOCl₂ (7.8 mL, 0.11 mol) in 60 mL of CH_2Cl_2 was added to 12b (5 g, 0.026 mol) in 150 mL of CH₂Cl₂ and the mixture stirred and refluxed for 24 h. Evaporation of the solvents under reduced pressure and washing with CH_2Cl_2 (2 × 200 mL) afforded the crude acid chloride, which was used without purification. A solution of the acid chloride in 150 mL of dry THF was added dropwise over 30 min at 0 °C to a stirred solution of 4.4 mL (0.031 mol) of Et₃N and CH₂N₂ (prepared from 9.7 g (0.045 mol) of N-methyl-N-nitroso-p-toluenesulfonamide (Diazald³¹)) in 150 mL anhydrous Et₂O. Stirring was continued for 3 h at 0 °C under N₂. The suspension was filtered and the precipitate washed with THF $(2 \times 50 \text{ mL})$ and Et₂O $(2 \times 50 \text{ mL})$. The solvents were concentrated under reduced pressure, and the residue was chromatographed on a dry silica gel column with benzene-CHCl₃ (1:1) to afford the diazo ketone, a light green oil: yield, 4.4 g (76%); IR (neat) 2095, 1630 cm⁻¹. The crude diazo ketone, 4.0 g (0.018 mol) in 100 mL of absolute EtOH, was refluxed and treated with a 10% solution of silver benzoate in Et_3N (0.5 mL) under N₂. A total of 15 mL was slowly added over 15 min. The mixture was refluxed an additional 1 h, cooled, and filtered through Celite. The filtrate was concentrated under reduced pressure, the residue taken up with Et_2O (100 mL), and the organic layer washed with 10% Na_2CO_3 (100 mL), saturated NaCl (2 × 50 mL), and dried (Na_2SO_4) . Evaporation yielded the crude ethyl spiro[4.6]undecane-2-acetate, 2.65 g (74%), which was saponified with NaOH (5.5 g, 0.14 mol) in 55 mL of MeOH and 110 mL of H_2O for 4 h. Evaporation of the MeOH and acidification with cold 10% HCl was followed by extraction with Et_2O (2 × 200 mL) and drying (Na₂SO₄). The product, after evaporation under reduced pressure and distillation (bp 160–163 °C (0.45 mm)), yield 1.82 g (64%), was a light yellow oil: NMR (CDCl₃) & 1.50 (br s, 18 H, protons on seven membered ring + CH_2 on C_1 , C_3 , and C_4), 2.3 (m, 3 H,

CHCH₂CO₂), 11.0 (s, 1 H, CO₂H); IR (Neat) 3000, 1690 cm⁻¹. Anal. (C₁₃H₂₂O₂) C, H.

Pharmacology. Initial evaluation for anticonvulsant activity was done by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological and Communicative Disorders and Stroke and included phases I, II, and V testing procedures, which have previously been described.³² These tests were performed in male Carworth Farms no. 1 mice. Phase I of the evaluation included three tests: maximal electroshock (MES), subcutaneous pentylenetetrazol (scMet), and the rotorod test for neurological toxicity (Tox). Compounds were suspended in 30% polyethylene glycol 400 and were administered by intraperitoneal injection at four dosage levels (30, 100, 300, and 600 mg/kg) at 30 min and 4 h before testing. Phase II testing quantitated the anticonvulsant activity and neurotoxicity by determining the median effective dose (ED_{50}) and median toxic dose (TD_{50}) for those compounds which protected in the MES and scMet tests. For the determination of the ED_{50} or TD_{50} , groups of 6-12 mice were given a range of doses of the test compounds until at least three points were established in the range of 10-90% seizure protection or minimal neurotoxicity. From a plot of the data, the respective ED_{50} , TD_{50} , and 95% confidence intervals were calculated by the method of Litchfield and Wilcoxon.³³ Phase V of the testing measured the ability of the compounds to provide protection against seizures induced by subcutaneous injection of the CD₉₇ of the following convulsant agents: Met (85 mg/kg), bicuculline (2.7 mg/kg), picrotoxin (3.15 mg/kg), and strychnine (1.2 mg/kg). As in phase II, the ED_{50} values were determined at the time of peak effect.

On the basis of the data obtained from the initial screening, additional tests were performed in this laboratory on compounds 11b, 12a, 12b, and valproic acid with use of Sprague–Dawley CF1 mice. These compounds were evaluated for their ability to protect against bicuculline- and picrotoxin-induced (3.75 and 4.0 mg/kg, respectively) seizures at their time of peak effect. This dose of bicuculline produces clonic and tonic seizures and death in 100% of the control animals tested. Picrotoxin at 4.0 mg/kg produces clonic convulsions in 100% of the control mice. Control animals in all phases of testing were injected with the solvent, 30%polyethylene glycol 400.

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