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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF STEREOISOMERIC TRANS-DECAHYDROQUINOLINE-5-CARBOXYLIC ACIDS AS GABAERGIC AGENTS

The Ohio State University  Ph.D.  1984

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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF STEREOISOMERIC TRANS-DECAHYDROQUINOLINE-5-CARBOXYLIC ACIDS AS GABAERGIC AGENTS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Raymond J. Patch, B.Sc.

****

The Ohio State University
1984

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Donald T. Witiak
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to Betty,
for her constant understanding
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My wife, Betty, for her love, encouragement, patience and understanding, especially during the final months
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INTRODUCTION

Subsequent to its detection in mouse brain tissue,\textsuperscript{1-3} much experimental work has been directed towards delineating the role of $\gamma$-amino- butyric acid (GABA) in the nervous system. Though first suspected by some as merely a metabolic product, GABA is now widely recognized as being a major inhibitory neurotransmitter in the vertebrate central nervous system (CNS) and in the invertebrate central and peripheral nervous systems.\textsuperscript{4}

Since the GABA neurosystem interacts closely with most other central neurotransmitter systems, its effects are prominent throughout the entire CNS. The maintenance of a coordinated balance between these transmitter systems is essential for proper CNS function. Thus, if one or more of these systems should become defective, serious neurological disorders could result. Impaired GABA function has been indicated in a number of diseased states including Huntington's chorea, Parkinsonism, epilepsy, spasticity and nervous depression. Although neither the underlying mechanisms by which these disorders originate nor the extent to which the impaired GABAergic system plays a role in these disorders are fully understood, several neurophysiological manifestations of these diseases have been noted.
A. GABA in Disease States

Huntington's Chorea

Huntington's disease is a genetic disorder characterized by dyskinesias (abnormal involuntary jerking movements) resulting from extensive neuronal loss in the region of the basal ganglia, more specifically, in the neostriatum. Examination of postmortem brain tissues of Huntington's patients revealed a degeneration (up to 50%) of both cholinergic and GABAergic neurons. The activity of choline acetyltransferase (CAT), the acetylcholine synthesizing enzyme, was found to be significantly decreased in the basal ganglia of these patients, as were the concentrations of GABA, and its synthesizing enzyme, glutamic acid decarboxylase (GAD). Brain dopamine levels in these patients were essentially unchanged except in those cases where the disease had progressed to a point where muscular rigidity had set in. Levels of dopamine were significantly higher in such cases.

Given this shift in balance between these interacting transmitter systems, therapy for Huntington's patients has involved treatment with dopamine antagonists. Therapy involving cholinergic drugs, namely physostigmine (an antagonist of the acetylcholine metabolizing enzyme, acetylcholine esterase), has not given consistent results. This, however, could be due to a reduced availability of cholinergic receptor sites resulting from the neuronal degeneration described above.

The observed decrease in GAD activity has indicated the use of GABA agonists as possible therapy. Earlier studies had suggested that while GABA neurons had degenerated, the actual densities of GABA
binding sites were at normal or even increased levels. However, later studies reported a decrease in GABA receptors in the striatum of Huntington's patients. Variable results reported by Iversen, et al. suggest that GABA binding site densities differ between individual patients and that GABA agonists might be of clinical use only to some of the Huntington's patients.

Parkinson's Disease

Unlike Huntington's chorea, Parkinson's disease is characterized by a slowness of movements and muscle rigidity. The major pathophysiological defect of this disease is believed to be a deficiency in the dopaminergic system involving marked loss of dopamine neurons in the substantia nigra. Recently it has become clear that other neuronal systems, in particular GABAergic and cholinergic systems, play an important contributing role. There appears to exist a complex reciprocal interrelationship between GABA and dopamine neurons within the basal ganglia with each regulating the other.

Studies on postmortem brain samples from Parkinson's patients also revealed changes in the GABAergic components. Decreased GAD activity was observed in many areas of the basal ganglia, however without a corresponding decrease in GABA content. Interestingly, this situation was reversed by prolonged treatment (> 1 year) with L-Dopa, after which time GABA levels were found to be normal. It was also observed that GABA receptor binding was reduced in the substantia nigra although
no correlations between the binding and duration or severity of the disease could be drawn.  

While the significance of these changes in GABA neurons of Parkinsonism remains unclear, there seems to be a consensus that the potential use of GABA agents in treating this disease warrants investigation. This is evident, especially in light of the fact that the effectiveness of current therapy (which involves treatment with the dopamine bioprecursor, L-Dopa) declines as the disease progresses.

Marsden has reported that GABA-dopamine interactions vary in different regions of the basal ganglia and has suggested the need for regionally-selective GABA agents. For example, in rodent globus pallidus, increased GABA activity mimics the effect of dopamine depletion (akinetic-rigid syndrome), whereas in the striatum there seems to be a correlation between increased dopamine activity and increased GABA turnover. Thus, a GABA antagonist might be useful if selective for the globus pallidus whereas a GABA agonist would have to be selective for the striatum. At present the development of such regionally selective GABA agents has not been realized.

Epilepsy

It has long been thought that GABA plays a functional role in some forms of epilepsy. In 1959, Hayashi reported the ability of GABA to arrest generalized seizures in dogs following electrical or chemical stimulation of the motor cortex. Dietary vitamin B₆ deficiency was found to be responsible for epileptic seizures in some infants.
Subsequent studies have shown a direct link between \( B_6 \) levels and GAD activity (pyridoxal phosphate is a coenzyme for GAD).\(^{21}\) Thus, with \( B_6 \) deficiency, GAD activity is reduced, as are brain concentrations of GABA. The reduction in inhibitory mechanisms resulting from this situation leads to the development of convulsions.\(^{22}\)

Compounds which either interfere with GAD activity or block GABA receptor sites are potent convulsants. In this context, Wood and Peesker have related brain excitability to a combination of decreased GAD activity and GABA concentration, the former being the more important factor.\(^{23}\) These investigators have further suggested that any agent capable of elevating brain GABA levels should potentially be an anticonvulsant, barring opposing effects.\(^{24}\) Whereas hydrazine increases GABA brain concentrations, it decreases GAD activity. The net result of these opposing effects is induced convulsions, presumably owing to the greater importance of decreased GAD activity.

A logical approach to the treatment of seizures has been to increase GABA concentration in the brain. Direct administration of GABA has proven inefficacious presumably due to the inability of GABA to permeate the blood-brain barrier. However, inhibition of GABA metabolism has met with some success.

Valproic acid (1) a clinically useful anticonvulsant,\(^{25}\) produces increased GABA levels in various brain regions\(^{26}\) as well as in the cerebrospinal fluid of epileptic patients.\(^{27}\) Its mechanism of action has been somewhat controversial. Some investigators suggest that valproic acid inhibits succinic semialdehyde dehydrogenase (SSAD),\(^{28,29}\)
an enzyme involved with GABA metabolism. Others propose that this acid inhibits GABA transaminase (GABA-T), the enzyme responsible for metabolizing GABA to succinic semialdehyde. Either proposal would be expected to give rise to increased GABA levels.

Numerous compounds have been designed to inhibit GABA-T. However, because GABA-T, like GAD, is also a pyridoxal phosphate-requiring enzyme, such agents must show a pronounced selectivity for GABA-T in order to be useful as anticonvulsants.

Tsui, et al. introduced L-α-amino-β-chloropropionic acid hydroxamide (2) as a selective inhibitor of GABA-T. This compound is more potent than valproic acid when assessed for its ability to elevate GABA concentration in mouse brain.

γ-Acetylenic GABA (3), an irreversible inhibitor of GABA-T in vitro and in vivo, was also shown to increase whole brain concentrations. Although protected mice against audiogenic-, electroshock- and some types of chemically-induced seizures, this compound was ineffective against seizures induced by picrotoxin or pentylenetetrazol. Besides being a potent GABA-T inhibitor, 3 has a weak, but significant inhibitory action on mouse brain GAD in vivo. Stereoselectivity of γ-acetylenic GABA enantiomers on the GABA-T and GAD enzymes has been reported. Whereas (S)-(+)γ-acetylenic GABA inhibits both GABA-T and GAD, the (R)-(−)-enantiomer is a selective inhibitor of rat brain GABA-T.
γ-Vinyl GABA (4) is also an irreversible inhibitor of GABA-T both in vitro and in vivo. Unlike γ-acetylenic GABA however, this compound demonstrated no inhibitory effect on rat brain GAD at concentrations as high as 10mM in vitro. The anticonvulsant profiles of these two compounds are similar, though the potency of 4 is less. ED\textsubscript{50} values of 3 and 4 for protection against audiogenic-induced seizures in mice were 41mg/kg and 990mg/kg, respectively. However, the duration of GABA-T inhibition in vitro by γ-vinyl GABA is considerably greater than of γ-acetylenic GABA (five days as compared to one day).

Gabaculine (5) is a specific irreversible inhibitor of mammalian GABA-T, whose potency has been reported to be two to three orders of magnitude greater than (±)-γ-acetylenic GABA in vitro. This compound also increases GABA levels in mouse brain and prevents some chemically-induced seizures, but is ineffective against electroshock-induced convulsions.
Spasticity

Spasticity is a disorder, often resulting from a neurological injury in which a hyperexcitability of reflex activity manifests itself in the form of uncontrolled muscular movements. Impaired GABA function has been implicated in this condition. It has been shown that there is a loss of presynaptic inhibition concomitant with the development of spinal spasticity. High levels of GABA were found in vertebrate spinal dorsal grey matter providing further evidence that GABA serves there as the inhibitory neurotransmitter. In that region, GABA inhibition is predominantly a presynaptic inhibition.

Smith, et al. studied the effects of experimentally-induced spasticity in dogs (brought about by mid-thoracic spinal cord transection) on the GABA system in the dorsal grey matter of the lumbar cord. Following an initial decrease in GABA content in this area, there was a gradual increase in content which correlated with the progressive development of spinal spasticity. Within twelve weeks of the transection, the GABA content had risen to 68% above control levels. Naftchi, et al. have obtained similar results with paraplegic cats. This paradoxical situation of increased GABA content and yet loss of presynaptic inhibition has been rationalized in terms of a diminished release of the transmitter from the interneurons mediating presynaptic inhibition.

Although other mechanisms are likely involved, it is clear that dysfunction of the GABA system in the spinal region plays a major role in spasticity. It would follow that therapeutic approaches to the
treatment of spasticity would be directed towards increasing pre-
synaptic inhibition, either by increasing GABA release or by direct
stimulation of the appropriate GABA receptors. In support of this
argument, the GABA analogue, baclofen (6) has found clinical usefulness
in the treatment of spasticity resulting from spinal lesions.

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CO}_2\text{H} \\
& \quad \text{Cl}
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\]

6

Depression

Recently, the role of GABA in depression has been suggested. Analysis of both the cerebrospinal fluid and plasma of depressed
patients revealed GABA levels which were significantly lower than normal. Lloyd, et al. have examined the effects of the GABA agonist,
progabide (7) on behavioral and biochemical indices used to predict antidepressant drug action. In both behavioral models studied, proga-
bide exerted an antidepressant action which could be blocked by the
GABA antagonist, bicuculline. Progabide was also effective in clinical
studies, where it significantly reduced the depressive symptoms in
eight out of eleven cases. DeFeudis has also hypothesized that GABA
agonists might be useful against some forms of nervous depression.

Release of corticotrophin releasing hormone (CRH), a hormone whose
production is thought to be involved in these depressive states, was shown to be inhibited by GABA.

Summary

GABA plays a major role in regulating CNS function. Impaired GABA systems have been observed in a number of neurological disorders. Evidence has also linked GABA function to aggression, feeding behavior\(^5\) and cardiovascular control.\(^{49}\) These results clearly demonstrate the need for further investigation of GABA biology and more importantly, the relationship of GABA to other transmitter systems. To understand the function of GABA in these disease states and to develop more effective therapeutic agents, the role of GABA as a neurotransmitter warrants discussion. Such a discussion follows.
B. GABA: Role as a Neurotransmitter

Three criteria are usually met in order for a substance to be considered a neurotransmitter. Generally, neurotransmitters are biosynthesized and stored in a particular set of neurons. Although such substances can be found throughout these cells, they are largely concentrated in synaptic vesicles within the nerve terminals. Release of the neurotransmitter from these vesicles by a secretory mechanism occurs in response to nerve activity. Following release into the synaptic cleft, the substance interacts with specific receptors on the surface of the target postsynaptic membrane, thereby causing a change in the postsynaptic cell's activity. Evidence suggests that GABA fulfills these general criteria.

Since GABA cannot cross the blood-brain barrier, it must be synthesized within the GABAergic neuron. The fundamental source of GABA from outside the neuron is thought to be glucose, which does cross the blood-brain barrier. Once inside the neuron, it follows the glycolysis pathway and is then converted to glutamic acid via the tricarboxylic acid (TCA) cycle, as outlined in Scheme 1. Decarboxylation of glutamate by glutamic acid decarboxylase (GAD) is then the predominant biosynthetic pathway for GABA synthesis. GABA transaminase (GABA-T) metabolizes GABA to succinic semialdehyde, which in turn is converted to succinic acid by the action of succinic semialdehyde dehydrogenase (SSAD). Succinic acid then re-enters the TCA cycle.
A direct relationship has been found between GAD activity and GABA localization throughout the vertebrate CNS. In contrast, there is no such parallel between the regional distributions of GABA and either GABA-T or SSAD activity. Therefore, GAD activity has served as a marker for the identification and localization of GABAergic neurons in the CNS.

High concentrations of GABA found in synaptosomal preparations of GABAergic neurons provide evidence that GABA is localized in the nerve terminal where it is thought to be stored in vesicles. Just as most neurohumoral release mechanisms are calcium-dependent, GABA release from nerve terminals and glial cells is also a calcium-dependent process. Potassium-evoked release of GABA from either of these cell preparations occurred only in the presence of calcium ions. Furthermore, specific release of GABA from cortical areas during synaptic inhibition has led to the conclusion that
GABA release is associated with inhibition of cortical cell firing.\textsuperscript{63}
Thus, during presynaptic nerve stimulation there is a net influx of calcium ions and subsequent release of GABA which results in inhibitory responses.

There are two known ionic mechanisms by which GABA decreases the responses of excitatory neurons. Postsynaptically,\textsuperscript{40} GABA increases the cell membrane's permeability to chloride ions. The influx of these ions alters the membrane's normal resting potential, making it more negative. Thus, when excitatory transmitter interacts with such a hyperpolarized membrane, its depolarizing effect is not sufficient to initiate the postsynaptic action potential, and hence, the excitatory response does not occur.

Presynaptically, the effect of GABA also is to increase the cell's permeability to chloride ions. In this instance, however, there is a net efflux of chloride ions out of the cell. The resulting depolarization reduces the amplitude of successive action potentials. Since the quantity of transmitter released is proportional to the amplitude of the action potential, the net effect of presynaptic inhibition is a reduction in the release of excitatory transmitter.\textsuperscript{71}

Whereas presynaptic GABA inhibition occurs primarily in the spinal cord and lower brain stem, the postsynaptic inhibitory action of GABA is prevalent in the higher brain areas.\textsuperscript{72} This postsynaptic hyperpolarization was shown to be identical to that produced by the endogenous inhibitor, thus supporting the role of GABA as an inhibitory neurotransmitter.\textsuperscript{73,74}
Changes in chloride ion permeability are brought about through the interaction of GABA molecules with specific receptor sites on the nerve membrane. In binding with its receptors, GABA induces a change in the associated ion channels which causes ion conductivity to increase. Elucidation of the nature of GABA-receptor interactions at the biochemical level requires an understanding of GABA receptors. The individual components of GABA receptor complexes will therefore be described.

C. GABA Receptor Complexes

Designation of a neurotransmitter role for a substance requires demonstration of specific receptor sites to which the substance binds thereby effecting its biological response. Such binding sites have been identified for GABA and have been found to be "complex structures composed of a variety of independent, but interrelated sites." It is now evident that different types of GABA receptors exist and that these receptors exhibit distinct pharmacological profiles. These distinctions have provided the basis for the subclassification of GABA receptors.

GABA<sub>A</sub> Receptors

Until recently, all known GABA receptors were shown to be bicuculline-sensitive; binding to these sites in vitro was competitively inhibited by the phthalide isoquinoline alkaloid, bicuculline (8), a
known GABA antagonist. However, the discovery of bicuculline-insensitive sites has demanded a more precise classification of GABA receptors. Therefore, the term "GABA $A$ receptors" refers to those sites which are bicuculline-sensitive.

Binding to GABA $A$ receptors is a sodium-independent process involving synaptic GABA receptors located on cell bodies, dendrites and axon terminals. These receptors mediate the classical inhibitory effects of GABA (i.e., postsynaptic hyperpolarizations and presynaptic depolarizations) and so are coupled to the chloride ion channel. Stimulation of GABA $A$ receptors produces a change in chloride ion conductance (and hence, cell polarity) resulting in the inhibitory response.
GABA<sub>B</sub> Receptors

GABA<sub>B</sub> receptors have only recently been identified. These receptors, which are only located on nerve terminals of non-GABAergic neurons, are bicuculline-insensitive. Inhibitory responses resulting from GABA<sub>B</sub> receptor stimulation are due to a decrease in the release of excitatory transmitter. This action has been attributed to a modification of calcium influx.

Unlike GABA<sub>A</sub> receptors, for which both selective agonists and antagonists have been found, only the former have been identified for GABA<sub>B</sub> receptors. Baclofen (6), initially designed to be a lipophilic GABA agonist, shows no binding ability in vitro for GABA<sub>A</sub> receptor sites. However, this compound is a selective GABA<sub>B</sub> receptor agonist, being equipotent with GABA at reducing evoked transmitter output and having the same affinity as GABA for this site. Furthermore, a stereoselective activity was found with (-)-baclofen being the active enantiomer both in receptor binding and in producing neuronal depression. No selective GABA<sub>B</sub> receptor antagonists have as yet been identified.

GABA Transport Systems

Uptake into neuronal elements by way of a membrane transport system represents a mechanism for the removal of most neurotransmitters from their synaptic clefts. Such a mechanism is believed to be the predominant means by which the action of GABA is terminated following its neuronal release.
Uptake systems for GABA have been identified in synaptic endings and in glial cells. Like other neurotransmitter uptake systems, the GABA uptake systems exhibit high affinity for the substrate and are both sodium- and temperature-dependent. Though neuronal uptake is thought to serve as a terminating mechanism of GABA action, the physiological significance of GABA uptake into glia is unclear. Not all GABA-containing glial cells in the nervous system are located in the vicinity of GABA-releasing nerve terminals, suggesting that the termination of GABA action may be secondary to a presently unknown primary function of glial uptake.

Pharmacologically, the two systems are distinct. Thus, L-2,4-diaminobutyric acid (DABA) preferentially inhibits neuronal GABA uptake, whereas β-alanine is more selective at inhibiting glial uptake. Such GABA uptake inhibitors are expected to be of clinical importance, since by increasing synaptic levels of GABA these compounds would serve as indirect GABA agonists.

GABA Receptor Ionophore

As mentioned previously, GABA_A receptors are coupled to chloride ion channels pharmacologically, if not physically. It is generally believed that stimulation of these receptors by GABA agonists induces a conformational change in the receptor complex such that the ion channel opens thereby allowing for a chloride ion flux. Compounds which act either directly or indirectly on these channels can be expected to influence GABAergic activity and the behavioral responses associated with such activity.
Benzodiazepines are known to bind to specific receptor sites, some of which are associated with GABA receptor-ionophore complexes. Binding of benzodiazepines to these GABA-related sites increases the frequency of the GABA-induced opening of the chloride ion channel. Interestingly, GABA, in binding to its receptor, increases the affinity of the benzodiazepine site for benzodiazepines and as such, might be considered as an allosteric enhancer of benzodiazepine binding. This effect can be reversed by the GABA receptor antagonist, bicuculline. Clearly the interactions between GABA and benzodiazepines are complex, but such interactions are consistent with their central depressant activity.

Barbiturates also enhance GABAergic responses in vitro, though their actions are weaker and occur at sites other than the benzodiazepine site. This enhancement is likely due to the ability of these compounds to prolong the lifetime of GABA-activated open ion channels. GABA does not influence barbiturate binding, however GABA binding to rat brain synaptosomes is enhanced by barbiturates. Distinctions between the GABA-related effects of sedative-hypnotic barbiturates and anticonvulsant barbiturates (i.e., pentobarbital and phenobarbital, respectively) have also been noted. At concentrations of 200 μM, pentobarbital was more potent than phenobarbital in its ability to enhance and prolong GABA responses in vitro.

Picrotoxinin, a known GABA receptor antagonist and potent convulsant, is also thought to act at the GABA ionophore, by preventing the opening of the chloride channel following GABA-receptor activation. Binding of picrotoxinin to brain membranes was found to
be competitively inhibited by barbiturates.\textsuperscript{98} Other similarly-acting convulsants include pentylenetetrazole (\textsuperscript{10}),\textsuperscript{99} isopropylbicyclophosphate (\textsuperscript{11}),\textsuperscript{99} t-butylbicyclophosphorothionate (TBPS) (\textsuperscript{12}),\textsuperscript{100} and the convulsant benzodiazepine, Ro5-3663 (\textsuperscript{13}),\textsuperscript{101} all of which have been shown to bind to the same site as picrotoxinin.

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9 & 10 & 11
\end{tabular}
\end{center}

D. Structure-activity Relationships

In order to study structure-activity relationships of the interactions of GABA with various binding sites, numerous GABA analogues have been prepared. Design of these compounds has mainly centered around modification of three GABAergic agents, namely, GABA, muscimol and nipecotic acid. Generally, the resultant activities of these
analogue have been mixed. However, in some instances, these modifications have produced compounds which act at selective recognition sites in GABA-mediated synapses. Such compounds can potentially serve as therapeutic agents and/or as tools for investigating GABA binding and the nature of GABA receptors.

Structure-activity relationships of GABAergic compounds have been extensively reviewed. In such studies, analogues are assessed for their abilities to bind to GABA receptors and to inhibit high affinity $[^3H]^{-}$GABA uptake into rat brain slices or synaptosomes (neuronal uptake) and cultured astrocytes cells (glial uptake). Binding of analogues to GABA receptors is usually demonstrated by their displacement of $[^3H]^{-}$GABA bound to rat brain membranes, or by their inhibition of sodium-independent binding of $[^3H]^{-}$GABA to these membranes. The importance of such binding studies has been emphasized by Olsen, et al., who observed a positive correlation between this receptor binding in vitro and agonist (or antagonist) activity in vivo.

Through the incorporation of unsaturation, ring structures and combinations thereof into the GABA pharmacophore, conformationally-restricted analogues of GABA have been produced which differ markedly from GABA with respect to potency and selectivity of action. For example, unsaturation at C2-C3 of GABA gives rise to cis and trans isomers of 4-aminocrotonic acid (cis- and trans-ACA; 14 and 15).
trans-ACA (IC$_{50} = 24$ μM) inhibited GABA uptake into rat brain slices essentially as well as GABA (IC$_{50} = 20$ μM). However, this isomer had more than twice the affinity for GABA receptors (IC$_{50} = 0.14$ μM) than does GABA (IC$_{50} = 0.34$ μM). On the other hand, cis-ACA did not significantly inhibit GABA uptake and was nearly a hundred times less potent than GABA in receptor binding studies (IC$_{50} = 25$ μM).

Cyclic GABA analogues of the type 16 (n = 1-3), have also been studied in these assays (Table 1). Selective inhibition of uptake was found with the six-membered ring analogues 16a, b; neither of these compounds demonstrated significant affinity for GABA receptors in vitro. cis-3-Aminocyclohexanecarboxylic acid (16a) was more than ten times as potent as its trans counterpart 16b when assessed for its ability to inhibit uptake. No such selective activity was observed with five-membered ring analogues 16c, d.
However, the cis isomer 16c was only slightly more active than the trans isomer 16d as an inhibitor of GABA uptake. In its ability to inhibit GABA receptor binding, trans-16d was approximately forty times more potent than cis-16c. Neither of the cyclobutane analogues, 16e, f significantly inhibited GABA uptake. Both isomers however, inhibited GABA binding. In contrast to the cyclopentane series wherein the trans isomer was the more potent of the two, cis cyclobutane 16e was more potent than trans-16f in this assay.

Table 1. C2-C4 Cyclic GABA Analogues

<table>
<thead>
<tr>
<th>GABA Analogue</th>
<th>( \text{IC}_{50} (\mu\text{M}) )</th>
<th>Binding</th>
<th>Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>0.34</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>cis-3-Aminocyclohexanecarboxylic Acid (16a)</td>
<td>n.s.</td>
<td></td>
<td>85</td>
</tr>
<tr>
<td>trans-3-Aminocyclohexanecarboxylic Acid (16b)</td>
<td>n.s.</td>
<td></td>
<td>1400</td>
</tr>
<tr>
<td>cis-3-Aminocyclopentane carboxylic Acid (16c)</td>
<td>35</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>trans-3-Aminocyclopentane carboxylic Acid (16d)</td>
<td>0.8</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>cis-3-Aminocyclobutanecarboxylic Acid (16e)</td>
<td>3.1</td>
<td></td>
<td>&gt;500</td>
</tr>
<tr>
<td>trans-3-Aminocyclobutanecarboxylic Acid (16f)</td>
<td>20</td>
<td></td>
<td>n.s.</td>
</tr>
</tbody>
</table>

a) Inhibition of sodium-independent binding to rat brain membranes
b) Inhibition of sodium-dependent high affinity GABA uptake into rat brain slices
c) n.s. = not significant

Other cyclic GABA analogues, represented by structures 17\textsuperscript{113} and 18\textsuperscript{114} (n = 1-4), showed significant stereoselective activity in GABA receptor binding and uptake assays (Table 2).
In receptor binding studies, cyclohexane and cyclopentane analogues (n = 3 and 4, respectively) of these series were inactive. Of the smaller ring compounds (n = 1 and 2), the trans isomers exhibited greater affinity for GABA receptors than the corresponding cis isomers. None of the compounds in either group inhibited GABA uptake at concentrations up to 100 μM.

Table 2. C₃-C₄ and C₂-C₃ Cyclic GABA Analogues

<table>
<thead>
<tr>
<th>GABA Analogue</th>
<th>Binding (IC₅₀ [μM])&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>0.037 (0.34)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>cis-2-Aminocyclopropylacetic Acid (17a)</td>
<td>100</td>
</tr>
<tr>
<td>trans-2-Aminocyclopropylacetic Acid (17b)</td>
<td>0.7</td>
</tr>
<tr>
<td>cis-2-Aminocyclobutylacetic Acid (17c)</td>
<td>49</td>
</tr>
<tr>
<td>trans-2-Aminocyclobutylacetic Acid (17d)</td>
<td>4.4</td>
</tr>
<tr>
<td>cis-2-Aminomethylcyclopropylcarboxylic Acid (18a)</td>
<td>n.s.&lt;sup&gt;b&lt;/sup&gt; (19)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>trans-2-Aminomethylcyclopropylcarboxylic Acid (18b)</td>
<td>0.4 (0.26)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>cis-2-Aminomethylcyclobutylcarboxylic Acid (18c)</td>
<td>n.s.&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>trans-2-Aminomethylcyclobutylcarboxylic Acid (18d)</td>
<td>25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Displacement of [³H]-muscimol from rat whole brain membranes  
<sup>b</sup> n.s. = not significant  
<sup>c</sup> Inhibition of sodium-independent binding of [³H]-GABA to rat brain membranes. See ref. 116
Structure-activity relationships of compounds 18a-d support the hypothesis of Nicholson, et al.\textsuperscript{117} concerning the active receptor conformation for GABA. According to these researchers, GABA adopts a partially extended conformation in which the $\alpha$-carbon and amino group are eclipsed when binding to its receptor. Thus, active trans isomers 18b and 18d may adopt such a geometry for receptor binding. Conversely, compounds 17a-d do not support this hypothesis. Although trans isomers in this series (17b and 17d) have the greater affinity for GABA receptors, these compounds cannot adopt the proposed "active conformation." For the less active cis isomers (17a and 17c) however, such a conformation is possible. It should be noted that Nicholson, et al. based their hypothesis on results obtained from planar molecules. The overall non-planar geometries of compounds such as 17 and 18 may give rise to steric interactions at the GABA receptor not present in Nicholson's model compounds. Thus, the hypothesis for the receptor-active conformation appears to be limited to planar molecules.

Bridging of the $C_2$ carbon of GABA to its amine function leads to analogues represented by structure 19.

\[ \text{HN} - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{CO}_2\text{H} \]

\[ 19 \]

Three such compounds, isonipecotic acid (19a), isoguvacine (19b) and $\beta$-proline (19c) have selective GABAergic activities.
Isonipecotic acid (19a) and isoguvacine (19b) are selective GABA receptor agonists in vitro and in vivo; neither compound has a significant effect on GABA uptake ($IC_{50} > 5000 \mu M$). On the other hand, $\beta$-proline (19c), which has little affinity for GABA receptors in vitro, is a weak, but selective inhibitor of glial uptake. Besides being viewed as a rigid GABA analogue, 19c might also be considered as a rigid analogue of $\beta$-alanine (20), which is a known selective uptake inhibitor mainly associated with glial uptake. Compared to $\beta$-alanine ($IC_{50} = 1000 \mu M$), (±)-19c is three times as active as an inhibitor of $[^3H]$-GABA uptake into astrocytes ($IC_{50} = 320 \mu M$).
R-(-)-nipecotic acid (21a) and guvacine (22), which also contain the β-alanine pharmacophore, are among the most potent inhibitors of neuronal (IC$_{50}$ = 5 μM and 8 μM, respectively) and glial (IC$_{50}$ = 30 μM and 25 μM, respectively) GABA uptake. S-(+)-nipecotic acid (21b) also inhibits GABA uptake, but is weaker than its enantiomer in both neuronal (IC$_{50}$ = 30 μM) and glial (IC$_{50}$ = 1000 μM) uptake inhibition.

![Structural modifications of these uptake inhibitors have generally led to less active compounds.](image)

For example, ring expansion (23, 24) or contraction (19c, 25) decreases uptake inhibitory ability, with the reduction in activity being more dramatic in the former cases.

![However, stereospecific incorporation of a hydroxyl substituent at the 4-position of nipecotic acid produced a compound, whose ability to inhibit GABA uptake into astrocytes (IC$_{50}$ = 10 μM) is three times greater than that of the parent compound.](image)
Muscimol (27) is a naturally-occurring substance which exhibits potent GABA receptor agonist properties. Its ability to bind to GABA receptors in vitro ($IC_{50} = 0.006 \mu M$) exceeds that of GABA ($IC_{50} = 0.033$),\textsuperscript{103,122} as does its ability to depress neuronal firing in vivo.\textsuperscript{123} This compound can be viewed as a structural analogue of GABA in which the carboxylate group and the C\textsubscript{2} and C\textsubscript{3} methylene units have been replaced by the rigid 3-isoxazolol moiety as shown below.

\begin{center}
\begin{tikzpicture}
  \node[anchor=west] at (0,0) {27};

  \begin{scope}[xshift=2.5cm]
    \node[anchor=west] at (0,0) {GABA};

    \draw (0,0) -- (0.5,0) -- (0.5,0.5) -- (0,0);
    \draw (0,0.5) -- (0,1); \node[anchor=west] at (0.5,0.75) {$OH$};
    \draw (0,0) -- (0,-0.5); \node[anchor=west] at (0.5,-0.25) {$O$};
    \node[anchor=west] at (-0.25,-0.5) {H\textsubscript{2}N};
  \end{scope}

  \begin{scope}[xshift=-2.5cm]
    \node[anchor=west] at (0,0) {26};

    \draw (0,0) -- (0.5,0) -- (0.5,0.5) -- (0,0);
    \draw (0,0.5) -- (0,1); \node[anchor=west] at (0.5,0.75) {$OH$};
    \draw (0,0) -- (0,-0.5); \node[anchor=west] at (0.5,-0.25) {$O$};
    \node[anchor=west] at (-0.25,-0.5) {H\textsubscript{2}N};
  \end{scope}

  \draw (0,0) -- (0,1) -- (1,1) -- (1,0) -- (0,0);
\end{tikzpicture}
\end{center}

In addition to its receptor binding capacity, muscimol also inhibits both neuronal ($IC_{50} > 1000 \mu M$) and glial ($IC_{50} = 2000 \mu M$) GABA uptake but is much weaker than nipecotic acid and guvacine in these assays.\textsuperscript{119,124} Like GABA, muscimol is a substrate for the neuronal transport carrier.\textsuperscript{122}
Numerous analogues of muscimol have been assessed for GABAergic activity. Most compounds resulting from modifications in the heterocyclic ring had decreased or no activity. However, two exceptions, thiomuscimol (28) and 4,5-dihydromuscimol (29) are essentially equi-potent with muscimol in vitro and in vivo; 28 is a selective GABA receptor agonist with no significant uptake inhibitory action.

![Chemical structures](image)

Bicyclic analogues of muscimol, which restrict the free rotation of the aminomethyl side chain have also been developed in an attempt to produce compounds with greater selectivities of action. THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; 30) emerged from these studies as a specific GABA agonist, exhibiting GABA receptor affinity similar to isoguvacine (29) (IC$_{50}$ = 2.6 μM and 1.4 μM, respectively). Like isoguvacine, THIP does not inhibit neuronal GABA uptake at concentrations up to 0.5 mM.
THPO (4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol; 31), a regioisomer of THIP, has very low affinity for GABA receptors (~12,000 times less active than muscimol).108 However, this compound is a selective inhibitor of glial uptake, with four times the ability of muscimol to inhibit GABA uptake into astrocytes. The ring homologue of THPO, THAO (5,6,7,8-tetrahydro-4H-isoxazolo[4,5-c]azepin-3-ol; 32) has activity very similar to THPO. 108

\[ \text{Figure 4} \]

As a result of structure-activity relationships of GABA and GABA-related compounds, several analogues have been obtained which possess selective GABAergic activities. Figures 1-3 summarize some of the more selective-acting agonists, uptake inhibitors and antagonists. Figure 4 is a compilation of non-selective potent GABAergic agents.
Figure 1. Selective GABA Receptor Agonists

Isonipecotic Acid$^a$

Isoguvacine$^a$

Baclofen$^c$

(GABA$_B$)

P4S$^a$

DH - P4S$^a$

IAA$^b$

PMSA$^a$

THIP$^a$

Thiomuscimol$^a$

---

a) Ref. 109
b) Ref. 103
c) Ref. 85
Figure 2. Selective GABA Uptake Inhibitors
Figure 3. Selective GABA Receptor Antagonists
Figure 4. Nonselective Potent GABAergic Agents

- GABA
- 3-APS
- Muscimol
- (RS)-Dihydromuscimol
- \(\beta\)-Guanidino propionic acid
- (S)-(+)\-3-Hydroxy GABA

\[\text{Figure 4. Nonselective Potent GABAergic Agents}^{109}\]
STATEMENT OF THE PROBLEM

GABA is a flexible molecule, able to exist in an infinite number of conformations. Numerous conformationally-restricted analogues of GABA have therefore been prepared in an effort to determine the optimum conformations by which GABA interacts with its various binding sites. Results from such studies have led some investigators to the hypothesis that GABA adopts a planar, eclipsed, partially extended conformation in binding with GABA_A receptors, as illustrated by \(^{33,117}\)

This conformation is inherent in the previously described selective GABA receptor agonists, THIP, isoguvacine and isonipecotic acid, but as previously discussed there are exceptions to this generality. Compounds which contain this GABA conformation do not inhibit GABA uptake and so it is thought that the "receptor-active" conformation differs from those which GABA assumes when interacting with its transport carriers.\(^{104}\)
Few of the known selective GABA uptake inhibitors can be formally considered as structural GABA analogues. For example, nipecotic acid, cis-4-hydroxynipecotic acid and guvacine are better viewed as cyclic analogues of β-alanine or δ-valeric acid. However, one uptake inhibitor that does intrinsically contain the GABA pharmacophore is cis-ACHA (16).

The preferred solution conformation of cis-ACHA is thought to be that in which the zwitterionic centers are situated equatorially from the cyclohexane chair (16a), rather than axially 16a'.

Bowery, et al. have proposed that intercharge distance may be an important factor relating to the degree of selectivity of action of various uptake inhibitors. Thus, in its diequatorial conformation, the approximate distance between the nitrogen and oxygen atoms of cis-ACHA (neuronal uptake) is 5.6 Å. For β-alanine, a selective inhibitor of glial uptake, this intercharge distance is approximately 3.8 Å. Nipecotic acid, which inhibits both neuronal and glial uptake has a corresponding charge distance between these two values (approximately 4.5 Å).
Since neither the cyclohexane ring nor the piperidine ring constitute a conformationally-rigid system, it is important to recognize that solution conformations of analogues incorporating these ring systems do not necessarily reflect their pharmacologically-active conformations. Therefore, structure-activity relationships based upon such flexible systems may not be totally meaningful. Nonetheless, such studies can be useful for the development of selective-acting compounds.

The decahydroquinoline-5-carboxylic acids present themselves as interesting targets for studying GABAergic activity. Such compounds can be viewed as a centrally-located GABA moiety contained by six methylene units as shown by structure 34.
The four diastereomers comprising this series include structures 35-38 (Figure 5). Whereas cis decahydroquinolines 35 and 36 are flexible molecules, trans isomers 37 and 38 are rigidly fixed. Because of the steric bulk introduced by the carbon skeleton around the GABA pharmacophore, these compounds are not necessarily expected to possess the intrinsic activities of GABA receptor agonists. Nonetheless, such GABA-containing compounds may retain affinity for various GABA recognition sites and as such, could serve as GABA receptor antagonists or inhibitors of GABA uptake. The topography of the GABA pharmacophore found in diequatorial 16a (selective GABA uptake inhibitor) is mimicked in cis-36a and trans-38. Likewise, a similar correlation can be seen between diaxial conformer 16a' and cis-36b. Of particular interest is trans-38, as this structure constitutes a rigid analogue of cis-ACHA (16a).

![Chemical Structures]

Figure 5. Decahydroquinoline-5-carboxylic Acids
Previously, the syntheses\textsuperscript{131} and preliminary pharmacological evaluations\textsuperscript{131,132} of cis-35 and 36 were reported. The aim of the present research has been to synthesize the two remaining stereoisomers of this series, namely trans-37 and 38. These syntheses along with preliminary pharmacological results obtained in vitro are presented in this dissertation.
RESULTS AND DISCUSSIONS

A. Synthetic Chemistry

The previously reported syntheses of cis decahydroquinolines 35 and 36\textsuperscript{131} employed both intra- and intermolecular Diels-Alder reactions as key steps in the reaction sequences (Scheme II).

Scheme II. Synthetic Routes to cis-35 and 36

Intramolecular Diels-Alder reaction of triene 39 leads almost exclusively to cis decahydroquinoline 40, accompanied by only a trace of the corresponding trans isomer. Oppolzer and Frostle\textsuperscript{133} have observed this high stereoselectivity in similar systems and have suggested that this
is due to the allowance of a greater degree of $\pi$-orbital overlap in the transition state leading to cis decahydroquinolines than in that leading to trans ring systems (Figure 6).

![CIS TRANS Diagram](image)

Figure 6. Intramolecular Diels-Alder Transition States

Given this preference for the formation of cis-decahydroquinoline ring systems, use of intramolecular Diels-Alder methodology for trans targets 37 and 38 would require extensive chemical manipulation, such as involvement of a masked carbonyl at position 4 (see retrosynthetic analysis, Scheme III). Recognizing complications involved in the epimerization of such systems,$^{134}$ we elected to pursue other possible routes.

Scheme III. Retroanalysis of Intramolecular Diels-Alder Approach
The use of intermolecular Diels-Alder adduct 41 as a means of generating the trans-decahydroquinoline ring system was also investigated (Scheme IV). Equilibration of 41 produced an inseparable mixture (2:1) of isomers 43 and 41, respectively. Wittig reaction with this mixture afforded the corresponding homologated products 44 and 42. Hydrogenation of this isomeric mixture followed by reaction with 3,5-dinitrobenzoyl chloride gave a mixture of decahydroquinolines 45 and 46 from which trans-45 could be separated by preparative thin layer chromatography. Hydrolysis of ester and amide functionalities afforded trans-37 as a hydrochloride salt.

Scheme IV. Intermolecular Diels-Alder Route to 37
Although cyclization of 42 leading to cis-35 and 36 proceeded in good yield (ca. 66%), similar treatment of 44 was a very poor-yielding (~6%) reaction. Subsequent hydrolysis proceeded only moderately (~53%) providing a disappointingly low yield of product 37.

Because of the ineffectual applicability of Diels-Alder methodology to the preparation of trans isomers 37 and 38, an alternative entry into the trans decahydroquinoline ring system was desired. The cis decahydroquinoline ring structure can be found in a variety of natural products (for example, pumiliotoxin C and gephyrotoxins). The trans decahydroquinoline framework has also been found in nature (various lycopodium alkaloids). Examination of the chemical literature revealed few examples of syntheses of trans decahydroquinolines, especially those substituted at the 5-position.

Verhapper and Eliel have reported that partial hydrogenation of quinolines 47 in the presence of strong acid leads to selective reduction of the benzene ring. Dissolving-metal reduction of these tetrahydroquinolines, 48, using sodium in ethanol leads predominantly (90%) to trans decahydroquinolines, 49, along with approximately 10% of the corresponding cis isomers, 50. Substituted quinolines give rise to the possibility of forming additional diastereomers resulting from the induction of other chiral centers.
While application of this method to quinoline-5-carboxylic acid (47a) might lead to trans-38, lack of stereochemical control makes this sequence of little use for the synthesis of 37, in which the epimerizable carboxyl function must assume the thermodynamically less favorable axial position.

![Chemical structures](image)

A means by which both diastereomers could be stereoselectively prepared was therefore required. To this end, we chose to explore the chemistry of trans-decahydroquinoline-2,5-dione (53a), which could be obtained by reduction of known vinylogous imide, 52. The method of Shono, et al. was used to synthesize 52, which involved condensation of acrylic acid with readily available (also commercially available) cyclohexenaminone 51.
Product isolation involved successive recrystallizations from methanol. Although these investigators report a yield of 95% for this conversion, in our hands optimum yields were only on the order of 50-55% of product which was sufficiently pure for subsequent reactions. Nonetheless, this method did provide a rapid and simple preparation of vinylogous imide 52.

trans-Decahydroquinoline-2,5-dione (53a), along with cis isomer 53b, has been synthesized previously by Mamose, et al.141 (Scheme V). Thus, hydrogenation of 52 over rhodium-alumina catalyst afforded cis-fused alcohol 54 which was oxidized to cis-decahydroquinoline-2,5-dione (53b) in 47.5% overall yield. Isomerization under either acidic or basic conditions provided an equilibrium mixture (57:43) of 53a and 53b, respectively.

Scheme V. Syntheses of Decahydroquinoline-2,5-diones 53a and 53b140
In our hands, hydrogenation of 52 over 10% Pd/C in methanol containing a small amount of 10% aqueous KOH (1 ml per 100 ml MeOH) directly produced a 1.8:1 mixture of 53a and 53b, respectively, in yields typically ranging between 70-80%. This reaction had to be carefully monitored so as not to allow overreduction of the ketone. The equilibrium distribution of 53a and 53b in this mixture was quantitated by measuring the integration of the H$_{8a}$ proton in the pmr spectrum. For trans-53a in CDCl$_3$, the H$_{8a}$ proton resonance signal appears as a doublet of triplets centered at $\delta$ 3.27, whereas for cis-53b, this signal occurs as a broad singlet at $\delta$ 4.05. Trans isomer 53a could be separated from this mixture by fractional crystallization from water.

Peterson olefination$^{142}$ of trans-53a was carried out by adding this compound to a cooled solution (0°C) of two equivalents of 2-lithio-2-trimethylsilyl-1,3-dithiane$^{143}$ in dry THF. After stirring at room temperature for 24 hours, reaction workup afforded ketenedithioacetal 55 in 83% yield. Attempts to hydrolyze this
ketenedithioacetal directly to corresponding carboxylic acids 56, using either HgO/ BF₃·Et₂O¹⁴⁴ or HgO/HBF₄¹⁴⁵ were unsuccessful. Therefore, conversion of this functionality to a carboxylic acid was carried out in a stepwise fashion.

Carey and Court¹⁴⁶ have effected the reduction of ketenedithioacetals via transfer hydrogenation. These reductions, which employed trifluoracetic acid (TFA) as the proton source and triethylsilane as hydride donor, were complete in approximately 24 hours. Similar reduction of 55 proceeded at a much slower rate; however, after 8 days at r.t., yields of 57 were 95%.

Proton NMR spectroscopy was of little diagnostic value in determining stereochemistry at the 5-position of 57. The H₅ resonance signal is obscured under a 14-proton multiplet which did not lend itself to simplification by various proton decouplings. However, based
upon the well-defined reaction mechanism involving a sulfur-stabilized carbonium-ion intermediate,\textsuperscript{146} the dithianyl ring likely assumes an equatorial position following protonation (intermediate 57\textsuperscript{a}). Hydride transfer from triethylsilane then leads to 57.

\[
\text{HN} \quad \text{S} \\
\circ \quad \circ \quad \circ \\
\text{H} \quad \text{H} \quad \text{S}
\]

57\textsuperscript{a}

Reduction of lactam 57 with lithium aluminum hydride in refluxing THF afforded crude amine 58 which was converted to Cbz-protected amine 59 in an overall yield of 88\%. Dethioketalization of 59 was accomplished by the method of Vedejs and Fuchs.\textsuperscript{147} Thus, a mixture of 59, along with two equivalents each of red mercuric oxide and BF\textsubscript{3}etherate in 15\% aqueous THF was refluxed overnight to afford aldehyde 60 in 89\% yield. Jones oxidation of 60 gave carboxylic acid 61 (73\%) which was deprotected by hydrogenolysis [40 psi; 10\% Pd/C; water:THF (1:1); (HCl)] to afford trans-38 hydrochloride in 83\% yield.
For the synthesis of trans-37, we again turned to trans-decahydroquinoline-2,5-dione (53a) as starting material. Thus, Wittig reaction was carried out by adding 53a neat to a solution of two equivalents of methylenetriphenylphosphorane in dry THF followed by refluxing for 3 hours. Unlike the Peterson olefination reaction which proceeded in 83% yield, Wittig reaction with 53a produced 62 in a maximum yield of only 53%. This yield was not influenced by the length of reaction time or
the amount of ylide used. Lactam reduction followed by amine protection afforded 95% of carbamate \( 64 \) under conditions similar to those employed for the conversion of \( 57 \) to \( 59 \).

\[
\begin{align*}
\text{53a} & \quad \text{Ph}_3\text{PCH}_2^- \quad + \quad \text{THF (53\%)} \\
\text{62} & \quad \text{1. LAH, THF, } \Delta \\
\text{64} & \quad \text{2. Cbz Cl (95\%)}
\end{align*}
\]

Diborane reduction of \( 64 \), presumably from the least sterically-hindered side of the molecule (Dreiding molecular models) provided the stereocontrol necessary for the preparation of target acid \( 37 \). Thus, hydroboration of \( 64 \) produced an essentially quantitative yield of axially-substituted alcohol \( 65 \). Interestingly, the bulky reducing agent 9-BBN (9-borabicyclo [3.3.1] nonane)\(^{148}\) did not undergo reaction with exocyclic olefin \( 64 \). Confirmation of the assigned stereochemistry of \( 65 \) was obtained by its ultimate conversion to the final product \( 37 \). (Spectral analysis of \( 65 \) provided no information concerning stereochemistry at the 5-position.)
In order to effect oxidation of alcohol 65 to carboxylic acid 66 without concomitant epimerization, mild oxidative methods were investigated. Corey suggested use of pyridinium dichromate (PDC) in DMF for such conversions. However, reaction with 65 was very sluggish; after two days and excess reagent (7 equivalents), the reaction mixture contained a substantial amount of starting alcohol, along with aldehyde and three other components.

Oxidation of 65 with ruthenium tetraoxide was somewhat more successful. Thus, using the modification of Sharpless, a catalytic amount of ruthenium trichloride hydrate (2.2 mol%) was added to a biphasic solution of 65 and sodium metaperiodate in carbon tetrachloride, acetonitrile and water (2:2:3). Reaction workup after 2 hours afforded crude carboxylic acid 66. No epimerized acid product was detected in this reaction. Deprotective hydrogenolysis of 66, as carried out for epimer 61, produced trans-37 hydrochloride in an overall yield of 45.6%.
B. \[^{1}\text{H}]-\text{NMR} \text{ Spectroscopy}

The 300 MHz proton resonance spectra of cis-35 and 36 in D\(_2\)O are found in figures 7 and 8, respectively. Resonance signal assignments were previously discussed in detail.\(^{131}\) The 300 MHz proton resonance spectrum of trans-38 is shown in figure 9. Axial proton H\(_5\) is strongly coupled to two adjacent axial protons and one equatorial proton. Hence, its resonance signal appears as an expected triplet (\(J \approx 11.2\) Hz) of doublets (\(J \approx 3.4\) Hz) centered at \(\delta \) 2.06. A similar pattern was observed for the resonance signal of H\(_{8a}\) which also results primarily from two diaxial couplings and one axial-equatorial coupling. This signal appears at \(\delta \) 2.75 and is partially overlapped by the H\(_{2a}\) resonance signal. The furthermost downfield signal (\(\delta \approx 3.20\)) was assigned to the equatorial proton H\(_{2e}\) resonance signal. The observed doublet, attributed to geminal coupling (\(J \approx 12.8\) Hz), exhibits further slight splitting and shoulders indicative of quartets. However, these couplings, owing to equatorial-axial and diequatorial interactions were too small to be accurately measured.

Figure 10 depicts the 300 MHz proton resonance spectrum of trans-37. Chemical shifts and observed coupling constants of the resonance signals assigned to H\(_{2e}\) and H\(_{2a}\) are essentially the same as those of trans-38. However, H\(_{8a}\), presumably deshielded by the axial carboxylate group, is downfield (\(\delta \approx 3.39\)) relative to the H\(_{8a}\) signal in trans-38. The resonance signal assigned to H\(_5\) having an equatorial conformation also is downfield (\(\delta \approx 2.68\)) relative to the H\(_5\) axial proton signal in trans-38. This broadened singlet for the H\(_5\) resonance signal in trans-37 reflects relatively smaller equatorial-axial and diequatorial couplings.
Figure 7. $[^1H]$-NMR Spectrum (300 MHz) of cis-35
Figure 8. $[^1H]$-NMR Spectrum (300 MHz) of cis-36
Figure 9. \(^{1}\text{H}\text{-NMR Spectrum (300 MHz)}\) of trans-38
Figure 10. $[^1\text{H}]$-NMR Spectrum (300 MHz) of trans-37
C. Preliminary Pharmacological Evaluation In Vitro

Cis isomers 35 and 36 had previously been assessed for GABA receptor affinity and for their ability to inhibit sodium-dependent GABA uptake into rat brain synaptosomes. These compounds have been re-examined in these and other assays in vitro, along with trans isomers 37 and 38.

Compounds 35-37 displayed no significant affinity for GABA receptors; at concentrations of 100 μM, these isomers inhibited binding of [3H]-GABA to rat brain membranes less than 10%. At this same concentration, trans-38 inhibited 43% of this binding. However, compared to bicuculline (IC50 = 5-10 μM) this receptor affinity is weak.

Since GABA receptor agonists are known to enhance benzodiazepine binding to brain membranes, as previously discussed, these compounds were also examined for such activity. None of the four isomers, 35-38, influenced either benzodiazepine binding or GABA-activated benzodiazepine binding at concentrations up to 100 μM.

When assessed for GABA receptor affinity, compounds 35-38 displayed no significant binding ability. Inhibition of [3H]-GABA binding to these receptors was less than 20% at concentrations of 100 μM.

These compounds also have little affinity for GABA transport carriers as no significant inhibition of GABA uptake was observed at concentrations up to 100 μM. In addition, isomers 35-38 had no affinity for the [35S]-TBPS binding site, suggesting that these compounds do not interact with the picrotoxinin site.
Preliminary studies examining the effects of cis-35 and 36 in vivo have also been reported. When injected directly into the substantia nigra, these isomers elicit CNS excitatory behavior in mice, as evidenced by the induction of convulsions and even death in the case of cis-35. These excitatory effects, like those of picrotoxinin, are completely reversed by the indirect GABA agonist, valproic acid, suggesting that these compounds interfere with some aspect of GABA-mediated transmission in vivo.

As previously mentioned, a positive correlation between receptor binding in vitro and activity in vivo has been demonstrated. Considering the poor binding activities of isomers 35 and 36 in vitro, it does not seem likely that these compounds are acting as antagonists of GABA receptors, GABA uptake or the chloride-ion channel in vivo. It is conceivable that the CNS excitatory responses observed in vivo could reflect a decrease in GABA release or synthesis or an interaction with a subset of GABA receptors not yet examined.

Trans isomers 37 and 38 display activity in vitro comparable to cis isomers 35 and 36, although trans-38 has weak GABA_A receptor affinity. Examination of the effects of trans-37 and 38 in vivo are currently underway.
EXPERIMENTAL

Melting points were determined in open capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Infrared spectra were recorded with a Beckman model 4230 spectrophotometer. Nuclear magnetic resonance spectra were recorded using either a Bruker WP-80, HX-90E or 300 MHz spectrophotometer. TMS (CDCl₃, DMSO) was used as internal standard. Chemical shifts are reported on the δ scale with peak multiplicities: br, broad; d, doublet; dd, doublet of doublets; ddd, doublet (doublet of doublets); dt, doublet of triplets; m, multiplet; q, quartet; s, singlet; t, triplet; and td, triplet of doublets. Mass spectra were recorded with a DuPont model 21-491 mass spectrometer with a model 21-094 data system. High resolution mass spectra were obtained with a Kratos MS-30 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.
4,6,7,8-Tetrahydro-2,5(1H,3H)-quinolinedione (52). The method of Shono, et al.\textsuperscript{139} was employed for the synthesis of 52. Thus, a mixture of 3-amino-2-cyclohexen-1-one (1.11 g; 0.01 mol) and acrylic acid (1.08 g; 0.015 mol) was slowly heated to 140°C with stirring under N\textsubscript{2}. Stirring was maintained for 3 h after which time the reaction mixture solidified. The solid was recrystallized 3 times from MeOH to afford 892 mg (54\%) of 10 as a very lightly colored green-yellow solid mp 198-200°C (Lit\textsuperscript{138} mp 200-201°C) (Lit\textsuperscript{139} mp 194-195°C); IR (KBr) 3200, 3120, 1695, 1635, 820 cm\textsuperscript{-1}; m/e 165 (M\textsuperscript{+}).

trans-Hexahydro-2,5(1H,3H)-quinolinedione (53a). A solution containing 52 (1.0 g; 6.06 mmol), 10\% aq. KOH solution (1 ml) and MeOH (100 ml) was hydrogenated over 300 mg of 10\% Pd/C at r.t. and 40 psi for 2 h. The solution was acidified to pH 2 with 10\% HCl solution, filtered and concentrated under reduced pressure to yield a solid. CHCl\textsubscript{3} (150 ml) was added, the mixture shaken and passed through a small plug of Celite. The CHCl\textsubscript{3} was removed under reduced pressure producing a white solid which was washed with Me\textsubscript{2}CO to afford 828 mg (81.8\%) of a mixture (1.8:1.0) of 53a and 53b, respectively. Isomer 53a was separated by fractional crystallization (H\textsubscript{2}O) affording colorless needles mp 229-230°C dec.; IR (KBr) 3190, 3060, 1710, 1650, 830 cm\textsuperscript{-1}; NMR (CDCl\textsubscript{3}, 90 MHz) \delta 1.2-2.7 (m, 11H), 3.27 (dt, 1H, J = 3.3 and 10.5 Hz, 8a-H), 7.0 (br.s, 1H, NH) m/e 167 (M\textsuperscript{+}). Anal. calcd for C\textsubscript{9}H\textsubscript{13}N\textsubscript{2}: C, 64.67; H, 7.78; N, 8.38. Found: C, 64.42; H, 7.98; N, 8.40.

trans-5-(1,3-Dithian-2-ylidene)octahydro-2(1H)-quinolinone (55). To an ice-cooled solution of 2-trimethylsilyl-1,3-dithiane (1.15 g; 0.006 mol) in dry THF (25 ml) was added n-BuLi [1.55 M in hexane (3.75...
mL, 0.006 mol). After stirring for 1/2 h at 0°C, solid \(53a\) (0.500 g; 0.003 mol) was added. The mixture was allowed to warm to r.t. and stirring was continued for 36 h after which time the reaction mixture was quenched with \(H_2O\) (25 mL) and extracted with \(CHCl_3\). The organic phase was washed with brine, dried \((MgSO_4)\), filtered and concentrated under reduced pressure affording a solid/oil mixture. This mixture was washed with hexane affording 672 mg (83.5%) of \(55\) as white needles \((EtOH)\) mp 222-229°C dec.; IR \((KBr)\) 3180, 3040, 2920, 2850, 1645 cm\(^{-1}\); NMR \((CDCl_3, 90 MHz)\) \(\delta 1.2-2.7\) (m, 14H), 2.7-3.1 [m, 4H, 2(CH\(_2\)S)], 3.35 (dt, 1H, \(J \approx 3.7\) and 10.5 Hz, 8a-H), 5.95 (br.s, 1H, NH) m/e 269 \((M^+)\). Anal. calcd for \(C_{13}H_{19}NOS_2\): C, 57.99; H, 7.06; N, 5.20; S, 23.79. Found: C, 58.14; H, 7.22; N, 5.21; S, 23.54.

\((4aa,5a,8ga)-5-(1,3-Dithian-2-yl)octahydro-2(1H)-quinolinone (57).\)

A solution of \(55\) (925 mg; 3.44 mmol), triethylsilane (1 mL) and trifluoroacetic acid (2.0 mL) in \(CH_2Cl_2\) (10 mL) was stirred at r.t. for 8 days. The reaction mixture was made alkaline with saturated aq. \(NaHCO_3\) solution and extracted with \(CH_2Cl_2\). The organic layer was dried \((CaSO_4)\), filtered and concentrated under reduced pressure affording 890 mg (95.4%) of \(57\) as fine white needles \((EtOH)\) mp 222-223°C; IR \((KBr)\) 3150, 3030, 2900, 1640 cm\(^{-1}\); NMR \((CDCl_3, 90 MHz)\) \(\delta 1.2-2.6\) (m, 14H), 2.7-3.2 [m, 5H, 8a-H, 2(CH\(_2\)S)], 4.38 (s, 1H, S-CH-S), 5.92 (br.s, 1H, NH) m/e 271 \((M^+)\). Anal. calcd for \(C_{13}H_{21}NOS_2\): C, 57.56; H, 7.75; N, 5.16; S, 23.62. Found: C, 57.75; H, 7.87; N, 5.14; S, 23.29.

\(Phenylmethyl-(4aa,5a,8ga)-5-(1,3-Dithian-2-yl)octahydro-1(2H)-quinolinecarboxylate (59).\) A mixture of \(57\) (900 mg; 3.32 mmol) and \(LiAlH_4\) (505 mg; 13.28 mmol) in 100 mL of dry THF was refluxed under \(N_2\).
After 20 h the reaction mixture was cooled to r.t., carefully quenched with Na₂SO₄·10H₂O and filtered. The filtered aluminum salts were washed with hot THF and the combined filtrates were concentrated under reduced pressure to afford 898 mg of a viscous oil containing (4aa,5a,8aa)-5-(1,3-Dithian-2-yl)decahydroquinoline (58). Crude 58 was taken up in 25 ml of THF to which 15 ml of 10% aq. KOH solution subsequently was added. Benzyl chloroformate (850 mg; 4.98 mmol) was then added to this biphasic solution which was vigorously stirred for 2 h. The mixture was acidified with 10% HCl, extracted with CH₂Cl₂, washed with brine, dried (MgSO₄) and concentrated under reduced pressure affording 2.3 g of a light yellow oil. The oil was chromatographed on silica gel (column) by elution with CHCl₃ yielding 1.14 g (88.1%) of 59 as a viscous, undistillable, colorless oil; IR (Neat) 2930, 2890, 2855, 1690, 750, 695 cm⁻¹; NMR (CDCl₃, 90 MHz) δ 0.8-2.3 (m, 14H), 2.7-3.0 [m, 4H, 2(S-CH₂)], 3.1-3.4 (m, 2H, 2ax-H), 3.4-3.7 (m, 2H, 2eq-H), 3.73 (observed center) (ddd, 1H, J = 3.5, 6.2 and 13.8 Hz, 2eq-H), 4.36 (s, 1H, S-CH-S), 5.12 (s, 2H, OCH₂), 7.34 (s, 5H, phenyl) m/e 391 (M⁺). Anal. calcd for C₂₁H₂₉NO₂S₂: C, 64.45; H, 7.42; N, 3.58; S, 16.37. Found: C, 64.31; H, 7.85; N, 3.45; S, 16.29.

Phenylmethyl-(4aa,5a,8aa)-5-formylocta hydro-1(2H)-quinolinecarboxylate (60). To a vigorously stirred suspension of red HgO (445 mg, 2.046 mmol) and BF₃·Et₂O (290 mg, 2.046 mmol) in 15% aq. THF (10 ml) was added a solution of 59 (400 mg, 1.023 mmol) in 2 ml of THF. After refluxing for 0.5 h the HgO dissolved and the reaction mixture turned colorless. Refluxing was continued for 24 h during which time a grey precipitate formed. The reaction mixture was cooled to r.t. and Et₂O
(10 ml) was added. The coagulated precipitate was triturated, washed with Et₂O and the combined washings were concentrated to a volume of 5 ml. H₂O (10 ml) was added and the mixture was extracted with CHCl₃, dried (MgSO₄) and concentrated under reduced pressure to afford 290 mg of a colorless oil. The oil was chromatographed on silica gel (column) by elution with CHCl₃ affording 275 mg (89.3%) of 60 as a colorless oil bp 115°C; IR (Neat) 2930, 2855, 2700, 1720, 1690, 745, 690 cm⁻¹; NMR (CDCl₃, 90 MHz) δ 0.8-2.3 (m, 12H), 3.18 (dt, 1H, J = 3.2 and 10.3 Hz, 8a-H), 3.3-3.8 (m, 2H, N-CH₂), 5.13 (s, 2H, OCH₂), 7.34 (s, 5H, phenyl), 9.56 (d, 1H, J = 3.2 Hz, CHO). Anal. calcd for C₁₇H₂₃NO₃: C, 71.76; H, 7.64; N, 4.65. Found: C, 71.63; H, 7.56; N, 4.65.

(4αα,5αα,8αα)-Octahydro-1,5(2H)-quinolinedicarboxylic Acid-1-phenylmethyl Ester (61). To a cooled solution (ice-bath) of 60 (175 mg; 0.581 mmol) in Me₂CO (15 ml) was added Jones reagent (diluted to 1.25 M Cr⁶⁺) dropwise until the orange color of the reagent persisted. After stirring for an additional 0.5 h, the excess chromic acid was quenched with i-PrOH and the solution decanted. The chromium salts were triturated with Me₂CO (5 times) and the combined washings were concentrated under reduced pressure. The residue was taken up in a saturated Na₂CO₃ solution and extracted with Et₂O. The aqueous layer was acidified with dilute H₂SO₄ solution and extracted with CHCl₃. The CHCl₃ layer was dried (MgSO₄) and concentrated under reduced pressure to afford 161 mg of a colorless oil which solidified on standing overnight. Recrystallization (Me₂CO/hexane) yielded 135 mg (73.2%) of 61 as fine colorless plates mp 124-125°C; IR (KBr) 3400-2400, 1700, 1680, 740, 695 cm⁻¹; NMR (CDCl₃, 90 MHz) δ 1.0-2.3 (m, 12H), 3.0-3.5 (m, 2H,
8a-H, 2ax-H), 3.7 (observed center) (ddd, 1H, J = 3.3, 6.0 and 13.5 Hz, 2eq-H), 5.13 (s, 2H, OCH₂), 7.34 (s, 5H, phenyl), 9.0 (br.s, 1H, CO₂H) m/e 317 (M⁺). Anal. calcd for C₁₈H₂₃NO₄: C, 68.14; H, 7.26; N, 4.42. Found: C, 68.24; H, 7.46; N, 4.50.

(4aa,5a,8aa)-Decahydroquinoline-5-carboxylic Acid (trans-38) Hydrochloride. A solution of 61 (375 mg; 1.183 mmol) in 60 ml of THF/H₂O (1:1) containing 0.4 ml of 10% HCl solution was hydrogenated over 125 mg of 10% Pd/C at r.t. and 40 psi for 2 h. After filtration, the solvent was removed under reduced pressure and the residue was dissolved in MeOH. The solution was passed through a small plug of Celite and concentrated. The residual semi-solid was recrystallized from MeOH/Et₂O affording 216 mg (83.2%) of 38 as white crystals mp 275-280°C dec.; IR (KBr) 3300-2300, 1730, 1560 cm⁻¹; NMR (D₂O, 300 MHz) δ 1.0-1.8 (m, 11H), 2.06 (dt, 1H, J = 3.4 and 11.2 Hz, 5-H), 2.6-2.9 (m, 2H, 8a-H, 2ax-H), 3.20 (br.d, 1H, J = 12.8 Hz, 2eq-H), with 6.30 (s, HOD). Anal. calcd for C₁₀H₁₆ClNO₂: C, 54.67; H, 8.20; Cl, 16.17; N, 6.38. Found: C, 54.30; H, 8.30; Cl, 16.24; N, 6.20.

trans-Octahydro-5-methylene-2(1H)-quinolinone (62). To a suspension of methyl triphenylphosphonium iodide (2.420 g, 5.988 mmol) in 50 ml of THF was added 1.6 M n-BuLi (3.75 ml; 5.988 mmol). The resulting solution was stirred under N₂ at r.t. for 4 h. Solid 53a (500 mg; 2.994 mmol) was added and the mixture refluxed for 3 h. After cooling to r.t., H₂O (25 ml) was added and the solution extracted with Et₂O, dried (MgSO₄) and concentrated under reduced pressure affording 1.23 g of a yellow solid/oil mixture. Purification by flash chromatography using EtOAc as eluent afforded 264 mg (53.4%) of 62 as white needles
(CHCl₅/hexane) mp 154.5-156°C; IR (KBr) 3190, 3080, 3060, 2940, 2870, 1690, 1660 cm⁻¹; NMR (CDCl₃, 90 MHz) δ 1.0-2.6 (m, 11H), 2.95 (dt, 1H, J = 3.3 and 10.0 Hz, 8a-H), 4.75 [d, with small (~1 Hz) allylic coupling 2H, J = 13.0 Hz, vinyl H], 6.06 (br.s, 1H, NH) m/e 165 (M⁺).

Phenylmethyl-trans-octahydro-5-methylene-1(2H)-quinolinecarboxylate (64). A mixture of 63 (260 mg, 1.576 mmol) and LiAlH₄ (240 mg, 6.30 mmol) in 50 ml of dry THF was refluxed for 5 h under N₂. After cooling to r.t., the reaction mixture was carefully quenched with Na₂S₂O₄•10H₂O and filtered. The aluminum salts were washed with hot THF and the combined filtrates were concentrated under reduced pressure affording 240 mg of a solid containing trans-Decahydro-5-methylenequinoline (63). Crude 63 was dissolved in 10 ml of THF followed by 2 ml of 10% eq. KOH solution. Benzylchloroformate (540 mg, 3.15 mmol) was then added and the resulting biphasic solution was stirred vigorously for 1 h. H₂O (10 ml) was added and the mixture extracted with Et₂O. The organic layer was washed with 10% HCl solution, dried (MgSO₄) and concentrated under reduced pressure to give a colorless oil which was chromatographed on silica gel (column) by elution with CHCl₃ yielding 428 mg (95.3%) of 64 as a colorless oil; IR (Neat) 2940, 2865, 1700, 1650, 700 cm⁻¹; NMR (CDCl₃, 90 MHz) δ 1.1-2.5 (m, 11H), 3.0-3.5 (m, 2H, 2ax-H, 8a-H), 3.80 (observed center) (ddd, 1H, J = 2.5, 6.5 and 13.5 Hz, 2eq-H), 4.62 [d, with small (~1 Hz) allylic coupling, 2H, J = 16.5 Hz, vinyl H], 5.13 (s, 2H, 0-CH₂), 7.33 (s, 5H, phenyl) m/e 285 (M⁺).
Phenylmethyl-(4αα,5β,8αβ)-octahydro-5-hydroxymethyl-1(2H)-quinolinecarboxylate (65). To a cooled (dry ice) solution of 64 (380 mg, 1.33 mmol) in dry THF (20 ml) was added B$_2$H$_6$ [0.98 M in THF (2 ml; 1.96 mmol)]. The mixture was allowed to warm to r.t. and stirring was maintained for 1 h after which time 6M NaOH solution (5 ml) was slowly added. H$_2$O$_2$ (30%; 3.5 ml) was added and the resulting solution was stirred vigorously overnight. K$_2$CO$_3$ was added to saturate the aqueous phase and the mixture was extracted with Et$_2$O. The combined Et$_2$O extracts were dried (MgSO$_4$) and concentrated under reduced pressure to afford 436 mg of a colorless oil. Purification by flash chromatography using CHCl$_3$ as eluent gave 400 mg (99%) of 65 as a colorless, viscous oil; IR (Neat) 3600-3100, 2930, 2870, 1680, 700 cm$^{-1}$; NMR (CDCl$_3$, 90 MHz) δ 1.0-2.2 (m, 12H), 2.8-4.0 (m, 6H), 5.12 (s, 2H, CH$_2$Ph), 7.34 (s, 5H, phenyl); m/e calcd (M$^+$) 303.1834, obsd 303.1856.

(4αα,5β,8αβ)-Octahydro-1,5(2H)-quinolinedicarboxylic Acid-1-phenylmethyl Ester (66). To a biphasic mixture of 65 (103 mg; 0.340 mmol) and NaIO$_4$ (255 mg; 1.190 mmol)$^{151}$ in CCl$_4$ (1.5 ml), CH$_3$CN (1.5 ml) and H$_2$O (2.25 ml) was added RuCl$_3$·3H$_2$O (2 mg; 2.2 mol%). After stirring vigorously for 2 h, CH$_2$Cl$_2$ (10 ml) was added and the solvent layers were separated. The aqueous layer was extracted (3 times) with CH$_2$Cl$_2$ and the combined organic extracts were dried (MgSO$_4$) and concentrated. The residue was dissolved in Et$_2$O, passed through a small plug of Celite and concentrated under reduced pressure affording 99 mg of an
oil. Purification by preparative TLC (CHCl₃/HOAc, 100:2) produced 77 mg of crude 66 as a viscous oil which was used directly without further purification.

(4αα,5β,8αβ)-Decahydroquinoline-5-carboxylic Acid (trans-37) Hydrochloride. A solution of 66 (77 mg; ~0.24 mmol) in 20 ml of THF/H₂O (1:1) containing 0.020 ml of conc. HCl solution was hydrogenated over 25 mg of 10% Pd/C at r.t. and 40 psi for 2 h. After filtration, the solvent was removed under reduced pressure and the residue was dissolved in a minimal amount of MeOH. This solution was passed through a small plug of Celite and concentrated. The residual semi-solid was recrystallized from MeOH/Me₂CO affording 34 mg (45.6%) of trans-37 as white crystals, mp 315-320°C dec.; IR (KBr) 3400-2200, 1720, 1590 cm⁻¹; NMR (D₂O, 300 MHz) δ 1.1-1.9 (m, 11H), 2.68 (br.s, 1H, 5-H), 2.79 (br.dt, 1H, Jea = 2.5 Hz, Jgem = Jaa = 12-13 Hz, 2ax-H), 3.19 (d, 1H, Jgem = 12.3 Hz, 2eq-H), 3.39 (br.dt, 1H, J = 3.4 and 11.4 Hz, 8a-H) with 4.30 (HOD). Anal. calcd for C₁₀H₁₈ClNO₂: C, 54.67; H, 8.20; Cl, 16.17; N, 6.38. Found: C, 54.86; H, 8.44; N, 6.37; Cl, 15.94.
BIBLIOGRAPHY


114. Ibid, 2563.


135. K. Tomita, unpublished results.


138. Ibid., 2734.


151. Substitution of HIO₆ for NaIO₄ led to a reduced reaction time (15 minutes) but did not affect the overall yield of this transformation.

152. We gratefully acknowledge Professor S. J. Enna for carrying out these pharmacological studies.
Appendix A - Reference 131

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Epimeric cis-Decahydroquinoline-5-carboxylic Acids: Effects on γ-Aminobutyric Acid Uptake and Receptor Binding in Vitro

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Houston, Texas 77025. Received January 19, 1981

The syntheses for two cis-decahydroquinoline-5-carboxylic acid epimers (1 and 2) which contain the α-aminobutyric acid (GABA) moiety [=N(CH)₂CO₂H]. While it is possible that these stereoisomers may serve as probes to study GABA-mediated neurotransmission, it would not be expected that such structures should have the intrinsic activity of a GABA agonist. cis-Decahydroquinoline epimers 1 and 2 are conformationally flexible, whereas trans isomers 3 and 4 are rigidly fixed. In the present report the syntheses for cis-1 and -2 are described, and evidence for their preferred solution conformations as structures 1a and 2a is presented. Additionally, preliminary biochemical data are discussed con-

The four diastereomers (1-4) of decahydroquinoline-5-

carboxylic acid are twisterionic species* which contain a γ-aminobutyric acid (GABA) moiety [=N(CH)₂CO₂H]. While it is possible that these stereoisomers may serve as probes to study GABA-mediated neurotransmission, it would not be expected that such structures should have the intrinsic activity of a GABA agonist. cis-Decahydroquinoline epimers 1 and 2 are conformationally flexible, whereas trans isomers 3 and 4 are rigidly fixed. In the present report the syntheses for cis-1 and -2 are described, and evidence for their preferred solution conformations as structures 1a and 2a is presented. Additionally, preliminary biochemical data are discussed con-


(2) Compounds were studied as their hydrochloride salts. Cyclic compounds were named according to Chemical Abstracts® convention wherein the substituent at the lowest carbon number (i.e., 4a in bicyclic compounds) is arbitrarily assigned the α notation and all other substituents are defined as α or β relative to this position.


Scheme I

![Scheme I](image)

Epiceric cis-Decahydroquinoline-5-carboxylic Acids


Scheme III

22 was converted to 24 via oxidation (pyridinium chlorochromate) to 25, followed by decarboxylation using triis(triphenylphosphine)rhodium(III) chloride. Alternatively, cis-1 was prepared by intermolecular [4 + 2] cycloaddition (Scheme II). Dienamide 7 underwent reaction with methyl 4-oxo-2(E)-hexenoate (8), affording 10 in approximately 65% isolated yield. Acid 21 was prepared by reaction of glycodehyde (25) with methyl (triphenylphosphoranylidene)acetate, followed by pyridinium chlorochromate oxidation of intermediate methyl 4-hydroxyacetone (26) (Scheme III). Compound 8 also

[12] Reduced yields were attributed to concurrent formation of substantial quantities of methyl 3-epicycroplaccitate.

(12) Reduced yields were attributed to concurrent formation of substantial quantities of methyl 3-epicycloplaccitate.
Table I. Effect of cis-1 HCl and cis-2 HCl on [3H]GABA Receptor Binding to Rat Brain Membranes

<table>
<thead>
<tr>
<th>compd</th>
<th>concn, µM</th>
<th>% displacement of specifically bound [3H]GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>0.01</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>muscimol</td>
<td>0.01</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>cis 1 HCl</td>
<td>100</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>cis 2 HCl</td>
<td>100</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

*Each value is the mean plus or minus the SEM of four to six separate determinations. The compounds were analyzed using a previously reported in vitro ligand binding assay for GABA receptors. Specifically (receptor) bound [3H]GABA was defined as the amount of isotope displaced from the brain membrane preparation by a saturating (1 mM) concentration of unlabeled GABA.

was prepared by the less expensive SeO₂ oxidation of methyl crotonate. Reaction of 7 with dimethyl 8 in refluxing toluene for 3 h afforded 10 in a yield similar to the one reported by Overman et al. for the preparation of 22 under similar conditions. Intermediate 22 also had been prepared in refluxing toluene. Wittig reaction using 10 and formaldehyde trimethylphosphoramide in toluene afforded 29 in 92% yield. Catalytic hydrogenation of 29 over Pd/C in MeOH afforded 30. Reaction of crude 30 with ethyl chloroformate yielded 31 in 46% overall yield. Reaction of 31 at -70 °C in THF using LDA afforded a mixture of 31 and 32 in a ratio of approximately 1:2. Epimer 33 was separated as pure colorless crystals, which were hydrolyzed to cis-2-HCl in 72% yield. Alkaline hydrolysis of 31 afforded 34 in 83% yield. Acid-catalyzed hydrolysis yielded 66% of cis-1-HCl. Alternatively, hydrolysis of 29 over Pd/C in MeOH containing 1 equiv of HOAc afforded crystalline 32 in 66% yield, which also served as a source of cis-1-HCl.

1H NMR Spectroscopy, Characterization and Solution Conformations of Analogues. Cycloadduct 5a was characterized by analysis of its 1H NMR spectrum and comparison with spectra of the reduced analogues 31, 34, and cis-1-HCl. Using standard synthetic reactions, 31 was converted to 32 identical in all respects with the compound prepared from cis-decyldihydroquinoline 39. Compound 32 showed the expected vinyl proton resonance signals at δ 5.36 and 5.76 with J = 11 Hz. The tentatively assigned allylic methine proton resonance signal at δ 4.77 exhibited a half-height width of 12 Hz. Hydrogenation of 32 over 10% Pd/C afforded deoxydihydroquinoline 31 in 92% yield. The 1H NMR spectrum for 31 was characteristic for the N-carboxylic cis-hydropyridine system (i.e., 38). Resonance signals and splitting patterns for H₃, H₄, and H₅ in 31 were virtually identical with those found in 38. Assuming a preferred equatorial conformation (31-38) of Et₂OCN⁻ to ring A, the broad singlet at δ 2.50 for 31 with half-height width of 7.0 Hz could readily be assigned to H₄. Acid 34 exhibited a 1H NMR spectrum closely related to enter 31 and could be obtained free of epimer by selective crystallization. Reaction of 34 with diazomethane yielded 35. Spectral data from benzyl carbamate derivative 35 further served to support structural assignments.

The 1H NMR spectrum of cis-1-HCl, obtained by acid-catalyzed hydrolysis of 34, exhibited characteristic proton resonance signals for H₃, similar to those observed in an authentic sample of 39. Analogues such as cis-1-HCl and 39 have been shown to exist mainly in that conformation wherein the NH function is axial to ring A(1a). In this case, H₃ is axial and strongly coupled to two adjacent axial protons. Thus, the resonance signal appears as the expected triplet (J = 10 Hz) of doublets (J = 4 Hz) at δ 2.56.

Compound 38, derived from authentic 39 by reaction with ethyl chloroformate, was identical in all respects with 35 prepared from 31 as follows: Reduction (DBAL) of 31 afforded hydroxymethyl derivative 36 in 85% yield. Oxidation (pyridinium chlorochromate) followed by de-carboxylation (Wilkinson's catalyst) afforded 37 (76% yield) and 38 (54% yield), respectively. For cis-2-HCl, the H3 resonance signal appeared as a doublet (J = 13 Hz) of triplets (J = 4 Hz) at δ 2.5, but the multiplet is qualitatively different than the H3 resonance signal for cis-1-HCl, since H3 is coupled to two equatorial and one axial proton rather than two axial and one equatorial proton as for cis-1-HCl. Clearly, 2b has no opportunity for 1,2-diaxial coupling with H3. The H3 resonance signal at δ 3.5 in cis-2-HCl is also a doublet (J = 12 Hz) of triplets (J = 4 Hz) having a qualitatively different multiplet than cis-2-HCl owing to axial rather than equatorial coupling. Thus, the preferred solution conformation for cis-2-HCl is 2a.

Pharmacological Results In Vitro. The effects of the HCl salts of cis-1 and cis-2 on [3H]GABA receptor binding to rat brain membranes are compared to GABA and muscimol in Table I. These stereoisomers are virtually inactive relative to the known GABA receptor agonists. However, some stereoselective activity was observed when these analogues were assessed for their ability to inhibit high-affinity [3H]GABA uptake into rat brain synaptosomes (Table II). Whereas cis-1 is inactive, cis-2 inhibited [3H]GABA uptake by approximately 20% at a concentration of 100 µM, making it over 10 times less potent than...
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GABA and nipeotic acid in this regard.

Discussion

Introduction of six lipophilic methylene groups into the GABA pharmacophore afforded stereoisomeric decahydroquinoline zwitters that rendered cis-1 inactive in vitro and epimer cis-2 a weak inhibitor of GABA uptake. Neither cis-1 nor 2 have appreciable affinity for GABA receptors in rat brain membranes. Smiseman et al. similarly observed that lipophilic trans-decalin analogues incorporating the acetylene moiety decreased muscarinic activity, with the 2,5-bisaxial isomer being most potent, but 100 times less active than acetylcholine as an agonist on the guinea pig ileum. Mays and Trigg have suggested that a newly induced activity for the trans-decalin analogues may not be due to a "failure to reproduce the geometry of receptor-bound acetylcholine, but to the incorporation of additional potential binding sites, specifically the large hydrocarbon skeleton." The high muscarinic potency of the trans-cyclopropyl analogue of acetylcholine supports this proposal. In the GABA series, trans-2-(aminomethyl)cyclobutanecarboxylic acid was approximately 15,000 times less potent than GABA for rat brain membrane receptors. Insertion of CH₂ groups into GABA or construction of cyclopentane stereoisomers also markedly reduced the relative affinity of these agents for sodium-independent receptor binding sites, whereas the trans-(aminomethyl)cyclobutanecarboxylic analogue closely approximated GABA in these assays.

In addition to altering potency, structural modification may markedly alter the mechanism of action of a compound. cis-2·HCl blocks GABA uptake and is conceivable that it may also cause a release of GABA from storage sites in certain cholinomimetic analogues release acetylcholine. Whereas it is possible that the relative differences in uptake inhibition observed between 1 and 2 will provide clues for further drug development, stereoselectivity in itself does not imply biological specificity. Clearly, further pharmacological testing will be necessary to establish this point. Since these species are zwittrions, future plans are to study all four isomers by direct-administration into the nucleus accumbens and substantia nigra (reticularis) using methodology described by Kuruvilla and Uretsky. Conformation 2a may be preferred for GABA uptake inhibition activity. Whereas the solution conformation for cis-1 favours 1a in D₂O, interaction of the axial NH with a receptor protein function may favor conformation 1b. Acylation of N in 39 favours such a conformational flip.


(31) Discussion using trans-3 and trans-4 having topographies of pharmacophores related to conformations 1b and 2a, respectively, are desired to further correlate stereosteric-activity relationships with inhibition of GABA uptake. Depending upon the results, resolved isomers and nonsteremic prodrugs will be prepared. It is possible that it may also cause a release of GABA from storage sites as certain cholinomimetic analogues release acetylcholine. Whereas it is possible that the relative differences in uptake inhibition observed between 1 and 2 will provide clues for further drug development, stereoselectivity in itself does not imply biological specificity. Clearly, further pharmacological testing will be necessary to establish this point. Since these species are zwittrions, future plans are to study all four isomers by direct-administration into the nucleus accumbens and substantia nigra (reticularis) using methodology described by Kuruvilla and Uretsky. Conformation 2a may be preferred for GABA uptake inhibition activity. Whereas the solution conformation for cis-1 favours 1a in D₂O, interaction of the axial NH with a receptor protein function may favor conformation 1b. Acylation of N in 39 favours such a conformational flip.

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The benzene (14 mg) was heated at 70°C in 15 mL of BuOH containing 2 mL of 0.5% aqueous EtOH solution. The reaction mixture was concentrated under reduced pressure, poured into ice-H₂O and extracted with EtOAc. The EtOAc layer was washed with H₂O and dried (Na₂SO₄). Removal of solvent afforded reduced pressure afforded 7 mg (91%) of 18 which was not further purified but used as such in the conversion to 20 and 22. NMR (CDCl₃): δ 1.21 (t, 3 H, J = 7 Hz, CH₃), 1.3-2.3 (m, 8 H, 7-H, 8-H), 4.09 (q, 2 H, J = 7 Hz, CH₂), 5.60 (s, 1 H, J = 12 Hz, 5-H), 5.92 (d, 1 H, J = 12 Hz, 6-H), 6.12 (s, 2 H, J = 2 Hz, 7-H, 8-H), 9.23 (s, 1 H, J = 5 Hz, 4-H)

The benzene (19 mg) was heated at 70°C in 15 mL of BuOH containing 2 mL of 0.5% aqueous EtOH solution. The reaction mixture was concentrated under reduced pressure, poured into ice-H₂O and extracted with EtOAc. The EtOAc layer was washed with H₂O and dried (Na₂SO₄). Removal of solvent afforded reduced pressure afforded 7 mg (91%) of 18 which was not further purified but used as such in the conversion to 20 and 22. NMR (CDCl₃): δ 1.21 (t, 3 H, J = 7 Hz, CH₃), 1.3-2.3 (m, 8 H, 7-H, 8-H), 4.09 (q, 2 H, J = 7 Hz, CH₂), 5.60 (s, 1 H, J = 12 Hz, 5-H), 5.92 (d, 1 H, J = 12 Hz, 6-H), 6.12 (s, 2 H, J = 2 Hz, 7-H, 8-H), 9.23 (s, 1 H, J = 5 Hz, 4-H)

The benzene (19 mg) was heated at 70°C in 15 mL of BuOH containing 2 mL of 0.5% aqueous EtOH solution. The reaction mixture was concentrated under reduced pressure, poured into ice-H₂O and extracted with EtOAc. The EtOAc layer was washed with H₂O and dried (Na₂SO₄). Removal of solvent afforded reduced pressure afforded 7 mg (91%) of 18 which was not further purified but used as such in the conversion to 20 and 22. NMR (CDCl₃): δ 1.21 (t, 3 H, J = 7 Hz, CH₃), 1.3-2.3 (m, 8 H, 7-H, 8-H), 4.09 (q, 2 H, J = 7 Hz, CH₂), 5.60 (s, 1 H, J = 12 Hz, 5-H), 5.92 (d, 1 H, J = 12 Hz, 6-H), 6.12 (s, 2 H, J = 2 Hz, 7-H, 8-H), 9.23 (s, 1 H, J = 5 Hz, 4-H)
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g. 3.1 m (1.0 mL) of EtOH was hydrogenated over 0.3 mol of 10% Pd/C at room temperature and 40 psi for 3 h. After the solution was filtered and concentrated under reduced pressure, the residue was chromatographed on silica gel (2 mm) and eluted with EtOAc:benzene (1:3). After concentration under reduced pressure, 0.62 g (81%) of compound cis-2 was obtained as a colorless oil (oil bath temperature 160 °C (1.5 mm)).

Method B: Hydrogenation of 29 (2.6 g, 10.5 mmol) in 100 mL of MeOH was carried out over 0.5 mol of 10% Pd/C at room temperature and 40 psi for 4 h. After filtration, the solvent was removed under reduced pressure and the residual oil was dissolved in 20 mL of acetone.

To the solution was added 2.0 g (18.0 mmol) of ethyl chloroformate and the mixture was stirred for 1 h at room temperature. The reaction mixture was poured into ice-H2O and extracted with EtOAc. The EtOAc layer was washed with H2O and dried (Na2SO4). After the solvent was removed under reduced pressure, the residual oil was distilled to afford 1.3 g (46% of 31 (oil bath temperature 170 °C (2 mmol): IR (KBr) 1800, 1730 cm⁻¹). NMR (CDCl3) δ 1.2-1.3 (13 H, J = 10 Hz, CH2), 1.2-2.1 (16 H, J = 13 Hz, 2H), 2.25 (d, J = 12 Hz, 8-H), 2.50 (s, 1 H, half-height width = 7 Hz, 5-H), 2.85 (t, 1 H, J = 13 Hz, 26-H), 3.70 (s, 2H, CHO), 3.59 (s, 1H), J = 11 Hz, 2o-H), 4.12 (q, 2 H, J = 7 Hz, CH3), 4.20 (d, 1 H, J = 12 Hz, 8-CH2); MS: 70 (20%), m/z 268 (M +). Anal. (C25H26NO3) C, H, N.

Method A: cis-Decahydroquiniline-5-carboxylic acid hydroxylactate (32). Hydrogenation of 29 (500 mg, 1.46 mmol) in 70 mL of MeOH containing 0.1 mol of glacial HCl was carried out over 0.1 mol of 10% Pd/C at room temperature and 40 psi for 4 h. After filtration, the solution was concentrated under reduced pressure to afford a colorless oil which was washed with acetone, yielding 0.22 (66%) of pure cis-32, m.p 144-145 °C. Anal. (C25H26NO3) C, H, N.

(4aR,5aR)-Decahydroquinoline-5-carboxylic acid (cis-1) Hydrochloride. A solution of 54 mg (0.20 mmol) of decamethylenedecanoic acid (cis-1) in 10 mL of MeOH was hydrogenated over 0.3 mol of 10% Pd/C and concentrated under reduced pressure to afford a colorless oil (oil bath temperature 160 °C (1.5 mm)).

(4aR,5aR)-Decahydroquinoline-5-carboxylic acid (cis-1) Ethyl ester (31). To a solution of cis-1 (5 mg, 0.023 mmol) in 5 mL of dry MeOH was added 0.1 mL of a 1 M solution of HCl in dry MeOH. After stirring for 30 min, the reaction was quenched by the addition of NaHCO3 solution and poured into ice-H2O. The H2O layer was acidified with dilute HCl and extracted with EtOAc. The EtOAc extract was dried (Na2SO4) and concentrated under reduced pressure to yield 0.14 g (52%) of 31 (m.p. 170 °C (2 mmol): IR (KBr) 1780, 1710 cm⁻¹). NMR (CDCl3) δ 1.2-2.1 (13 H, J = 10 Hz, CH2), 1.2-2.1 (16 H, J = 13 Hz, 2H), 2.32 (d, J = 13 Hz, 2o-H), 2.15 (br s, 1 H, half-height width = 7 Hz, 5-H), 2.80 (m, 1 H, J = 11 Hz, 26-H), 3.39 (d, 1 H, J = 11 Hz, 2o-H), 4.12 (q, 2 H, J = 7 Hz, CH3), 4.20 (d, 1 H, J = 12 Hz, 8-CH2); MS: 70 (10%), m/z 268 (M -18). Anal. (C25H24NO3) C, H, N.

Methyl (4aR,5aR)-Decahydroquinoline-5-carboxylic acid (cis-1) Ethyl ester (31). A mixture of 0.22 g (0.63 mmol) of compound cis-1 and 0.20 g (0.63 mmol) of benzyl chloroformate in 10 mL of dry THF was stirred for 4 h at room temperature. The reaction mixture was concentrated under reduced pressure to afford 45 mg (96%) of compound cis-2: m.p. 138-140 °C (1 mm): IR (KBr) 3430 (M+); 1780, 1710 cm⁻¹. NMR (CDCl3) δ 1.2-2.4 (12 H, J = 10 Hz, CH2), 1.2-2.5 (m, 11 H, J = 10 Hz, 2H), 2.51 (s, 1 H, half-height width = 9 Hz, 5-H), 2.85 (d, 1 H, J = 13 Hz, 8-CH2), 4.05 (d, 1 H, J = 12 Hz, 8-CH2). Anal. (C31H32NO3) C, H, N.
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\(J = 13 \text{ Hz}, 2 \text{o}, \text{H}, 4.45 \text{ (d, m, 1 H, J = 12 Hz, 8aa, H)}, \text{MS (70 eV), m/e 317 (M^+)}.\)

(4aa,5aa,8aa) - 1-(Ethoxycarbonyl)-5-(hydroxymethyl)-decahydروquinoline (36). To a solution of 31 (106 mg, 0.71 mmol) in toluene (10 mL) was added 1 mL of DIBAL (25\% solution in toluene) at -60 °C. After 0.5 h, the mixture was decomposed with excess saturated ammonium chloride solution and poured into ice-H\(_2\)O. The H\(_2\)O layer was extracted with EtOAc and the EtOAc layer was washed with H\(_2\)O and dried (Na\(_2\)SO\(_4\)). Removal of the solvent under reduced pressure afforded 145 mg (85\%) of a colorless oil (36) which was not further purified but used as such in subsequent reactions. IR (neat) 3400, 1660 cm\(^{-1}\); NMR (CDCl\(_3\)) \(1.23 \text{ (t, 3 H, J = 7 Hz, CH}_2\text{CH}_3\), 1.2-2.0 (m, 12 H), 2.4 (s, 1 H, OH), 2.80 (t, m, 1 H, J = 15 Hz, 2H), 3.65 (d, 2 H, J = 7 Hz, CH\(_2\)OH), 3.9 (d, 1 H, 2 o-H), 4.17 (q, 2 H, J = 7 Hz, CH\(_2\)CO), 8aa-H is hidden under the CH\(_2\)CH\(_3\) signals.

(4aa,5aa,8aa) - 1-(Ethoxycarbonyl)decahydroyquinoline (37). A mixture of 36 (37 mg, 0.20 mmol) and triphenylphosphine-chloroformate (10 mg) in toluene (10 mL) was stirred at room temperature for 1 h and excess EtOAc was added. The solvent was removed from the precipitate by decantation. The EtOAc extract was washed with dilute H\(_2\)SO\(_4\), aqueous Na\(_2\)CO\(_3\), and H\(_2\)O and dried (Na\(_2\)SO\(_4\)). Removal of the solvent under reduced pressure afforded 12 mg (54\%) of a colorless oil (oil bath temperature 130 °C (1.0 mm)). IR (neat) 1690 cm\(^{-1}\); NMR (CDCl\(_3\)) 1.16 (t, 3 H, J = 7 Hz, CH\(_2\)CH\(_3\)), 1.1-2.2 (m, 13 H), 2.77 (t, m, 1 H, J = 15 Hz, 2H), 3.9 (d, 1 H, 2 o-H), 4.07 (q, 2 H, J = 7 Hz, CH\(_2\)CH\(_2\)), 8aa-H is hidden under the CH\(_2\)CH\(_3\) signals. Upon standing at room temperature, the aldehyde 37 [MS (70 eV), m/e 239 (M^+)] air oxidizes to carboxylic acid 34 [m/e 225 (M^+)].

(4aa,5aa) - 1-(Ethoxycarbonyl)decahydroyquinoline (38). A solution of 37 (35 mg, 0.16 mmol) and tritylhydrophilic acid (150 mg) in toluene (10 mL) was heated at 170 °C for 40 min. The reaction mixture was chromatographed over silica gel using EtOAc--benzene (1:1) as eluent. Following removal of the solvent under reduced pressure, 38 (18 mg, 54\%) was obtained as a colorless oil (oil bath temperature 180 °C (1.0 mm)). This compound was identical in all respects with 38 prepared as follows: to a solution of cis-decahydroyquinoline (39) (from 68 mg of cis-decahydroyquinoline HCICO\(_2\) in acetone (10 mL) was added K\(_2\)CO\(_3\) (0.2 g) and ethyl chloroformate (0.1 g) at room temperature. The mixture was stirred for 3 h, poured into ice-H\(_2\)O and extracted with EtOAc. The EtOAc extract was washed with dilute HC\(_2\)O, aqueous Na\(_2\)CO\(_3\), and H\(_2\)O and dried (Na\(_2\)SO\(_4\)). Removal of the solvent under reduced pressure afforded 60 mg (73\%) of 38 as a colorless oil (oil bath temperature 130 °C (1.0 mm)). IR (neat) 1690 cm\(^{-1}\); NMR (CDCl\(_3\)) 1.16 (t, 3 H, J = 7 Hz, CH\(_2\)CH\(_3\)), 1.1-2.2 (m, 13 H), 2.77 (t, m, 1 H, J = 15 Hz, 2H), 3.9 (d, 1 H, 2 o-H).

Acknowledgment. We thank the Sankyo Co., Ltd., Tokyo, Japan, for support of Dr. K. Tomita in these laboratories for 2 years.
STEREOSTRUCTURE-ACTIVITY RELATIONSHIPS OF THE GABA-CONTAINING CIS-DECAHYDROQUINOLINE-5-CARBOXYLIC ACIDS; INTRACEREBROVENTRICULAR STUDIES IN MICE

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ABSTRACT

Two cis-decahydroquinoline-5-carboxylic acids, cis-1 and 2 were investigated for their pharmacological activities in vivo. Intracerebroventricular administration of either agent elicited convulsant activity in mice. This convulsant effect can be antagonized by pretreating the mice with n-dipropylacetate (valproate). Our results suggest that cis-1 may act indirectly as a partial γ-aminobutyric acid (GABA) agonist in vivo. On the other hand, both cis-1 and 2, at higher concentrations, exhibit properties of a GABA-antagonist, but this effect seems not to be due to GABA receptor binding.

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**INTRODUCTION**

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter found in high concentration in the mammalian central nervous system (CNS) (Johnston, 1978; Enna and Maggi, 1979). Abnormalities of this GABA neurotransmitter system have been implicated in various neurological disorders (Barbeau, 1973; Bennett et al., 1979; Enna, 1980, 1981). Consequently, the development of drugs capable of selectively activating the GABA system has potential therapeutic value in man. Furthermore, such compounds could have major significance as probes of the GABA neurotransmitter system.

Two lipophilic cis-decahydroquinoline-5-carboxylic acid epimers (1 and 2) (Fig. 1) contain the zwitterionic =N(C)\text{2}C_{6}H (GABA) moiety and were previously synthesized to assess GABAergic stereostructure-activity relationships (Witiak et al., 1981). In D_2O, conformations 1a and 2a are preferred. Pharmacological studies in vitro revealed that these isomers have little affinity for GABA receptors relative to GABA agonists, but exhibit weak stereoselective activity when assessed for their ability to inhibit high-affinity $[^{3}$H] GABA uptake in rat brain synaptosomes. Whereas cis-1 was inactive, cis-2 inhibited $[^{3}$H] GABA uptake by approximately 20% at a concentration of 100 uM, making it over 10 times less potent than GABA or nipecotic acid in this regard (Witiak, et al., 1981).
Figure 1. cis-1 (1a + 1b) and cis-2 (2a + 2b) epimers

Owing to their zwitterionic character, absorption, distribution and penetration of these substances across the blood brain barrier could be markedly influenced. To circumvent such problems, we administered diastereomers cis-1 and 2 by direct intracerebroventricular (ICV) injection. Since the nigrostriatal and mesolimbic dopaminergic neurons are implicated in the regulation of
locomotor activities are their activities are regulated by GABAergic neurons (Anden and Stock, 1973; Moore and Wuerthele, 1979), we sought to determine the stereoselective effects of these compounds on apomorphine-induced climbing behavior (Protais et al., 1979; Fung et al., 1982). In this article we summarize the results of these studies.

MATERIALS AND METHODS

Animals. Male ICR mice (Harlan) weighing between 25-35 g were used. They were allowed free access to food (Purina Lab. Chow) and water. All mice were housed in plastic cages (10/cage) in a room maintained at 23±1°C with an automatic 12-hr. light-dark cycle. All behavioral studies were conducted between 12-5 p.m.

Intracerebroventricular (ICV) Drug Injections. Mice were anesthetized with chloral hydrate (430 mg/kg, ip.) and an incision was made in a longitudinal direction. A hole was made in the left side of the skull (1.2 mm lateral to bregma) for the ICV administration of various compounds at a later time. The incision was closed with a wound clip.

Freehand ICV injection was performed according to Fung and Uretsky (1982). Under halothane anesthesia, saline, cis-1 (20-100 ug) or cis-2 (10-100 ug) in a volume of 5 ul was injected into the left ventricle of the mouse via a 10 ul Hamilton syringe. The syringe was fitted with a polyethylene cuff so that only the distal
2.5 mm of the needle was exposed. This "free hand" injection was performed over a period of 20 seconds and the needle was held in place for an additional 10-15 seconds before withdrawing it from the skull. The incision was then closed with a wound clip. All mice recovered from the halothane anesthesia within 3 minutes after the ICV injection. The location of drug in the brain was confirmed by examining the stain produced by the injection of a 10% aqueous methylene blue solution intraventricularly in a group of six animals.

**Behavioral Assessment.** After ICV administration of the various drugs, each animal was put into a 1-liter beaker and observed for behavioral changes for 45 minutes. In other studies, mice were pretreated with either saline or n-dipropylacetate (valproate) 30 minutes prior to ICV drug administration.

Apomorphine-induced climbing behavior was employed to measure dopaminergic function (Anden and Stock, 1973; Castall et al., 1978). Animals were observed in wire mesh metal cages that were 15 cm. high, 12 cm. in diameter and covered at the top and bottom with metal plates. All mice were allowed to adapt to these cages for one hour prior to the experiment.

Apomorphine (1.5 mg/kg, sc.) was given five minutes after ICV administration of various drugs and the climbing behavior of the mice was observed for the subsequent 30 minutes. The recorded climbing index (Cl) is the percent of time spent in climbing during the 30 minute period following the first climb.
Drugs. Picrotoxin was purchased from Sigma Chemical Company and was dissolved in saline solution. Apomorphine HCl (Merck Company Inc.) was dissolved in 0.1% sodium metabisulfite. Sodium n-dipropylacetate was obtained from Abbott Laboratory. For ICV injection, experimental and control drugs were administered in a volume of 5 µl. For systemic drug injection, n-dipropylacetate was administered in 0.1 ml/10 g body weight of the mouse.

RESULTS

Effect in Mice of ICV Administration of Cis-1 or 2. Animals showed clonic and tonic seizure within 5 minutes of ICV administration of either cis-1 or 2. The potency of these agents in eliciting convulsant activity was compared to picrotoxin, a GABA antagonist (Table 1). A stereoselective effect on lethality was observed wherein cis-1 caused a significant number of deaths and cis-2 caused none up to 100 µg.

Effect of Pretreatment with n-Dipropylacetate. All mice appeared sedated after the 30 minute pretreatment with n-dipropylacetate (400 mg/kg, ip.). This compound inhibited the convulsant activity elicited by picrotoxin as well as either of the cis diastereomers (Table 2). Both clonic and tonic convulsions as well as lethality in the case of cis-1 were completely reversed.
### Table 1

Effect of ICV Administration of Saline, Cis-1 or Cis-2 on Mice

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Clonic Seizure</th>
<th>Tonic Seizure</th>
<th>Lethality</th>
</tr>
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<tbody>
<tr>
<td>Saline</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
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<tr>
<td>Cis-1 (ug)</td>
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<td>100</td>
<td>5/5</td>
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<tr>
<td>Cis-2 (ug)</td>
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<td>10</td>
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<td>Picrotoxin (ug)</td>
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<tr>
<td>0.6</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
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<tr>
<td>0.125</td>
<td>1/5</td>
<td>1/5</td>
<td>1/5</td>
</tr>
<tr>
<td>0.05</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

a Under halothane anesthesia, saline, cis-1, cis-2 or picrotoxin was injected in a volume of 5 ul into the left ventricle of mice. Animals were observed for seizure activity 3 minutes post ICV drug administration.

b Ratio indicates the number of mice showing the indicated behavior to the total number of mice tested.

c Significantly different from control $p < 0.05$ (chi square analysis).
### Table 2

**Effect of n-Dipropylacetate (400 mg/kg, ip.) in reversing the convulsant effects of picrotoxin, Cis-1 or Cis-2**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Clonic Seizure</th>
<th>Tonic Seizure</th>
<th>Lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (ip.) + saline (ICV)</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Saline (ip.) + cis-1 (100 ug, ICV)</td>
<td>5/5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5/5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5/5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>n-dipropylacetate (400 mg/kg, ip.) + cis-1 (100 ug, ICV)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Saline (ip.) + cis-2 (100 ug, ICV)</td>
<td>5/5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5/5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/5</td>
</tr>
<tr>
<td>n-dipropylacetate (400 mg/kg, ip.) + cis-2 (100 ug, ICV)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Saline (ip.) + picrotoxin (0.6 ug, ICV)</td>
<td>6/6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5/6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4/6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>n-dipropylacetate (400 mg/kg, ip.) + picrotoxin (0.6 ug, ICV)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Animals were pretreated with saline or n-dipropylacetate (400 mg/kg, ip.) 30 minutes prior to the ICV administration of various agents. Animals were observed for convulsant activity for 45 minutes post ICV drug injections.

<sup>b</sup> Ratio indicates the number of mice showing the indicated behavior to the total number of mice examined.

<sup>c</sup> Significantly different from respective control groups p < 0.05 (chi square analysis).
### Table 3

**Effect of ICV Administration of Cis-1, Cis-2 or Saline on Apomorphine-induced Climbing Behavior**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Climbing Index (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>83 ± 4</td>
</tr>
<tr>
<td>Cis-1 (20 ug)</td>
<td>51 ± 10(^b)</td>
</tr>
<tr>
<td>Cis-1 (10 ug)</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>Cis-2 (25 ug)</td>
<td>72 ± 8</td>
</tr>
</tbody>
</table>

\(^a\) Under halothane anesthesia, saline, cis-1 or cis-2 in a volume of 5 ul was injected into the left ventricle of the mouse. Five minutes later, apomorphine (1.5 mg/kg, sc.) was given and the climbing index measured.

\(^b\) p < 0.05 (Mann-Whitely "U" test) when compared to saline-treated controls. n = 5-6 in each group.
Effect of ICV Administration of Cis-1 and 2 Upon Apomorphine-Induced Climbing Behavior. The effect of ICV administration of these compounds on apomorphine-induced climbing behavior is presented in Table 3. At doses that did not elicit convulsions, only cis-1 was effective in inhibiting apomorphine-induced climbing behavior in mice.

DISCUSSION

Diastereomers cis-1 and 2 were investigated as probes for GABA-mediated neurotransmission. However, neither isomer was expected to exhibit the significant intrinsic activity of a GABA agonist (O'Donnell et al., 1980) since insertion of the large lipophilic hydrocarbon skeleton about the GABA pharmacophore likely would preclude interaction with GABA receptors in a manner necessary for expression of intrinsic activity (Witiak et al., 1981).

Nonetheless, in vivo we have observed that both diastereomers exhibit a dose-dependent CNS stimulation manifested by clonic and tonic seizures. Such CNS stimulatory activity could be reflective of a GABA antagonist. Furthermore, the GABA transaminase inhibitor, n-dipropylacetate, a clinically proven anticonvulsant, reverses the convulsant effects of both experimental drugs as well as picrotoxin, a GABA antagonist which acts on the GABA receptor-ionophore system. Studies employing more than 50 drugs have supported a positive correlation between Na-independent binding to GABA receptors in vitro
and their agonist (or antagonist) activity in vivo (Olsen et al., 1979). Therefore, it seems that these diastereomers, at concentrations above 40-50 μg, do not inhibit the specific GABA binding site per se, but rather may be antagonizing some other component of the receptor-ionophore system. The stereoselective effect of cis-1 vs. cis-2 on lethality (Table 1) is likely reflective of a stronger convulsant activity for the former compound.

Particularly interesting was the stereoselectivity observed for the two decahydroquinoline diastereomers in the apomorphine-induced climbing assay. It is known that GABA agonists, administered by ICV injection, effectively inhibit such climbing behavior (Fung et al., 1982). At doses which did not cause convulsions, only cis-1 (20 μg) inhibited the effect elicited by apomorphine suggesting that this compound may act as a partial GABA agonist. Although the inhibitory effect was weak, the activity is particularly significant since in vitro only cis-2 blocked high affinity [3H]-GABA uptake into rat brain synaptosomes by 20% at 100 μM concentrations (Witiak et al., 1981). Since cis-1 neither blocks such [3H]-GABA uptake nor has appreciable affinity for GABA receptors in vitro, the agonist effect observed in vivo could be due to some other GABAergic mechanism such as release of GABA from storage sites (Witiak et al., 1981). Clearly, further work is necessary to differentiate this possibility from the possibility that there may be more than one pharmacological type of GABA receptor (Enna and DeFrance, 1980) for which there are different stereochemical and bulk requirements.
Structural requirements for partially conformationally constrained GABA agonists showing high affinity for GABA binding sites in rat brain in vitro and neurophysiological potency in vivo are found in analogues such as 3-hydroxy-5-aminomethylisoxazole- (muscimol), 1,2,5,6-tetrahydropyridine-4-carboxylic acid- (isoguvacine), trans-1-aminocyclopentane-3-carboxylic acid, and 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol (THIP) (Greenlee et al., 1978). In all of these structures the "GABA pharmacophore" adopts a partially extended relatively flat conformation not found in structures such as 1a and 1b wherein the N and CO₂H groups assume a nonplanar 1,3-equatorial-axial disposition relative to one of the cyclohexane rings. Therefore, if cis-1 functions as an indirect GABA agonist, such activity may be related to conformations 1a or 1b. Conformationally constrained trans-decahydroquinoline analogues may serve to further define topographical requirements (i.e., 1a or 1b) for indirectly acting agonists (Witiak et al., 1981). Such studies are in progress.

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REFERENCES

Anden, N.E. and Stock, G. (1973). Inhibitory effect of


