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Structure-Activity Relationships of Alkyl-Substituted γ-Butyrolactones and Succinimides

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SUMMARY

Derivatives of γ-butyrolactone (GBL) substituted on both the α- and the β-position were synthesized and tested for their effects on behavior in mice, on the electroencephalograph (EEG) and blood pressure of paralyzed-ventilated guinea pigs, and on the electrical activity of incubated hippocampal slices. Several compounds, including α,α,β,β-tetramethyl GBL (TMGBL), α-hydroxy-β,β-dimethyl GBL, *endo*-bicyclo[2.2.1]hept- and *endo*-bicyclo[2.2.2]oct-5-ene-2-hydroxymethyl-3-carboxylic acid lactone, produced convulsive seizures in mice and epileptiform EEG discharges in guinea pigs identical with those produced by β-ethyl-β-methyl GBL. Neuronal activity in hippocampal slices was also markedly activated by TMGBL. Seizures and epileptiform discharges produced by these α,β-substituted GBLs were prevented by α-ethyl-α-methyl GBL and ethosuximide but not by phenytoin. A succinimide with the same alkyl substitutions as TMGBL, α,α,α′,α′-tetramethylsuccinimide (TMSM), had convulsant properties and a response to anticonvulsant drugs identical with those of TMGBL. A model for the hypothetical site of action of alkyl-substituted GBLs and succinimides explaining the structure-activity relationships is presented. We propose that the absolute requirements for activity of the compounds are (a) a carbonyl oxygen atom on a heterocyclic ring adjacent to an oxygen or a nitrogen atom with an ionizable hydrogen atom and (b) suitable alkyl substituents occupying at least one of the positions α, β, or γ to the carbonyl. Alkyl substitution at the α- and/or γ-position, but not at the β-position, results in a compound which is anticonvulsant. Alkyl substitution at the β-position produces compounds which are convulsant. Thus, it is the presence or absence of alkyl substituents at the β-position that dictates whether a GBL or succinimide will be convulsant or anticonvulsant. Possibly alkyl-substituted GBLs and succinimides act at γ-aminobutyric acid-regulated chloride channels, as does picrotoxinin, which has a β-alkyl-substituted GBL moiety that is essential for its activity.

INTRODUCTION

In preceding studies (1, 2) we examined GBLs[2] substituted with alkyl groups at the α-, β-, or the γ-position. The γ-substituted GBLs were largely inactive. The β-substituted compounds proved to be potent convulsants, producing seizures very distinct from those produced by unsubstituted GBL. The α-substituted compounds possessed substantial anticonvulsant activity very similar to the anti-absence (petit mal) antiepileptic drugs. In addition, the α-substituted GBLs prevented the seizures induced by the β-substituted GBLs. This intriguing difference in activity between two such chemically similar agents prompted a more detailed study of the structure-activity relationships of this class of compounds.

In order to elucidate these structure-activity relationships, it was necessary to determine the activity of α,β-substituted GBLs. In an effort to provide a direct comparison with previously characterized α- and β-compounds and to avoid complications caused by optically active isomers, we synthesized and tested TMGBL (see Fig. 1). We also compared the effects of hydrophobic versus hydrophilic substitutions at the α-position. In addition, since the α-substituted GBLs had activities and potencies so similar to those of the α-substituted succin-

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[2] The abbreviations used are: GBL, γ-butyrolactone; TMGBL, α,α,β,β-tetramethylethyl-γ-butyrolactone; TMSM, α,α,α′,α′-tetramethylsuccinimide; ESM, ethosuximide; PHT, phenytoin; β-DMGBL, β,β-dimethyl-γ-butyrolactone; β-EMGBL, β-ethyl-β-methyl-γ-butyrolactone; α-DMGBL, α,α-dimethyl-γ-butyrolactone; α-EMGBL, α-ethyl-α-methyl-γ-butyrolactone; PMSM, pentamethylsuccinimide; α-OH-β-DMGBL, α-hydroxy-β,β-dimethyl-γ-butyrolactone; NHCL, *endo*-bicyclo[2.2.1]hept-5-ene-2-hydroxymethyl-3-carboxylic acid lactone; OHCL, *endo*-bicyclo[2.2.2]oct-5-ene-2-hydroxymethyl-3-carboxylic acid lactone; EEG, electroencephalograph(ic); GABA, γ-aminobutyric acid.

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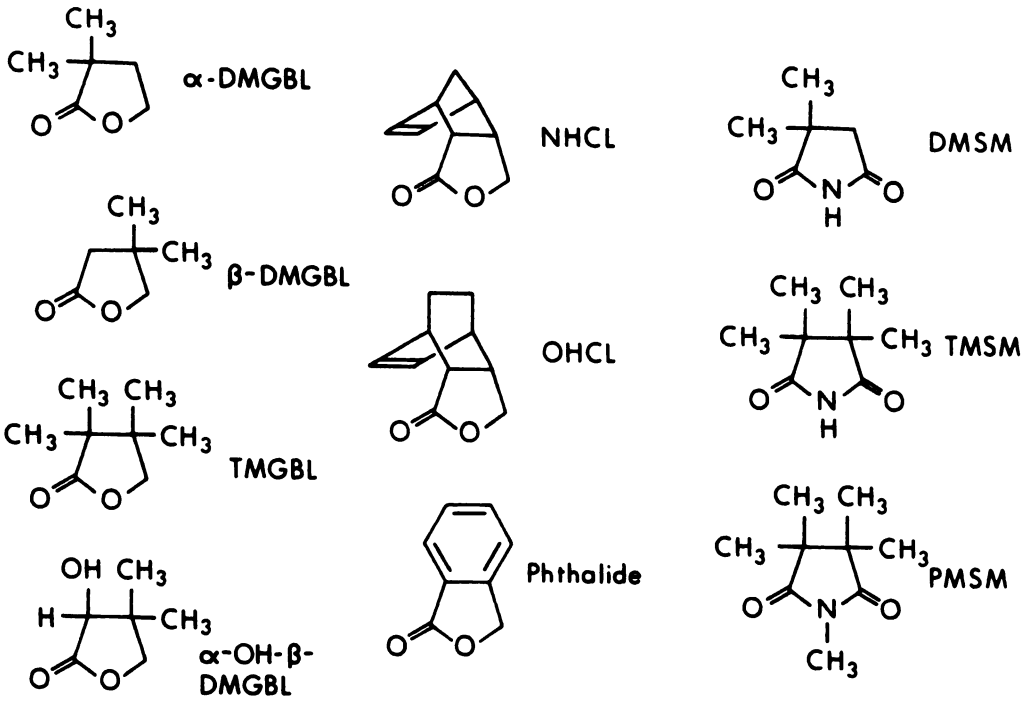

FIG. 1. *Chemical structures of some α-, β-, and α,β-substituted GBLs and α- and α,α′-substituted succinimides*

imides, it was important to compare the activity of TMSM with that of the corresponding GBL, TMGBL.

Finally, with the data presently available from all of the alkyl-substituted GBLs and succinimides, we suggest a relationship of structure to activity in these compounds. The resulting model may also apply to picrotoxinin, another convulsant drug with a β-alkyl-substituted GBL moiety which is essential for its activity.

MATERIALS AND METHODS

Drugs and Chemicals

ESM (Zarontin) and PHT (Dilantin) were obtained from Parke-Davis (Morris Plains, N. J.). DL-Pantolactone (α-hydroxy-β,β-dimethyl-γ-butyrolactone) was obtained from Chemical Procurement Laboratories (College Point, N. Y.). Phthalide was obtained from Aldrich Chemical Company (Milwaukee, Wisc.). TMSM was obtained from ICN (Cleveland, Ohio). β-DMGBL, α-EMGBL, and NHCL were prepared as previously described (1, 2).

Chemical Syntheses

General chemical methods. All chemical methods were identical with those previously reported (1).

Synthesis of TMGBL and OHCL. Tetramethylsuccinonitrile (Parish) was converted to tetramethylsuccinic anhydride by the method of Thiele and Heuser (3). The anhydride was then converted to TMGBL by the method of Bailey and Johnson (4), using $NaBH_4$. OHCL was synthesized from the corresponding anhydride (Aldrich) by the same method. Both had IR and proton magnetic resonance spectra consistent with the assigned structures.

Synthesis of PMSM. TMSM was *N*-methylated by treatment with methyl iodide in sodium methoxide for 24 hr at room temperature. The NaI was filtered off and the filtrate was concentrated and distilled. The product was a clear, colorless liquid, b.p. 100–104° (1.2 mm Hg). The IR spectra showed two strong carbonyl absorptions at 1780 and 1710 cm^{-1} and no N—H absorption. The proton NMR in $CDCl_3$ showed the following: δ 1.17 (*s*, 12, α- and α′-CH_3 *x* 4); 2.93 (*s*, 3, —N—CH_3).

Testing of Behavioral and Electrophysiological Effects

Effects on behavior in mice. Drug administration and recording of behavioral markers was carried out as previously described (2).

Effects on the EEG of paralyzed-ventilated guinea pigs. This technique has been described in detail in a preceding report (1).

Effects on incubated hippocampal slices. This technique has also been previously described (1).

RESULTS

Effects on behavior in mice. Mice receiving injections of α,β-substituted GBLs or TMSM had seizures identical with those produced by β-EMGBL and β-DMGBL. These were characterized by an initial myoclonic twitch, followed rapidly by a generalized clonic seizure. At sufficiently high doses, a tonic seizure then followed and usually resulted in death. The time course varied among the different compounds (Table 1), but increasing doses produced the same qualitative effects as did β-EMGBL; i.e., each behavioral marker occurred earlier, and a higher percentage of animals displayed that marker. The percentage of animals which displayed a tonic seizure was dose-dependent. By this criterion, the CD_{50} for TMGBL was 0.43 mmole/kg. This represented a 4-fold higher potency than that of β-DMGBL, which had a CD_{50} of 1.6

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TABLE 1

Time course and dose-response of TMGBL, α-OH-β-DMGBL, β-DMGBL, NHCL, OHCL, and TMSM

Compound	Dose		Time[a] to			
	mg/kg	mmoles/kg	First clonic seizure		Tonic seizure	
			sec			
TMGBL	25.0	0.18	—	(0/4)	—	(0/4)
	50.0	0.35	53 ± 6	(3/4)	179	(1/4)
	75.0	0.53	67 ± 24	(4/4)	147 ± 35	(3/4)
	100.0	0.70	30 ± 2	(2/2)	52 ± 12	(2/2)
	200.0	1.41	20 ± 1	(2/2)	35 ± 12	(2/2)
α-OH-β-DMGBL	250	1.92	—	(0/2)	—	(0/2)
	750	5.77	340 ± 131	(3/3)	—	(0/3)
	1000	7.69	246 ± 135	(3/3)	1275	(1/3)
	1500	11.5	85 ± 10	(3/3)	287 ± 174	(3/3)
	2000	15.4	101 ± 28	(3/3)	39 ± 126	(3/3)
β-DMGBL	100	0.88	105	(1/4)	—	(0/4)
	150	1.30	52 ± 6	(4/4)	—	(0/4)
	225	2.00	32 ± 1	(4/4)	82 ± 17	(4/4)
	500	4.40	28 ± 3	(4/4)	37 ± 8	(4/4)
NHCL	50.0	0.33	73 ± 8	(4/4)	—	(0/4)
	70.0	0.47	50 ± 4	(4/4)	179 ± 45	(4/4)
	100.0	0.67	40 ± 2	(4/4)	104 ± 11	(4/4)
OHCL	15.0	0.09	44 ± 3	(2/4)	—	(0/4)
	20.0	0.12	44 ± 3	(4/4)	—	(0/4)
	30.0	0.18	34 ± 3	(4/4)	100 ± 9	(4/4)
	50.0	0.30	29 ± 1	(2/2)	72 ± 38	(2/2)
	75.0	0.46	28 ± 1	(2/2)	71 ± 10	(2/2)
TMSM	7.0	0.04	—	(0/4)	—	(0/4)
	12.0	0.08	156 ± 29	(4/4)	—	(0/4)
	15.0	0.10	339 ± 113	(4/4)	1372 ± 313	(3/4)
	20.0	0.13	112 ± 25	(4/4)	376 ± 52	(4/4)
	25.0	0.16	81 ± 6	(4/4)	176 ± 6	(4/4)
	50.0	0.32	89 ± 5	(4/4)	129 ± 15	(4/4)
	100.0	0.64	40 ± 3	(4/4)	61 ± 10	(4/4)

[a] Times indicate the period elapsed between the injection of the compound and occurrence of the particular event. Numbers in parentheses indicate the number responding versus the number receiving injections. Values are means ± standard error of the mean.

mmoles/kg. In contrast, α-OH-β-DMGBL had a CD_{50} of 8.4 mmoles/kg and thus was 5-fold less potent than β-DMGBL.

The bicyclic compounds, NHCL and OHCL, had CD_{50} values of 0.39 and 0.15 mmoles/kg, respectively. TMSM was the most potent, with a CD_{50} of 0.09 mmoles/kg. However, the time course of seizures was much slower at threshold doses of TMSM than at threshold doses of TMGBL or the other convulsant lactones (Table 1).

Table 2 shows the effects of a α-EMGBL, ESM, and PHT on seizures induced by α,β-substituted GBLs and TMSM. As with the β-substituted derivatives (1, 2), α-EMGBL and ESM were about equally effective in preventing the tonic phase of the seizure. However, again as with the β-substituted compounds, PHT exacerbated the clonic phase of the seizure and protected none of the animals from death.

Effects on the EEG of paralyzed-ventilated guinea pigs. The epileptiform discharges produced by TMGBL (Fig. 2) were essentially identical with those produced by β-substituted GBLs (1). These discharges progressed through all of the same stages with a time course similar to that previously described in detail for β-EMGBL and β-DMGBL. In addition, TMGBL-induced discharges were prevented by pretreatment with α-EMGBL (Fig. 2).

Two other bicyclic α,β-substituted GBLs, NHCL and

TABLE 2

Effect of anticonvulsants on seizures induced by α,β-substituted GBLs and TMSM

Convulsant	Fraction protected[a]					
	α-EMGBL (mg/kg)		ESM (mg/kg)			PHT, 50 mg/kg
	250	350	250	375	500	
TMGBL (75 mg/kg)	4/4	—	4/4	—	4/4	0/4
NHCL (70 mg/kg)	—	4/4	—	4/4	—	0/4
OHCL (30 mg/kg)	—	3/4	—	4/4	—	0/4
TMSM (25 mg/kg)	6/6	—	3/3	—	2/2	0/3

[a] Fraction surviving 30 min after convulsant challenge.

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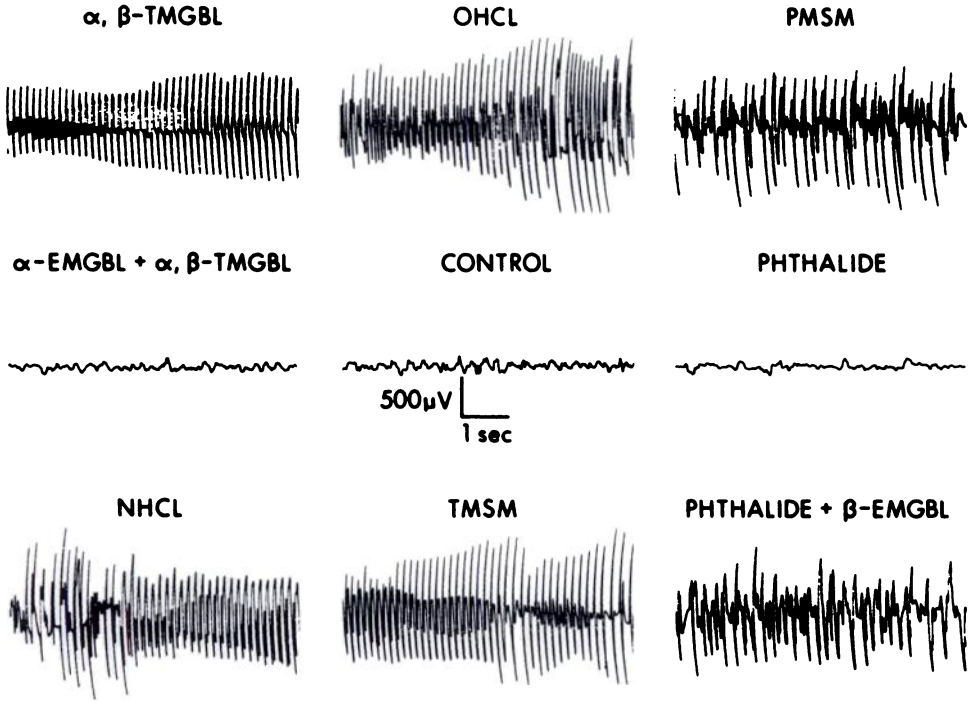

FIG. 2. *Effects of α,β-substituted GBLs and α,α-tetrasubstituted succinimides on the guinea pig EEG*

Left. *Top*, 20 sec after TMGBL (10 mg/kg i.v.); *middle*, 25 sec after TMGBL (10 mg/kg i.v.) given 10 min after pretreatment with α-EMGBL (250 mg/kg i.v.); *bottom*, 20 sec after NHCL (30 mg/kg i.v.).

Center. *Top*, 30 sec after OHCL (3 mg/kg i.v.); *middle*, control recording; *bottom*, 25 sec after TMSM (10 mg/kg i.v.).

Right. *Top*, 19 min after PMSM (100 mg/kg i.v.); *middle*, 5 min after phthalide (2000 mg/kg i.v.); *bottom*, 60 sec after β-EMGBL (50 mg/kg) given 6 min after phthalide (2000 mg/kg i.v.).

OHCL (Fig. 1), also produced the same types of epileptiform discharges (Fig. 2). These discharges were also prevented by pretreatment with α-EMGBL (data not shown). In contrast, phthalide, an aromatic α,β-substituted GBL, proved to be inactive even at a dose of up to 2000 mg/kg (Fig. 2). This compound was then tested for anticonvulsant activity but was not able to prevent β-EMGBL-induced discharges (Fig. 2).

In a preceding study (2), the succinimide corresponding to α-DMGBL, i.e., DMSM (Fig. 1), was shown to possess very similar anticonvulsant activity. In the present study, the succinimide corresponding to TMGBL, TMSM (Fig. 1), proved to have very similar convulsant activity (Fig. 2) which was also prevented by α-EMGBL. The *N*-methyl derivative of this compound, PMSM (Fig. 1), also had convulsant activity. However, these two compounds had important differences in potency and onset of action. Whereas a 10 mg/kg dose of TMSM produced epileptiform activity within 25 sec (Fig. 2), a 25 mg/kg dose of PMSM did not cause any epileptiform activity. At a dose of 100 mg/kg, PMSM induced a brief period of epileptiform activity within 30 sec and then, over the next 15–20 min, 2-sec bursts of activity occurred more and more frequently until the first extended, generalized, high-frequency seizure occurred (Fig. 2). This pattern then recurred over the next 60–90 min, with a generalized high-frequency discharge occurring approximately once every 4 min.

The cardiovascular effects of the α,β-substituted GBLs were identical with those of the β-substituted GBLs (1, 2). For example, a 20 mg/kg dose of TMGBL increased the blood pressure from 80/50 to 125/85. Treatment with α-EMGBL (250 mg/kg) returned the blood pressure to a normal range, lowering it to 70/45. Pretreatment with α-EMGBL lowered the basal pressure and attenuated the TMGBL-induced increase just as previously reported for β-EMGBL (2).

Effects on electrical activity of incubated hippocampal slices. Activity was evoked and recorded as previously described (1). Below 10 μM, TMGBL had no effect. At a concentration of 10μM the duration of the evoked potential was increased to 60–80 msec but there was little effect on spontaneous activity. From 100 μM to 1 mM TMGBL, evoked potentials were again increased, and spontaneous paroxysmal discharges of several millivolts lasting 60–100 msec appeared in two of three experiments. This complex activity, commonly referred to as epileptiform discharges, occurred at a rate of ~10/min. This effect of 100 μM TMGBL was antagonized by α-EMGBL. A concentration of 1 mM α-EMGBL had little effect, but 10 mM totally suppressed the excitation.

DISCUSSION

Previous studies (1, 2) demonstrated that GBLs substituted with alkyl groups in the β-position have convulsant activity and GBLs substituted in the α- and/or γ-position have anticonvulsant activity. In order to understand more fully the structure-activity relationships of alkyl-substituted GBLs, we also examined GBLs substituted in both the α- and the β-positions. The present results show that these α,β-substituted GBLs have convulsant activity very similar to that of the β-substituted GBLs. TMGBL, NHCL, OHCL, and α-OH-β-DMGBL produced seizures in mice identical with those observed

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with β-EMGBL, although there were differences in potency. TMGBL, NHCL, and OHCL were further tested in guinea pigs and produced epileptiform discharges and cardiovascular effects identical with those produced by β-EMGBL. Finally, TMGBL was tested in incubated hippocampal slices and again excitatory effects identical with those seen with β-EMGBL were observed. In addition, all of the effects of α,β-substituted GBLs were blocked by α-EMGBL and ESM but not by PHT.

We previously observed (2) that a GBL substituted in the α-position with an α,β-exocyclic double bond (α-IPGBL) was inactive. In this study, we found that phthalide, a GBL substituted in both the α- and the β-position with exocyclic double bonds, was also inactive. However, because we have not tested a compound with an exocyclic double bond in only the β-position, we can only conclude that such a double bond in the α-position seems to render the compound inactive.

Sites of action of GBLs. Comparison of TMGBL with α-DMGBL and β-DMGBL yields some insights into the sites of action of all of these agents. If the alkyl-substituted GBLs acted at two separate sites—one for convulsants and one for anticonvulsants—then TMGBL would be expected to act at both of these sites. This could result in an inactive compound or a convulsant or an anticonvulsant of lesser potency than either β-DMGBL or α-DMGBL. However, if there is only one site which mediates both the convulsant and anticonvulsant actions of alkyl-substituted GBLs, TMGBL could, in addition, be a more active convulsant or anticonvulsant than either β-DMGBL or α-DMGBL, respectively. Thus, the finding that TMGBL is a more active convulsant than β-DMGBL strongly supports the idea for one common site of action.

We previously observed (2) a close similarity between the activities of α-substituted GBLs and succinimides. In this study, we compared TMGBL with the corresponding succinimide, TMSM. The succinimide again had activity very similar to, and even more potent than, that of the identically substituted GBL. Seizures in mice and epileptiform discharges in paralyzed-ventilated guinea pigs and incubated hippocampal slices (data not shown) were indistinguishable from those induced by TMGBL. The response to anticonvulsant agents was also the same; i.e., the effects of TMSM were blocked by α-EMGBL and ESM but not by PHT. TMSM also had the same cardiovascular effects as did TMGBL and β-EMGBL.

Structure-activity relationships. In this and previous studies (1, 2) we demonstrated that GBLs and succinimides have convulsant or anticonvulsant properties depending upon their substituents at the α-, β-, and γ-positions of GBLs or the α- and α′-positions of succinimides. These data led to the following conclusions concerning the structure-activity relationships and a hypothetical model describing the topography of the site of action for these compounds (Fig. 3).

The interior of the site is occupied by any structure which will present the peripheral sites, labeled *C*, *H*, α, β, and γ, with the proper substituents. So far, we have only studied five-membered heterocyclic rings, but six-membered rings may also fit. The position labeled *C* is occupied by a carbonyl oxygen atom in all of the compounds studied; thus we are unable to state whether or not it is essential.

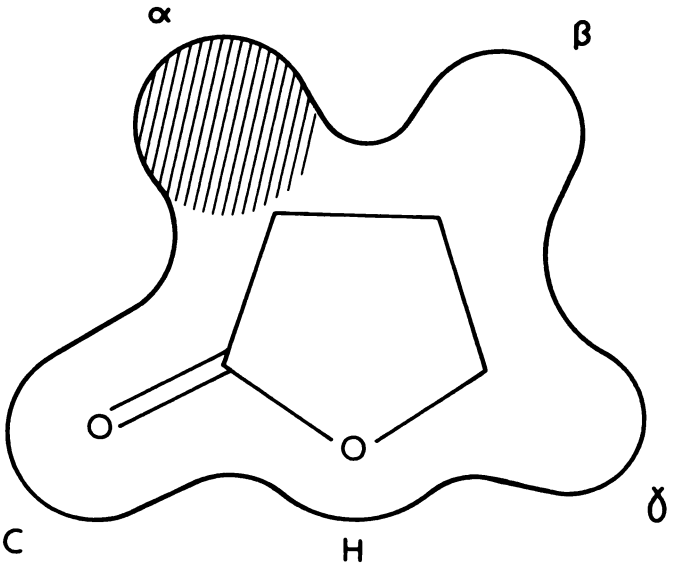

FIG. 3. *Proposed model of the alkyl-substituted GBL and succinimide binding site*

The *shaded area* in the α-position indicates that the α-pocket lies out of the plane of the figure. See text for explanation.

Position H is occupied by a heteroatom such as oxygen or nitrogen. It appears to be necessary for this heteroatom to have two free electron pairs. Thus, if nitrogen is to occupy this site it seems to be necessary to have an ionizable hydrogen, i.e., an unsubstituted imide nitrogen. This is supported by three observations. First, *N*-methylation (as in PMSM) greatly decreases activity. Although this could be due to an increased steric bulk, it may also involve the removal of the ionizable hydrogen which exists in TMSM. Second, although DMSM is an active anticonvulsant, preliminary results show that α,α-dimethyl-γ-butyrolactam is inactive. Also, while ESM, α-EMGBL, and β-EMGBL are all very active, β-ethyl-β-methyl-γ-butyrolactam is much less active by our convulsant and anticonvulsant criteria.[3] These lactams are intermediate in structure between GBLs and succinimides. However, the hydrogen on these cyclic amides has a pK_a of approximatley 17 relative to water (5), while ESM has a pK_a of 9.1 (6). Thus, although succinimides are only 2–5% ionized at physiological pH, they are nevertheless 10^8 times more ionizable than lactams. Third, preliminary results show that β-β-dimethylglutarimide is convulsant, whereas the corresponding compound with the —NH— replaced by —CH_2—, 5,5-dimethyl-1,3-cyclo-hexanedione, is inactive.[3] Therefore, we conclude that an atom with electronic characteristics similar to those of an oxygen or unsubstituted imide nitrogen is necessary at position *H*.

Position α may or may not be occupied by groups other than hydrogen. If it is, a hydrophobic substituent will increase activity and a hydrophilic group will decrease activity, respectively, (e.g., compare TMGBL and α-OH-

[3] W. E. Klunk, D. F. Covey, and J. A. Ferrendelli, unpublished observations.

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β-DMGBL with β-DMGBL). In addition, the orientation and degree of saturation of the α-substituent is important; i.e., the presence of an exocyclic α,β-unsaturated bond in the plane of the ring seems to destroy activity (thus the *shaded area* in Fig. 3).

Position β seems to be the factor which determines the activity of the compound. It also appears to be involved in determining potency. Thus, if the β-position has the proper alkyl substituent(s), the drug will be a convulsant. If there is no substituent here, the drug will be an anticonvulsant if it meets the other criteria for activity; otherwise, it will be inactive.

Position γ is relatively indiscriminant. It may be occupied by hydrogen atoms, alkyl groups, or a carbonyl oxygen atom. Alkyl groups appear to confer some degree of activity when no other alkyl groups are present at the α-position, as evidenced by the low anticonvulsant activity of γ-EMGBL (2). However, comparison of α,γ-DEMGBL with α-EMGBL (2) indicates that γ-alkyl groups do not increase activity over that conferred by hydrogen atoms when alkyl groups are present on the α-position. Neither hydrogen atoms nor carbonyl oxygen atoms confer activity, as is evidenced by the inactivity of unsubstituted GBL or succinimide.

The requirements for activity, then, are (a) a carbonyl oxygen atom in position *C*; (b) an oxygen or nitrogen atom with a ionizable hydrogen atom in position *H*; and (c) suitable alkyl substituents occupying at least one of the α-, β-, or γ-positions. Assuming that conditions a and b are met, if there are proper alkyl substituents at the α- and/or γ-positions and *no* substituent at the β-position, then the compound will be anticonvulsant. If there is a proper alkyl substituent at the β-position, then the compound will be a convulsant. Thus, it is the presence or absence of alkyl substituents at the β-position that dictates whether a GBL or succinimide will be convulsant or anticonvulsant.

While direct comparison of the α- an α′-positions of TMSM to the α- and β-positions of TMGBL is straightforward, the symmetry of the succinimide molecule makes it uncertain whether the alkyl substituents on the α-position of ESM should correspond to the alkyl substituents on the α-position or the β-position of GBL. The fact that ESM is an anticonvulsant similar to α-EMGBL suggests that it is oriented with its alkyl groups in the same position as α-EMGBL at the site of action, but this argument is circular. In order to address this problem directly, we looked at compounds with heterocyclic rings similar to those of ESM but with alkyl substituents frozen in either the α- or the β-position. One such class of compounds comprises the glutarimides (a six-membered cyclic imide as opposed to the the five-membered succinimides). The glutarimides have three carbon atoms subject to substitution: equivalent α- and α′-positions as well as a distinct β-position. Substitution with an ethyl and a methyl group at this β-position produced Bemegride, a commonly known convulsant (7) similar to β-EMGBL. In contrast, substitution at the α-position (with an ethyl and a phenyl) produces glutethimide, an anticonvulsant used clinically in Switzerland and Hungary.

A second class of compounds similar to succinimides with distinct α- and β-positions includes the γ-butyrolactams, or 2-pyrrolidones. These are five-membered cyclic amides exactly like succinimides except that one carbonyl

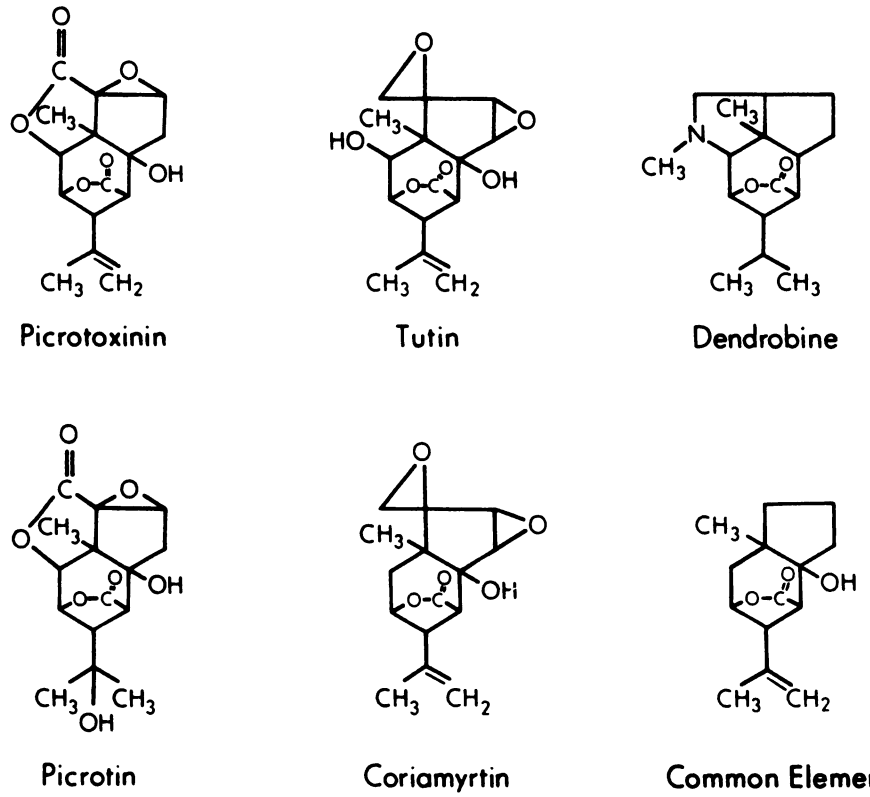

FIG. 4. *Chemical structures of some picrotoxinin-like plant neurotoxins and their common structural element*

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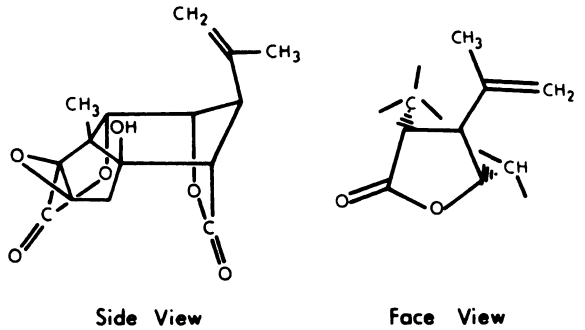

FIG. 5. *Three-dimensional configuration of picrotoxinin as determined from X-ray crystallography of α_1-bromopicrotoxinin (10)*

Left. View from the carbonyl and α-carbon side of the essential lactone ring which lies to the *right side* of the molecule. Right. Face view of this lactone ring showing the β-position of the isopropenyl group.

is reduced to a methylene group. Our preliminary results show that substitution with an ethyl and a methyl group at the β-position produces a convulsant drug. However, differences in acidity between the amide and imide hydrogen (see above) prevent direct further comparison of these lactams with the succinimides. Finally, TMSM itself provides a way to ensure that alkyl substituents are in the area which corresponds to the β-position of GBLs regardless of the orientation of the succinimide ring. As stated above, this compound is a convulsant. Thus, whenever a "succinimide-like" molecule has an alkyl substituent restricted to what corresponds to the β-position of a GBL, it is convulsant. The one compound in which alkyl substituents are restricted to the α-position, glutethimide, is an anticonvulsant. Therefore we believe that the orientation of the alkyl groups of ESM coincide with those of α-EMGBL at their site of action.

Upon examining other potent convulsants, we found that picrotoxinin, the active component of picrotoxin, has a β-alkyl-substituted lactone ring which appears to be absolutely essential for activity (Fig. 4). Picrotin has a hydroxyl group on the β-substituent of this lactone ring and is a much less potent convulsant than picrotoxinin, which has a β-isopropenyl group. Hydrolysis of this lactone ring destroys all activity (8). In addition, Jarboe *et al.* (8) have suggested that only the cyclohexanecarboxylic acid lactone along with the β-lactone substituent and the bridgehead hydroxyl are the essential structural components. This conclusion is derived in part from the fact that other picrotoxinin-like plant neurotoxins (Fig. 4) have these components but do not possess the second lactone ring present in picrotoxinin and have the oxirane ring in different locations (or missing altogether). Kuwano *et al.* (9) have shown that even less of the picrotoxinin skeleton is needed, as 8-isopropyl-6-oxabicyclo[3.2.1] octan-7-one was shown to be active. We further suggest that only the lactone ring with proper β-substituents is necessary.

If this hypothesis is valid, then all of the alkyl-substituted lactone and succinimide convulsants and anticonvulsants may work at the same site as picrotoxinin. Thus, picrotoxinin should fit the requirements of our proposed site of action. A side view of the X-ray structure of picrotoxinin (10) is shown in Fig. 5 and clearly shows the highly exposed nature of this essential lactone ring. A face view of this lactone ring shows that it is substituted in each of the α-, β-, and γ-positions. Fitting this ring into our model in Fig. 3 would put the lactone oxygen in position *H*, the carbonyl oxygen in position *C*, a hydrogen and two methylene groups of the cyclopentane ring (out of the plane of the lactone ring) in position α, the isopropenyl group in position β, and a hydrogen and a methylene group of the cyclohexane ring in position γ. Picrotoxinin, therefore, fits our model well as a convulsant. It should also be noted here that in our studies as well as those of others (2, 11) picrotoxin had the same response to ESM, PHT, and α-EMGBL as did β-EMGBL, TMGBL, and TMSM. The remainder of the picrotoxinin molecule is also undoubtedly important in determining how well it binds and contributes to its greater convulsant potency.

In conclusion, we have found that the alkyl-substituted GBLs are potent neuropharmacological agents. Structure-activity studies lead to the suggestion that they may share a common site of action with the succinimides, and possibly picrotoxinin. The convulsant lactones and succinimides would present alkyl groups to the β-position of the receptor site (Fig. 3). If this site is the same as the proposed site of action of picrotoxinin, i.e., the GABA-regulated chloride channel (11), then these alkyl groups would lead to blockade of GABA-induced chloride conductance. The anticonvulsants would act at the same site but present no alkyl groups, and thus would cause no blockade of chloride conductance or may even potentiate the action of GABA. The alkyl-substituted GBLs represent a new class of anticonvulsant and convulsant agents which have potential not only in the clinical therapy of epilepsy, but also in understanding how other anticonvulsant drugs work. This understanding could lead to the design of more specific and effective therapeutic agents.

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