Synthesis and Structure–Activity Studies of Alkyl-Substituted γ-Butyrolactones and γ-Thiobutyrolactones: Ligands for the Picrotoxin Receptor

Daniel J. Canney, Katherine D. Holland, Jeffrey A. Levine, Ann C. McKeon, James A. Ferrendelli, and Douglas F. Covey*

Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110. *Received May 24, 1990*

A series of γ-butyrolactones and γ-thiobutyrolactones possessing a variety of alkyl groups and alkyl-substitution patterns was prepared and evaluated for anticonvulsant and convulsant activity. Behavioral studies performed on these compounds suggest that maximal anticonvulsant activity (against maximal electroshock and pentylenetetrazol) results when three or four carbon atoms are present at the α-position. For convulsant potency, a similar dependence on the size of the alkyl chain at the β-position was observed. Additional γ-dimethyl groups were found to increase the convulsant potency of a β-substituted compound and to cause an α-substituted anticonvulsant to become a convulsant. In general, sulfur for oxygen heteroatom substitution in the α-substituted lactones resulted in improved anticonvulsant potency and spectrum of activity. Binding of these compounds to the picrotoxin site of the GABA receptor complex was demonstrated with a [^{35}S]-*tert*-butylbicyclophosphorothionate radioligand binding assay. Measurements of brain concentrations for selected compounds supports a hypothesis that correlates binding to the picrotoxin site with the pharmacological effects of these compounds.

Preliminary structure–activity studies performed on alkyl-substituted γ-butyrolactones and γ-thiobutyrolactones have demonstrated that these compounds exhibit either convulsant or anticonvulsant activity, depending on the pattern of alkyl substitution.[1-4] The α-alkyl-substituted compounds are efficacious *anticonvulsant* agents capable of protecting animals from seizures induced by pentylenetetrazol (PTZ) and, in some cases, maximal electroshock (MES). In contrast, β-alkyl-substituted analogues are potent *convulsants* producing seizures that are behaviorally similar to those induced by PTZ. On the basis of two lines of evidence, it was hypothesized that these agents produce their pharmacological effects via interactions at the picrotoxin binding site of the GABA receptor/chloride ionophore complex. First, the compounds competitively inhibit the binding of *tert*-butylbicyclophosphorothionate ([^{35}S]TBPS), a radioligand used to measure binding to the picrotoxin receptor.[5-7] Binding studies with representative lactone and thiolactone derivatives using radioligands for the benzodiazepine ([^{3}H]flunitrazepam) and GABA ([^{3}H]muscimol) receptors have demonstrated that these compounds have little or no affinity for these sites.[6] The second line of evidence, derived from electrophysiological experiments, clearly demonstrates that these agents modulate chloride conductances through GABA receptor/ionophore channels in voltage-clamped spinal cord neurons and hippocampal neurons.[8-10]

In order to further investigate the structure–activity relationships for these classes of compounds, we have developed efficient and versatile synthetic routes for γ-butyro- and γ-thiobutyrolactones with various alkyl groups and alkyl-substitution patterns from readily prepared olefinic esters. These structurally diverse lactone deriva-

Scheme I

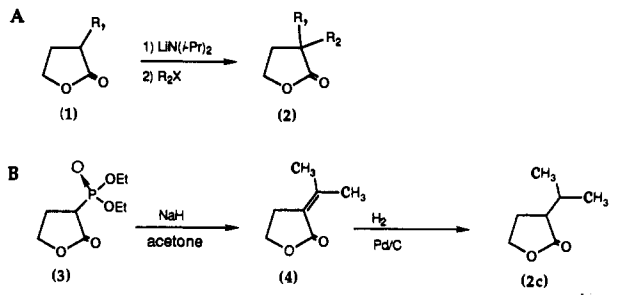


Table I. Olefinic Ester Precursors (6a–e) Utilized in the Synthesis of Alkyl-Substituted γ-Butyrolactones As Outlined in Scheme II

	substituents					
no.	R	R_1	R_2	R_3	bp, °C/mmHg	% yield
6a	Et	*i*-Pr	H	H	88–90/31	83
6b	Me	*t*-Bu	H	H	84–85/28	78
6c	Me	Et	Me	H	84–86/41	85
6d	Me	Et	Et	H	108/57	75
6e	Me	Et	Me	Me	71–73/15	76

Table II. The Anticonvulsant Activity, Neurotoxicity, and [^{35}S]TBPS Binding Data for α-Substituted Lactones 2a–i and α,γ-Substituted Lactone 8 Prepared As Described in Scheme II

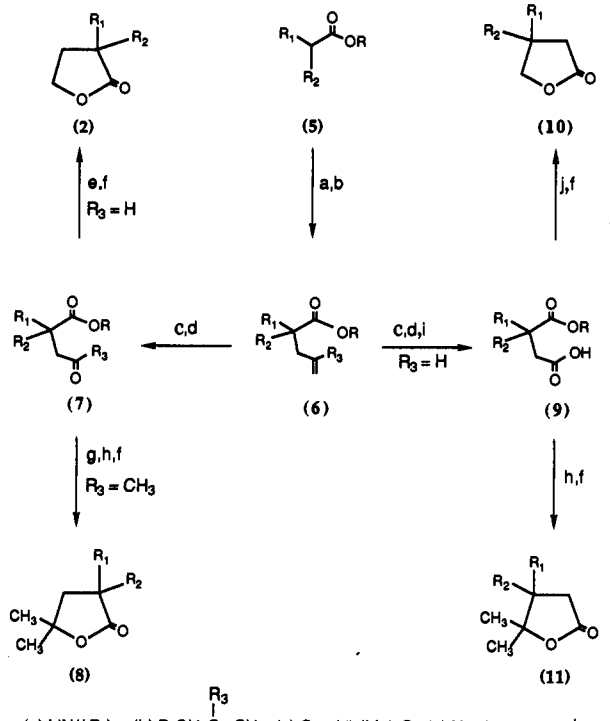
			anticonvulsant potency: ED_{50}[a]			TBPS binding,[b]
no.	R_1	R_2	PTZ	MES	rotorod toxicity: TD_{50}[a]	mM IC_{50}
2a	Me	H	>500 [24]	>500 [20]	>500 [15]	>10
2b	Et	H	>500 [35]	>500 [10]	>500 [44]	8.5 ± 0.4
2c	*i*-Pr	H	150 [85] (119–184)	>400 [44]	>400 [125]	2.0 ± 0.1
2d	*t*-Bu	H	145 [50] (118–171)	375 [35] (298–575)	>500 [50]	0.99 ± 0.09
2e	Me	Me	>500 [90]	>500 [65]	>500 [155]	9.2 ± 0.7
2f	Et	Me	259 [45] (230–291)	>500 [54]	621 [80] (481–1215)	2.3 ± 0.2
2g	Et	Et	191 [55] (172–211)	463 [40] (403–567)	416 [100] (372–492)	0.75 ± 0.05
2h	Me	*i*-Pr	198 [50] (171–232)	>500 [40]	278 [90] (239–329)	0.85 ± 0.07
2i	*i*-Pr	*i*-Pr	convulsant[a,c] [20] 137 (108–184)		not determined	0.22 ± 0.02
8	Et	Me	convulsant[a,c] [30] 117 (98–150)		not determined	0.55 ± 0.035

[a] Dosed are reported as mg/kg. Numbers in brackets are the number of animals tested. Numbers in parentheses are the 95% fiducial limits. [b] Binding data are presented as the mean ± SEM of three experiments performed in quadruplicate. [c] The number reported is the CD_{50} value for clonic convulsions.

(1) Klunk, W. E.; Covey, D. F.; Ferrendelli, J. A. *Mol. Pharm.* **1982**, *22*, 431.
(2) Klunk, W. E.; Covey, D. F.; Ferrendelli, J. A. *Mol. Pharm.* **1982**, *22*, 438.
(3) Klunk, W. E.; Covey, D. F.; Ferrendelli, J. A. *Mol. Pharm.* **1982**, *22*, 444.
(4) Levine, J. A.; Ferrendelli, J. A.; Covey, D. F. *J. Med. Chem.* **1986**, *29*, 1996.
(5) Levine, J. A.; Ferrendelli, J. A.; Covey, D. F. *Biochem. Pharmacol.* **1985**, *23*, 4187.
(6) Holland, K. D.; McKeon, A. C.; Rothman, S. M.; Covey, D. F.; Ferrendelli, J. A. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 578.
(7) Weissman, B. A.; Burke, T. R.; Rice, K. C.; Skolnick, P. *Eur. J. Pharmacol.* **1984**, *105*, 195.
(8) Holland, K. D.; Ferrendelli, J. A.; Covey, D. F.; Rothman, S. M. *J. Neurosci.* **1990**, *10*, 1719.
(9) Baker, K.; Yang, J.; Covey, D. F.; Clifford, D. B.; Zorumski, C. F. *Neurosci. Lett.* **1988**, *87*, 133.
(10) Clifford, D. B.; Baker, K.; Yang, J.; Covey, D. F.; Zorumski, C. F. *Brain Res.* **1989**, *484*, 102.

0022-2623/91/1834-1460$02.50/0

Scheme II



(a) $LiN(i\text{-}Pr)_2$; (b) $BrCH_2C(R_3)=CH_2$; (c) O_3; (d) $(Me)_2S$; (e) $NaBH_4$; (f) H_3O^+; (g) KOH/EtOH; (h) CH_3MgBr; (i) $H_2Cr_2O_7$, acetone; (j) $AlH(i\text{-}Bu)_2$.

tives were then evaluated in behavioral studies (convulsant or anticonvulsant activity and toxicity) and in [^{35}S]TBPS binding assays. Changes in binding affinity and potency produced by substitution of the ring heteroatom (sulfur for oxygen) were also evaluated. Finally, brain concentrations of representative compounds were measured by GC and correlated with their convulsant and anticonvulsant potencies and their ability to inhibit TBPS binding.

Chemistry

Standard reaction methods (Scheme I) could be utilized to prepare many of the desired α-substituted lactones. Other target compounds required the use of different synthetic routes (Scheme II) using readily prepared olefinic esters (Table I). Those compounds with primary alkyl substituents (Table II, compounds **2b,e–g**) could be readily prepared by direct alkylation of γ-butyrolactone (**1**; Scheme I, path A, R_1 = H), or a monosubstituted γ-butyrolactone (**1**, R_1 = methyl or ethyl), using standard alkylation techniques. However, when secondary alkyl halides were employed these alkylation reactions gave unsatisfactory yields. α-Isopropyl-γ-butyrolactone (**2c**) could be prepared in good yield as shown in Scheme I, path B. The Wittig–Horner reagent, [α-(O,O-diethylphosphono)-γ-butyrolactone, **3**],[11] was treated (in benzene) with NaH followed by addition of acetone to afford α-isopropylidene-γ-butyrolactone (**4**).[12] Catalytic hydrogenation of **4** in ethanol using 10% Pd/C yielded **2c**.

Because neither of these methods could be utilized in the preparation of α-*tert*-butyl-γ-butyrolactone or other target compounds, a more general, alternative approach was devised. We have recently reported the reaction pathways shown in Scheme II which provide easy access to a wide variety of lactones possessing various alkyl groups and alkyl-substitution patterns.[13] This initial step involves alkylation of the appropriately substituted ester (**5**) with an allylic halide. The olefinic esters thus formed (**6**), serve as common precursors to each of the desired substitution patterns.

The α-substituted lactones (Table II) were synthesized from the olefinic esters (Table I, compounds **6a–e**) by ozonolysis. Ozonolysis of **6** (R_3 = H) in methanol/methylene chloride (1:1) afforded the acetal of **7** (R_3 = H), which was reduced (without purification) with $NaBH_4$. The reaction mixture containing the resultant hydroxy ester was refluxed gently for 1 h, water added, and the mixture refluxed for an additional hour. Acidic workup to effect ring closure afforded the α-substituted lactone **2**. Alternatively, compound **8** (Table II), substituted at both the α- and γ-positions, was obtained via ozonolysis of the substituted olefinic ester **6** (R_3 = CH_3) in methylene chloride containing 8% acetic acid. Hydrolysis of keto ester **7** (R_3 = CH_3) in ethanolic KOH, followed by treatment of the resultant keto acid with CH_3MgBr and an acidic workup, provided tetrasubstituted lactone **8**.

Preparation of β-substituted derivatives (Table III) also involved cleavage of the common olefinic precursor **6** (R_3 = H) by ozonolysis. Treatment of the resulting aldehydic (or acetal) ester with Jones' reagent afforded half-ester **9**. Subsequent reduction of the ester to the corresponding alcohol with DIBAH at −78 °C, followed by acidic workup to effect ring closure, yielded the β-substituted lactone **10**. Similarly, the β,γ-substituted derivative **11** (Table III) was readily obtained via a reaction involving half-ester **9** and CH_3MgBr followed by an acidic workup.

The α-substituted thiolactones (Table IV, compounds **12a–h**) and the β-substituted thiolactone (Table III, compound **13**) were synthesized as described previously by treating the corresponding lactones with potassium thioacetate in *N,N*-dimethylacetamide.[4] The α-substituted derivatives were allowed to react for 4 h, while the β-substituted compound required a reaction time of 24 h. Compound **12i** was prepared by direct alkylation of compound **12c**.

Table III. Convulsant Potency and [^{35}S]TBPS Binding Data for β-Substituted Lactones **10a–c**, β,γ-Substituted Lactone **11**, and β-Substituted Thiolactone **13**

no.	R_1	R_2	R_3	X	convulsant potency: CD_{50}[a]	TBPS binding,[b] μM IC_{50}
10a	*i*-Pr	H	H	O	15 [40] (10–19)	42 ± 1
10b	*t*-Bu	H	H	O	5.5 [25] (2.5–7.5)	13 ± 2
10c	Et	Me	H	O	20 [25] (12–29)	55 ± 5
11	Et	Me	Me	O	8.2 [20] (5–13)	22 ± 5
13	Et	Me	H	S	3.5 [54] (2.3–4.4)	8.6 ± 0.5

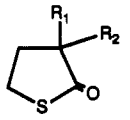
[a] CD_{50} values are for clonic seizures. Doses are reported as mg/kg. Numbers in brackets are the number of animals tested. Numbers in parentheses are the 95% fiducial limits. [b] Binding data are presented as the mean ± SEM of three experiments performed in triplicate.

Results

The lactones and thiolactones described here were first screened for their ability to produce seizures in mice. Those compounds that did not exhibit convulsant activity were screened for their ability to protect animals against

(11) Falsone, G.; Wingen, U. *Tetrahedron Lett.* **1989**, *30*, 675.
(12) Minami, T.; Niki, I.; Agawa, T. *J. Org. Chem.* **1974**, *39*, 3236.
(13) Canney, D. J.; Levine, J. A.; Lu, H-F.; Covey, D. F. *Synth. Commun.* **1990**, *20*, 2065.

Table IV. Anticonvulsant Potency, Neurotoxicity, and [^{35}S]TBPS Binding Data for α-Substituted Thiolactones **12a–i**



no.	R_1	R_2	anticonvulsant potency: ED_{50}[a] PTZ	MES	rotorod toxicity: TD_{50}[a]	TBPS binding,[b] mM IC_{50}
12a	Me	H	convulsant[a,c] [29] 91 (67–118)		not determined	4.4 ± 0.4
12b	Et	H	414 [54] (354–429)	232 [64] (200–265)	>500 [29]	1.5 ± 0.1
12c	*i*-Pr	H	145 [50] (122–203)	>500 [64]	>500 [120]	0.26 ± 0.03
12d	*t*-Bu	H	244 [80] (194–327)	>500 [35]	>500 [90]	0.19 ± 0.02
12e	Me	Me	208 [50] (171–248)	369 [50] (312–449)	220 [115] (194–246)	0.92 ± 0.09
12f	Et	Me	128 [78] (74–149)	219 [55] (195–245)	244 [147] (227–268)	0.33 ± 0.2
12g	Et	Et	104 [35] (82–171)	374 [35] (320–492)	285 [60] (250–342)	0.26 ± 0.02
12h	Me	*i*-Pr	154 [50] (130–182)	>500 [60]	>500 [110]	0.14 ± 0.01
12i	*i*-Pr	*i*-Pr	>500 [40]	>500 [25]	>500 [50]	0.074 ± 0.006

[a] Doses are reported as mg/kg. Numbers in brackets are the number of animals tested. Numbers in parentheses are the 95% fiducial limits. [b] Binding data are presented as the mean ±SEM of three experiments performed in quadruplicate. [c] The number reported is the CD_{50} value for clonic convulsions.

seizures induced by PTZ and MES (ED_{50}).[14] The neurotoxic effects of these compounds were assessed with the rotorod toxicity test.[15] All mice given intraperitonal injections (0.01 mL/g body weight) of the vehicle (30% polyethylene glycol) used to administer the test compounds were unprotected from the convulsant agents and displayed no rotorod toxicity. Both convulsant and anticonvulsant agents were also evaluated in radioligand binding assays using [^{35}S]TBPS,[5,16] a radioligand for the picrotoxin site on the GABA receptor.[17]

Many of the α-substituted lactones (**2c,d,f–h**) studied were found to exhibit anticonvulsant activity against PTZ-induced convulsions (Table II). At doses below 400 mg/kg, only one compound (**2d**) was effective against MES seizures. When evaluated by the rotorod test, the α-substituted lactones, with the exception of **2i** and **8**, were found to be relatively nontoxic (Table II). The anticonvulsant potency of the alkyl-substituted γ-butyrolactones was found to be highly dependent on the size and branching of the alkyl side chain(s). The most potent compounds in the series were found to be those possessing a total of three or four carbons at the α-position of the lactone ring. Those carbons could be contained in a branched alkyl group (three or four carbons; **2c,d,h**) or in *n*-alkyl groups containing one or two atoms each (**2f,g**). The compounds with only one (**2a**) or two (**2b,e**) carbon atoms at the α-position provided virtually no protection from PTZ- or MES-induced seizures, while the diisopropyl (**2i**) and α,γ-substituted (**8**) derivatives were convulsants producing clonic–tonic seizures in mice. The rank order of anticonvulsant potency for these lactones, as defined by the dose required to protect 50% of mice from PTZ-induced seizures (ED_{50}), was found to be **2d** ~ **2c** > **2g** ~ **2h** > **2f** ≫ **2a**, **2b**, **2e**.

In radioligand binding studies, the lactones were shown to inhibit the binding of TBPS in a concentration-dependent manner. The IC_{50} values obtained for these lactone derivatives ranged from 0.22 to 9.2 mM (Table II). Those compounds (**2a,b,e**) exhibiting very low affinity for the picrotoxin receptor (IC_{50} > 3 mM) in the binding assays possessed no anticonvulsant activity. Conversely, the compounds (**2c,d,f–h**) found to protect animals from PTZ-induced seizures were shown to inhibit TBPS binding at concentrations below 3 mM (Table II). The ability of the lactones to displace the radioligand also appears to be related to the total number of carbon atoms present in the alkyl side chains, so that as the number of carbon atoms increases, the IC_{50} values decrease. The IC_{50} values obtained for anticonvulsant compounds with three carbon atoms (**2c** and **2f**) were 2 and 2.3 mM, respectively. Compounds **2g**,**2h**, and **2d**, all possessing a total of four carbons in their alkyl side chains, had IC_{50}'s of 0.75, 0.85, and 0.99 mM, respectively. Finally, compound **8**, with a total of five carbons (α- and γ-positions), and compound **2i**, with a total of six carbon atoms (α-position), were found to have even higher affinities. The IC_{50} values for these compounds were 0.55 and 0.22 mM, respectively. However, as mentioned above, compounds **2i** and **8** were found to have convulsant activity.

The effect of heteroatom substitution in the lactone ring was also investigated. The thiolactones were tested as described above for the lactones. The heteroatom substitution often resulted in increased potency against PTZ-induced seizures, as well as a broader spectrum of anticonvulsant activity, including protection from MES seizures. For example, lactones **2b** and **2e** exhibited no anticonvulsant properties at doses up to 500 mg/kg, while the corresponding thiolactones **12b** and **12e** protected animals against both PTZ- and MES-induced seizures (Table IV). Additionally, unlike lactone **2f** which was only effective against PTZ-induced seizures, thiolactone **12f** was found to possess anticonvulsant activity against both seizure models studied here. For compounds **12e–g** the TD_{50} was either lower than or not significantly different from the ED_{50} for protection against MES seizures. By contrast, the increase in potency and the broader spectrum of activity observed for compound **12b** was achieved without any detectible increase in toxicity.

The neuroactive properties of the α-substituted thiolactone analogues were dependent on the size and branching of alkyl substituents (Table IV) in a manner similar to that observed for the α-alkyl-substituted lactones. Exceptions to the trend observed for the lactones include thio-derivatives **12a** and **12i**. Injection of compound **12a** into mice produced sedation followed by seizures characterized by explosive running episodes. Thiolactone **12i**, in contrast to the convulsant lactone **2i**, displayed no convulsant or anticonvulsant activity.

The thiolactones were also evaluated in radioligand binding studies and found to inhibit TBPS binding in a concentration-dependent manner. In every case, these compounds were found to have significantly higher affinity for the picrotoxin receptor than the corresponding lactones. The IC_{50} values for the α-substituted thiolactones were in the range of 0.074–4.4 mM (Table IV). As in the lactone series, the affinity of the thiolactones in the binding assay appeared to correlate with the total number of carbon atoms at the α (or at both α and γ) position. Furthermore, a strong correlation was observed between the ED_{50} doses

(14) Swinyard, E. A.; Woodhead, J. H. *Antiepileptic Drugs*, 2nd ed.; Raven Press: New York, 1982; p 111.
(15) Dunham, N. W.; Miya, T. S. *J. Am. Pharm. Assoc.* **1957**, *47*, 208.
(16) Triffiletti, R. R.; Snowman, A. M.; Snyder, S. H. *Eur. J. Pharmacol.* **1985**, *106*, 441.
(17) Squires, R. F.; Casida, J. E.; Richardson, M.; Saederup, E. *Mol. Pharm.* **1983**, *23*, 326.

Table V. Brain Concentrations of Compounds 2f, 12f, and 10c following Intraperitonal Injections of an ED_{50} or CD_{50} Dose As Determined by Gas Chromatography

time following injection, min	brain concentrations,[a] mM		
	2f[b]	12f[c]	10c[d]
5	2.97 ± 0.47	0.35 ± 0.06	0.075 ± 0.011
10	1.83 ± 0.26	0.37 ± 0.07	ND[e]
20	1.35 ± 0.13	0.29 ± 0.06	ND
30	1.21 ± 0.10	0.2 ± 0.03	ND
45	0.88 ± 0.10	ND	ND

[a] Brain concentrations at each time point represent the mean values (±SEM) measured in three animals. Duplicate injections were made of each sample. [b] ED_{50} dose administered was 259 mg/kg. [c] ED_{50} dose administered was 128 mg/kg. [d] CD_{50} dose administered was 25 mg/kg. Animals were sacrificed at the time of clonic seizures, or within 5 min of the injection, whichever came first. [e] ND = not determined.

(mM/kg) for compounds **12b**–h and the corresponding IC_{50} values in the binding experiments ($r = 0.898$, $p > 0.01$, $n = 7$).

β-Substituted lactones and a thiolactone also were tested in behavioral studies and the TBPS binding assay. Intraperitonal injections of these compounds (Table III, compounds **10a–c**,**11** and **13**) all produced seizures characterized by myoclonic twitches, clonic seizures, and tonic convulsions. The median convulsant doses (CD_{50}) ranged from 3.5 to 20 mg/kg. Like the α-substituted lactones, the potency of the β-substituted compounds increased as the size of the β-substituent increased. The increase in potency produced by heteroatom substitution (compare **10c** and **13**) is also consistent with that observed for the α-substituted lactones. The addition of γ-methyl groups increased convulsant potency (compare **10c** and **11**). The rank order of potency of the β-substituted compounds as displacers of [^{35}S]TBPS was the same as the order of convulsant potency (**13** > **10b** > **11** > **10a** ~ **10c**).

In order to determine the concentrations of these lactones attained in the brain following peripheral administration, the brain concentrations of **2f** and **12f** were measured as a function of time (Table V). In addition, the concentration of **10c** was determined. The amounts of **2f** and **12f** found in brain (1.21 ± 0.1 and 0.2 ± 0.03 mM, respectively) at the time of convulsant challenge (30 min) are in the range of their IC_{50} concentrations (2.3 and 0.33 mM, respectively) in the binding assay. The amount of **10c** in brain (75.4 ± 11 μM) at the time of clonic–tonic convulsions is also consistent with its IC_{50} value (55 μM) in the binding experiment.

Discussion

The goal of the present structure–activity study was to prepare alkyl-substituted γ-butyrolactones and γ-thiobutyrolactones and to evaluate the target compounds in behavioral, toxicity, and binding studies. In previous structure–activity studies with substituted γ-butyrolactones, succinimides, and structurally related heterocycles, Klunk et al.[3] proposed that heterocyclic compounds with alkyl substituents α and/or γ, but not β, to the carbonyl would be anticonvulsants. Alkyl substituents at the β-position confer convulsant properties, regardless of the presence or absence of other groups in the molecule. Levine et al.[4] found that oxygen for sulfur substitution in the ring resulted in increased potency and a broader spectrum of activity. Finally, Holland et al.[18] reported that alkyl-substituted cyclohexanones and cyclopentanones have convulsant and anticonvulsant properties similar to those observed for the γ-butyro- and γ-thiobutyrolactones. Therefore, previous studies with substituted heterocyclic compounds, as well as cyclic ketones, suggest that the position of alkyl groups relative to the ring carbonyl has dramatic effects on their pharmacological activity. Additionally, while the nature of the ring heteroatom is important, it is not requisite for neurological activity of these small cyclic molecules.

Our work supplies facile routes for the synthesis of structurally diverse lactone derivatives possessing a variety of alkyl groups and substitution patterns. We have utilized these methods to prepare a wide variety of γ-butyrolactone and γ-thiobutyrolactone analogues and evaluated the effects of changes in alkyl groups and alkyl-substitution patterns on their pharmacological activity and binding affinity. In both series of compounds, the α-substituted derivatives were generally anticonvulsants, while the β-substituted or β,γ-substituted compounds were convulsants. This study has demonstrated that the convulsant and anticonvulsant potency and binding affinity of these lactone and thiolactone derivatives is highly dependent on the position, size, and branching of these substituents.

Of those α-substituted compounds investigated in this work, some interesting exceptions to past observations include compounds **2a**,**2b**,**2i**,**8**,**12a**, and **12i**. Compounds **2a** and **2b** had no anticonvulsant activity against either PTZ or MES. These compounds may experience rapid metabolism following ip administration. It is known that γ-butyrolactone is hydrolyzed in vivo by blood and liver lactonases.[19] The active metabolite of γ-butyrolactone, γ-hydroxybutyrate, then produces nonconvulsive seizures in animals.[20,21] Unlike animals receiving γ-butyrolactone, animals receiving **2a** and **2b** showed no overt behavioral changes, implying either that the metabolic fate of these agents is different from that of the unsubstituted lactone or that the hydroxy-acid metabolites of these compounds are inactive. Alternatively, their lack of activity may be better explained by their poor affinity for the receptor.

Compound **12a** was the only α-substituted thiolactone producing convulsant behavior in the animal tests. Thio-derivative **12a** displayed poor affinity for the receptor in the binding assay and produced convulsions which were characterized by uncontrolled running fits rather than the clonic–tonic seizures observed for the other convulsants studied here. The open-ring form of γ-thiobutyrolactone, 4-mercaptobutyric acid, is a competitive inhibitor of glutamate decarboxylase, the enzyme responsible for GABA biosynthesis.[22] The behavioral effects of this enzyme inhibitor are also characterized by running fits,[23] suggesting that the convulsant properties observed for **12a** may be attributed to the ring-opened form of the drug.

Compound **8** produced convulsions when administered to mice which were qualitatively similar to those induced by PTZ and by the β-substituted compounds. The nature of these convulsions and the affinity of **8** for the picrotoxin receptor suggests that this compound exerts its effects via interactions at this site. These observations are contrary to those previously reported for α-ethyl-α-methyl-γ-ethyl-γ-methyl-γ-butyrolactone. Klunk et al.[2] found that this α,α,γ,γ-tetrasubstituted lactone protected animals from tonic seizures induced by PTZ (100 mg/kg). These data suggest that the transition from two (γ,γ-dimethyl) to three carbons (γ-ethyl-γ-methyl) at the γ-position of

(18) Holland, K. D.; Naritoku, D. K.; McKeon, A. C.; Ferrendelli, J. A.; Covey, D. F. *Mol. Pharm.* **1990**, *37*, 98.
(19) Fishbein, W. N.; Bessman, S. P. *J. Biol. Chem.* **1966**, *241*, 4835.
(20) Snead, O. C. *Neurology* **1978**, *28*, 636.
(21) Snead, O. C. *Life Sci.* **1977**, *20*, 1935.
(22) Meldrum, B. S. *Int. Rev. Neurobiol.* **1975**, *17*, 1.
(23) Sprince, H. *Biol. Psychiatry* **1969**, *1*, 301.

this molecule changes its pharmacological activity from convulsant to anticonvulsant. Alternatively, it is conceivable that the compound reported by Klunk et al. is a very weak convulsant agent with poor affinity for the picrotoxin receptor (weak partial agonist). At a dose of 450 mg/kg, the $\alpha,\alpha,\gamma,\gamma$-tetrasubstituted lactone examined by Klunk et al. produced a 15–20 min period of severe hyperexcitability, followed by ataxia and sedation. Perhaps the doses of this compound administered by Klunk et al. in their protection experiment were not high enough to produce convulsions in the animals, but were sufficiently high for this weak agonist to successfully protect the animals from the potent convulsant properties of PTZ, a picrotoxin agonist.[24]

Compound **2i** was also found to be a convulsant. Like compound **8**, the convulsions were characterized by myoclonic twiches and clonic–tonic seizures as described for the β-substituted γ-butyro- and γ-thiobutyrolactones and PTZ. The relatively high affinity of this compound, and the type of seizure it induced, suggests that the convulsant activity of **2i** is due to interactions with the picrotoxin receptor. It is conceivable that steric interactions between the bulky isopropyl groups at the α-position and the receptor necessitate a different binding orientation for this analogue such that this compound is recognized as a picrotoxin agonist. Enders et al.[25-27] have reported the convulsant properties of α-spirocyclohexyl-γ-butyrolactone, while we have observed weak anticonvulsant properties in the corresponding α-spirocyclopentyl- and α-spirocyclobutyl-γ-butyrolactone analogues.[28] Similar changes in the binding orientation of these semirigid molecules resulting from the decreased steric bulk at the α-position might also explain the transition from convulsant to anticonvulsant activity in these lactones. Finally, it is interesting to note that changing the heteroatom in the ring (**2i** to **12i**) produces a compound with good affinity for the picrotoxin receptor but devoid of either convulsant or anticonvulsant activity. An explanation for this puzzling result is not yet apparent to us.

The behavioral studies performed in mice suggest that α-alkyl-substituted γ-butyrolactones are relatively nontoxic as assessed by the rotorod test and that a minimum of three carbon atoms at the α-position are requisite for anticonvulsant activity. Similar structural requirements for anticonvulsant activity were observed for the α-alkyl-substituted γ-thiobutyrolactones. In general, the thiolactones were also found to be more potent and to have a broader spectrum of anticonvulsant activity than the corresponding lactones. Our data also suggest that in order to maintain anticonvulsant activity, there is a maximum number of carbons permitted at the α-position. As illustrated by the α-diisopropyl analogues, six carbons at this position resulted in either a reduction in anticonvulsant potency (**12i**) or convulsant activity (**2i**). While we cannot rule out the possibility that five or more carbons could be present as *n*-alkyl and/or different branched groups, six carbons contained in two isopropyl groups are clearly not tolerated at the α-position. As discussed above, the convulsant properties of eight demonstrate that the presence of γ-alkyl groups on α-substituted compounds can drastically alter the pharmacological properties of an α-substituted γ-butyrolactone.

While significantly fewer β-substituted compounds have been prepared, the data reported here and elsewhere[1-4] suggest that convulsant potency and binding affinity are dependent on the size of the alkyl group at the β-position. In the one example studied here, the presence of alkyl substituents at the γ-position, in addition to β-ethyl and β-methyl substituents, also appears to increase convulsant potency.

With a GC method, we also have determined the brain concentrations of compounds **2f**,**12f**, and **10c**. These studies show that the compounds rapidly enter the central nervous system (CNS) following peripheral administration (within 5 min of an ip injection). The concentrations observed in brain closely approximated the IC_{50} values for these compounds in the [^{35}S]TBPS binding assay. Similar concentrations of these drugs have also been shown to modulate GABA currents in electrophysiological experiments.[8,10] Therefore, these lactone derivatives readily enter the CNS following ip injection of an ED_{50} or CD_{50} dose and reach concentrations in brain which are very similar to the concentrations required to demonstrate effects of these compounds on the GABA receptor complex in vitro.

In conclusion, structure–activity studies involving α-alkyl-substituted γ-butyro- and γ-thiobutyrolactones indicate that maximal anticonvulsant activity and binding affinity results when three or four carbon atoms are present at the α-position. For the convulsant compounds, there appears to be a similar dependence on the size of the alkyl chain at the β-position. The carbons can be present either as a single branched substituent or as two *n*-alkyl chains. Methyl groups at the γ-position, in addition to β-substituents, appear to increase convulsant potency and binding affinity. The addition of γ-methyl groups to an α-substituted lactone resulted in convulsant activity. Sulfur for oxygen heteroatom substitution generally resulted in improved anticonvulsant potency, spectrum of activity, and binding affinity. Additionally, in the α-substituted thiolactone series, a strong correlation was observed between the ED_{50} doses (mM/kg) for compounds **12b–h** and the corresponding IC_{50} values in the binding experiments.

The compounds described here are the first molecules shown to be capable of modulating GABA-mediated currents in opposing ways via interactions at the picrotoxin receptor site. Representative compounds have been described as picrotoxin agonists (**10c**), antagonists (**2f**), and inverse agonists (**12f**).[8] The α-alkyl-substituted γ-butyro- and γ-thiobutyrolactones represent a novel class of compounds with potential therapeutic utility as anticonvulsant drugs. In fact, Ferrendelli et al.[29] have recently demonstrated that the potency and spectrum of anticonvulsant activity of **12f** compares favorably with those of valproate and ethosuximide, two very widely used anticonvulsant drugs. Finally, the compounds reported here are also invaluable tools with which to further study the complex allosteric interactions occurring between receptor sites on the GABA receptor complex.

Experimental Section

A. Materials. α-Methyl-γ-butyrolactone, γ-butyrolactone, and other reagents used in syntheses were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification unless otherwise indicated. Reagent-grade and HPLC-grade solvents were obtained from Baxter Healthcare Corp.

(24) Ramanjaneyulu, R.; Ticku, M. *Eur. J. Pharmacol.* 1984, *98*, 337.
(25) Enders, A.; Vigelius, W. D.; van Wessem, G. C. *Arzneim. Forsch.* **1960**, *10*, 243.
(26) Enders, A. *Arch. Int. Pharmacodyn. Ther.* **1960**, *127*, 285.
(27) Enders, A.; Vigelius, W. D.; van Wessem, G. C. *Naturwissenschaften* **1960**, *47*, 84.
(28) Canney, D. J.; Covey, D. F. unpublished observations.
(29) Ferrendelli, J. A.; Holland, K. D.; McKeon, A. C.; Covey, D. F. *Epilepsia* **1989**, *30*, 617.

(Muskegon, MI). Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories (Woburn, MA). Tetrahydrofuran (THF) was dried by distillation from sodium benzophenone ketyl. Benzene was distilled from sodium and stored over 4A molecular sieves. Thin-layer chromatography (TLC) was performed on Analtech (Newark, DE) precoated (250 mm) silica gel GF plates, and the spots were detected with I_2 vapor and/or UV light. Dry column-grade silica gel obtained from Universal Scientific (Atlanta, GA) was used for dry-column chromatography (DCC). Compounds **2f**, **10c**, and **13** used as the internal standards in the GC determinations were synthesized as described in the synthetic portion of this work and exhibited satisfactory spectroscopic data. IR spectra were obtained (neat) with a Perkin-Elmer 1710 FT-IR spectrophotometer. ^1H and ^{13}C spectra were obtained on a Varian XL-300 multinuclear spectrometer equipped with a 5-mm probe. Samples were dissolved in $CDCl_3$, and chemical shifts are reported as δ values with the chloroform resonance as the internal reference (δ = 7.24). The multiplicity is defined by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The relative peak heights of the resonances are reported as integers after the multiplicity. UV spectra were recorded in 95% EtOH on a Beckman DU-8 spectrophotometer. Microanalyses were carried out by Micro-Analysis, Inc. (Wilmington, DE) and Galbraith Labs, Inc. (Knoxville, TN). High-resolution mass spectroscopic (HRMS) data were recorded with a VGZAB-SE double-focusing mass spectrometer. Accurate mass assignments were made by manual peak matching against appropriate reference ions in the spectrum of perfluorokerosene. A Waters Model M6000A liquid chromatograph equipped with an Econosil silica (5 μm) cartridge (Alltech/Applied Science) was used for analytical HPLC separations. Catalytic hydrogenations were carried out on a Parr hydrogenation apparatus. Ozonolysis reactions were carried out with a Model T-408 Welsbach Ozonator (Philadelphia, PA). Bulb-to-bulb distillations were performed using an Aldrich Kugelrohr apparatus. Brain concentration analyses were performed with a Hewlett-Packard 5890A GC on an Ultra 1 capillary column (0.2 mm i.d., 0.11 μm film thickness, 25 m length). A Varian Model 3700 gas chromatograph equipped with a glass column packed with 1% SP2401 on 80/100 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) was utilized to monitor the progress of reactions and assess the purity of products.

B. Chemistry. α-Isopropyl-γ-butyrolactone (2c). As described by Minami et al.,[12] the anion of α-(*O,O*-diethylphosphono)-γ-butyrolactone[11] was prepared by treating 24.6 g (111 mmol) of **3** with 2.9 g (120 mmol) of NaH in dry benzene (200 mL) at 50–60 °C. Reaction over 4 h with dry acetone (15 mL) at 50–60 °C yielded a gummy precipitate. The reaction was stopped, the organic layer was decanted off, and the gummy residue was taken up in 0.6 N HCl (750 mL). The aqueous layer was extracted with 2 portions (each 250 mL of benzene, and the combined organic extract was washed with water (250 mL) followed by saturated NaCl solution (250 mL). After drying over anhydrous Na_2SO_4, the solvent was removed by evaporation under reduced pressure to yield crude α-isopropylidene-γ-butyrolactone (**4**) as a pale yellow oil. Catalytic hydrogenation at 45 psi in the presence of 10% Pd/C in ethanol, followed by vacuum distillation, yielded 10.2 g (72%) of **2c** as a colorless liquid: bp 86–88 °C (3.2 mmHg); ^1H NMR and IR spectra were found to be identical with those reported.[30]

General Procedure for Alkylation Reactions. The reactions were performed in oven-dried glassware under a nitrogen atmosphere. Diisopropylamine was refluxed over calcium hydride and then distilled under a nitrogen atmosphere prior to use. A three-neck, 500-mL round-bottomed flask was fitted with a magnetic stir bar, an addition funnel, and a rubber septum. The flask was charged with dry THF (200 mL) followed by diisopropylamine (21.2 mL, 151 mmol). After cooling the solution to 0 °C, *n*-butyllithium (60.4 mL of a 2.5 M solution, 151 mmol) was added and the reaction mixture was stirred for 30 min. The solution was then chilled to −78 °C and subsequently treated with the appropriately substituted ester or γ-lactone (151 mmol), dissolved in dry THF (10 mL). After 30 min, 1.3 equiv of the appropriate alkyl halide dissolved in 0.5 equiv of hexamethylphosphoramide (HMPA) was added from the dropping funnel. When addition was complete, stirring was maintained at −78 °C for 4 h. The reaction was then allowed to warm to room temperature and stirred overnight. The following morning, the mixture was quenched by careful addition of 10% HCl (100 mL) followed by dropwise addition of concentrated HCl until acidic (pH = 1–2). The phases were separated and the aqueous layer was extracted with 3 portions (100 mL) of hexanes. The combined organic extract was washed with water, 5% $NaHCO_3$, and saturated NaCl solution (50-mL portions of each). The organic solution was dried over anhydrous $MgSO_4$, and concentrated in vacuo to yield the desired crude products as slightly yellow oils. Purification of individual compounds are reported below with yields and spectroscopic data.

Ethyl 2-(1-Methylethyl)-4-pentenoate (6a). The oil recovered from the reaction was twice vacuum distilled to yield pure **6a** (82.5%) as a colorless oil: bp 88–90 °C (31 mmHg); ^1H NMR δ 5.65–5.80 (m, 1, =CH), 5.05–4.94 (m, 2, $=CH_2$), 4.10 (q, 2, *J* = 7 Hz, OCH_2), 2.35–2.12 (m, 3, $=CHCH_2$ and CHC=O), 1.91–1.78 (m, 1, $CH(CH_3)_2$), 1.22 (t, 3, *J* = 7 Hz, OCH_2CH_3), 0.92 (d, 3, *J* = 7 Hz, $CHCH_3$), 0.89 (d, 3, *J* = 7 Hz, $CHCH_3$); IR 3080 ($=CH_2$), 1734 (C=O), 1643 (C=C) cm^{-1}. Spectroscopic data are consistent with literature values.[31]

Methyl 2-(1,1-Dimethylethyl)-4-pentenoate (6b). The oil recovered from the reaction was vacuum distilled to yield the product (78%) as a colorless oil. A second distillation yielded an analytical sample: bp 84–85 °C (28 mmHg); ^1H NMR δ 5.73–5.6 (m, 1, =CH) 5.03–4.91 (m, 2, CH_2), 3.60 (s, 3, OCH_3), 2.34–2.16 (m, 3, $=CHCH_2$ and CHC=O), 0.93 (s, 9, $(CH_3)_3$); IR 3080 ($=CH_2$), 1737 (C=O), 1643 (C=C) cm^{-1}. Anal. ($C_{10}H_{18}O_2$) C, H.

Methyl 2-Ethyl-2-methyl-4-pentenoate (6c). The product was vacuum distilled to yield **6c** (85%) as a colorless oil. A second distillation yielded an analytically pure sample: bp 84–86 °C (41 mmHg); ^1H NMR δ 5.71–5.59 (m, 1, =CH), 5.01–4.98 (m, 2, $=CH_2$), 3.62 (s, 3, OCH_3), 2.38–2.09 (m, 2, $=CHCH_2$), 1.71–1.40 (m, 2, CH_2CH_3), 1.07 (s, 3, CH_3), 0.78 (t, 3, *J* = 7 Hz, CH_2CH_3); IR 3078 ($=CH_2$), 1735 (C=O), 1641 (C=C) cm^{-1}. Anal. ($C_9H_{16}O_2$) C, H.

Methyl 2,2-Diethyl-4-pentenoate (6d). Following workup as described above, the product was twice distilled to yield **6d** (75%) as a colorless oil: bp 108 °C (57 mmHg); ^1H NMR δ 5.66–5.55 (m, 1, =CH), 5.04–4.97 (m, 2, $=CH_2$), 3.62 (s, 3, OCH_3), 2.28 (d, 2, *J* = 8 Hz, $=CHCH_2$), 1.55 (q, 4, *J* = 8 Hz, CH_2CH_3), 0.71 (t, 6, *J* = 8 Hz, CH_2CH_3); IR 3078 ($=CH_2$), 1732 (C=O), 1641 (C=C) cm^{-1}. Anal. ($C_{10}H_{18}O_2$) C, H.

Methyl 2,4-Dimethyl-2-ethyl-4-pentenoate (6e). Chromatography on silica gel using 20% ethyl acetate in hexane as eluent followed by vacuum distillation yielded **6e** (76%) as a colorless oil: bp 71–73 °C (15 mmHg); ^1H NMR δ 4.81–4.77 (m, 1, =CH), 4.63 (br s, 1, =CH), 3.66 (s, 3, CH_3), 2.49 (d, 1, *J* = 14 Hz, $=CCH_2$), 2.09 (d, 1, *J* = 14 Hz, $=CCH_2$), 1.80–1.65 (m, 1, CH_2CH_3), 1.64 (s, 3, $=CCH_3$), 1.44–1.30 (m, 1, CH_2CH_3), 1.09 (s, 3, CH_3), 0.82 (t, 3, *J* = 7 Hz, CH_2CH_3); IR 3076 (=CH), 1734 (C=O), 1647 (C=C) cm^{-1}; HRMS *m/z* 170.13091 $C_{10}H_{18}O_2$ requires 170.13068.

α-Ethyl-γ-butyrolactone (2b). γ-Butyrolactone was alkylated with ethyl iodide. Chromatography on silica gel using 20% ethyl acetate in hexane as eluent followed by vacuum distillation yielded **2b** (12%) as a colorless oil: bp 84–86 °C (4.0 mmHg). Spectroscopic data obtained for this compound were in excellent agreement with reported values.[32]

α,α-Dimethyl-γ-butyrolactone (2e). α-Methyl-γ-butyrolactone was alkylated with methyl iodide. Chromatography on silica gel using 20% ethyl acetate as eluent followed by vacuum distillation yielded **2e** (47%) as a colorless oil: bp 64–66 °C (3.2 mmHg). Spectroscopic data were found to be in excellent agreement with reported values.[30,33]

α-Ethyl-α-methyl-γ-butyrolactone (2f). α-Methyl-γ-butyrolactone was alkylated with ethyl iodide. The product was twice distilled to yield pure **2f** (75%) as a colorless oil: bp 75–76

(30) Ishii, Y.; Osakada, K.; Ikariya, T.; Saburi, M.; Yoskikawa, S. *J. Org. Chem.* **1986**, *51*, 2034.

(31) Randad, R. S.; Kulkarni, G. H. *Synth. Commun.* **1985**, *15*, 311.

(32) Zenk, P. C.; Wiley, R. A. *Synthesis* **1984**, 695.

(33) Doyle, M. P.; Dow, R. L. *Synth. Commun.* **1980**, *10*, 881.

°C (2.5 mmHg). Spectroscopic data obtained for **2f** were in excellent agreement with reported values.[33]

α-Isopropyl-α-methyl-γ-butyrolactone (2h). α-Methyl-γ-butyrolactone was alkylated with isopropyl iodide. The product was vacuum distilled to yield **2h** (69%) as a colorless oil: bp 65–73 °C (0.2 mmHg); ^{1}H NMR 4.22–4.17 (m, 2, γ-CH_2), 2.24–2.17 (m, 2, β-CH_2), 1.94–1.76 (m, 1, C*H*(CH_3)$_2$), 1.19 (s, 3, CH_3), 0.91 (d, 3, J = 7 Hz, CH(C*H*$_3$)$_2$), 0.88 (d, 3, J = 7 Hz, CH(C*H*$_3$)$_2$); IR 1767 (C=O) cm^{-1}. Anal. ($C_8H_{14}O_2$) C, H.

α,α-Diisopropyl-γ-butyrolactone (2i). α-Isopropyl-γ-butyrolactone was alkylated with isopropyl iodide. Purification by HPLC on silica gel (10 mm × 25 cm) using 5% acetone in hexane as eluent (6 mL/min) followed by bulb-to-bulb distillation [pot temperature, 55 °C (3 mm Hg)] yielded **2i** (13%) as a colorless liquid: ^{1}H NMR δ 4.20 (t, 2, J = 8 Hz, γ-CH_2), 2.13–2.03 (m, 4, β-CH_2 and C*H*(CH_3)$_2$), 0.99 (d, 6, J = 7 Hz, CH(C*H*$_3$)$_2$), 0.94 (d, 6, J = 7 Hz, CH(C*H*$_3$)$_2$); IR 1764 (C=O) cm^{-1}. Anal. ($C_{10}H_{18}O_2$) C, H.

α,α-Diisopropyl-γ-thiobutyrolactone (12i). α-Isopropyl-γ-thiobutyrolactone was alkylated with isopropyl iodide. Fractional vacuum distillation yielded the product (21%) as a colorless oil: bp 86 °C (0.3 mmHg); ^{1}H NMR δ 3.19 (t, 2, J = 7 Hz, γ-CH_2), 2.18 (t, 2, J = 7 Hz, β-CH_2), 2.08–1.99 (m, 2, C*H*(CH_3)$_2$), 0.93 (d, 6, J = 7 Hz, CH(C*H*$_3$)$_2$), 0.91 (d, 6, J = 7 Hz, CH(C*H*$_3$)$_2$); IR 1691 (C=O) cm^{-1}; UV λ_{max} 237 (log ε 3.60). Anal. ($C_{10}H_{18}OS$) C, H, S.

Preparation of α-Substituted γ-Butyrolactones. Those α-substituted-γ-butyrolactones which were not readily prepared by the direct alkylation method used above were synthesized as previously described for compounds **2d** and **8**.[13] The oils recovered from these reactions were vacuum distilled. Any further purification measures that were necessary for individual compounds are reported below with their yields and spectroscopic data.

α-Isopropyl-γ-butyrolactone (2c). The spectroscopic data obtained for **2c** when prepared by this route were identical with the data reported previously for this compound.[30]

α-Ethyl-α-methyl-γ-butyrolactone (2f). Vacuum distillation of the oil recovered from the reaction yielded **2f** (65%) as a colorless oil: bp 75–76 °C (2.5 mmHg). The spectroscopic data obtained for this compound, as prepared by this route, were identical with previously reported values.[33]

α,α-Diethyl-γ-butyrolactone (2g). The product was vacuum distilled to yield pure **2g** (72%) as a colorless oil: bp 99–101 °C (3.0 mmHg); ^{1}H NMR δ 4.19 (t, 2, J = 8 Hz, γ-CH_2), 2.08 (t, 2, J = 8 Hz, β-CH_2), 1.58 (q, 4, J = 7 Hz, C*H*$_2$CH$_3$), 0.88 (t, 6, J = 7 Hz, CH_2C*H*$_3$); IR 1764 (C=O) cm^{-1}. Anal. ($C_8H_{14}O_2$) C, H.

Preparation of β-Substituted γ-Butyrolactones. The β-substituted lactone derivative were synthesized as previously described for compounds **10b** and **11**.[13] The oils recovered from these reactions were vacuum distilled. Any further purification measures that were necessary for individual compounds are reported below with their yields and spectroscopic data.

β-Isopropyl-γ-butyrolactone (10a). The oil recovered from the reaction was vacuum distilled to yield a low-boiling fraction [bp 64–90 °C (0.4 mmHg)], containing unoxidized aldehyde, and a high-boiling fraction [bp 105–115 °C (0.4 mmHg)] containing the carboxy ester. The fraction containing the half-ester was treated as previously described[13] and vacuum distilled to yield **10a** (37%) as a colorless oil: bp 69–72 °C (0.3 mmHg). Spectroscopic data obtained for this compound were in good agreement with reported values.[30]

β-Ethyl-β-methyl-γ-butyrolactone (10c). The product was chromatographed on silica gel using 15% ethyl acetate in hexane as eluent followed by vacuum distillation to yield pure **10c** (62%) as a colorless oil: bp 78–80 °C (3 mmHg). Spectroscopic data obtained for **10c** were in agreement with previously reported values.[33,34]

General Procedure for the Preparation of γ-Thiobutyrolactones. The thiobutyrolactones, with the exception of compound **12i**, were synthesized as described previously for α-substituted derivatives.[4] Briefly, the appropriate γ-butyrolactone (69.4 mmol) was treated with potassium thioacetate (109 mmol) in *N,N*-dimethylacetamide (50 mL) at 150–160 °C for 4 h for the α-substituted derivatives. The β-substituted compound was also prepared by this method with a reaction time of 24 h. The reaction mixture was partitioned between hexane (200 mL) and water (200 mL) and the aqueous layer further extracted with hexane. The combined organic layer was washed (H_2O and saturated NaCl) and dried over Na_2SO_4, and the solvent evaporated under reduced pressure to yield the crude products as red oils.

α-Methyl-γ-thiobutyrolactone (12a).[35] The product was twice vacuum distilled to yield pure compound **12a** (44%) as a pale yellow oil: bp 71–73 °C (4.0 mmHg); ^{1}H NMR δ 3.34–3.26 (m, 2, γ-CH_2), 2.58–2.42 (m, 2, β-CH_2), 1.96–1.82 (m, 1, α-CH), 1.19 (d, 3, J = 7 Hz, CH_3); IR 1692 (C=O) cm^{-1}; HRMS m/z 116.0266 C_5H_8OS requires 116.0296. Anal. (C_5H_8OS) C, H.

α-Ethyl-γ-thiobutyrolactone (12b).[36] The product was vacuum distilled to yield pure **12b** (75%) as a pale yellow oil: bp 85–87 °C (3.6 mmHg); ^{1}H NMR δ 3.34–3.28 (m, 2, γ-CH_2), 2.55–2.34 (m, 2, β-CH_2), 2.02–1.81 (m, 2, C*H*$_2$CH$_3$), 1.52–1.37 (m, 1, α-CH) 0.99 (t, 3, J = 7.5 Hz, CH_2C*H*$_3$); IR 1692 (C=O) cm^{-1}. Anal. ($C_6H_{10}OS$) C, H, S.

α-Isopropyl-γ-thiobutyrolactone (12c). The product was vacuum distilled to yield compound **12c** (83%) as a pale yellow oil: bp 62–64 °C (0.3–0.4 mmHg); ^{1}H NMR δ 3.26–3.22 (m, 2, γ-CH_2), 2.42–1.99 (m, 4, α-CH, β-CH_2, and C*H*(CH_3)$_2$), 1.0 (d, 3, J = 8 Hz, CH(C*H*$_3$)$_2$), 0.84 (d, 3, J = 8 Hz, CH(C*H*$_3$)$_2$); IR 1692 (C=O) cm^{-1}; UV λ_{max} 236 nm (log ε 3.51). Anal. ($C_7H_{12}OS$) C, H, S.

α-*tert*-Butyl-γ-thiobutyrolactone (12d). Chromatography on silica gel using 5% ethyl acetate in hexane as eluent followed by double vacuum distillation yielded pure **12d** (60%) as a pale yellow oil: bp 69–71 °C (0.3 mmHg); ^{1}H NMR δ 3.18–3.13 (m, 2, γ-CH_2), 2.39–2.01 (m, 3, α-CH and β-CH_2), 0.99 (s, 9, C(CH_3)$_3$); IR 1694 (C=O) cm^{-1}; UV λ_{max} 236 nm (log ε 3.54). Anal. ($C_8H_{14}OS$) C, H, S.

α,α-Dimethyl-γ-thiobutyrolactone (12e). The product was twice distilled to yield compound **12e** (69%) as a pale yellow oil: bp 70–71 °C (4.0 mmHg). Spectroscopic data for this compound were in good agreement with literature values.[37]

α-Ethyl-α-methyl-γ-thiobutyrolactone (12f). The product was chromatographed on silica gel using 10% ethyl acetate in hexane as eluent and distilled to yield pure **12f** (81%) as a colorless oil: bp 55–56 °C (0.4 mmHg). Spectroscopic data were in excellent agreement with previously reported values.[4]

α,α-Diethyl-γ-thiobutyrolactone (12g). Chromatography on silica gel using 2.5% ethyl acetate in hexane as eluant and vacuum distillation yielded pure **12g** (60%) as a pale yellow oil: bp 76–78 °C (0.5 mmHg); ^{1}H NMR δ 3.20 (t, 2, J = 7 Hz, γ-CH_2), 2.11 (t, 2, J = 7 Hz, β-CH_2), 1.62–1.45 (m, 4, C*H*$_2$CH$_3$), 0.85 (t, 6, J = 7 Hz, CH_2C*H*$_3$); IR 1691 (C=O) cm^{-1}; UV λ_{max} 235 nm (log ε 3.60). Anal. ($C_8H_{14}OS$) C, H, S.

α-Isopropyl-α-methyl-γ-thiobutyrolactone (12h). The product was chromatographed on silica gel using 2.5% ethyl acetate in hexane as eluent and vacuum distilled to yield compound **12h** (74%) as a pale yellow oil: bp 67 °C (0.4 mmHg); ^{1}H NMR δ 3.23–3.18 (m, 2, γ-CH_2), 2.31–2.24 (m, 2, β-CH_2), 1.99 (m, 1, C*H*(CH_3)$_2$, 1.09 (s, 3, CH_3), 0.90 (d, 3, J = 7 Hz, CH(C*H*$_3$)$_2$), 0.87 (d, 3, J = 7 Hz, CH(C*H*$_3$)$_2$); IR 1695 (C=O) cm^{-1}; UV λ_{max} 235 nm (log ε 3.64). Anal. ($C_8H_{14}OS$) C, H, S.

β-Ethyl-β-methyl-γ-thiobutyrolactone (13). The product was chromatographed on silica gel using 10% ethyl acetate in hexane as eluent followed by vacuum distillation to yield pure **13** (62%) as a colorless oil: bp 70–72 °C (1.5 mmHg). Spectroscopic data were found to be identical with literature values.[4]

C. Quantitation of Lactones in Brain Tissue Using Gas Chromatography. The appropriate dose of test compound was injected ip into female CF-1 mice. The animals were sacrificed and decapitated at the desired time. The brain stem and cerebellum were removed by crude dissection, and the remaining brain tissue (cerebral cortex) was immediately frozen on dry ice and

(34) Tomioka, K.; Cho, Y.-S.; Sato, F.; Koga, K. *J. Org. Chem.* **1988**, *53*, 4094.

(35) Gorski, R. A.; Wolber, G. J.; Wemple, J. *Tetrahedron Lett.* **1976**, 2577.

(36) Aboul-Enein, H. Y.; Al-Badr, A. A.; Ibrahim, S. E., Ismail, M. *Pharm. Acta Helv.* **1980**, *55*, 228.

(37) Baas, J. L.; Davies-Fidder, A.; Huisman, H. O. *Tetrahedron* **1966**, *22*, 285.

weighed. To avoid loss of extraction solvents and/or test compounds due to evaporation, further sample preparation was performed in a cold room at 4 °C. The brains were manually homogenized with 10 volumes (by tissue weight) of extraction solvent. The solution used for the extractions was a 2:1 mixture of chloroform and methanol and contained **10c** (50 μM), **13** (29 μM), or **2f** (12.5 μM) as internal standards for **2f**, **12f**, and **10c**, respectively. The homogenate was centrifuged for 30 min at 1500*g* and the lower phase recovered and stored at 4 °C prior to GC analysis. For the time-course experiments, doses of **2f** (275 mg/kg), **12f** (128 mg/kg), or **10c** (25 mg/kg) were administered and the animals (n = 3) were sacrificed at the times stated in Table V. Analyses were performed with a Hewlett-Packard 5890A GC on an Ultra 1 capillary column (0.2 mm i.d., 0.11 μm film thickness, 25 m length). Helium was used as the carrier gas (1 mL/min) and splitless injection was employed. Operating temperatures were as follows: injection port, 150 °C; detector, 280 °C; oven temperature program, 80–120 °C at 5 °C/min for compounds **2f** and **10c** or 105–180 °C at 5 °C/min for compound **12f**. The detector response ratios for the internal standards and the test compounds were validated for linearity and brain concentrations were determined from the ratio of the integration (HP 3393A integrator) of the flame-ionization signal of the test compound and the internal standard with subsequent correction for detector response and tissue wet weight (specific gravity assumed to be 1.0 g/mL).

D. Pharmacology. Materials. Picrotoxinin, PTZ, and polyethylene glycol-400 (PEG) were purchased from Sigma Chemical Co. (St. Louis, MO); both unlabeled and ^{35}S-labeled TBPS were purchased from New England Nuclear (Boston, MA).

Neurological Effects. Female CF-1 strain mice (Harlan, 6–8 weeks old) were maintained on a 12:12 light–dark cycle with food and water available ad lib. Drug screening was accomplished by methods based on those of Swinyard and Woodhead.[14] Test compounds were dissolved in 30% polyethylene glycol and given by intraperitoneal (ip) injections in a volume of 0.01 mL/g of body weight. Following the injection of the test compound, the mice were observed for 30 min and any seizure activity was noted. Compounds that did not induce seizures were tested for anticonvulsant activity by examining their ability to block seizures caused by PTZ or maximal electroshock (MES).

Maximal electroshock seizures were induced by a 60-Hz alternating current of 50 mA delivered via corneal electrodes for 0.2 s with an electroshock stimulator (Wahlquist Instrument Co., Salt Lake City, UT). Sodium chloride (0.9%) drops were placed on the animals' eyes before application of the corneal electrodes. Seizure protection was defined as the failure to demonstrate tonic hind limb extension past 90°. Pentylenetetrazol (85 mg/kg) was administered as a 0.85% solution in 0.9% NaCl and the mice were observed for 30 min for seizure activity. Protection was defined as the absence of clonic seizures.

Neurotoxic effects were assessed with the rotorod toxicity test.[15] In this test the mouse was placed on a 1 in. diameter rod rotating at 6 rpm. The compound was considered to cause toxicity if it caused the mouse to fall from the rotating rod twice during the 10-min testing period. The ED_{50} and TD_{50} values were determined by log probit analysis of the dose–response data.[38]

[^{35}S]TBPS Binding. [^{35}S]TBPS binding was performed according to previously described methods.[5,16] The cerebral cortex from female Sprague–Dawley rats (250–300 g) was removed over ice immediately following decapitation. The brains were homogenized in 20 mL of ice-cold 0.32 M sucrose/g of tissue and centrifuged at 1000*g* for 10 min. The supernatant was carefully decanted and centrifuged at 150000*g* for 30 min. The resulting pellet was resuspended in 20 vol of ice cold deionized water and centrifuged at 150000*g* for 30 min. The pellet was resuspended in 20 vol of 50 mM Tris–citrate buffer (pH 7.5) and centrifuged at 50000*g* for 30 min. The resulting pellet was resuspended in 20 vol of 50 mM Tris–citrate buffer. The membrane suspensions were stored at −70 °C and were thawed and resuspended in 50 mM Tris-citrate buffer immediately prior to use. The protein concentration was determined according to the Lowry method.[39]

For binding assays, 100 μL of rat brain membranes was added to a solution containing 50 μL of [^{35}S]TBPS (specific activity 60–85 Ci/mmol) in 1 M NaBr and 50 μL of the test compound dissolved in 50 mM Tris–citrate buffer. The final [^{35}S]TBPS concentration was 2 nM. The samples were incubated in triplicate for 90 min at 25 °C, diluted with 0.9% NaCl (3 mL), rapidly filtered through Whatman GF/B filters, and washed twice with 0.9% NaCl (3 mL). Filter-bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined as that observed in the presence of 10 μM unlabeled TBPS or 100 μM unlabeled picrotoxinin.

The determination of the drug concentration that displaced 50% of specifically bound [^{35}S]TBPS and curve fitting of sigmoidal concentration–displacement curves were accomplished by log probit analysis using at least five different drug concentrations.[38] The IC_{50} values determined for PTZ and picrotoxinin were 759 ± 53 μM (n = 3) and 310 ± 40 nM (n = 3), respectively. Squires et al.[17] previously reported IC_{50} values of 560 ± 49 μM and 190 ± 21 nM for PTZ and picrotoxinin, respectively.

Acknowledgment. We thank Hwang-Fun Lu for her assistance with portions of the synthetic work described herein. This work was supported by NIH grants NS07129, NS14834, and GM07805. Assistance was also provided by the Washington University High Resolution NMR (NIH 1 S10 RR00204 and a gift from the Monsanto Co.) and Mass Spectrometry Service Facilities (NIH RR00954).

Registry No. 1, 96-48-0; **2a**, 1679-47-6; **2b**, 13888-01-2; **2c**, 1608-63-5; **2d**, 131424-13-0; **2e**, 3709-08-8; **2f**, 31004-76-9; **2g**, 50994-87-1; **2h**, 132462-11-4; **2i**, 132462-12-5; **3**, 2907-85-9; **4**, 24186-31-0; **6a**, 21244-42-8; **6b**, 131424-10-7; **6c**, 131424-09-4; **6d**, 42997-97-7; **6e**, 131424-11-8; **8**, 131424-14-1; **10a**, 10547-88-3; **10b**, 22530-95-6; **10c**, 50598-34-0; **11**, 131424-12-9; **12a**, 1679-47-6; **12b**, 76780-78-4; **12c**, 111605-31-3; **12d**, 132462-13-6; **12e**, 4951-37-5; **12f**, 103620-92-4; **12g**, 132462-14-7; **12h**, 132462-15-8; **12i**, 111605-32-4; **13**, 103620-94-6.

(38) Finney, D. J. *Probit Analysis*, 3rd ed.; Cambridge University Press: London, 1971.
(39) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.