Para-Substituted N-Acetyl-L(S)- and -D(R)-α-amino-N-phenylsuccinimides and -glutarimides. Substituent Effects on Stereoselective Anticonvulsant Activity[1,†]

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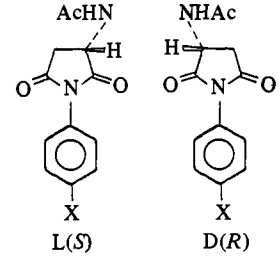
Acetyl-D(R)- and -L(S)-N-(para-substituted phenyl)succinimides (1-5) and -glutarimides (6-10) were synthesized from amino acids of known absolute configuration and subjected to a battery of standard anticonvulsant testing procedures in mice. The neurotoxic doses (TD_{50}'s), anticonvulsant potencies (MES and sc MET ED_{50}'s), protective indexes (PI = TD_{50}/ED_{50}), and effects on minimal seizure threshold (iv MET) were compared with similar values concomitantly determined for clinically useful anticonvulsants. Several compounds compare favorably with drugs of clinical significance. These analogs, which contain an acetamido group and chiral center α to one of the imide C=O groups, exhibit stereoselective biological activity; the magnitude of the activity difference between isomers is a function of the substituent on the phenyl ring. Some succinimides having the L(S) absolute configuration enhanced seizure susceptibility and decreased seizure threshold; for the most part succinimides and glutarimides having the D(R) configuration exhibit activity which is equal to or greater than the activity observed for L(S) anticonvulsants.

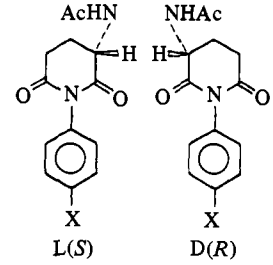
For the ultimate purpose of examining quantitative as well as qualitative stereostructure-activity relationships of enantiomorphic anticonvulsants of known absolute configuration, we desired a series of related compounds which (1) compared favorably with well-known clinically useful drugs and (2) readily could be synthesized from available precursors of known absolute configuration. The latter requirement is particularly important if correlations of biological activity with thermodynamically derived substituent constants[2] are to be investigated within a large group of pure *S* or *R* enantiomers. Stereoselective synthesis of a series of drugs has the advantage over classical resolution of *dl* mixtures if the absolute configuration of drugs prepared is easily determined because of the nature of the synthesis, and the synthesis is less time consuming and expensive.

Since many clinically useful anticonvulsants (standard drugs in Table I) are neutral or weakly acidic heterocycles which contain an imide function, we anticipated the neutral succinimides [L(S) or D(R) **1-5**] and glutarimides [L(S) or D(R) **6-10**] would exhibit anticonvulsant activity. Further, since these compounds are analogs of aspartic and glutamic acid they may act as antagonists of amino acid metabolism or receptor site activation in the CNS. It is known that during various experimentally induced convulsions the enzymes which reduce the concentration of glutamic acid (glutamate decarboxylase and glutamine synthetase) are inhibited in the CNS, while those which increase the concentration of glutamic acid (glutaminase and glutamate dehydrogenase) form glutamic acid to an increased extent.[3] This results in a considerable increase in free glutamic acid in the brain. It has been proposed that glutamic acid, which exerts a strong excitatory effect upon the nerve cell, possibly constitutes the chemical stimulus which is largely responsible for the convulsive seizure. In addition to glutamic acid, α-aminobutyric acid, alanine, aspartic acid, and glutamine also show a rise in concentration during experimentally induced seizures.[3]

For these reasons, it seemed likely that compounds **1-10**, which contain a chiral center α to one of the imide C=O groups, would satisfy the two requirements listed above. In this communication the toxicity, anticonvulsant activity against minimal and maximal seizures, and the protective index (PI) of the semirigid succinimides and more flexible glutarimides are compared with the results obtained for standard drugs in the same biological system. In a subsequent communication we will consider the quantitative analysis of a larger series of these agents.

Synthetic Aspects. Succinimides **1-5** were synthesized from either L(S)- or D(R)-aspartic acid hydrobromide (**11**). Glutarimides **6-10** were prepared in a similar manner by employing L(S)- or D(R)-glutamic acid (**18**) as the starting amino acid. Optical rotatory dispersion (ORD) and circular dichroism (CD) spectra were investigated (see Experimental Section) to determine whether racemization took place during the conversion of these amino acids to the respective imides **1-10**.





1, X = H
2, X = Cl
3, X = Me
4, X = NO_2
5, X = MeO

6, X = H
7, X = Cl
8, X = Me
9, X = NO_2
10, X = MeO

For the preparation of succinimides **1-5**, L(S)- or D(R)-aspartic acid hydrobromide (**11**) was converted to *N*-acetyl-L(S)- or -D(R)-aspartic acid anhydride (**12**) by reaction with Ac_2O.[4] It is important that this reaction, including removal of the Ac_2O-HOAc, be carried out in <0.5 hr and that the resulting aspartic acid anhydride be dried immediately under reduced pressure and at room temperature in the presence of a drying agent. If these precautions are not taken racemization occurs. Reaction of anhydride **12** with various para-substituted anilines in ethanol at room temperature afforded a mixture of α- and β-anilides (**13-17**). For steric reasons, it is most likely that the β-anilide forms a major component of the mixture.[4] The sharp melting point of the products also indicated only one isomer was present after recrystallization. Cyclization with crude or recrystallized anilide was accomplished by reaction with acetic acid anhydride at 90-95° for 45 min to 1 hr.

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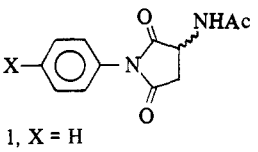
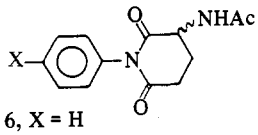
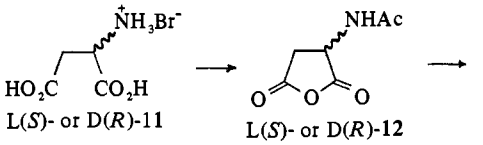
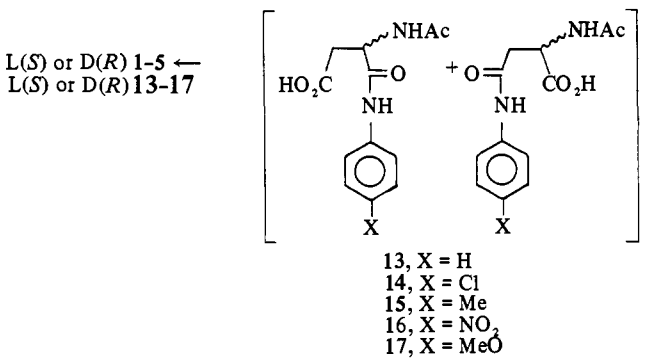
Table I. Anticonvulsant Activity of Standard and Experimental Drugs[a,b]

Compd name or no.	TPE,[c] min	TD_{50}, mmoles/kg	Maximal electroshock seizures (MES) ED_{50}, mmole/kg	TD_{50}/ED_{50}	Subcutaneous metrazole seizures (sc MET) ED_{50}, mmole/kg	TD_{50}/ED_{50}	Seizure threshold (iv MET) Dose[h]	Ratio
Phenobarbital[d] (Na salt)	30	0.38 (0.35-0.41)	0.078 (0.057-0.10)	4.80 (3.48-6.62)	0.042 (0.030-0.059)	8.89 (6.35-12.45)	$0.5TD_{50}$	2.31 (1.93-2.74)
Diphenylhydantoin[d] (Na salt)	60	0.27 (0.24-0.29)	0.030 (0.024-0.038)	8.80 (6.88-11.30)	Ineffective		$0.5TD_{50}$	0.96 (0.84-1.10)
Trimethadione[d]	5	7.48 (6.99-7.96)	4.92 (4.18-5.81)	1.52 (1.27-1.82)	1.75 (1.39-2.19)	4.28 (3.37-5.44)	$0.5TD_{50}$	2.97 (2.53-3.49)
Paramethadione	5	2.39 (2.02-2.82)	1.24 (1.05-1.47)	1.92 (1.51-2.44)	0.42 (0.35-0.52)	5.64 (4.34-7.33)	$0.5TD_{50}$	2.91 (2.49-3.35)
Phensuximide	5	1.37 (1.27-1.49)	0.39 (0.35-0.43)	3.51 (3.31-3.72)	0.43 (0.37-0.49)	3.21 (2.97-3.47)	$0.5TD_{50}$	2.69 (2.27-3.19)
Methsuximide	5	0.64 (0.58-0.71)	0.21 (0.17-0.26)	3.10 (2.44-3.94)	0.17 (0.15-0.20)	3.71 (3.09-4.45)	$0.5TD_{50}$	2.03 (1.74-2.35)
Ethosuximide[d]	5	2.16 (1.86-2.51)	4.33 (3.86-4.80)	0.50 (0.41-0.61)	0.52 (0.43-0.62)	4.19 (3.33-5.28)	$0.5TD_{50}$	2.21 (1.89-2.59)
								
1, X = H								
D(*R*)	60	8.10 (7.59-8.62)	3.79 (3.47-4.16)	2.14 (1.93-2.38)	1.42 (1.22-1.67)	5.70 (4.75-6.84)	$0.5TD_{50}$	2.07 (1.54-2.69)
L(*S*)	75	>10.78	5.39 (4.53-6.42)		Ineffective to 4.31		5.39	1.20 (1.08-1.33)
2, X = Cl								
D(*R*)	45	1.86 (1.73-2.00)	1.54 (1.35-1.75)	1.21 (1.03-1.42)	0.77 (0.70-0.84)	2.41 (2.10-2.78)	$0.5TD_{50}$	1.28 (1.06-1.50)
L(*S*)	45	1.46 (1.29-1.73)	Ineffective[e] to 3.75		Ineffective[e] to 0.75		$0.5TD_{50}$	0.84 (0.74-0.96)
3, X = Me								
D(*R*)	60	3.62 (3.38-3.87)	Ineffective[e] to 4.07		Ineffective[e] to 4.07		$0.5TD_{50}$	0.91 (0.79-1.06)
L(*S*)	30	1.87 (1.65-2.12)	Ineffective[e] to 0.81		Ineffective[e] to 0.81		$0.5TD_{50}$	0.71 (0.58-0.86)
4, X = NO_2 D(*R*)[f] or L(*S*)[f]								
5, X = MeO								
D(*R*)	45	>5.73	Ineffective to 5.73		Ineffective to 3.82		3.82	1.17 (0.995-1.36)
L(*S*)	45	>7.63	Ineffective to 7.63		Ineffective to 3.82		3.82	1.08 (0.89-1.29)
								
6, X = H								
D(*R*)	30	1.77 (1.62-1.93)	0.50 (0.45-0.54)	3.57 (3.19-4.00)	0.71 (0.53-0.96)	2.49 (1.82-3.41)	$0.5TD_{50}$	1.66 (1.39-1.96)
L(*S*)	15	1.69 (1.49-1.91)	0.58 (0.48-0.69)	2.92 (2.35-3.62)	0.77 (0.67-0.88)	2.18 (1.82-2.62)	$0.5TD_{50}$	1.61 (1.42-1.82)
7, X = Cl								
D(*R*)	90	1.25 (1.15-1.35)	0.26 (0.22-0.30)	4.79 (4.06-5.65)	0.77 (0.56-1.04)	1.63 (1.22-2.22)	$0.5TD_{50}$	0.994 (0.85-1.17)
L(*S*)	150	1.71 (1.52-1.93)	0.53 (0.47-0.58)	3.24 (2.82-3.73)	0.60 (0.50-0.70)	2.91 (2.35-3.61)	$0.5TD_{50}$	1.19 (0.93-1.51)
8, X = Me								
D(*R*)	45	2.00 (1.75-2.28)	1.23 (0.90-1.67)	1.63 (1.17-2.27)	1.83 (1.26-2.65)	1.09 (0.74-1.61)	$0.5TD_{50}$	1.16 (0.98-1.35)
L(*S*)	45	4.08 (3.16-5.26)	1.62 (1.24-2.10)	2.52 (1.74-3.65)	1.69 (1.30-2.20)	2.41 (1.66-3.49)	$0.5TD_{50}$	2.21 (1.66-2.80)
9, X = NO_2								
D(*R*)	60	9.79 (7.50-12.82)	1.36 (0.98-1.87)	7.22 (4.72-11.05)	1.51 (1.04-2.12)	6.48 (4.10-10.30)	$0.5TD_{50}$	1.49 (1.30-1.70)
L(*S*)	210	8.07 (6.32-10.31)	2.47 (2.19-2.80)	3.26 (2.45-4.34)	5.50 (4.01-7.53)	1.47 (0.98-2.21)	$0.5TD_{50}$	1.38 (1.13-1.65)
10, X = MeO								
D(*R*)	60	>18.00[g]	2.63 (2.08-3.36)	>6.85	2.23 (1.48-3.41)	>8.07	3.62	1.17 (0.95-1.40)
L(*S*)	30	>18.00[g]	3.39 (2.97-3.90)	>5.32	3.41 (2.38-4.87)	>5.32	3.62	1.41 (1.21-1.63)

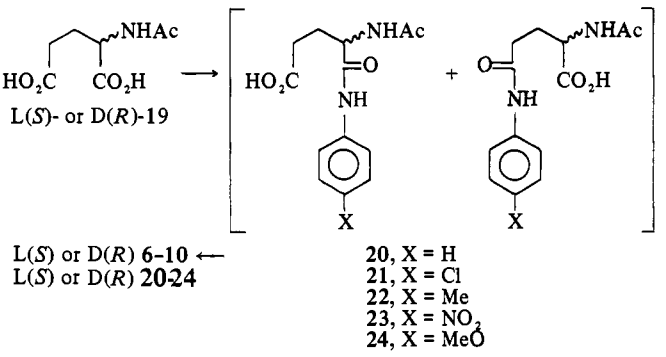
Footnotes to Table I

[a]All compounds were administered ip to CF-1 male mice (Carworth Farms). [b]Numbers in parentheses refer to 95% confidence limits, as calculated by the method of Litchfield and Wilcoxon. [c]Time of peak neurotoxic effect as measured by a rotarod apparatus. [d]Except for these drugs which were dissolved in H_2O, all other compounds were administered in a methyl cellulose vehicle. [e]These compounds lower, rather than raise, electroshock and chemoshock seizure threshold; seizures induced by both methods are exacerbated upon administration of these drugs. [f]These isomers formed a gummy residue with methyl cellulose suspending agent and could not be tested. [g]These isomers were very slowly absorbed after ip injection; hence high doses were required for demonstration of neurotoxic effects. [h]When not given as fraction of TD_{50} dose is given in mmoles.





Glutarimides of known absolute configuration were synthesized by a modified route. L(*S*)- or D(*R*)-glutamic acid (**18**) was converted to the *N*-acetyl derivative **19** by reaction with Ac_2O in boiling water. Reaction of L(*S*)- or D(*R*)-**19** with the appropriate para-substituted aniline in the presence of dicyclohexylcarbodiimide (DCC) in pyridine at 0° afforded a mixture of α- and γ-anilides (**20–24**). If this reaction is run at room temperature base-catalyzed racemization occurs owing to the presence of pyridine solvent. The resulting anilide mixture, which was removed from the insoluble dicyclohexylurea by filtration, was heated with Ac_2O at 90° for 45 min to effect cyclization to the crystalline imides **6–10**.



Biological Results. The biological results for standard drugs and optically pure succinimides and glutarimides are shown in Table I. Only the acetyl-D(*R*)- or -L(*S*)-α-amino-*N*-(*p*-nitrophenyl)succinimides (**4**) could not be investigated biologically owing to the formation of a gummy residue with with the methyl cellulose suspending agent. In Table I experimental succinimides and glutarimides are compared with various standard drugs in terms of time of peak effect (TPE), neurotoxicity (TD_{50}), protection against maximal electroshock seizures (MES) relative to potency (mmole/kg) and PI (TD_{50}/ED_{50}), protection against minimal or subcutaneous metrazole seizures (sc MET) relative to potency and PI, and the ability of doses equivalent to $0.5TD_{50}$ (when known) to alter iv metrazole seizure threshold (iv MET).

Generally, the TPE for the standard drugs was much more rapid than the TPE for all experimental succinimides and glutarimides. This is likely a reflection of the insolubility of the experimental drugs in aqueous media; upon gross observation these compounds could be found in crystalline form in the peritoneal cavity long after the TPE. A greater range for the TPE was observed for the optically pure glutarimides than the succinimides. However, no readily discernible relationship exists between the absolute configuration or various para substituents and the TPE.

Relative to neurotoxicity (defined in terms of TD_{50}), two isomers, namely acetyl-D(*R*)-α-amino-*N*-(*p*-chlorophenyl)-succinimide (**2**) and acetyl-L(*S*)-α-amino-*N*-(*p*-methylphenyl)succinimide (**3**), exhibit a median neurotoxic dose similar to ethosuccinimide. However, the D(*R*) enantiomorphs **2** and **3** are less toxic than their corresponding L(*S*) antipods. Both enantiomorphs of **1** (X = H) and **5** (X = MeO) exhibit considerably less neurotoxicity than the standard succinimides (phensuximide, methsuximide, and ethosuximide). All experimental succinimides also are less toxic than phenobarbital and diphenylhydantoin. Trimethadione exhibits less neurotoxicity than D(*R*)- or L(*S*)-**2** and -**3**. Paramethadione also is less neurotoxic than D(*R*)- or L(*S*)-**2**, but is equitoxic with L(*S*)-**3** and more toxic than D(*R*)-**3**. While the unsubstituted and *p*-MeO analogs (**1** and **5**, respectively) are clearly the least toxic, only acetyl-D(*R*)-α-amino-*N*-phenylsuccinimide (**1**) and the D(*R*) Cl analog **2** are effective anticonvulsant drugs. Stereoselective toxicity was observed for these analogs; D(*R*)-**1**, L(*S*)-**2**, and L(*S*)-**3** are significantly more toxic than their respective antipodes.

These data can be compared with TD_{50} values obtained for the experimental glutarimides. Except for ethosuximide, trimethadione, and paramethadione, the neurotoxicity exhibited for all glutarimide antipodes is equal to or less than the standard drugs. In this series insertion of NO_2 (**9**) and MeO (**10**) groups into the para position of the phenyl ring afforded analogs exhibiting less neurotoxicity than the Cl (**7**), Me (**8**), and unsubstituted (**6**) compounds. Significant stereoselective neurotoxicity differences were observed for the Cl (**7**) and Me (**8**) analogs; the D(*R*) antipodes showed significantly greater neurotoxicity than their respective L(*S*) isomers. For other glutarimide analogs stereoselective neurotoxic differences are not significant. However, in both the D(*R*)- and L(*S*)-glutarimide series the order of decreasing toxicity of these compounds as judged from the TD_{50} is similar. In other words for the D(*R*) series Cl > H ~ Me > NO_2 > MeO in order of decreasing toxicity; for the L(*S*) series Cl ~ H > Me > NO_2 > MeO. Interestingly, in the succinimide series the order of decreasing toxicity for the D(*R*) antipodes is Cl > Me > MeO > H; for the L(*S*) antipodes the order is Cl ~ Me > MeO or H. In other words, in the succinimide series the unsubstituted analogs are among the least toxic drugs while in the glutarimide series the unsubstituted compounds are among the most toxic.

In the succinimide series only the D(*R*) unsubstituted (**1**) and Cl (**2**) analogs have anticonvulsant activity (measured in

terms of ED_{50}). These analogs were effective against both maximal (MES) and minimal (sc MET) seizures and provided evidence of significantly decreasing CNS excitability. The TD_{50}/ED_{50} ratio (PI's) against MES for D(*R*) unsubstituted succinimide **1** is in the same general range as the corresponding ratios for the standard succinimide and oxazolidinedione derivatives, but does not compare favorably with phenobarbital or diphenylhydantoin. Conversely, several isomers in the succinimide series demonstrated the ability to produce central stimulation as manifested by an increase in seizure susceptibility (iv MET ratio significantly less than 1.00). The L(*S*) Cl (**2**) and Me (**3**) antipodes exhibited a particularly significant increase in central stimulation. Both isomers having MeO groups (**5**) have neither anticonvulsant nor central stimulating activity.

In the glutarimide series those isomers having the D(*R*) absolute configuration also are more potent than their L(*S*) antipodes against MES, but only in the case of the Cl (**7**) and NO_2 (**9**) analogs are these differences significant. In this series the order of decreasing potency follows the order of decreasing neurotoxicity; *i.e.*, for the D(*R*) antipodes Cl > H > Me ~ NO_2 > MeO and for the L(*S*) antipodes Cl ~ H > Me > NO_2 > MeO. Against MES all glutarimides (**6–10**) have a PI significantly greater than one. However, the D(*R*) NO_2 (**9**) and MeO (**10**) analogs have the best PI. These compounds compare favorably against MES with any of the standard drugs in terms of their MES TD_{50}/ED_{50} ratios. Except for the Me-substituted glutarimide **8**, which has a PI equivalent to the D(*R*) unsubstituted (**1**) and Cl (**2**) succinimides, all other glutarimides have more favorable PI's (MES TD_{50}/ED_{50}) than the anticonvulsant succinimides.

Against sc MET-induced seizures stereoselective activity was observed for the unsubstituted (**1**) and Cl (**2**) succinimides and for the NO_2 (**9**) glutarimide. For these compounds the D(*R*) antipode exhibits the greater potency. However, the succinimides (**1** and **2**) exhibit considerably greater stereoselective anticonvulsant action than glutarimide **10**. Such stereoselectivity is especially evident in compound **2** where the D(*R*) Cl-substituted succinimide protects against sc MET and elevates seizure threshold (iv MET), whereas the corresponding antipode facilitates the expression of sc MET seizures and increases seizure susceptibility (iv MET). In the glutarimide series a good correlation was observed between TD_{50} and MES ED_{50}, but the correlation between TD_{50} and sc MET ED_{50} was less striking. In the glutarimide series the order of decreasing potency (sc MET ED_{50}) for the D(*R*) isomers is Cl ~ H > Me > NO_2 > MeO; for the L(*S*) antipode Cl ~ H > Me > NO_2 > MeO. Again, the PI for the D(*R*) NO_2 (**9**) and MeO (**10**) glutarimides compare favorably with the standard drugs. Whereas the other glutarimides are effective, their PI's in this procedure are generally less than for the standard drugs. It is interesting to note that with one exception (compound **8**; metrazole-induced seizure) for all experimental compounds which possess anticonvulsant activity the D(*R*) antipode demonstrated potency equal to or greater than the corresponding L(*S*) isomer.

Discussion

In an overall comparison of the anticonvulsant efficacy of these experimental compounds with diphenylhydantoin it would appear that the D(*R*) NO_2 (**9**) and D(*R*) or L(*S*) MeO (**10**) glutarimides compare favorably when evaluated against MES. Moreover, with the exception of D(*R*) Me (**8**) the remaining glutarimides appear to demonstrate considerable selective anticonvulsant activity against maximal seizures. With respect to anticonvulsant activity against sc MET the D(*R*) NO_2 (**9**) and D(*R*) and L(*S*) MeO (**10**) glutarimides and the D(*R*) unsubstituted succinimide **1** compare favorably with standard drugs which are known to protect against the expression of minimal seizures. *A priori* it seems to us that these compounds should be investigated in greater depth and their clinical efficacy as anticonvulsants determined. Further, additional analogs should be prepared for purposes of carrying out quantitative structure-activity studies and optimizing the PI and TPE. From this limited series it is of interest to note that the PI against MES for the glutarimides follows the same pattern [MeO ≥ NO_2 > Cl ≥ H ≥ Me] for both the L(*S*) and D(*R*) enantiomorphs. However, the PI against sc MET is different for the two isomeric series. For the D(*R*) enantiomorphs MeO ≥ NO_2 > H > Cl ≥ Me; for the L(*S*) series MeO > Cl ≥ Me ≥ H > NO_2.

While an explanation for these observations must await further investigation, qualitative analysis of our results leads us to suggest that these compounds are likely affecting or binding to more than one active or allosteric site in the CNS and that differences in binding modes may be altered markedly by the nature of the substituent.[5] The most dramatic example of the influence of substituents was observed in the succinimide series where introduction of Cl or Me groups into the para position of the phenyl ring, a position far removed from the asymmetric center, decreased the PI relative to the unsubstituted D(*R*) analog and afforded L(*S*) analogs which lowered, rather than raised, electroshock and chemoshock seizure threshold.

These data may be compared with the activity reported for other isomeric compounds. Enantiomorphs of phenetureide are equipotent anticonvulsants.[6] In the case of mesantoin a twofold difference in anticonvulsant potency exists between *d* and *l* isomers.[7] In the case of pipradrol the *S* isomer is approximately twice as active as the *R* enantiomorph against electroshock-induced seizures.[8] These isomers not only have different anticonvulsant potencies, but some are stimulants; (+)-phenetureide and *R*-pipradrol possess stimulant activity while their antipodes do not.

The results of these studies underscore the importance of considering steric as well as hydrophobic and electronic parameters when designing new drugs and optimizing the PI within a series of analogs. However, quantitative correlation of biological data with physical parameters must await the biological evaluation of a greater number of compounds.

Experimental Section§

N-Ac-D(*R*)- or -L(*S*)-Asp anhydride (12) was prepared according to methods previously published.[9]

N-Ac-D(*R*)- or -L(*S*)-Glu (19) was prepared according to methods previously published affording crystals (88%), mp 198–200°; lit.[10] mp 199°.

General Method for Preparation of L(*S*)- or D(*R*)-Aspartic Acid Para-Substituted Anilides (13–17). The desired para-substituted aniline (0.1 mole) dissolved in 75 ml of absolute ethanol was added to a solution of *N*-acetyl-L(*S*)- or -D(*R*)-Asp anhydride (0.1 mole) in 25 ml of absolute ethanol. A semisolid formed after stirring at room temperature for 15 min. The mixture was dissolved in 10% Na_2CO_3 and extracted with ether to remove the excess amine. The aqueous solution was acidified (pH 2.0) with dilute HCl; the precipitated anilide was filtered and crystallized from methanol. The yields and melting points of compounds prepared are reported in Table II.

§Melting points were determined using a calibrated Thomas-Hoover melting point apparatus. Ir spectra were recorded utilizing a Perkin-Elmer 257 spectrophotometer. Optical rotatory dispersion (ORD) and circular dichroism (CD) spectra were taken utilizing the Durham-Jasco ORD/CD instrument. Elemental analyses were performed by Clark Microanalytical Labs, Urbana, Ill.

General Method for the Preparation of *N*-Acetyl-L(*S*)- or -D(*R*)-Glu Para-Substituted Anilides (20–24). The anilides were prepared from 19 by a modification of a method reported in the literature.[11] *N*-Acetyl-L(*S*)- or -D(*R*)-Glu (18.9 g, 0.1 mole) and DCC (22.8 g, 0.1 mole) were dissolved in pyridine (100 ml) and stirred at 0°. To this solution was added 0.1 mole of the desired para-substituted aniline. The mixture was stirred for 24 hr at 0°, the dicyclohexylurea was removed by filtration, and the solution was concentrated under reduced pressure. The residue was made basic (pH 7.4) by addition of 10% Na_2CO_3 solution. The remaining insoluble dicyclohexylurea was removed by filtration; the filtrate was acidified and extracted with ethyl acetate. The ethyl acetate solution was dried (Na_2SO_4), filtered, and concentrated under reduced pressure affording the desired anilide in a crystalline state. All the anilides were easily recrystallized from methanol. The physical properties for these compounds (likely mainly γ-anilides) are found in Table II.

Table II. *N*-Acetyl-L(*S*)- and -D(*R*)-aspartic and -glutamic Acid Anilides

Compound	Mp, °C	% yield
13-L(*S*)	190–191	75.5
D(*R*)	191–192	80.0
14-L(*S*)	182–184	55.7
D(*R*)	182–184	71.0
15-L(*S*)	181–182	56.2
D(*R*)	183–184	61.0
16-L(*S*)	189–191	56.0
D(*R*)	189–191	65.0
17-L(*S*)	194–196	78.4
D(*R*)	192–195	53.5
20-L(*S*)	183–184	23.1
D(*R*)	183–184	24.0
21-L(*S*)	189–190	56.0
D(*R*)	190–191	48.5
22-L(*S*)	194–195	28.0
D(*R*)	195–196	30.2
23-L(*S*)	200–202	48.0
D(*R*)	200–203	40.0
24-L(*S*)	188–190	24.0
D(*R*)	188–190	24.5

General Method for the Preparation of *N*-Acetyl-L(*S*)- or -D(*R*)-α-amino-*N*-para-substituted-phenylsuccinimides (1–5) and -glutarimides (6–10). The L(*S*)- or D(*R*)-aspartic or -glutamic acid para-substituted anilides (0.1–0.2 mole) were dissolved in 10–15 times their weight of acetic anhydride. Anilides generally dissolved within 15 min but were heated for an additional 30–45 min to ensure reaction completion. The acetic acid anhydride was removed under reduced pressure, the residue dissolved in benzene–ethanol (3:1), and the resulting solution was decolorized with charcoal, filtered, and cooled to afford crystalline imide. These compounds were recrystallized from MeOH–$CHCl_3$ (1:1). The physical properties for imides (**1**–**10**) are found in Table III.

Table III. *N*-Acetyl-L(*S*)- and -D(*R*)-α-amino-para-substituted-phenylsuccinimides and -glutarimides

Compound	Mp, °C	% yield	Formula	Analyses
1[a]-L(*S*)				
D(*R*)				
2-L(*S*)	189–191	69.0	$C_{12}H_{11}O_3N_2Cl$	C, H, N, Cl
D(*R*)	189–192	62.7		
3-L(*S*)	199–200	82.3	$C_{13}H_{14}O_3N_2$	C, H, N
D(*R*)	200–201	76.5		
4-L(*S*)	183–186	69.0	$C_{12}H_{11}N_3O_5$	C, H, N
D(*R*)	182–184	73.0		
5-L(*S*)	187–188	64.5	$C_{13}H_{14}O_4N_2$	
D(*R*)	187–188	76.5		C, H, N
6-L(*S*)	190–192	90.0	$C_{13}H_{14}O_3N_2$	
D(*R*)	190–192	89.0		C, H, N
7-L(*S*)	206–207	75.0	$C_{13}H_{13}O_3N_2Cl$	
D(*R*)	206–207	68.0		C, H, N, Cl
8-L(*S*)	195–197	90.0	$C_{14}H_{16}O_3N_2$	
D(*R*)	196–197	74.0		C, H, N
9-L(*S*)	212–213	56.0	$C_{13}H_{13}O_5N_3$	C, H, N
D(*R*)	212–214	57.0		
10-L(*S*)	208–210	62.5	$C_{14}H_{16}O_4N_2$	
D(*R*)	208–210	71.0		C, H, N

[a] See ref 4.

Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD) Data. ORD and CD spectral data were obtained to determine whether racemization took place during the conversion of α-amino acids to the para-substituted aspartic and glutamic acid imides. The ORD spectra for succinimides **2**–**5** are similar to those reported for the respective D(*R*) and L(*S*) antipodes **1**.[4] The CD data for all succinimides and glutarimides are found in Table IV. The ORD spectra for glutarimides **6**–**8** and **10**, which have not been reported previously, are shown in Figures 1–4. Similar ORD curves could not be obtained for the *p*-NO_2 analog **9** because this compound absorbs strongly in the region 310–315 mμ.

Table IV. Circular Dichroism Values at Selected Wavelengths for L(*S*)- and L(*R*)-Succinimides and Glutarimides

Compound no.	Concentration, g/ml D(*R*)	Concentration, g/ml L(*S*)	Wavelength, mμ	[θ] D(*R*)	[θ] L(*S*)
1	0.00091	0.00264	260	110	−120
			250	430	−500
			245	810	−880
2	0.0019	0.00068	260	110	−130
			255	140	−190
			250	280	−250
			248	300	−280
3	0.00104	0.00320	280	110	−120
			270	160	−180
			265	200	−230
			260	240	−260
			250	380	−390
5	0.00189	0.00116	270	100	−100
			260	120	−140
			250	200	−180
			248	410	−420
6	0.00236	0.00282	278	200	−200
			274	0.0	0.0
			270	−100	100
			260	−1600	1450
7	0.00183	0.00188	285	100	−100
			279	0.0	0.0
			270	−1200	800
			265	−2000	1800
			250	−5000	4000
			240	−6000	5800
8	0.00337	0.00187	286	300	−300
			276	0.0	0.0
			270	−700	700
			265	−1500	1600
			260	−2300	2600
			255	−3500	4000
10	0.00187	0.00257	295	−250	250
			287	0.0	0.0
			280	400	−400
			270	1300	−1300
			260	2600	−2400
			250	3700	−4000

Whereas the ORD curves for acetyl-L(*S*)-α-amino-*N*-phenylsuccinimides exhibit negative Cotton effects with troughs near 245 mμ, the acetyl derivatives of L(*S*)-α-amino-*N*-phenylglutarimides exhibit a weak intensity peak at 255–262 mμ, cross-over at 244–245 mμ, and a high intensity trough of negative sign at 216–222 mμ. The ORD spectra for the D(*R*) analogs are of opposite sign and, within experimental error, are equal in magnitude. The glutamic acid anilide precursors to the imides which are of the L(*S*) configuration exhibit plain curves of negative sign and show a transition in the region 275–295 mμ. The shift of the plain curve observed for the anilide to the lower wavelength trough observed for the imide characterizes the anilide to imide conversion and substantiates the applicability of the synthesis for the preparation of acetyl-L(*S*)- or -D(*R*)-α-amino-*N*-para-substituted-phenylglutarimides.

Pharmacology. The experimental animals employed were 18–25 g, adult male albino mice of the CF-1 strain (Carworth Farms). All compounds were administered by the intraperitoneal route in aqueous solution or in 0.5% methylcellulose suspension, controls receiving the vehicle alone.

Neurotoxicity Testing. The mean neurotoxic dose (TD_{50}) was determined for each drug. The end point for minimal neurotoxicity was muscular incoordination, based on the inability of the animal to remain on a horizontal rod rotating at 6 rpm for 1 min. A des-

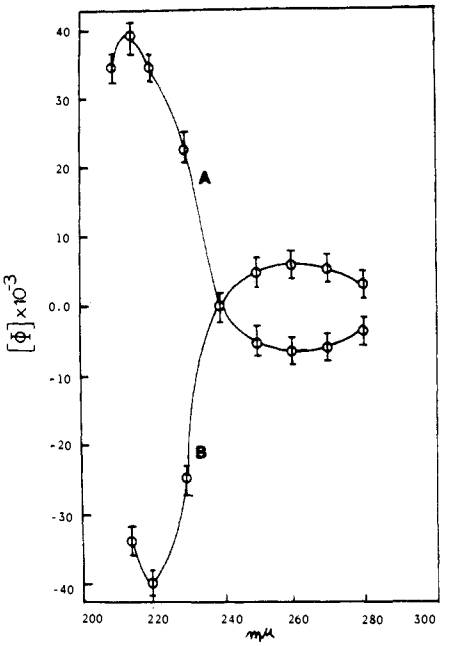


Figure 1. ORD spectra in MeOH of acetyl-D(*R*)- and -L(*S*)-α-amino-*N*-phenylglutarimide (6). A = D(*R*) enantiomorph (*c* 0.026); B = L(*S*) enantiomorph (*c* 0.028).

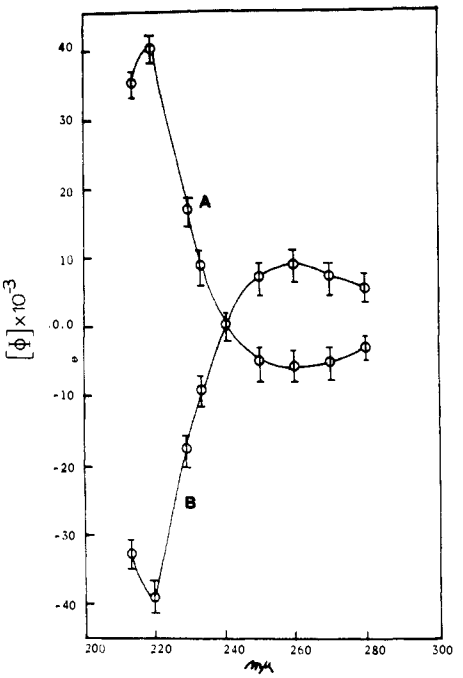


Figure 2. ORD spectra in MeOH of acetyl-D(*R*)- and -L(*S*)-α-amino-*N*-*p*-methylphenylglutarimide (8). A = D(*R*) enantiomorph (*c* 0.025); B = L(*S*) enantiomorph (*c* 0.035).

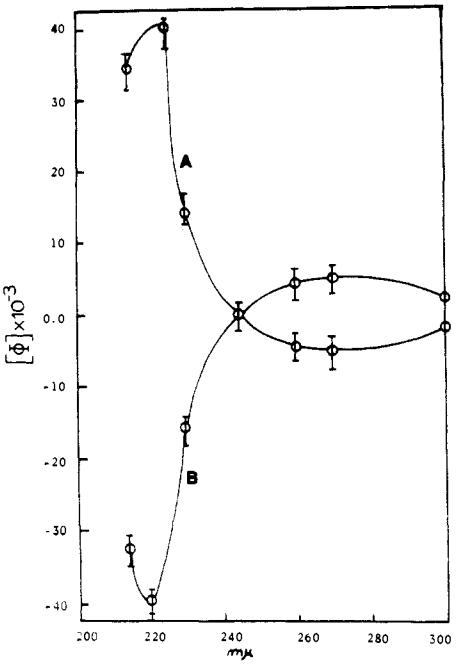


Figure 3. ORD spectra in MeOH of acetyl-D(*R*)- and -L(*S*)-α-amino-*N*-*p*-methoxyphenylglutarimide (**10**). A = D(*R*) enantiomorph (*c* 0.023); B = L(*S*) enantiomorph (*c* 0.031).

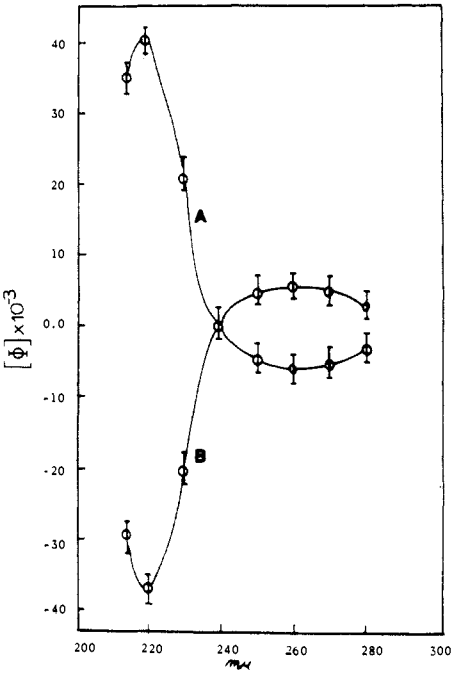


Figure 4. ORD spectra in MeOH of acetyl-D(*R*)- and -L(*S*)-α-amino-*N*-*p*-chlorophenylglutarimide (7). A = D(*R*) enantiomorph (*c* 0.031); B = L(*S*) enantiomorph (*c* 0.027).

cription of the apparatus and experimental procedure employed has been published previously.[12] This test and subsequent anticonvulsant procedures were used to determine the time of peak activity for each agent.

Anticonvulsant Activity. Anticonvulsant potencies (ED_{50}'s) were determined by two tests (one electrical and one chemical). The test based on electrically induced seizures measured the ability of a drug to prevent the hindleg tonic-extensor component of the maximum electroshock seizures evoked by supramaximal current (MES test; 50 mA alternating current, 0.2-sec stimulus duration, corneal electrodes) while the test based on chemically induced seizures measured the ability of a drug to afford complete protection against convulsions induced by the subcutaneous injection of pentylenetetrazol (85 mg/kg; sc MET test). The details of the various procedures, the end points in mice, and the characteristics of the electroshock apparatus have been described elsewhere.[13-15]

For the determination of the ED_{50} or TD_{50}, groups of 6–12 mice were given various doses of the drug until at least three points were established in the range between 0 and 100% seizure protection or minimal neurotoxicity. The results obtained were plotted on logarithmic probability paper and a regression line was fitted to the plotted points by eye. From this plot of the data, the respective ED_{50}, TD_{50}, 95% confidence limits, and protective indices (PI = TD_{50}/ED_{50}) were calculated by the method of Litchfield and Wilcoxon.[16]

The ability of selected doses (usually $0.5TD_{50}$) of these drugs to alter minimal seizure threshold was determined employing a timed intravenous infusion of pentylenetetrazole (iv MET test). A description of the utility of this technique as an indicator of the level of central nervous system excitability and the details concerning the computation of confidence intervals for the threshold ratios (the mean mg/kg of convulsant required to produce clonic

seizure in drug-treated animals divided by the mean mg/kg of convulsant required to reach the same end point in controls) have been reported previously by Wolf and Stock.[17]

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Cycloalkane Spiroheterocyclic Compounds. 9. 8-(1,2,3,4-Tetrahydro-2-naphthyl)-2-oxo-1-oxa-3,8-diazaspiro[4.5]decanes and Related Compounds[1]

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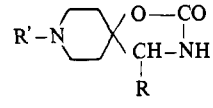
Several new 2-oxo-1-oxa-3,8-diazaspiro[4.5]decanes with 2-indanyl, 2-tetralyl, phenylcyclohexyl, and phenylcycloheptyl substitution on N-8 were prepared from the corresponding cyclanones. Other groups (2-indanylmethyl, 2-tetralylmethyl) were introduced in the same position by means of their halogenated derivatives. Some 8-(2-tetralyl) compounds were synthesized from 1-(2-tetralyl)-4-piperidone. The 2-tetralyl derivatives were found to be the most analgetic and adrenolytic. The relations between these activities and the structure of the substituent are discussed.

Among the 2-oxo-1-oxa-3,8-diazaspiro[4.5]decanes (**1**) described in a previous work,[1] the derivatives which contain an aralkyl group in position 8 were shown to be the most interesting. The best pharmacological activities (antiarrhythmic and analgetic) were obtained with R′ = $C_6H_5CH_2CH_2$ (**1a**) or $C_6H_5(CH_2)_3$ (**1b**).



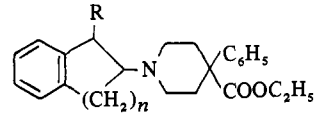
1a, R′ = $C_6H_5CH_2CH_2$
1b, R′ = $C_6H_5CH_2CH_2CH_2$

It appears that the aralkyl chain plays a definite role in ascribing to each of these two derivatives its own pharmacological profile. **1a** is mainly analgetic and central nervous system depressant, and **1b**, weakly analgetic, exerts good antiarrhythmic and hypotensive activities. The differences between their sites of action may be related to various orientations of the phenyl ring in relation to the piperidine or to the oxazolidine cycle which is perpendicular to the medium plane of piperidine.

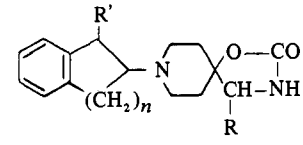
We wanted to assign a restricted conformation to this structure by replacing the aralkyl group by 2-indanyl (**3a**) or 2-tetralyl (**3b**) or a benzocycloheptyl ring (**3c** and **47**).

A similar hypothesis had previously led to derivatives of normeperidine **2**, as potential analgetics and antitussives.[2] 2-Indanylamine itself was found to be endowed with analgetic properties.[3]

Molecular models show that the plane of the aromatic

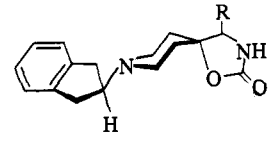


2, *n* = 1 or 2

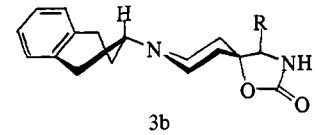


3a, *n* = 1
3b, *n* = 2
3c, *n* = 3

ring in **3b** is roughly parallel to the axis of the bond between the piperidine and the saturated cycle of tetralin, which takes the most likely half-chair conformation.[4] This gives to the molecule an elongated shape which cannot be taken by **3a**. Moreover, on account of the distance between the phenyl ring and the N atom, we could expect that **3b** would have pharmacological characteristics nearer those of **1a** than **1b**.



3a



3b

We then tried to obtain compounds more strictly related to **1b**, either by replacing the flexible chain $(CH_2)_n$ by cyclohexyl, substituted with C_6H_5 in various positions (type **4**), or by removing the indanyl or tetralyl groups from the N atom with a CH_2 link (type **5**).

In one example (compound **52**), the piperidine ring was involved in a benzo[*a*]quinolizine structure, which is known