THEORETICAL AND SPECTROSCOPIC STUDIES
OF ANTICONVULSANT DRUGS

by

Peter Ronald Andrews

Submitted to the University of Melbourne for the Degree of Doctor of Philosophy, December, 1969.
Parts of the work described here have been published as

follows:

Sections 3.1 and 3.2, P. R. Andrews,

Sections 3.3 and 3.4, P. R. Andrews,
J. Med. Chem., 12, 761 (1969);

Section 2.6, P. R. Andrews,
J. Mol. Struct., in press

Some of the material in Chapters 4 and 5 is being prepared for
publication.
I am grateful to H. F. Andrews, C. G. Barraclough, A. S. Buchanan, R. D. Harcourt, G. H. Scott and A. Shulman for their encouragement and advice during the course of this work, and to the Commonwealth of Australia for financial support.
# Table of Contents

## Introduction

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter One</td>
<td>The Scene</td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>Epilepsy and the Central Nervous System</td>
<td>4</td>
</tr>
<tr>
<td>1.2</td>
<td>Possible Sites of Action of Anticonvulsant Drugs</td>
<td>7</td>
</tr>
<tr>
<td>1.3</td>
<td>Aims and Outline of this Work</td>
<td>12</td>
</tr>
</tbody>
</table>

## Chapter Two | Theoretical Preliminaries

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Choice of Molecular Orbital Method</td>
<td>14</td>
</tr>
<tr>
<td>2.2</td>
<td>Choice of Drugs</td>
<td>22</td>
</tr>
<tr>
<td>2.3</td>
<td>Choice of Molecular State</td>
<td>24</td>
</tr>
<tr>
<td>2.4</td>
<td>Molecular Geometries</td>
<td>26</td>
</tr>
<tr>
<td>2.5</td>
<td>Molecular Conformations</td>
<td>31</td>
</tr>
<tr>
<td>2.6</td>
<td>An Interesting Radical</td>
<td>34</td>
</tr>
</tbody>
</table>

## Chapter Three | Theoretical Results and Discussion

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Dipole Moments</td>
<td>51</td>
</tr>
<tr>
<td>3.2</td>
<td>Atomic Charges</td>
<td>54</td>
</tr>
<tr>
<td>3.3</td>
<td>Biologically Active Centre Hypothesis</td>
<td>57</td>
</tr>
<tr>
<td>3.4</td>
<td>The Common Bonding Group</td>
<td>69</td>
</tr>
</tbody>
</table>
INTRODUCTION

Once upon a time, people who suffered from grand mal were fed mustard, and weasels' blood, and some had their skulls perforated. Grand mal, the falling sickness, had been known since the time of Hippocrates, but even the empirical Greeks resorted to mystical explanations for the thunderclap onset of a grand mal fit, and the churchmen and surgeons of the Middle Ages were of the opinion that a glass of human urine, preferably provided by the first witness to the seizure, would hasten the departure of the offending demon. Although logically reasonable, this and other treatments failed to alleviate grand mal or any of the related cerebral conditions which are now known collectively as epilepsy, and it was not until 1857 that the first useful anticonvulsant drug, potassium bromide, was introduced.

The bromides were moderately effective, and remained the major treatment until early this century, when the therapeutic value of barbiturates was discovered. The number of useful organic anticonvulsants has since grown rapidly and nearly seventy per cent of all epileptic patients could be rendered seizure-free and socially functional with currently available treatment.

Precise information on the prevalence of epilepsy is not available,
but an incidence of approximately 0.5 per cent is generally accepted\textsuperscript{3, 4}. The thirty per cent of epileptics for whom available medication is unsatisfactory represent a significant population; roughly 3000 people in Melbourne. The search for useful anticonvulsant drugs therefore continues, and is assisted by any contribution to our understanding of their mode of action. It is in the latter area that I hope this work will take its place.

The techniques employed here are normally labelled physical chemistry, but the problem is more biological. There is a substantial language barrier between the two disciplines, especially when coming from the physico-chemical side, so wherever possible I have avoided using biological terms.

An outline of the essential biology is given in Chapter 1, together with a survey of anticonvulsant activity. The survey is not a detailed review of the anticonvulsant drug literature, but a discussion of some broad principles of anticonvulsant action, particularly among clinically useful drugs. A basis of this type is vital if physico-chemical techniques are to be usefully applied.

With the exception of Section 2.6 the physico-chemical terms used are now quite common in the biological literature. The offending section is not essential to the main argument of the thesis, and the biologically
inclined reader may prefer to move on to Chapter 3. Discussion of the physico-chemical techniques and their recent biological applications is reserved for the chapters where they are employed.
CHAPTER ONE

1.1 Epilepsy and the Central Nervous System

As you read these words a series of signals is transmitted from eye to brain. The brain in turn transmits a signal to eye muscle, saying 'Read on', or to other muscles saying 'Throw it away and make some coffee'. To provide such versatility of response, a complex set of interconnecting pathways is required. It is known as the central nervous system, and is made up of nerve cells, or neurones, and the junctions between them, called synapses.

Like other cells, neurones have a thin outer membrane which is commonly thought to consist of a bimolecular layer of lipid sandwiched between two monolayers of protein\(^5\), although several alternative structures have been proposed over the past decade\(^6\). The membrane regulates influx and efflux of molecules and ions, maintaining an excess of sodium ions outside the cell, and of potassium ions inside. In the resting state there is a potential difference of 50 to 100 mV. across the membrane.

The transmission of an impulse along a nerve fibre results when a part of the membrane is depolarized. A transient reversal of the membrane potential occurs, during which sodium ions enter the cell,
and potassium ions leave. The ionic currents depolarize the next portion
of the membrane, and the impulse is transmitted at velocities ranging
from 0.1 to 100 metres per second. The slight excess of sodium ions
left inside the cell is pumped out by an energy-consuming process known
as active transport, which is itself catalysed by sodium ions.

The transmission of the impulse across a synapse to another nerve
cell is often achieved chemically by the release of a substance which
can depolarize part of the postsynaptic membrane. This synaptic
transmitter is then destroyed by a specific enzyme, and the synapse
restored to the resting state. Similarly, the signal can be transmitted
from a nerve to a muscle cell.

In the central nervous system (CNS) the integration of many
converging signals determines whether discharge will occur. The
signals come from both excitatory and inhibitory neurones, which either
depolarise or stabilise the postsynaptic membrane by releasing different
synaptic transmitters. Inhibitory transmitters may also act on the
excitatory neurone, and so reduce the quantity of excitatory transmitter
released. Acetylcholine is a known excitatory transmitter in the CNS,
and dopamine and noradrenaline may be inhibitory transmitters.
Glutamic acid and \( \gamma \)-aminobutyric acid (GABA) are suspected central
excitatory and inhibitory transmitters respectively.
In addition to the direct transmitter effects, an initial enhancement and subsequent depression of synaptic transmission result from rapid repetitive stimulation, and are called post-tetanic potentiation (PTP) and post-tetanic depression respectively\textsuperscript{12}.

The interplay of all these mechanisms may have led you to ask your wife to make the coffee.

In epilepsy, the functioning of the CNS is perturbed by a seizure focus. Single cells in the seizure foci of epileptic patients show occasional bursts of high frequency activity\textsuperscript{13}, and there is an apparent suppression of inhibitory mechanisms. The enhancement of these conditions by PTP can result in convulsive activity characteristic of the seizure focus. Examples are petit mal, where brief attacks of loss of consciousness may occur hundreds of times each day, and psychomotor epilepsy, which is manifested by periods of confused behaviour\textsuperscript{2}.

The spread of convulsive activity to unaffected parts of the brain is normally prevented by existing inhibitory mechanisms, but minor physiological changes may be sufficient to trigger activation of the entire brain\textsuperscript{2}. The subsequent major convulsions and prolonged postseizure depression of CNS functions are the outward manifestations of grand mal epilepsy.

Drugs which control seizures could function by preventing the
excessive discharge of the seizure focus, or by making unaffected regions less susceptible to it. Possible sites of action are discussed in the next section.

1.2 Possible Sites of Action of Anticonvulsant Drugs

One of the most widely used anticonvulsant drugs is 5-ethyl-5-phenylbarbituric acid, which, in common with other anticonvulsant barbiturates, also belongs to the broad class of nonselective CNS depressants. At anaesthetic concentrations, depressants reduce metabolic oxygen consumption\textsuperscript{14}, indicating that they inhibit respiration (the process in which food metabolites are oxidised to CO\textsubscript{2} and H\textsubscript{2}O, with associated storage of energy). Systematic elimination of alternative sites indicates that the inhibition of the respiratory chain occurs between the coenzyme nicotinamide adenine dinucleotide and the enzyme flavoprotein\textsuperscript{15}, but the exact site is still uncertain\textsuperscript{16, 17}. However the high
concentration required suggests that the direct action on the respiratory chain is irrelevant to anticonvulsant activity, especially since the application of the convulsant drug β-methyl-β-ethylglutarimide to rat liver mitochondria, albeit in lethal concentrations, inhibits the respiratory chain in the same region. At lower concentrations of anticonvulsants no respiratory decrease is observed in isolated brain tissue. However, if the tissue is stimulated with high frequency alternating current, as a substitute for normal excitation, the respiration rate doubles as additional energy is used. Anticonvulsants markedly inhibit this response, but probably by decreasing the energy requirements of the cell rather than by direct action on the respiratory chain, since no decrease in available energy levels is observed. Stimulation of the active extrusion of sodium ions is a possible mechanism, but any other process which inhibits discharge of the neurone, and the subsequent energy requirements of active
transport, is equally plausible.

Some indication of possible sites of action has been obtained by numerous studies of the influence of anticonvulsants on artificially induced convulsions, and by direct measurements of pre- and post-synaptic potentials in model systems.

With the aid of these results, and sacrificing a wealth of detail, two broad classes of activity may be discerned among the clinically useful anticonvulsants. They are typified by 5, 5-diphenylhydantoin and 3, 5, 5-trimethyloxazolidine-2, 4-dione. The latter compound is demethylated metabolically to 5, 5-dimethyloxazolidine-2, 4-dione, which is probably the more active form\textsuperscript{21, 22}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{compound_images.png}
\caption{Chemical structures of 5, 5-diphenylhydantoin and 3, 5, 5-trimethyloxazolidine-2, 4-dione.}
\end{figure}

Diphenylhydantoin does not quell activity in the seizure focus\textsuperscript{2}, but prevents seizure activity reaching its maximum level by stabilizing membrane thresholds and reducing PTP\textsuperscript{23}. Both actions could be due
to stimulation of sodium efflux from neurones\textsuperscript{24}, but this now seems unlikely\textsuperscript{25}. Related activity is exhibited by anticonvulsants containing an aromatic group, most of which abolish the maximal (tonic) phase of electroshock induced seizures. These compounds are particularly active against grand mal.

Trimethyloxazolidine-2, 4-dione decreases susceptibility to induced convulsions, and inhibits activity in seizure foci. PTP is unaffected, but post-tetanic depression is intensified\textsuperscript{26} and could explain both effects. The drug completely prevents seizures induced by pentylenetetrazole (pentamethylenetetrazole), which acts by reducing neuronal recovery time\textsuperscript{27}. Anticonvulsants with similar action to trimethyloxazolidine-2, 4-dione generally contain short alkyl groups and are most useful against petit mal.

\[ \text{pentylenetetrazole} \]

Several useful anticonvulsant drugs have both classes of activity, in differing degrees, and many also exhibit the depressant action typical
of 5-ethyl-5-phenylbarbituric acid. It must be emphasized that this classification disregards the quite individual properties, particularly side-effects, of many clinically useful drugs, and ignores those anticonvulsants which have not found widespread clinical use. Among the latter group are compounds which appear to act by raising brain \( \text{CO}_2 \) or \( \text{GABA} \) levels, but neither of these effects is observed in commonly prescribed anticonvulsants.

The important outcome of the preceding classification is the existence of two rather distinct types of clinical activity, which may be related to the observed activity against seizures induced by electro-shock and pentylentetrazole respectively. However, regardless of the precision of this relationship, the tests on induced seizures provide quantitative comparisons of the drugs, which can be used as significant criteria of activity.

A further outcome is the general impression that synaptic effects are partly responsible for the activity of all the useful anticonvulsants, and this is strengthened by the recent demonstration of competitive activity between numerous pairs of CNS active drugs, many with markedly different structures, using titration techniques in intact mice. Among the drugs studied were the \( \beta,\beta \)-disubstituted glutarimides, the activities of which vary qualitatively with the length
of an alkyl substituent group[^32].

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>R</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = H</td>
<td>inactive</td>
</tr>
<tr>
<td>R = CH₃, C₂H₅</td>
<td>convulsant</td>
</tr>
<tr>
<td>R = n-C₃H₇</td>
<td>dual activity</td>
</tr>
<tr>
<td>R = n-C₄H₉, n-C₅H₁₁</td>
<td>anticonvulsant</td>
</tr>
</tbody>
</table>

These results led to a hypothesis that both convulsants and anticonvulsants act by modifying the architecture of the protein or lipid components, or both, of synaptic membrane, with subsequent effects on the release or action of synaptic transmitters[^37,^38]. It seems likely that the highly polar bonding group shown below, which is present in most clinically useful anticonvulsants and also some convulsant drugs, will be directly involved in any conformational changes produced.

### 1.3 Aims and Outline of this Work

The first aim of the work described here is to find out whether any relationship exists between the polarity of the bonding group and activity.
against either electroshock or pentylenetetrazole seizures. Molecular orbital calculations on a group of anticonvulsant drugs are used for this purpose. These calculations are also extended to the $\beta$-glutarimide series, to determine the nature of the bonding group in drugs with qualitatively different activities.

Perkow has suggested that the electron density at a specific atom, termed the biologically active centre (BAC), provides a yardstick for the prediction of convulsant and anticonvulsant activity. Despite the lack of any obvious biological basis, this hypothesis has been extended in sixteen separate papers to include drugs with sedative, analgesic, spasmylytic, antiallergic, local anaesthetic, diuretic, anticoagulant and laxative activity, and chemotherapeutic agents with insecticidal, bacteriostatic and weed killing applications. The calculations presented here also provide a direct and critical test for this rather general hypothesis.

Following the molecular orbital studies, and partly as a result of them, the binding of some of the drugs to model substrates is investigated using infrared spectroscopy.
CHAPTER TWO

2.1 Choice of Molecular Orbital Method

Most molecular orbital (MO) studies of biochemical\textsuperscript{41-43} and therapeutic\textsuperscript{44-51} problems have employed simple Hückel theory\textsuperscript{52} or related techniques which consider only \(\pi\)-electrons explicitly. They assume that the \(\sigma\) and \(\pi\) systems may be treated separately, and generally the \(\sigma\)-electron distribution is not considered. Unfortunately, there is no unambiguous way of checking the calculated \(\pi\)-electron distribution, since it cannot be used alone to predict any observable molecular property. In spite of this, many of the studies mentioned have related variations in biological activity to very small changes in calculated atomic charges or energy levels. In the case of the bacteriostatic sulphonamides, it has recently been observed\textsuperscript{53} that although the calculated atomic charges correlate with observed biological activity\textsuperscript{44}, they are not related to the results of more accurate calculations\textsuperscript{54}.

For the molecules being considered here, which are neither conjugated nor planar, the separation of \(\sigma\)-and \(\pi\)-electrons is quite unreasonable, so a method which considers all electrons simultaneously is required. Several such methods are now available, the most widely used being the extended Hückel theory\textsuperscript{55} (EHT) and the complete neglect
of differential overlap method\textsuperscript{56-58} (CNDO/2).

In the present study, the primary requirement of an MO method is satisfactory prediction of atomic charges, which is usually assessed from the accuracy of calculated dipole moments. The EHT appears to exaggerate the charge distribution\textsuperscript{43,55}, so that predicted dipole moments are generally too large\textsuperscript{59}. Iteration improves the calculated dipoles\textsuperscript{60}, but is not always effective\textsuperscript{61}. Studies aimed at improving the prediction of many physical properties by the CNDO/2 method have focussed attention on some of the approximations employed\textsuperscript{62,63}, and particularly on the evaluation of semiempirical parameters\textsuperscript{64-66}, but have not significantly improved the calculation of dipole moments, which is already extremely good\textsuperscript{67-69}. The original form of CNDO/2 has also proved successful in predicting infrared intensities\textsuperscript{70} and inner electron binding energies\textsuperscript{71}, which again depend on charge distribution. The method is less suitable for calculating hyperfine coupling constants\textsuperscript{72}, where the unpaired spin distribution around an atom is important, but a slightly extended version, intermediate neglect of differential overlap\textsuperscript{73} (INDO), accounts for this problem\textsuperscript{74,75} without otherwise effecting the results\textsuperscript{72}. Several other all electron treatments\textsuperscript{76-78} are now available, but have not been so widely tested, and at this stage CNDO/2 seems the obvious choice for calculating atomic charges.
Also important in this project is the prediction of molecular conformation, for which the CNDO/2 method has proved moderately successful\textsuperscript{72,79}. However for biologically interesting molecules EHT has been extensively employed\textsuperscript{80-86}, and gives results in good agreement with experiment\textsuperscript{84,86}, provided that all likely conformations are considered\textsuperscript{86}. EHT is also potentially capable of handling larger molecules than CNDO/2 without additional computer storage. For these reasons it was decided that both CNDO/2 and EHT should be used here. An important advantage of this approach is that the significance of slight variations in atomic charges can be assessed by comparing the two sets of calculations prior to any attempt to correlate these variations with biological activity.

In both CNDO/2 and EHT the molecular orbitals $\psi_1$ are expressed as a linear combination of $N$ atomic orbitals $\phi_v$:

$$\psi_i = \sum_{\nu} \phi_v \phi_{\nu i} \quad \text{i = 1, 2, \ldots, N,} \quad (2.1)$$

the $\phi_v$ employed being the 2s and 2p Slater orbitals for first row atoms and the 1s Slater orbital for hydrogen. In each case the set of molecular orbitals which minimises total energy is given by the secular equations
\[ \sum_{\nu} F_{\mu \nu} c_{\nu i} = S_{\mu \nu} c_{\nu i} \xi_i, \quad i = 1, 2 \ldots N. \]  

(2.2)

For nontrivial solutions of (2.2) the vanishing of the \( N \times N \) secular determinant

\[ | F_{\mu \nu} - \xi S_{\mu \nu} | = 0 \]  

(2.3)

is required, and provides \( N \) eigenvalues \( \xi_i \) and \( N \) sets of molecular orbital coefficients \( c_{\nu i} \). The difference between the two methods lies in the nature of the terms \( F_{\mu \nu} \) and \( S_{\mu \nu} \).

CNDO/2 is based on self-consistent field (SCF) theory, where the motion of each electron is governed by the effective field (charge distribution) of the whole system. In SCF theory the linear equations (2.2) were derived by Roothaan, and the term \( F_{\mu \nu} \), which is a combination of one-electron and two-electron components, depends on the calculated MO coefficients (hence SCF). The Roothaan equations are suitable for small systems, but further approximations are required before they can be applied to large molecules. CNDO/2 achieves this by neglect of differential overlap \( (S_{\mu \nu} = 0 \) when \( \mu \neq \nu \)) and penetration integrals, plus the restriction that the method should be independent of orthogonal transformation of the atomic orbital basic set. The terms \( F_{\mu \nu} \) and \( F_{\mu \nu} \) are then given by

\[ F_{\mu \nu} = F_{\mu \nu}^{\mu} = F_{\mu \nu}^{\nu} \]  

(2.4)
\[ F_{\mu\mu} = U_{\mu\mu} + \left( P_{AA} - \frac{1}{2} P_{\mu\mu} \right) \gamma_{AA} - \sum_{B \neq A} Q_B \gamma_{AB} \]  \hspace{1cm} (2.4)

and

\[ F_{\mu\nu} = \beta_{AB}^0 S_{\mu\nu} - \frac{1}{2} P_{\mu\nu} \gamma_{AB} \]  \hspace{1cm} (2.5)

where \( P_{\mu\nu} \) is the charge density and bond order matrix

\[ P_{\mu\nu} = 2 \sum_{i}^{\text{occ}} c_{\nu i} c_{\mu i} \]  \hspace{1cm} (2.6)

\( P_{AA} \) is the total charge density on atom A, and \( Q_B \) is the net charge on atom B,

\[ Q_B = Z_B - P_{BB} \]  \hspace{1cm} (2.7)

where \( Z_B \) is the core charge on atom B.

The electron-repulsion integral \( \gamma_{AB} \), which is an average repulsion between an electron on atom A and another on atom B, and the overlap integral \( S_{\mu\nu} \) are evaluated from standard formulae. \( U_{\mu\nu} \) is a measure of the energy of the atomic orbital \( \phi_{\mu} \), which is obtained from observed atomic energy levels, and \( \beta_{AB}^0 \) is given by

\[ \beta_{AB}^0 = \frac{1}{2} \left( \beta_A^0 + \beta_B^0 \right) \]  \hspace{1cm} (2.8)

where \( \beta_A^0 \) and \( \beta_B^0 \) have been determined empirically to give a close fit to accurate SCF calculations on diatomic molecules. The molecular dipole moment \( \mu \) is obtained from the population matrix \( P_{\mu\nu} \) using
\[ \mu_z = 2.5416 \sum_A Q_A z_A - 7.3370 \sum_A (Z'_A)^{-1} P_{2s}(A), 2p_z(A) \]  
(2.9)

and \[ \mu^2 = \mu_x^2 + \mu_y^2 + \mu_z^2 \]  
(2.10)

where \( z_A \) is the z-coordinate of atom A (in atomic units) and \( Z'_A \) is the orbital exponent. The two terms in equation (2.9) arise from net charge densities and atomic polarization respectively. Finally, the total energy is obtained by summing electronic energies and nuclear repulsion terms.

The EHT lacks the theoretical basis of CNDO/2, since it does not take electron repulsion into account. It is therefore termed a 'one-electron' method and \( F_{\mu \nu} \) is approximated \(^{55} \) by

\[ F_{\mu \nu} = 0.5K(F_{\rho \mu} + F_{\nu \nu})S_{\mu \nu} \]  
(2.11)

where \( F_{\rho \mu} \) and \( F_{\nu \nu} \) are the valence state ionization potentials (VSIP) of \( \phi_{\rho} \) and \( \phi_{\nu} \). These are calculated using spectroscopic atomic ionization potentials and valence state energies taken from recognized tabulations \(^{90, 91} \). For example, the s-electron VSIP of \( N(sp^4, V_3) \) to \( N^+(p^4, V_2) \) is given by

\[ I_s(N(sp^4, V_3)) = I_N + E(N^+(p^4, V_2)) - E(N(sp^4, V_3)) \]  
(2.12)

where \( I_N \) is the ionization potential of the nitrogen atom and the E terms
are valence state energies. The VSIP values used here were weighted averages over the several possible valence states, and are given in Table 2.1, together with those used by others.

Table 2.1  VSIP Values (eV)

<table>
<thead>
<tr>
<th>Orbital</th>
<th>VSIP Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hoffmann $^{55,92}$</td>
</tr>
<tr>
<td>$H_{1s}$</td>
<td>13.6</td>
</tr>
<tr>
<td>$C_{2s}$</td>
<td>21.4</td>
</tr>
<tr>
<td>$C_{2p}$</td>
<td>11.4</td>
</tr>
<tr>
<td>$N_{2s}$</td>
<td>26.0</td>
</tr>
<tr>
<td>$N_{2p}$</td>
<td>13.4</td>
</tr>
<tr>
<td>$O_{2s}$</td>
<td>35.3</td>
</tr>
<tr>
<td>$O_{2p}$</td>
<td>17.8</td>
</tr>
</tbody>
</table>

A value of 1.75 was chosen $^{55}$ for K in equation (2.11) because it gives good results for total energy, which is taken as the sum of the one-electron orbital energies. Unlike the CNDO/2 method EHT retains all the overlap integrals $S_{\mu\nu}$ in equation (2.3) and these are calculated from standard formulae$^{89}$. Consistently with this approach, the charge density and bond order matrix is given by
\[ P_{\mu\nu} = 2 \sum_{i}^{\text{occ}} c_{\mu i} c_{\nu i} S_{\mu\nu}, \]  

(2.13)

and the molecular dipole can be calculated by applying equations (2.9) and (2.10). Because of the form of the charge density matrix (2.13), the first term in equation (2.9) now also incorporates homopolar bond moments, which vanished in the zero differential overlap approximation.

From the preceding description the EHT emerges as an empirical method, whose only real justification is that it works. It is interesting to note, however, that the estimated values of \( P_{\mu\nu} \) closely approximate more rigorous SCF matrix elements \(^{94}\), and indeed, the SCF expressions can be reduced to those of EHT by applying some comparatively minor approximations \(^{95}\). The EHT prediction of total energy has been specifically rationalized by several authors \(^{55, 96, 97}\).

The CNDO/2 calculations were done with a Fortran IV program written by G. A. Segal and obtained from Quantum Chemistry Program Exchange (QCPE), Indiana, U.S.A. The program handled up to 72 atomic orbitals, which was sufficient for most of the molecules of interest here. An EHT program by R. Hoffmann was also obtained from QCPE but was limited to 68 atomic orbitals. Using this program as a basis two EHT programs were written which handle 80 and 96 atomic orbitals respectively, without requiring additional computer facilities. In both
cases atomic coordinates are calculated within the program so that input data is considerably simplified. These programs have been sent to QCPE for distribution to interested parties, and will not be reproduced here.

2.2 Choice of Drugs

In choosing a set of drugs for this study several factors were taken into account.

The molecular orbital calculations must clearly be made on the active molecular form of the drug, so details of metabolism, dissociation, isomerism, conformation and molecular geometry are desirable. Values for dipole moments would be very useful, as they provide an excellent check on the results of the calculations. Also, since these results are to be compared with anticonvulsant action, activity measurements using the same techniques are essential. Finally, if the comparison is to be quantitative, the distribution of the drug in the test system must also be known.

With the exception of the $\beta$-substituted glutarimides, the compounds shown in Table 2.2 were chosen on the basis of these considerations, with preference being given to drugs which have proved useful clinically. The $\beta$-glutarimides were included because they display such an
### Table 2.2 Drugs Studied

**Compound** | **X** | **Y** | **R** | **R₁** | **R₂**
--- | --- | --- | --- | --- | ---
Barbituric acid | - | CO-NH | H | H | H
5, 5-Diethylbarbituric acid | - | CO-NH | C₂H₅ | C₂H₅ | H
5-Ethyl-5-phenylbarbituric acid | - | CO-NH | C₂H₅ | C₆H₅ | H
5-Ethyldantoin | - | NH | C₂H₅ | H | H
5-Phenylhydantoin | - | NH | C₂H₅ | C₆H₅ | H
5-Ethyl-5-phenylhydantoin | - | NH | C₂H₅ | C₆H₅ | H
5, 5-Diphenylhydantoin | - | NH | C₂H₅ | C₆H₅ | H
Succinimide | - | CH₂ | H | H | H
1-Methylsuccinimide | - | CH₂ | H | H | CH₃
3-Pheynlsuccinimide | - | CH₂ | C₂H₅ | H | H
3-Methyl-3-phenylsuccinimide | - | CH₂ | CH₃ | C₆H₅ | H
3-Ethyl-3-phenylsuccinimide | - | CH₂ | C₂H₅ | C₆H₅ | H
3-Diphenylsuccinimide | - | CH₂ | C₂H₅ | C₆H₅ | H
5, 5-Dimethyloxazolidine-2, 4-dione | - | O | CH₃ | CH₃ | H
3, 5, 5-Trimethyloxazolidine-2, 4-dione | - | O | CH₃ | CH₃ | CH₃
3, 5-Dimethyl-5-ethyl oxazolidine- 2, 4-dione | - | O | C₂H₅ | CH₃ | CH₃
Glutarimide | CH₂ | CH₂ | H | H | H
N-Methylglutarimide | CH₂ | CH₂ | H | H | CH₃
β-Methylglutarimide | CH₂ | CH₂ | CH₃ | H | H
β,β-Dimethylglutarimide | CH₂ | CH₂ | CH₃ | CH₃ | H
β-Methyl-β-ethylglutarimide | CH₂ | CH₂ | CH₃ | C₂H₅ | H
β-Methyl-β-n-propylglutarimide | CH₂ | CH₂ | CH₃ | n-C₃H₇ | H
β-Methyl-β-n-butylglutarimide | CH₂ | CH₂ | CH₃ | n-C₄H₉ | H
α-Ethyl-α-phenylglutarimide | (CH₂)₂ | C₂H₅ | C₆H₅ | H
interesting range of activity with only slight changes in structure.

A generalized structural formula for the entire set is shown at the top of Table 2.2. The biologically active centre proposed by Perkow for these compounds is marked with an asterisk.

2.3 Choice of Molecular State

Many anticonvulsants are administered as N-methylated derivatives of the species which is thought to be active, and the methyl group is subsequently removed metabolically\(^2\),\(^{21}\). Other known metabolic products do not exhibit related activity\(^2\), so the test group remains as shown in Table 2.2. The N-methylated compounds are retained for their dipole moments, and also because there is less quantitative data available for the demethylated oxazolidine-2,4-diones.

Ionization of a drug provides another alternative molecular state. Experimental pK\(_a\) values, and calculated percentages of ionized forms at physiological pH (7.4) are given in Table 2.3. Although the barbiturates are extensively ionized, they are known to act solely in the neutral form in some instances\(^{101,102}\). Their close activity relationship with the glutarimides, whose active species is surely neutral, indicates that nonionized barbiturates are also involved in anticonvulsant activity. The succinimides and 5,5-dimethyloxazolidine-2,4-dione, which are
Table 2.3 Ionization Data

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Percentage Ionized</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5, 5-Diethylbarbituric acid</td>
<td>7.8</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylbarbituric acid</td>
<td>7.3</td>
<td>56</td>
<td>21</td>
</tr>
<tr>
<td>Glutarimide</td>
<td>11.2</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td>Succinimide</td>
<td>9.4</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>3-Phenylsuccinimide</td>
<td>8.7</td>
<td>5</td>
<td>99</td>
</tr>
<tr>
<td>3-Methyl-3-phenylsuccinimide</td>
<td>8.8</td>
<td>4</td>
<td>99</td>
</tr>
<tr>
<td>3-Ethyl-3-phenylsuccinimide</td>
<td>8.8</td>
<td>4</td>
<td>99</td>
</tr>
<tr>
<td>3, 3-Diphenylsuccinimide</td>
<td>8.3</td>
<td>11</td>
<td>99</td>
</tr>
<tr>
<td>5, 5-Dimethyloxazolidine-2, 4-dione</td>
<td>6.2</td>
<td>94</td>
<td>21</td>
</tr>
<tr>
<td>Hydantoin</td>
<td>9.1</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>5, 5-Dimethylhydantoin</td>
<td>9.2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylhydantoin</td>
<td>8.5</td>
<td>7</td>
<td>21</td>
</tr>
</tbody>
</table>

predominantly nonionized and ionized respectively, also have similar activity spectra, but there is no definite evidence concerning the active form. For consistency it is assumed that the nonionized species are active, but the results for 5, 5-dimethyloxazolidine-2, 4-dione are consequently less certain. The ionization constants for the hydantoin derivatives
clearly favour the nonionized drugs as the active forms.

In each of the drugs studied ionization is partly due to the tautomeric equilibrium between the lactam (\(-\text{CO-NH-}\)) and lactim (\(-\text{C(OH)=N-}\)) forms, since the latter tautomer dissociates readily. However infrared\(^{99,103-5}\) and NMR\(^{106}\) evidence suggest that the lactams are strongly favoured in the neutral species. The dipole moments calculated here (Chapter 3) for the lactam forms strengthen this conclusion.

The presence of asymmetrical carbon atoms in several anticonvulsants allows the separation of optical isomers, but the observed anticonvulsant activity of the two isomers is almost identical\(^{107}\). Furthermore, since optical isomers are mirror images of each other, molecular orbital calculations will give the same results in both cases. This situation does not apply for geometrical changes, which have a direct influence on the calculated orbitals. The choice of molecular geometries and conformational states is considered in the next two sections.

2.4 Molecular Geometries

With the exception of succinimide, accurate experimental geometries were not available for the drugs when the work was begun, so ring geometries were derived from a study of related molecules. For example
the geometry used for the barbiturate ring was based upon the crystal structures\textsuperscript{108} of the compounds shown in Figure 2.1. The bonds and angles containing the same atomic groups as those in the barbiturate ring were weighted according to the similarity of their environments and then averaged. Minimal adjustments were made to the average values to form a closed ring. The validity of this procedure is demonstrated in Table 2.4 where the calculated geometry is compared with recently published crystal structures of 5-methyl-5-phenylbarbituric acid\textsuperscript{109}, 5-cycloheptene-5-ethylbarbituric acid\textsuperscript{110} and 5-ethyl-5-phenylbarbituric acid in a 1:2 crystal complex with 8-bromo-9-ethyladenine\textsuperscript{111}. It is apparent that the carbon-carbon single bonds ($C_2 - C_3$ and $C_3 - C_4$) should be a little longer, with the $C_2 - C_3 - C_4$ angle being consequently decreased, but in general the differences are insignificant.

The crystal structures in Figure 2.1 were included in the set used to calculate the glutarimide ring geometry, and those of hydantoin and oxazolidine-2,4-dione were based on individual series of known 5-membered ring structures. All the rings, except glutarimide, were assumed to be planar, with the $C = O$ and $N - H$ bonds bisecting external angles. The calculated ring geometries are given in Figure 2.2.

The bond lengths and angles used for the substituent groups were
Figure 2.1  Structures Related to Barbiturate Ring

diketopiperazine  cyanuric acid

dialuric acid  uracil  2-pyridone

6-carbamoyl-3-pyridazone  xanthazole
Table 2.4 Calculated and Experimental Barbiturate Geometries

![Barbiturate Geometries Diagram]

<table>
<thead>
<tr>
<th>Bond Lengths (Å)</th>
<th>Calculated</th>
<th>Experimental</th>
<th>Ref. 109</th>
<th>Ref. 110</th>
<th>Ref. 111</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₁ - C₁</td>
<td>1.355</td>
<td>1.37</td>
<td>1.41</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>C₁ - N₂</td>
<td>1.355</td>
<td>1.40</td>
<td>1.37</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>N₂ - C₂</td>
<td>1.378</td>
<td>1.40</td>
<td>1.38</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>C₂ - C₃</td>
<td>1.477</td>
<td>1.53</td>
<td>1.55</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>C₃ - C₄</td>
<td>1.477</td>
<td>1.52</td>
<td>1.52</td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td>C₄ - N₁</td>
<td>1.378</td>
<td>1.38</td>
<td>1.39</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>C₁ - O₁</td>
<td>1.24</td>
<td>1.22</td>
<td>1.21</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>C₂ - O₂</td>
<td>1.24</td>
<td>1.22</td>
<td>1.20</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>C₄ - O₃</td>
<td>1.24</td>
<td>1.22</td>
<td>1.23</td>
<td>1.21</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bond Angles</th>
<th></th>
<th></th>
<th>Ref. 109</th>
<th>Ref. 110</th>
<th>Ref. 111</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₁ - C₁ - N₂</td>
<td>118.0</td>
<td>116.8</td>
<td>117.6</td>
<td>118.3</td>
<td></td>
</tr>
<tr>
<td>C₁ - N₂ - C₂</td>
<td>124.5</td>
<td>124.5</td>
<td>126.7</td>
<td>124.8</td>
<td></td>
</tr>
<tr>
<td>N₂ - C₂ - C₃</td>
<td>119.0</td>
<td>117.5</td>
<td>118.5</td>
<td>117.8</td>
<td></td>
</tr>
<tr>
<td>C₂ - C₃ - C₄</td>
<td>115.0</td>
<td>113.1</td>
<td>112.7</td>
<td>110.3</td>
<td></td>
</tr>
<tr>
<td>C₃ - C₄ - N₁</td>
<td>119.0</td>
<td>118.6</td>
<td>121.3</td>
<td>118.9</td>
<td></td>
</tr>
<tr>
<td>C₄ - N₁ - C₁</td>
<td>124.5</td>
<td>126.3</td>
<td>123.0</td>
<td>123.5</td>
<td></td>
</tr>
</tbody>
</table>
published\textsuperscript{108} average values; C - C, 1.54 Å; C - C (aromatic), 1.39 Å; N - C, 1.47 Å; C - H, 1.09 Å; N - H, 1.00 Å; sp\textsuperscript{3}, 109°28'; sp\textsuperscript{2}, 120°.

Figure 2.2 Calculated Ring Geometries

![Hydantoin](image)

![Oxazolidine-2, 4-dione](image)

![Succinimide](image)

![Glutarimide](image)
2.5 Molecular Conformation

Initially, calculations of molecular conformation using EHT were envisaged for several representative drugs, but the degree of conformational freedom in the molecules, together with their size, made the computation time required prohibitive. Fortunately the results of preliminary calculations provided reasons for eliminating this procedure.

Conformations were calculated for 5-ethylhydantoin and 5-phenylhydantoin, and a projected conformation of 5-ethyl-5-phenylhydantoin was derived from these computed conformations and that of propyl benzene. The results were presented at a seminar during which molecular models were distributed among the participants, who were asked to predict likely conformations. With the exception of a group comprising two pharmacologists and a theoretical chemist, intuition proved equally effective with EHT and several hours of computer time. The dissenting group had considered possible changes in conformation due to interactions with biological substrates, and thus provided a timely reminder of the major assumptions underlying this and many other molecular approaches to drug action.

A second, and more rigorous, argument was supplied by the
Table 2.5  Conformation of 5-Ethylhydantoin

![Chemical structure diagram]

<table>
<thead>
<tr>
<th>Dihedral Angles</th>
<th>Atomic Charges</th>
<th>Relative Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta$</td>
<td>$\phi$</td>
<td>BAC</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.206</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>0.225</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
<td>0.209</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>0.224</td>
</tr>
<tr>
<td>240</td>
<td>0</td>
<td>0.213</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>0.222</td>
</tr>
<tr>
<td>0</td>
<td>180</td>
<td>0.210</td>
</tr>
<tr>
<td>60</td>
<td>180</td>
<td>0.220</td>
</tr>
<tr>
<td>120</td>
<td>180</td>
<td>0.207</td>
</tr>
<tr>
<td>180</td>
<td>180</td>
<td>0.220</td>
</tr>
<tr>
<td>240</td>
<td>180</td>
<td>0.214</td>
</tr>
<tr>
<td>300</td>
<td>180</td>
<td>0.218</td>
</tr>
</tbody>
</table>
calculated atomic charges, which are shown for 5-ethylhydantoin in Table 2.5. The charges on the atoms in the bonding group are seen to be almost independent of conformation, while that at the BAC is effectively constant when compared to the variations between different molecules (Chapter 3). Knowledge of which conformation is most

![Skeletal Conformations for β-Glutaramides](image)

- glutarimide
- β-methylglutarimide
- β,β-dimethylglutarimide
- β-methyl-β-ethylglutarimide
- β-methyl-β-n-propylglutarimide
- β-methyl-β-n-butylglutarimide
favourable energetically is therefore comparatively unimportant, and conformations used for other drugs were chosen using molecular models. In doing so the computed conformations of the ethyl and phenyl derivatives of hydantoin were borne in mind, and so far as possible the conformations chosen were consistent throughout the set of drugs. As an example, the skeletal conformations chosen for the β-glutarimides, which have most degrees of freedom, are shown in Figure 2.3.

2.6 An Interesting Radical

The release of hydroxyl radicals in the presence of formamide results in abstraction of a hydrogen atom, leaving a radical with formula \( \text{CH}_2\text{NO}^- \), which is related to the bonding group found in the drugs being studied here. Calculations on this radical may therefore provide some feeling for the nature of the bonding group on an atomic level. However the primary aim of the calculations discussed in this section was to resolve a current debate on the structure of the radical.

The electron spin resonance (ESR) spectrum of \( \text{CH}_2\text{NO}^- \) has been assigned\(^{112,113}\) to configuration I on the basis of hyperfine coupling constants predicted by EHT calculations, although EHT is not really suitable for open-shell problems. On the other hand, comparison with the spectra of related species led\(^{114,115}\) to the conclusion that the
The geometry of formamide (Figure 2.4) has been determined accurately from the microwave spectrum\textsuperscript{116}, and the observed dimensions of the N - C - O core were used for each configuration of the radical. This approximation cannot be rigorously justified, but it was necessary if computation time was to be kept within reasonable limits. The positions of the hydrogen atoms in the three configurations were obtained by minimising total energy. For example, in configuration I the N - H\textsubscript{b} bond length and the C - N - H\textsubscript{a} and C - N - H\textsubscript{b} bond angles were initially held at the observed formamide values, and calculations made for a number of N - H\textsubscript{a} bond lengths. Interpolation of the computed energies to a minimum gave an improved N - H\textsubscript{a} bond length, which was then held constant while the C - N - H\textsubscript{a} angle was varied to give a new minimum energy. This procedure was continued until mutually consistent values.
were obtained for all four variables. The energy curves defining the final position of $H_a$ in configuration I are shown in Figure 2.5, and the bond lengths and angles obtained for all configurations are given in Table 2.6, with corresponding experimental values for formamide.

The computed total energies at different bond lengths or angles were also used to calculate vibrational frequencies, $\nu$. In the classical harmonic approximation \(^{117}\)

$$\nu = \frac{11.57 V^{1/2}}{r} \times 10^{13} \text{ sec}^{-1}$$

where $V$ is the increase in energy (a.u.) which occurs when the hydrogen atom is displaced to a point $r$ Å from the energy minimum. In each case, $\nu$ was determined for several displacements in either direction, so that agreement or otherwise between calculated values could be used to verify or correct the position and energy of the minimum. The calculated vibrational frequencies are presented in Table 2.7, and are
Figure 2.5  Potential Energy Curves Determining Position of $H_a$ in Configuration I
Table 2.6  Calculated Geometries

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Bond Angles</th>
<th>Bond Lengths (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNH</td>
<td>NCH</td>
</tr>
<tr>
<td>I {H_a, H_b}</td>
<td>122.9 ( \pm 1.0° )</td>
<td>1.062 ( \pm 0.01 )</td>
</tr>
<tr>
<td>II</td>
<td>123.9 ( \pm 1.0° )</td>
<td>1.065 ( \pm 0.01 )</td>
</tr>
<tr>
<td>III</td>
<td>109.0 ( \pm 1.0° )</td>
<td>121.6 ( \pm 1.0° )</td>
</tr>
<tr>
<td>Experimental</td>
<td>120.5°</td>
<td>103.9°</td>
</tr>
</tbody>
</table>

(formamide)

Table 2.7  Calculated Vibrational Frequencies \( (10^{13} \text{ sec}^{-1}) \)

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Stretching</th>
<th>Bending</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH</td>
<td>CH</td>
</tr>
<tr>
<td>I {H_a, H_b}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>14.91 ( \pm 1.0 )</td>
<td>13.61 ( \pm 1.0 )</td>
</tr>
<tr>
<td>III</td>
<td>14.66 ( \pm 1.0 )</td>
<td>13.60 ( \pm 1.0 )</td>
</tr>
<tr>
<td>Experimental</td>
<td>10.64, 10.35</td>
<td>3.56</td>
</tr>
</tbody>
</table>

(formamide)
quite similar to those observed\textsuperscript{116} in formamide.

The minimum total energies were used to calculate a Boltzmann distribution over the three configuration, which is given in Table 2.8. It is apparent that configurations II and III are energetically favoured, but this distribution is relevant only if they are in equilibrium with configuration I, which in turn depends on the magnitude of the energy barriers to transitions between the configurations.

Table 2.8 Boltzmann Distribution

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Energy (a.u.)</th>
<th>Boltzmann (300\textdegree K)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Relative</td>
</tr>
<tr>
<td>I</td>
<td>-38.4021</td>
<td>0.0164</td>
</tr>
<tr>
<td>II</td>
<td>-38.4171</td>
<td>0.0014</td>
</tr>
<tr>
<td>III</td>
<td>-38.4185</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Transitions between configurations were initially assumed to involve the movement of either or both protons around the circumference of the lamina shown in Figure 2.6, where the numbers represent clockwise distances (in $\text{Å}$) from an arbitrary origin. The N - H and C - H bond lengths used were those calculated above, and the O - H bond length was taken as 0.960 $\text{Å}$. Total energies were computed for
118 configurations, and are summarised by an energy contour map (Figure 2.7) in which eight minima appear, including configurations I, II and III. The deepest minimum, which corresponds to configuration III, was taken as the energy zero. Because both protons were restricted to the same closed curve, they return to their original positions approximately every 12 Å, and the potential energy surface repeats itself. Furthermore, each configuration occurs twice per cycle, so that configuration I appears a second time in Figure 2.7, with the protons having moved around the circuit until their positions are reversed. The
Figure 2.7  Energy Contour Map for Movement of Protons around Lamina in Figure 2.6
remaining four minima, which are energetically most improbable, are configurations where one proton is associated with the oxygen atom.

Transitions which effectively involve only one proton are marked in Figure 2.7, and the energy barriers for these transitions are given in Figure 2.8. They range from 0.04 to 0.18 a.u. (25 - 113 kcal/mole), and are classically insurmountable. A better reaction pathway, and thus lower energy barriers, could probably be found in the molecular plane, but the differences seem unlikely to be large. However it is possible that significantly lower reaction pathways may lie outside the molecular plane.

Nonplanar reaction pathways were considered for the one-proton transitions I ↔ II and II ↔ III. The I ↔ III transition was excluded because the planar results above indicate that the same result can be achieved, but more easily, by combining the other two transitions.

There is no obvious alternative to the original reaction pathway between configurations I and II, so an entire reaction surface was obtained by rotating the original pathway around the C - N bond axis. The best reaction path is marked on the resulting contour map (Figure 2.9), and involves the proton moving up to 80° from the molecular plane near the centre of the C - N bond, with a barrier height of approximately 0.04 a.u.
Figure 2.8  Energy Barriers
Figure 2.9  Contour Map for I ↔ II Nonplanar Transition
For the II ↔ III transition the nonplanar reaction path chosen was the semicircle with $\angle \text{CNH} = 109^\circ$, which is approximately the equilibrium angle calculated for both configurations (see Table 2.6). The calculated energy barrier, which is quite small, is shown in Figure 2.10, and two nonplanar configurations are seen to be more stable than configurations II or III.

Both the I ↔ II and II ↔ III nonplanar barriers are considerably lower than their planar counterparts, and it was thought worthwhile to calculate transition rates for quantum-mechanical proton tunnelling through these barriers.

The minimum tunnelling energy, E, for each reaction path was taken as the energy of the upper minimum plus the zero point energy for one proton. The zero point energy used, 0.0028 a.u., corresponds to the average of the calculated bending frequencies, $3.69 \times 10^{13}$ sec$^{-1}$.

Figure 2.10  Nonplanar II ↔ III Transition
It was assumed that all tunnelling protons were at the minimum tunnelling energy, which amounts to assuming that an accessible excited state lies at or near this level, and that higher states have negligible populations. The tunnelling probability, $g$, may then be approximated by

$$g = \exp(-2s) \quad (2.15)$$

and

$$s = \left(\frac{2\pi}{\hbar}\right) \left(\int [2m(V(x)-E)]^{1/2} \, dx\right) \quad (2.16)$$

where $x$ is the distance along the reaction path, $V(x)$ is the barrier height, and $m$ is the mass of a proton. The integral was evaluated graphically over the classically forbidden section of the reaction path. The average tunnelling time, $t$, required for a given proton to make the transition is related to the tunnelling probability by

$$t = (gN\nu)^{-1} \text{ seconds} \quad (2.17)$$

where $N$ is the Boltzmann fraction of the protons in the appropriate configuration which are at the minimum tunnelling energy, and $\nu$ is the frequency of incidence of these protons at the barrier. This was assumed to be the average bending frequency, $3.69 \times 10^{13} \text{ sec}^{-1}$.

The tunnelling probabilities and times are given in Table 2.9, where calculations on the planar transitions are included for comparison. It is apparent that only the II $\leftrightarrow$ III nonplanar transition is fast enough to
Table 2.9  Tunnelling Times

<table>
<thead>
<tr>
<th>Transition</th>
<th>Tunnelling Probability, g</th>
<th>Tunnelling Time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Forward</td>
<td>Backward</td>
</tr>
<tr>
<td>I ↔ II</td>
<td>$5 \times 10^{-45}$</td>
<td>$5 \times 10^{30}$</td>
</tr>
<tr>
<td>I ↔ III</td>
<td>$5 \times 10^{-160}$</td>
<td>$6 \times 10^{145}$</td>
</tr>
<tr>
<td>II ↔ III</td>
<td>$3 \times 10^{-36}$</td>
<td>$8 \times 10^{21}$</td>
</tr>
<tr>
<td>I ↔ II</td>
<td>$2 \times 10^{-32}$</td>
<td>$1 \times 10^{19}$</td>
</tr>
<tr>
<td>(nonplanar)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II ↔ III</td>
<td>$2 \times 10^{-4}$</td>
<td>$1 \times 10^{-10}$</td>
</tr>
<tr>
<td>(nonplanar)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

occur during the lifetime of the radical, and all transitions to or from configuration I are energetically forbidden. Free rotation in configuration I is also unlikely, since tetrahedral and other nonplanar alternatives have higher total energies, and calculations based on a $[\text{CNH}]$ angle of $123^\circ$ yield an energy barrier (Figure 2.11) of approximately 0.03 a.u. (19 kcal/mole) to rotation around the C - N bond. This barrier is comparable to the observed activation energy for internal rotation in dimethyl formamide, which is 20.5 kcal/mole.

On the basis of these results, it is concluded that if the radical is formed by abstracting a hydrogen atom from nitrogen, an equilibrium
between II, III and intermediate configurations would result. If, on the other hand, the hydrogen attached to carbon is abstracted, the radical would be confined to the energetically unfavourable configuration I.

In order to distinguish between the two alternatives it was decided that hyperfine coupling constants should be calculated for each configuration.

\[
\begin{align*}
\text{Relative Energy, a.u.} & \\
0.00 & \\
0.01 & \\
1 & \\
2 & \\
3 & \\
\end{align*}
\]

Figure 2.11  Rotation Barrier in Configuration I

As pointed out in Section 2.1, the CNDO/2 method is sometimes inadequate\textsuperscript{72} for calculating coupling constants, because it ignores one centre exchange integrals, and INDO was therefore used. In common with CNDO/2, the INDO method is spin unrestricted, so that the wave function obtained is not a pure spin doublet, but includes components with spin quartet or higher multiplicity. This source of error is not of major importance\textsuperscript{121}, but the spin quartet function was nevertheless annihilated using the approximate equations of Amos and Snyder\textsuperscript{122}, which ignore the less important high-multiplicity functions. The procedure was programmed by the author and Mrs. M.J. Scarlett,
using the CNDO/2 program as a basis.

Hydrogen and nitrogen coupling constants, $a_H$ and $a_N$, were obtained from the calculated molecular orbitals using the expressions

$$a_H = 539.86 \rho_H \text{ gauss} \quad (2.18)$$

and

$$a_N = 379.34 \rho_N \text{ gauss} \quad (2.19)$$

where $\rho_H$ and $\rho_N$ are the unpaired spin densities in the hydrogen 1s and nitrogen 2s orbitals. The constants in equation (2.18) and (2.19) were derived empirically from calculations on a number of radicals.

Computed coupling constants for configurations I, II and III, as well as energy-weighted averages for the II $\leftrightarrow$ III equilibrium, are given in Table 2.10, together with experimental values. The values for configuration I fit the observed ESR spectrum well, but neither configurations II and III, nor the II $\leftrightarrow$ III equilibrium values, give reasonable agreement.

The predicted coupling constants thus provide positive evidence that the radical is in configuration I, and the impassable barrier between this form and the lower energy configurations, II and III, provides the additional information that hydrogen abstraction takes place at the formyl carbon. The latter conclusion concurs with a recent study of isotope effects in the gas phase attack by methyl radical on formamide.
The energy calculations also show that hydrogen abstraction from the nitrogen atom would produce a more stable radical, in which free rotation around the C - N bond would occur.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Hyperfine Coupling Constants (gauss)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>I</td>
<td>14.2</td>
</tr>
<tr>
<td>II</td>
<td>-1.1</td>
</tr>
<tr>
<td>III</td>
<td>-0.1</td>
</tr>
<tr>
<td>II ↔ III</td>
<td>3.1</td>
</tr>
<tr>
<td>Experimental</td>
<td>±21.8</td>
</tr>
</tbody>
</table>
CHAPTER THREE

3.1 Dipole Moments

Before comparing the predictions of the MO calculations with biological activity, it is valuable to investigate their validity by comparing the two sets of results with each other, and with experimental data. In order to give some generality to this comparison five additional, but structurally dissimilar, molecules were included. They were carbon dioxide, methane, formamide, methanol and toluene, and in each case experimental geometries (references shown) were used for the calculations.

Dipole moments calculated by the EHT and CNDO/2 methods are compared with available experimental values in Table 3.1. The exaggeration of the charge distribution by EHT, which was mentioned earlier, is immediately apparent in the predicted dipole moments which are much too large. The CNDO/2 method shows the qualitative trends in molecule and their correct quantitative relationship between the three, closely investigated using the lea.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Calculated Dipole</th>
<th>Exptl. Dipole</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EHT</td>
<td>CNDO/2</td>
<td></td>
</tr>
<tr>
<td>Barbituric acid</td>
<td>0.43</td>
<td>0.61</td>
<td>1.04</td>
</tr>
<tr>
<td>5, 5-Diethylbarbituric acid</td>
<td>0.53</td>
<td>0.71</td>
<td>1.13</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylbarbituric acid</td>
<td>1.27</td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>Succinimide</td>
<td>6.40</td>
<td>2.05</td>
<td>1.47</td>
</tr>
<tr>
<td>1-Methylsuccinimide</td>
<td>5.61</td>
<td>1.88</td>
<td>1.61</td>
</tr>
<tr>
<td>3, 5, 5-Trimethyloxazolidine -2, 4-dione</td>
<td>3.73</td>
<td>2.01</td>
<td>1.74</td>
</tr>
<tr>
<td>3, 5-Dimethyl-5-ethyloxazolidine -2, 4-dione</td>
<td>3.73</td>
<td>2.05</td>
<td>1.69</td>
</tr>
<tr>
<td>Glutarimide</td>
<td>9.18</td>
<td>3.10</td>
<td>2.58</td>
</tr>
<tr>
<td>N-Methylglutarimide</td>
<td>8.46</td>
<td>3.03</td>
<td>2.70</td>
</tr>
<tr>
<td>$\beta$-Methyl-$\beta$-ethylglutarimide</td>
<td>9.45</td>
<td>3.34</td>
<td>2.92</td>
</tr>
<tr>
<td>$\alpha$-Ethyl-$\alpha$-phenylglutarimide</td>
<td>8.67</td>
<td></td>
<td>2.83</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.16</td>
<td>0.27</td>
<td>0.43</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.60</td>
<td>1.98</td>
<td>1.71</td>
</tr>
<tr>
<td>Formamide</td>
<td>10.13</td>
<td>3.86</td>
<td>3.48</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Methane</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
being fitted to the best possible straight line. Both \( \text{CO}_2 \) and \( \text{CH}_4 \) were excluded from this analysis, as their dipole moments are zero due to molecular symmetry, and are correctly predicted for precisely the same reason.

The CNDO/2 dipole moments are related to the corresponding experimental values by

\[
\mu_{\text{CNDO/2}} = 1.25 \mu_{\text{Expt.}} - 0.27 \quad (\delta_m = 0.42, \quad \delta_c = 0.87) \quad (3.1)
\]

where \( \delta_m \) and \( \delta_c \) are the standard deviations in slope and intercept respectively. The small value of \( \delta_m \) and the slope of 1.25 vouch for both the qualitative and general quantitative accuracy of CNDO/2. On the other hand, the value of the intercept, which should ideally be zero, and the standard deviation \( \delta_c \) illustrate the specific inaccuracies of the method. These may be partly due to errors in the experimental dipoles, most of which were measured in benzene or dioxane, where significant deviations from gas phase results are to be expected\(^{130}\).

A similar relationship exists for EHT, where

\[
\mu_{\text{EHT}} = 3.68 \mu_{\text{Expt.}} - 1.65 \quad (\delta_m = 0.42, \quad \delta_c = 0.87) \quad (3.2)
\]

and the value of this method for qualitative predictions is also illustrated by the comparatively small value of \( \delta_m \). The quantitative results are
about three times too large, and the deviations from this general rule may be partly explained by the same arguments as those above. It is interesting to note that exceptionally low values are calculated for the barbiturates, where three C = O groups are symmetrically placed around the ring, so that the large charge separations involved in these groups make very little contribution to the calculated dipole moment. This effect may also explain the low dipole moments obtained for the barbiturates using CNDO/2, as opposed to all the other C = O containing molecules, for which the CNDO/2 predictions are fractionally high.

As would be anticipated from equations (3.1) and (3.2), a straight line relationship also holds between the CNDO/2 and EHT results, where

$$\mu_{\text{EHT}} = 2.99 \mu_{\text{CNDO/2}} - 0.92 \quad (\xi_m = 0.27, \xi_c = 0.62), \quad (3.3)$$

and the close qualitative similarity of the two methods is again apparent in the value of $\xi_m$. Equations (3.1), (3.2) and (3.3) thus provide a positive demonstration of the excellent qualitative agreement between the dipole moments predicted by EHT and CNDO/2, and those observed experimentally.

3.2 Atomic Charge

Molecular dipole moments are dependent on net atomic charges
through equation (2.9), and a linear relationship between the EHT and CNDO/2 predictions of atomic charges might therefore be expected. However this expectation relies on the assumption that the two theories discriminate equally between different atoms, and ignores the possibility of cancellation of opposing differences. In view of the different molecular types considered here, the latter possibility seems remote, but is undoubtedly worth testing by direct comparison of the calculated charges.

A least squares analysis was done on the net charges predicted by EHT and CNDO/2 for the 198 different atoms in the molecules listed in Table 3.1. Many of the atoms have the same nearest neighbours, and to avoid undue emphasis on particular environments, average values were used in all these cases. The set was thus reduced to 24 atoms, comprising fourteen carbons, four oxygens, three nitrogens and three hydrogens. The average charges ranged from -1.33 to 1.99 for EHT, and from -0.34 to 0.54 for CNDO/2. The two sets of data are plotted against each other in Figure 3.1, and are related by

\[ Q_{EHT} = 3.37 Q_{CNDO/2} + 0.02 \quad (\delta_m = 0.16, \quad \delta_c = 0.04) \]  

where the low values of \( \delta_m \) and \( \delta_c \), and the near zero value of the intercept, indicate a rather smooth qualitative relationship between
Figure 3.1  EHT and CNDO/2 Charges for Atoms with Different Nearest Neighbours
the two. They also imply that different atomic types are equally favoured by both methods, but the clusters of nitrogen and oxygen atoms immediately above and below the line of best fit in Figure 3.1 represent slight imperfections in this relationship. Nevertheless, least squares analysis for the fourteen carbon atoms alone gives the almost identical equation

\[ Q_{\text{EHT}} = 3.40 Q_{\text{CNDO/2}} + 0.00 \ (\xi_m = 0.23, \ \xi_c = 0.06). \] (3.5)

The combination of equations (3.4) and (3.5) demonstrates an excellent correlation between the atomic charges predicted by EHT and CNDO/2, but only for the case where the atoms are in distinctly different environments. The atoms of interest here, namely the BAC and those in the bonding group, do not fall into this category, and indeed those in the bonding group have identical nearest neighbours in all of the molecules considered. The ability or otherwise of EHT and CNDO/2 to follow the same trends for these atoms will be considered in the next two sections, where the atomic charges will also be compared with observed biological activity.

3.3 Biologically Active Centre Hypothesis

The calculated net charges on the BAC are given in Table 3.2,
<table>
<thead>
<tr>
<th>Compound</th>
<th>Charge on BAC</th>
<th>ES</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Charge on BAC</td>
<td>ES</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>EHT</td>
<td>CNDO/2</td>
<td></td>
</tr>
<tr>
<td>3, 5-Dimethyl-5-ethyl-oxazolidine-2, 4-dione</td>
<td>0.602 0.118</td>
<td>2.55</td>
<td>134</td>
</tr>
<tr>
<td>3, 5, 5-Trimethyl-oxazolidine-2, 4-dione</td>
<td>0.618 0.122</td>
<td>6.85</td>
<td>134</td>
</tr>
<tr>
<td>5, 5-Dimethyl-oxazolidine-2, 4-dione</td>
<td>0.616 0.126</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5, 5-Diphenylhydantoil</td>
<td>0.329</td>
<td>0.04</td>
<td>135</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylhydantoil</td>
<td>0.330 0.085</td>
<td>0.19</td>
<td>135</td>
</tr>
<tr>
<td>5-Phenylhydantoil</td>
<td>0.206 0.061</td>
<td>0.90</td>
<td>136</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylbarbituric acid</td>
<td>0.050</td>
<td>0.10</td>
<td>135</td>
</tr>
<tr>
<td>5, 5-Diethylbarbituric acid</td>
<td>0.048 -0.063</td>
<td>1.01</td>
<td>137</td>
</tr>
<tr>
<td>3, 3-Diphenylsuccinimide</td>
<td>0.044</td>
<td>0.18</td>
<td>138</td>
</tr>
<tr>
<td>3-Ethyl-3-phenylsuccinimide</td>
<td>0.047</td>
<td>0.29</td>
<td>138</td>
</tr>
<tr>
<td>3-Methyl-3-phenylsuccinimide</td>
<td>0.065 -0.022</td>
<td>0.53</td>
<td>138</td>
</tr>
<tr>
<td>3-Phenylsuccinimide</td>
<td>-0.080 -0.043</td>
<td>1.70</td>
<td>138</td>
</tr>
<tr>
<td>Succinimide</td>
<td>-0.197 -0.061</td>
<td>&gt;4</td>
<td>138</td>
</tr>
</tbody>
</table>

Table 3.2 BAC Charges and Electroshock Activity (ES)
as well as the dose of each drug (in millimoles per kilogram body weight) which protects 50% of a group of mice from the tonic extensor phase of a convolution induced by the supramaximal electroshock method.\(^{133}\)

The BAC charges predicted by EHT and CNDO/2 are plotted in Figure 3.2, and least squares analysis of these values leads to the significant correlation

\[
Q_{\text{EHT}} = 3.37 \, Q_{\text{CNDO/2}} + 0.10 \quad (\xi_m = 0.56, \, \xi_c = 0.04) \, , \quad (3.6)
\]

so that either set of charges is suitable for comparison with biological activity. In fact EHT is more useful in this instance, since four of the molecules were too large for the CNDO/2 program, and the following discussion is based on the EHT results.

It is apparent from the plot (Figure 3.3) of electroshock activity (ES) against the BAC charges predicted by EHT, that no direct relationship exists between the two, and this is confirmed by the least squares result.

\[
ES = 2.89 \, Q_{\text{EHT}} + 1.03 \quad (\xi_m = 2.40, \, \xi_c = 0.71) \, . \quad (3.7)
\]

However this equation does not allow for variations in the ability of the different drugs to reach their site of action, which is influenced by both ionization and liposolubility. Indeed the hypnotic activity within related groups of barbiturates shows a parabolic dependence on
lipophilic character\textsuperscript{139}, with the ideal liposolubility being high enough to allow permeation through the capillaries of the CNS\textsuperscript{140}, but low enough to prevent the molecule being excessively localized in fat.
Figure 3.3  BAC Charge and Electroshock Activity
Both degree of ionization and lipophilic character can be accounted for by considering isoelectronic derivatives of hydantoin and succinimide. The dissociation constants of these compounds are almost identical (see Table 2.3) and the change in ring structure from \(-\text{NH}\)- for the hydantoins to \(-\text{CH}_2\)- for the succinimides is unlikely to have a significant effect on liposolubility. Comparison of the pairs of drugs bearing the same substituents shows that although each hydantoin derivative is a little more active than the isoelectronic succinimide, the correspondence between the two series is extremely close. However the charge at the BAC for the hydantoin derivatives lies between +0.206 and +0.330, which is distinctly different from the range of -0.080 to +0.047 observed for the succinimides. It is apparent that this large difference does not correspond to a significant change in activity, and the BAC charge therefore cannot be used to predict the activity of different chemical classes against electroshock induced seizures.

It is also interesting to examine the trends in activity within each chemical class. For the hydantoins, increasing activity is superficially accompanied by an increase in positive charge on the BAC, and with the exception of the methylphenyl derivative the same trend pertains to the succinimide series. However variations in lipophilic character must again be taken into account. The relative liposolubility of the
individual members of each series may be estimated using the generally recognized \(^{139}\) additivity of substituent effects which operates for many organic/aqueous partition coefficients. The increase in liposolubility due to the substituents of interest here falls in the order \(^{141, 142}\)

\[
\text{phenyl} > \text{ethyl} > \text{methyl} > \text{hydrogen}
\]

so that the lipophilic character of the three hydantoin derivatives can be summarised by

\[
diphenyl > \text{ethylphenyl} > \text{phenyl},
\]

and that of the succinimides by

\[
diphenyl > \text{ethylphenyl} > \text{methylphenyl} > \text{phenyl} > \text{hydrogen}.
\]

In both series precisely the same sequence applies to activity against electroshock, so that the increase in activity could be interpreted simply as an approach towards ideal lipophilic character. The same increase in activity with liposolubility can be seen for the two barbiturates where the BAC charge is effectively constant, and for the oxazolidine-2,4-diones where the charge on the BAC is higher for the less active compound. The partial success of the BAC hypothesis in predicting the electroshock activity of the succinimides and hydantoins
can be ascribed to the fact that the substituent groups employed are not only more lipophilic, but also more electronegative than hydrogen atoms, so the removal of a substituent results in a less positive BAC charge. This relationship cannot be extended to the individual substituents, since methyl groups for example, are more electronegative but less liposoluble than the other substituents. The high BAC charge but low activity of methylphenylsuccinimide, and the opposing changes in BAC charge and activity of the oxazolidine-2, 4-diones are direct results of this distinction. Thus, although the charge at the BAC may sometimes appear to follow the trends in activity of compounds from a particular chemical class, the correlation could prove nothing more than the existence of an imperfect relationship between the BAC charge and liposolubility.

In summary, the charge at the BAC of compounds from the same chemical class, or from different chemical classes, does not represent a reasonable criterion for activity against convulsions induced by electroshock.

As discussed in Chapter 1, the actions of a drug against convulsions induced by electroshock and those induced by pentylenetetrazole are often distinctly different. The BAC charges discussed above are
therefore reproduced in Table 3.3, where they may be compared with
the number of rats (out of five) protected, at a specific dose (in
millimoles/kilogram body weight), from convulsions induced by the
subcutaneous administration of 93 mg./kilogram of pentylenetetrazole\textsuperscript{135}.

The variation in the number of animals protected by each drug
precludes direct least squares analysis of all the data, but the method
can be applied to those compounds which protect the whole group. The
BAC charges predicted by EHT are plotted with the observed activity
against pentylenetetrazole (PA) in Figure 3.4, and the line of best
fit is given by

\[
PA = 1.24 \, Q_{\text{EHT}} + 0.75 \quad (\xi_m = 1.22, \; \xi_c = 0.43) ,
\]  

(3.8)

where the values of $\xi_m$ and $\xi_c$ indicate that almost any other line
would have been equally suitable. Once again, the variations in
activity must be partly due to differences in degree of ionization and
liposolubility, but these factors become irrelevant upon consideration
of the difference in BAC charge of 0.698 between trimethyloxazolidine-
2,4-dione and phenylsuccinimide, which both protect all the animals in
approximately the same dosage. When this range in charge is compared
to the difference of 3.32 between the carbon charges calculated by EHT
for the very extreme cases of methane ($Q_c = -1.33$) and carbon dioxide
Table 3.3  BAC Charges and Pentylenetetrazole Activity (PA)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Charge in BAC</th>
<th>PA</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3, 5-Dimethyl-5-ethyloxadizidine -2, 4-dione</td>
<td>0.602</td>
<td>0.118</td>
<td>5/0.80 134</td>
</tr>
<tr>
<td>3, 5, 5-Trimethyloxazolidine -2, 4-dione</td>
<td>0.618</td>
<td>0.122</td>
<td>5/1.75 134</td>
</tr>
<tr>
<td>5, 5-Dimethyloxazolidine -2, 4-dione</td>
<td>0.616</td>
<td>0.126</td>
<td></td>
</tr>
<tr>
<td>5-Ethyl-5-phenylhydantoin</td>
<td>0.330</td>
<td>0.085</td>
<td>5/2.44 134</td>
</tr>
<tr>
<td>5-Phenylhydantoin</td>
<td>0.206</td>
<td>0.061</td>
<td>3/2.82 134</td>
</tr>
<tr>
<td>5, 5-Diphenylhydantoin</td>
<td>0.329</td>
<td></td>
<td>0/1.97 135</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylbarbituric acid</td>
<td>0.050</td>
<td></td>
<td>5/0.21 135</td>
</tr>
<tr>
<td>3-Ethyl-3-phenylsuccinimide</td>
<td>0.047</td>
<td></td>
<td>5/0.32 138</td>
</tr>
<tr>
<td>3-Methyl-3-phenylsuccinimide</td>
<td>0.065</td>
<td>-0.022</td>
<td>5/0.34 138</td>
</tr>
<tr>
<td>3-Phenylsuccinimide</td>
<td>-0.080</td>
<td>-0.043</td>
<td>5/1.42 138</td>
</tr>
<tr>
<td>3, 3-Diphenylsuccinimide</td>
<td>0.044</td>
<td></td>
<td>0/1.97 138</td>
</tr>
<tr>
<td>Succinimide</td>
<td>-0.197</td>
<td>-0.061</td>
<td>0/5.05 138</td>
</tr>
</tbody>
</table>

(Q_c = 1.99), it is apparent that most organic compounds would contain carbon atoms in the 'active' range, so that the BAC hypothesis is once more unsatisfactory for predicting the relative activity of different chemical classes.
The influence of the BAC within a related series of drugs is again illustrated by the hydantoin and succinimide derivatives. A relatively large dose of 5-ethyl-5-phenylhydantoin protects the whole group of animals, but a similar dose of the diphenyl derivative, whose BAC charge is almost identical, provides no protection. On the other hand, 5-phenyl hydantoin gives a measure of protection but the BAC charge is markedly different. Similarly, diphenylsuccinimide is inactive at six times the
dosage required for complete protection with ethylphenylsuccinimide although it bears approximately the same charge at the BAC, while phenylsuccinimide is fully effective despite a change in sign of the BAC charge.

In conclusion, there seems no doubt that the activities of drugs within a chemical class, or from different chemical classes, against seizures induced by pentylenetetrazole are not dependent on the calculated charge at the BAC.

Before leaving this subject, it is interesting to compare the variation in charge at the BAC with the qualitative change in activity displayed by the $\beta$-glutarimides$^{32}$. Inspection of Table 3.4, where

<table>
<thead>
<tr>
<th>Compound</th>
<th>BAC Charge (CNDO/2)</th>
<th>Type of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutarimide</td>
<td>0.033</td>
<td>Inactive</td>
</tr>
<tr>
<td>$\beta$-Methylglutarimide</td>
<td>0.053</td>
<td>Inactive</td>
</tr>
<tr>
<td>$\beta, \beta'$-Dimethylglutarimide</td>
<td>0.069</td>
<td>Convulsant</td>
</tr>
<tr>
<td>$\beta$-Methyl-$\beta'$-ethylglutarimide</td>
<td>0.064</td>
<td>Convulsant</td>
</tr>
<tr>
<td>$\beta$-Methyl-$\beta'$-n-propylglutarimide</td>
<td>0.058</td>
<td>Dual Action</td>
</tr>
<tr>
<td>$\beta$-Methyl-$\beta'$-n-butyglutarimide</td>
<td>0.060</td>
<td>Anticonvulsant</td>
</tr>
</tbody>
</table>

the glutarimide BAC charges are tabulated, shows that the charge on
the BAC remains almost constant as activity changes from convulsant to anticonvulsant, and the changes which are present are not systematically related to the differences in activity.

The preceding results for the $\beta$-glutarimides, taken in conjunction with those for the clinically useful drugs, demonstrate that the charge on BAC is unimportant in determining the qualitative activity of convulsant or anticonvulsant drugs, and is unrelated to quantitative activity against seizures induced by either electroshock or pentylene-tetrazole. The biologically active centre hypothesis is therefore quite untenable in this instance, and the results inevitably cast some doubt on the validity of the hypothesis in other systems where the same basic assumptions have been employed\textsuperscript{40}.

3.4 The Common Bonding Group

As mentioned in Chapter 1, there is a molecular fragment potentially capable of forming multiple hydrogen bonds which occurs in all of the drugs considered except the N-methylated oxazolidine-2, 4-diones, and the latter drugs are metabolically demethylated to compounds containing the same group. The structure of this common bonding group (CBG) is shown in Figure 3.5, where the atoms which could be directly
involved in hydrogen bonding are $O_1$, $H_1$ and $O_2$. The ability of these atoms to participate in hydrogen bonds will be partly determined by the magnitude of their net charges, which are presented for the clinically useful drugs in Table 3.5. The charges calculated by EHT and CNDO/2 are compared in Figure 3.6 ($O_1$ and $O_2$) and Figure 3.7 ($H_1$). There is obviously no close relationship between the two sets of results, and the best least squares fit is given by

$$Q_{EHT} = 0.40 Q_{\text{CNDO/2}} - 1.19 \quad (\xi_m = 0.18, \ \delta_c = 0.06) \quad (3.9)$$

for the oxygen atoms and

$$Q_{EHT} = -0.48 Q_{\text{CNDO/2}} + 0.40 \quad (\xi_m = 0.22, \ \delta_c = 0.03) \quad (3.10)$$

for the hydrogens. In both cases the value of the intercept, which should ideally be zero, is of the same order as the EHT charges, whose observed
<table>
<thead>
<tr>
<th>Compound</th>
<th>Atomic Charges (EHT)</th>
<th>Atomic Charges (CNDO/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₁</td>
<td>H₁</td>
</tr>
<tr>
<td>3, 5-Dimethyl-5-ethyloxazolidine-2, 4-dione</td>
<td>-1.327</td>
<td>-1.313</td>
</tr>
<tr>
<td>3, 5, 5-Trimethyloxazolidine-2, 4-dione</td>
<td>-1.327</td>
<td>-1.312</td>
</tr>
<tr>
<td>5, 5-Dimethyloxazolidine-2, 4-dione</td>
<td>-1.327</td>
<td>0.323</td>
</tr>
<tr>
<td>5, 5-Diphenylhydantoin</td>
<td>-1.317</td>
<td>0.322</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylhydantoin</td>
<td>-1.326</td>
<td>0.322</td>
</tr>
<tr>
<td>5-Phenylhydantoin</td>
<td>-1.335</td>
<td>0.322</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylbarbituric acid</td>
<td>-1.311</td>
<td>0.332</td>
</tr>
<tr>
<td>5, 5-Diethylbarbituric acid</td>
<td>-1.329</td>
<td>0.332</td>
</tr>
<tr>
<td>3, 3-Diphenylsuccinimide</td>
<td>-1.331</td>
<td>0.321</td>
</tr>
<tr>
<td>3-Ethyl-3-phenylsuccinimide</td>
<td>-1.340</td>
<td>0.321</td>
</tr>
<tr>
<td>3-Methyl-3-phenylsuccinimide</td>
<td>-1.340</td>
<td>0.321</td>
</tr>
<tr>
<td>3-Phenylsuccinimide</td>
<td>-1.350</td>
<td>0.322</td>
</tr>
<tr>
<td>Succinimide</td>
<td>-1.353</td>
<td>0.321</td>
</tr>
</tbody>
</table>
Figure 3.6  Carbonyl Oxygen Atom Charges

Figure 3.7  Imide Hydrogen Atom Charges
variations are accounted for by the large uncertainties in slope, $s_m$. Equations (3.9) and (3.10) plainly state that for the atoms in the CBG, which always have the same nearest neighbours, no correlation exists between the charges calculated by EHT and CNDO/2, so either one or both of the two sets of data is unsatisfactory for comparison with biological activity. The evidence outlined in Chapter 2 suggests that CNDO/2 would be preferable, but only the EHT calculations could be carried out for all of the molecules. Fortunately, the following discussion of the CBG charges and observed activity leads to conclusions which circumvent the problem of choosing between the CNDO/2 and EHT results.

For convenient comparison of CBG charges with activity, the individual charges have been combined to give

$$Q_T = Q_{H_1} - Q_{O_1} - Q_{O_2},$$

(3.11)

which provides an estimate of the overall hydrogen bonding capacity of the CBG to a suitable substrate. The values of $Q_T$ obtained using equation (3.11) are given in Tables 3.6 and 3.7, where they may be compared with activities against electroshock and pentylenetetrazole. In each case the activity of 5, 5-dimethyloxazolidine-2, 4-dione has been assumed to equal that of trimethyloxazolidine-2, 4-dione, and the units
Table 3.6  Total CBG Charge and Electroshock Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total CBG Charge, $Q_T$</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EHT</td>
<td>CNDO/2</td>
</tr>
<tr>
<td>5, 5-Diphenylhydantoin</td>
<td>2.980</td>
<td>0.04</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylbarbituric acid</td>
<td>2.986</td>
<td>0.10</td>
</tr>
<tr>
<td>3, 3-Diphenylsuccinimide</td>
<td>3.005</td>
<td>0.18</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylhydantoin</td>
<td>2.989</td>
<td>0.929</td>
</tr>
<tr>
<td>3-Ethyl-3-phenylsuccinimide</td>
<td>3.013</td>
<td>0.29</td>
</tr>
<tr>
<td>3-Methyl-3-phenylsuccinimide</td>
<td>3.013</td>
<td>0.876</td>
</tr>
<tr>
<td>3-Phenylsuccinimide</td>
<td>3.025</td>
<td>0.875</td>
</tr>
<tr>
<td>5-Phenylhydantoin</td>
<td>2.998</td>
<td>0.879</td>
</tr>
<tr>
<td>5, 5-Diethylbarbituric acid</td>
<td>3.003</td>
<td>0.848</td>
</tr>
<tr>
<td>3-Phenylsuccinimide</td>
<td>3.025</td>
<td>0.875</td>
</tr>
<tr>
<td>Succinimide</td>
<td>3.027</td>
<td>0.898</td>
</tr>
<tr>
<td>5, 5-Dimethyloxazolidine-2, 4-dione</td>
<td>2.963</td>
<td>870</td>
</tr>
</tbody>
</table>

and references for all the activity data are the same as those given in the preceding section. In neither Table 3.6 nor Table 3.7 is there any indication of a relationship between the relevant activity measurement and either set of calculated total charges. In fact, the overall variation in $Q_T$ is extremely small, and indicates that the ability of the CBG to form hydrogen bonds is about the same for all of these clinically useful
Table 3.7  Total CBG Charge and Pentylenetetrazole Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total CBG Charge, $Q_T$</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EHT CNDO/2</td>
<td></td>
</tr>
<tr>
<td>5-Ethyl-5-phenylbarbituric acid</td>
<td>2.986</td>
<td>5/0.21</td>
</tr>
<tr>
<td>3-Ethyl-3-phenylsuccinimide</td>
<td>3.013</td>
<td>5/0.32</td>
</tr>
<tr>
<td>3-Methyl-3-phenylsuccinimide</td>
<td>3.013 0.876</td>
<td>5/0.34</td>
</tr>
<tr>
<td>3-Phenylsuccinimide</td>
<td>3.025 0.875</td>
<td>5/1.42</td>
</tr>
<tr>
<td>5,5-Dimethyloxazolidine-2,4-dione</td>
<td>2.963 0.870</td>
<td>(5/1.75)</td>
</tr>
<tr>
<td>5-Ethyl-5-phenylhydantoin</td>
<td>2.989 0.929</td>
<td>5/2.44</td>
</tr>
<tr>
<td>5-Phenylhydantoin</td>
<td>2.998 0.879</td>
<td>3/2.82</td>
</tr>
<tr>
<td>5,5-Diphenylhydantoin</td>
<td>2.980</td>
<td>0/1.97</td>
</tr>
<tr>
<td>3,3-Diphenylsuccinimide</td>
<td>3.005</td>
<td>0/1.97</td>
</tr>
<tr>
<td>Succinimide</td>
<td>3.027 0.898</td>
<td>0/5.05</td>
</tr>
</tbody>
</table>

If hydrogen bonding through the CBG were the sole means of attaching the drugs to their site of action, then the foregoing results would provide evidence for the view that quantitative variations in activity depend on the conformational changes which the drug induces in a biological substrate. For example the degree of ionization, lipophilic character and CBG charges of diphenylsuccinimide and ethyl-
phenylsuccinimide are roughly equivalent, so their ability to reach the site of action, and to bond to it, should also be similar. A further variable must therefore be invoked to explain the very different activities of these drugs against pentylenetetrazole, and an obvious possibility is that different conformational changes are induced due to the differences in shape and conformation of the substituents. On the other hand, there is no reason to suppose that the CBG is alone in determining the strength of bonding to any one substrate, and the point charges and dipoles elsewhere in the ring, as well as van der Waals bonding by the substituent groups, almost certainly play some part in determining the relative activity of each drug. Nevertheless the difference in activity of the two succinimides, which is paralleled by the corresponding pair of hydantoins, is so distinct that the potential importance of conformational effects cannot be disregarded. For the present, however, the only conclusion which can reasonably be drawn from these results is that the hydrogen bonding ability of the CBG is fairly constant, and that individual differences in quantitative activity are apparently determined by other properties of the molecule, and particularly the substituent groups.

In view of the preceding conclusion, the bonding ability of the CBG in drugs with qualitatively different action is of great interest. The net
charges calculated using CNDO/2 for the atoms in the CBG, and the resulting values of $Q_T$, are given for the $\beta$-glutarimide series in Table 3.8. Once again the CBG charges vary only slightly from drug to drug, and the differences do not follow any obvious pattern.

Particularly notable are the $\beta$-methyl-$\beta$-ethyl and $\beta$-methyl-$\beta$-n-butyl derivatives, for which the calculated atomic charges are almost identical, but which have convulsant and anticonvulsant activity respectively. The net charges on other atoms in these two molecules are shown in Figure 3.8, where it can be seen that all the atoms except those in the altered alkyl chain retain almost exactly the same charges. The change in activity between the two glutarimides must therefore be due to the intermolecular rather than intramolecular influence of the longer alkyl chain, which includes increased van der Waals bonding and hence liposolubility, and increased molecular volume. These effects may marginally alter both the ability of the drugs to reach potential sites of action and the strength of the bonding to some substrates, but the predominant influence seems likely to be on the conformational changes which they might induce during or after the bonding process. However the only conclusion which can confidently be drawn is once again that the bonding of the drugs through the CBG remains effectively constant, and qualitative changes in activity are due to the direct influence of
Table 3.8  CBG Charges and Qualitative Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Atomic Charges (CNDO/2)</th>
<th>Activity$^{32}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$O_1$</td>
<td>$H_1$</td>
</tr>
<tr>
<td>Glutarimide</td>
<td>-0.337</td>
<td>0.140</td>
</tr>
<tr>
<td>$\beta$-Methylglutarimide</td>
<td>-0.323</td>
<td>0.143</td>
</tr>
<tr>
<td>$\beta,\beta$-Dimethylglutarimide</td>
<td>-0.340</td>
<td>0.139</td>
</tr>
<tr>
<td>$\beta$-Methyl-$\beta$-ethylglutarimide</td>
<td>-0.356</td>
<td>0.139</td>
</tr>
<tr>
<td>$\beta$-Methyl-$\beta$-n-propylglutarimide</td>
<td>-0.342</td>
<td>0.142</td>
</tr>
<tr>
<td>$\beta$-Methyl-$\beta$-n-butylglutarimide</td>
<td>-0.354</td>
<td>0.139</td>
</tr>
</tbody>
</table>
Figure 3.8 Atomic Charges and Qualitative Activity

The molecule shown is the anticonvulsant, $\beta$-methyl-$\beta$-n-butylglutarimide. The corresponding atomic charges for the convulsant, $\beta$-methyl-$\beta$-ethyl glutarimide, are given in brackets.
the substituent groups.

Combining this conclusion with the preceding discussion of clinically useful drugs provides the general conclusion that all of the drugs studied here are potentially capable of forming multiple hydrogen bonds of approximately the same strength through the CBG, and that variations in both qualitative and quantitative activity appear to be largely dependent on the effects of substituent groups, which will influence the ability of the drugs to reach, bond to, and deform their biological substrates.

Potential substrates for the drugs are discussed in the next chapter, where some methods which could be used to study drug-substrate interactions are also reviewed, and the method chosen for use here is outlined.
CHAPTER FOUR

4.1 Choice of Substrates

Although the range of polar and lipophilic groups in the drugs studied makes them capable of some bonding to almost any molecule, their ability to form multiple hydrogen bonds through the CBG indicates a preference for substrates with similar characteristics. In particular, strong bonds should occur with molecules containing adjacent hydrogen bond acceptors and donors, as observed in the drugs themselves. With this stipulation in mind, possible substrates were sought in both the respiratory chain and cell membrane, in the hope of elucidating inhibition of respiration and testing the possibility that anticonvulsant activity could be due to the effect of conformational changes on membrane properties.

The molecular species which make up the respiratory chain have not all been identified\textsuperscript{143}, but as discussed in Chapter 1 the locus of direct inhibitory action on the respiratory chain by some barbiturates and glutarimides has been narrowed\textsuperscript{15,18} to a region which includes the coenzymes nicotinamide-adenine dinucleotide (NAD) and flavin-adenine dinucleotide (FAD). The presence of adenine in both of these molecules, together with the structural similarity of the drugs and uracil, which
nicotinamide-adenine dinucleotide

flavin-adenine dinucleotide
pairs specifically with adenine in ribonucleic acids, suggests that the adenine moiety could be the operative substrate for the drugs in the respiratory chain.

\[
\text{uracil}
\]

The structural relationship between uracil and the barbiturates has inevitably been noticed by others, and shortly after this work was begun experimental evidence for strong and specific hydrogen bond formation between adenine and some barbiturate derivatives was published \(^{111,144}\), with the suggestion that the biological activity of the barbiturates might be due to their disruption of the coenzyme FAD \(^{145}\). Although these results clearly establish the suitability of adenine as a potential substrate they do not define the types of activity in which it might be involved, since the barbiturates display rather diverse biological effects. It was therefore thought valuable to investigate the interactions of adenine with the \(\beta\)-glutarimide series.

The use of the dinucleotide coenzymes as test substrates is unsatisfactory, because the presence of potential bonding groups in the nicotinamide and flavin nucleotides, as well as the phosphate linkages,
would make quantitative interpretation of experimental data extremely difficult. The problem can be partly overcome by employing model dinucleotides with methylene linkages\textsuperscript{146}, but in the work described here the interactions between the drugs and adenine were completely specified by using 9-ethyladenine as test substrate. The ethyl group

$$\text{9-ethyladenine}$$

in this molecule protects the nitrogen atom which is substituted in both coenzymes and also in the nucleic acids.

In the cell membrane the peptide linkage is an obvious substrate, and could provide an anchor for the drug while the lipophilic groups exert a conformational influence on the underlying lipid layer or on the protein itself. In either case the intimate relationship between protein and lipid could lead to significant changes in the permeability of the membrane. Direct experimental measurements on the bonding of drugs to bulk protein are not uncommon\textsuperscript{147, 148}, but for a quantitative determination of binding at a particular molecular site a smaller test system has to be used.
The smallest protein units containing a peptide bond are the dipeptides, and one of these, N-carbobenzoxy-L-alanyl-L-tyrosine ethyl ester, was considered as a possible substrate. However the properties of each dipeptide naturally depend on the component amino acids, so that the interaction of the drugs with different dipeptides could vary widely, and to extrapolate results from one to another is unreasonable.

An alternative is offered by the secondary amides, some of which have recently been shown\textsuperscript{149} to increase the solubility of barbiturates, and may therefore be presumed to interact with them significantly. The simplicity of secondary amides also advocates their use as test substrates, and a further reason arises from a consideration of the nature of the peptide linkage.

It has been known for some time that the peptide bond of proteins favours the trans configuration for helix formation\textsuperscript{150}, and it is

\begin{center}
\begin{tikzpicture}
\draw (0,0) -- (0,0.5) -- (0.5,0.5) -- (0.5,0) -- (0,0);
\draw (0,0.5) -- (0,0.3);\end{tikzpicture}
\quad H
\end{center}

\begin{center}
\begin{tikzpicture}
\draw (0,0) -- (0,0.5) -- (0.5,0.5) -- (0.5,0) -- (0,0);
\draw (0,0.5) -- (0,0.3);\end{tikzpicture}
\quad O
\end{center}

trans peptide bond \quad cis peptide bond

sometimes assumed that this invariably applies to all peptides\textsuperscript{151,152}. However, although the evidence indicates\textsuperscript{153} that the linkage
is predominantly trans, there is no reason to suppose that the cis form cannot occur, as indeed it does in a number of amides\textsuperscript{154}. If a small proportion of the peptide bonds in a protein molecule were in the cis configuration at any time, the drugs studied here might preferentially stabilize this form by bonding across the peptide linkage. The feasibility of this proposition can be tested if secondary amides are chosen as the test system, since in many cases there is an equilibrium between cis and trans forms\textsuperscript{155}. The two compounds chosen for this work were acetanilide and N-phenylurethane, which are predominantly in the trans

\[
\begin{align*}
\text{CH}_3 & \quad \text{N} & \quad \text{H} \\
\text{O} & \quad \text{C} & \quad \text{N} & \quad \text{C}_6\text{H}_5 \\
& \quad \text{C}_6\text{H}_5 &
\end{align*}
\]

acetanilide

\[
\begin{align*}
\text{C}_2\text{H}_5\text{O} & \quad \text{N} & \quad \text{H} \\
\text{O} & \quad \text{C} & \quad \text{N} & \quad \text{C}_6\text{H}_5 \\
& \quad \text{C}_6\text{H}_5 &
\end{align*}
\]

N-phenylurethane

and cis configurations respectively\textsuperscript{155}.

4.2 Choice of Experimental Method

In recent years the specific pairing\textsuperscript{156} between the nucleosides (Figure 4.1) of the purine and pyrimidine bases, adenine and thymine,
Figure 4.1  Nucleoside Pairing
adenine and uracil, and guanine and cytosine has received widespread attention. In particular, model systems have frequently been employed in which the sugar component is replaced by a lipophilic side chain, as in 9-ethyladenine. A survey of the methods and results of these studies provides an excellent basis for choosing a method to investigate bonding between the drugs and 9-ethyladenine, and possibly their interactions with other substrates, because much of the published work on the nucleosides has been concerned with the role of hydrogen bonding in specific base pairing. Hydrogen bonding has also been claimed as the major stabilizing factor in the nucleic acid helices, but the stabilization of vertical stacking by electrostatic and dispersion forces has been equally strongly supported. Although hydrogen bonding is the issue of interest here, both factors are clearly important in nucleic acids, and shades of the controversy will inevitably appear in the following discussion, which begins with a brief account of some theoretical approaches to the problem.

The contributions to helix stability from electrostatic interactions and London dispersion forces have been calculated in the monopole and dipole approximations, and demonstrate that both stacking and planar bonding make significant contributions. The specific pairing of the bases is correctly predicted, and proton
tunnelling to tautomeric forms in the base pairs has been related to natural mutation rates\textsuperscript{119,161}. Despite these successes in explaining observed results, the theoretical methods have not been proved sufficiently accurate to replace experimental measurements, especially when the differences between very similar molecular species are being investigated. The experimental methods which have proved most popular are NMR and infrared spectroscopy and also X-ray crystallography. These and a number of less common techniques will be discussed individually.

The NMR chemical shift of a proton involved in a short-lived intermolecular complex is a weighted average of the shifts for the free and associated states, and the predictable effects of concentration and temperature can therefore be used to study the association\textsuperscript{162}. Hydrogen bonding between bases results in a small downfield shift, but the shift to higher field observed with parallel stacking of bases is much larger and makes the occurrence of hydrogen bonding difficult to establish\textsuperscript{163}. Stacking interactions have been extensively studied in aqueous solutions\textsuperscript{163-5}, and were shown\textsuperscript{164} to be strongest for the purine bases, although quantitative results were not obtained. In solvents where solute-solvent bonding is less important, the base pairing due to hydrogen bonding has been measured\textsuperscript{166-7} and some equilibrium constants calculated\textsuperscript{168}, but the uncertainty introduced by the opposing effects of hydrogen
bonding and stacking remains a severe handicap to accurate quantitative results. Measurement of relaxation times could potentially overcome this problem by providing a quantitative integration of all molecular interactions, and has recently been used to study enzyme inhibition.\textsuperscript{169}

Hydrogen bonding can be observed without interference using infrared spectroscopy, where it results in a change in the light frequencies absorbed by both the N-H and C=O groups, the former being more significant. Association constants may then be calculated from the relative intensities due to the free and associated forms, and have provided quantitative measures of the specific pairing between nucleosides in several nonaqueous solvents.\textsuperscript{170-3} The method has been employed to investigate the possibility of mutations due to bonding of nucleoside tautomers,\textsuperscript{174} and was also used for the study of barbiturate-adenine interactions discussed in Section 4.1.

Attempts to grow mixed crystals between different bases and nucleosides have generally resulted in the usual base pairs or self-associations,\textsuperscript{175} but the molecular orientations have not been identical to those found in the nucleic acids for any but the guanine-cytosine pair,\textsuperscript{180-1} and a complex between the noncomplementary bases cytosine and uracil has also been observed.\textsuperscript{182} As well as being rather time-consuming X-ray crystallography offers no quantitative data and is not
of great value here.

Other methods which have provided qualitative results include ultraviolet spectroscopy\textsuperscript{183-4} where molecular interactions give rise to various intensity changes by preventing random orientation of transition moments, the binding of nucleosides by complementary bases in an ion-exchange column\textsuperscript{185}, and solubility effects\textsuperscript{186}. Both the latter method\textsuperscript{187} and measurement of heats of dilution\textsuperscript{188} can also be used to calculate association constants if the compounds involved are available in large quantities. An ultrasonic attenuation technique has recently been employed to obtain forward and reverse rate constants for the association of nucleoside pairs\textsuperscript{189}, and for the interaction of adenine with the barbiturates\textsuperscript{190}, but the method requires prior knowledge of the relevant association constants.

From the preceding survey the infrared method emerges as the least ambiguous and best tested technique for accurately measuring association constants of hydrogen-bonded complexes, and it was therefore chosen for the present study. The original equations\textsuperscript{172} for calculating association constants from infrared intensity data have been modified and extended for this work, and are described in detail in the next section.
4.3 Description of Method

The association constant, $K_X$, for the dimerization of a compound $X$ is given by

$$K_X = \frac{C_{xx}}{(C_x)^2} \quad (4.1)$$

where $C_x$ and $C_{xx}$ are the monomer and dimer concentrations, and the total concentration is

$$C_t = C_x + 2C_{xx} \quad (4.2)$$

The molecules of interest here contain an N-H group and one or more H-bond accepting atoms. The N-H stretching frequency changes significantly upon H-bonding, so that the absorbance $A$ at the nonbonded N-H frequency decreases with increasing dimerization. For a cyclic dimer, both N-H groups in the dimer will participate, and we can write

$$A = a_X C_x r \quad (4.3)$$

where $a_X$ is the extinction coefficient at the nonbonded N-H frequency and $r$ is the cell path length. However for an open dimer one N-H group will be nonbonded, so that

$$A = a_X(C_x + C_{xx})r \quad (4.4)$$
Combining equations (4.1), (4.2) and (4.3) gives

$$A = \left(\frac{a_x^2 r^2}{2K_x} \right) (C_t/A) - \frac{a_x r}{2K_x} ,$$

whereas from (4.1), (4.2) and (4.4) it follows that

$$A = \left(\frac{1}{K_x} - C_t \right) \left(\frac{a_x^2 r^2}{4} \right) (C_t/A) - \left(\frac{1}{K_x} - 4C_t \right) \left(\frac{a_x r}{4} \right) .$$

A plot of $A$ against $C_t/A$ will therefore lead to a straight line for a cyclic dimer, but not for an open one. In the absence of thermodynamic data this difference provides a useful test for the type of dimerization. In the important cyclic case, the slope ($m$) and intercept ($c$) of equation (4.5) can be used to calculate $a_x$ and $K_x$ from the expressions

$$a_x = \frac{-m}{cr} ,$$

and

$$K_x = \frac{m}{2c^2} .$$

For a mixture of two compounds, $X$ and $Y$, three different dimers may occur: $XX$, $YY$ and $XY$. The relevant association constants are then defined as

$$K_x = \frac{C_{XX}}{C_x^2} ,$$

$$K_y = \frac{C_{YY}}{C_y^2}$$
and \( K_{xy} = \frac{C_{xy}}{C_x C_y} \) . \hspace{1cm} (4.9)

and the total concentration, \( C_t \), of compound X is given by

\[
C_t = C_x + 2C_{xx} + C_{xy} .
\] \hspace{1cm} (4.10)

For a cyclic dimer equation (4.3) also holds, and combination with (4.9) and (4.10) gives

\[
A = \left(1/(C_y/C_x) K_{xy} + 2K_x\right) \left(a_x^2 r^2 \cdot (C_t/A) - a_x r\right)
\] \hspace{1cm} (4.11)

Now, provided \((C_y/C_x)\) remains constant, \( K_{xy} \) and \( a_x \) can be determined from a plot of \( A \) against \( C_t/A \). This condition will apply if \( K_x = K_y \) and an equimolar mixture of X and Y is employed. The factor \((C_y/C_x)\) is then approximately unity, so that

\[
a_x = \frac{-m}{cr}
\] \hspace{1cm} (4.12)

and \( K_{xy} = \frac{m}{c^2} - 2K_x \) \hspace{1cm} (4.13)

The agreement between the values of \( a_x \) calculated by equation (4.7) and (4.12) provides a first check on this approximation, and the value of \( K_{xy} \) can be further verified by repeating the calculations using the nonbonded absorbance of compound Y. In this case the equation (4.11) becomes
\[
A = \frac{1}{(C_x/C_y)K_{xy} + 2K_y)}\langle a_y^2 r^2 \rangle (C_t/A - a_y r) \tag{4.14}
\]

in which we set \(C_x/C_y = 1\), thus reversing the approximation. Equations (4.12) and (4.13) then become

\[
a_y = -\frac{m}{c r} \tag{4.15}
\]

and
\[
K_{xy} = \frac{m}{c^2} - 2K_y \tag{4.16}
\]

If straight lines are obtained for both plots of \(A\) versus \(C_t/A\), and the calculated \(K_{xy}\) values are in reasonable agreement, then we may conclude that a 1:1 cyclic dimer is formed. As a final check the ratio \(C_x/ C_y\) may be determined from the calculated association constant. For an equimolar mixture

\[
C_t = C_y + 2C_{yy} + C_{xy} \tag{4.17}
\]

and combining equations (4.9), (4.10) and (4.17) gives

\[
K_{xy} = 16C_{xy}K_xK_y/\left((-1 + \sqrt{1 - 8K_x(C_{xy} - C_t)}) \left(-1 + \sqrt{1 - 8K_y(C_{xy} - C_t)}\right)\right) \tag{4.18}
\]

where all terms other than \(C_{xy}\) are known. Successive approximations give a value of \(C_{xy}\), from which \(C_x\) and \(C_y\) may be calculated using equations (4.9) and (4.10), and (4.9) and (4.17) respectively.

The nonbonded N - H absorbance was determined by subtracting the absorbance due to the solvent (measured in the same cell) from
that observed. Where other peaks absorbed significantly at the nonbonded N - H frequency their contribution was similarly treated. This procedure was necessarily iterative, since the contribution of the nearby peaks was generally determined by the association constant being calculated.

The line of best fit for plots of \( A \) against \( C_t / A \) was determined by least squares analysis on the assumption that \( C_t / A \) could be measured much more accurately than \( A \). This assumption is unreasonable, but necessary if a confidence limit is to be estimated for the results.

The statistical variance in the slope, \( (\delta_m)^2 \), and intercept, \( (\delta_c)^2 \), as well as the covariance of slope and intercept, \( (\delta_{mc})^2 \), were calculated by standard methods. In these terms, the 95% confidence limits for slope and intercept are \( m \pm 2 \delta_m \) and \( c \pm 2 \delta_c \), and the extinction coefficient, \( a \), is given by

\[
a = \frac{m}{cr} \pm 2 \delta_a \quad (4.19)
\]

where

\[
\delta_a^2 = \frac{m^2}{c^2 r^2} \left( (c^2 \delta_m^2 - 2mc \delta_{mc}^2 + m^2 \delta_c^2)/m^2 c^2 \right) \quad (4.20)
\]

There is no corresponding expression for the limiting values of the association constants. They were calculated by rewriting equations (4.8), (4.13) and (4.16) in terms of the extinction coefficient and intercept, and then substituting extreme values for these quantities. If anything,
the resulting error estimates were too large.

The least squares analysis, calculation of extinction coefficients and association constants, and determination of confidence limits were combined in a single iterative computer program, which also plotted $A$ against $C_t/A$ and calculated the concentration of each component in mixtures of varying total concentration.

4.4 Materials and Experimental Techniques

Various $\beta$-glutarimides were supplied by both Dr. A. Shulman of the Pharmacology Department, Melbourne University, and the Nicholas Institute, Sherbrooke, Victoria. Nicholas Pty. Ltd. provided a sample of dimethylglutaric acid anhydride, from which $\beta,\beta$-dimethylglutarimide was prepared using the method of Abe$^{191}$. Samples of N-methyl-5-ethyl-5-phenylbarbituric acid and other drugs were donated by Parke-Davis and Company, Abbott Australasia Pty. Ltd., Sterling Pharmaceuticals Pty. Ltd. and Drug Houses of Australia Ltd.

The two amides, acetanilide and N-phenylurethane were purchased from the British Drug Houses, Ltd., and K. and K. Laboratories, Inc., New York, respectively, and 9-ethyladenine from Cyclo Chemical Co., Los Angeles. Mr. P. Scheelings of the Chemistry Department, Melbourne
University, prepared the dipeptide N-carbobenzoxy-L-alanyl-L-tyrosine ethyl ester.

Each of these compounds was recrystallized from chloroform which was also chosen as solvent for the spectral studies. Although this choice prevents the observation of competitive binding to the substrates or drugs by water or other molecules, it has the advantage that chloroform is nearly transparent at the N-H stretch frequency, and does not form strong hydrogen bonds. Spectroscopic quality chloroform, free of ethyl alcohol or other preservatives, was purchased from Mathieson, Coleman and Bell, Los Angeles.

Spectra were recorded with a Unicam SP100 (Mark 2) spectrophotometer, which was potentially the best instrument available for accurate intensity measurements. However, despite two overhauls, transmission intensities were not reproducible to better than 0.5% of incident light. To overcome this problem, the machine was generally operated with a slit width of approximately 330 μ and the frequency drive disengaged. The intensity of transmitted light was then recorded at each required frequency in turn for a period of two or three minutes. In some cases the presence of several overlapping peaks made a continuous record of the spectrum necessary, and this was obtained by repeatedly scanning the range from 3300 cm⁻¹ to 3500 cm⁻¹ at a speed
of 40 cm\(^{-1}\)/minute, and subsequently averaging the traces obtained.

To ensure constant conditions an RIIC microcell with NaCl windows, a path length of 1mm and a capacity of 0.06 ml was used for the spectra of both solvent and solutions. The cell blocked part of the light beam so reproducible placement was essential, and was achieved with a steel shim, which was machined to size and fitted into the holder beside the cell. The temperature in the cell well ranged from 28° to 30° C under operating conditions, whereas room temperature was maintained at 22° C. Each solution was therefore placed in the cell well for several minutes before making an intensity measurement, and this was repeated after a further fifteen minutes.

To avoid possible variations in chart placement and paper expansion the SP100 records wavenumber marker pips. These were calibrated against the lines in the \(\nu_1\) band of ammonia, which extend from 3200 cm\(^{-1}\) to 3500 cm\(^{-1}\) and have been accurately located\(^1\)\(^9\)\(^3\).

Most of the compounds studied were available only in small quantities, so wherever possible accurate and airtight micro equipment was employed. For each compound, or equimolar mixture of compounds, a chloroform solution of known concentration was prepared in a septum sealed standard flask. Solutions of varying concentrations were then made up by mixing small quantities of stock solution with solvent in
airtight penicillin vials obtained from the Commonwealth Serum Laboratories. The transfer from flask to vial was effected using Hamilton microanalytical syringes with Chaney adaptors, which allow delivery of precise volumes of solution. Syringes were also used to transfer the solution from vial to cell, and preliminary measurements were made to ensure that these procedures gave reproducible results. Similar checks were made on cell cleaning techniques, and in each case intensity measurements were reproducible to within the limits imposed by the instrument.
CHAPTER FIVE

5.1 Self-association of Drugs

It has been observed that the infrared spectra of $\beta,\beta$-dimethyl glutarimide and $\beta$-methyl-$\beta$-n-heptylglutarimide are very nearly identical, and that self-association of these and related $\beta$-glutarimides by hydrogen bonding should therefore be similar. This qualitative observation was confirmed by the spectra obtained here, but the actual equilibrium constants for the self-associations of the drugs are also needed before drug-substrate association constants can be calculated from equation (4.13). They are evaluated in this section, and the corresponding constants for the substrates, which appear in equation (4.16), are determined in Section 5.2.

The distinct vibrational frequencies absorbed due to the bonded and nonbonded stretching of N - H bonds participating in hydrogen bond formation may be assigned by observing the effects of solution and concentration on their apparent extinction coefficients. Hydrogen bonding increases with concentration, so that the broad peaks associated with bonded forms become stronger as concentration is increased. Nonbonded absorptions are correspondingly weakened, and may disappear entirely in the spectrum of the solid. As an example, the case of $\beta,\beta$-dimethyl glutarimide will be discussed in detail, and the corresponding results
for the other $\beta$-glutarimides tabulated at the end of the section.

The spectrum of solid $\beta, \beta'$-dimethylglutarimide in a nujol mull, and that of a 0.1 M solution in chloroform, are shown in Figure 5.1, where several qualitative differences are immediately apparent. The bands in the solution spectrum at 3000, 3600 and 3680 cm$^{-1}$ originate from the solvent, but the other peak missing from the solid spectrum, that at 3371 cm$^{-1}$, becomes weaker as the concentration is increased, and is assigned to the nonbonded N-H stretching frequency of the monomer. On the other hand, the shoulders at 3080 and 3180 cm$^{-1}$ are associated with the bonded N-H stretching vibrations, as they become more significant at higher concentrations and are strongest in the spectrum of the solid. Peaks associated with bonded and nonbonded N-H stretching in most of the other molecules studied in this chapter were assigned on the basis of similar considerations, and these cases will not be discussed individually.

The absorbance of the peak at 3371 cm$^{-1}$ was measured over the concentration range 0.008 M to 0.044 M, and a plot of $A$ against $C_t/A$ is shown in Figure 5.2. Least squares analysis of these results leads to the straight line

$$A = 46.4 \left(\frac{C_t}{A}\right) - 3.2 \quad (\delta_m = 3.0, \quad \delta_c = 0.2), \quad (5.1)$$

where the values of $\delta_m$ and $\delta_c$ indicate that this line accounts for all
Figure 5.1  Infrared Spectra of β,β-Dimethylglutarimide

Transmittance (%)

Frequency (cm⁻¹)

0.1 M in CHCl₃

Nujol Mull
Figure 5.2 ε,ε-dimethylglutarimide Self-association
the experimental data. According to equations (4.5) and (4.6) a straight line will occur only if a cyclic dimer is formed or if $C_t$ is negligible compared to $1/K$. In this instance, equation (4.8) gives a value of 2.3 M$^{-1}$ for $K$, which is large enough to make $C_t$ significant, so that the results are consistent with the formation of the cyclic dimer:

![Chemical Structure](image)

However, the conclusion is weakened by the scatter of points in Figure 5.2, which is typical of that encountered throughout this work, and is a consequence of the excessive noise levels discussed earlier.

The direct outcome of this problem may be seen in the calculated confidence limits for the extinction coefficient and association constant, which are given in Table 5.1, as well as the corresponding results for the other $\beta$-glutarimides.

The qualitative similarities in the spectra of these compounds are explained by the almost identical extinction coefficients and association...
Table 5.1  Self-association of Drugs

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutarimide</td>
<td>0.01, 0.04</td>
<td>3369 ± 3 142 ± 2 1.8 (1.6, 2.2)</td>
</tr>
<tr>
<td>(\beta,\beta)-Dimethylglutarimide</td>
<td>0.01, 0.04</td>
<td>3371 ± 3 146 ± 2 2.3 (2.0, 2.7)</td>
</tr>
<tr>
<td>(\beta)-Methyl-(\beta)-ethylglutarimide</td>
<td>0.01, 0.08</td>
<td>3371 ± 3 151 ± 3 2.8 (2.5, 3.2)</td>
</tr>
<tr>
<td>(\beta)-Methyl-(\beta)-n-propylglutarimide</td>
<td>0.01, 0.03</td>
<td>3372 ± 3 149 ± 2 2.4 (2.1, 2.8)</td>
</tr>
<tr>
<td>(\beta)-Methyl-(\beta)-n-butylglutarimide</td>
<td>0.01, 0.04</td>
<td>3372 ± 3 146 ± 1 2.0 (1.9, 2.2)</td>
</tr>
<tr>
<td>(\beta)-Methyl-(\beta)-n-pentylglutarimide</td>
<td>0.01, 0.04</td>
<td>3372 ± 3 151 ± 3 2.4 (1.9, 3.0)</td>
</tr>
<tr>
<td>N-Methyl-5-ethyl-5-phenylbarbituric acid</td>
<td>3381</td>
<td>2.3</td>
</tr>
</tbody>
</table>
constants throughout the series, and no significant trend can be observed in the values obtained.

The self-association constant for N-methyl-5-ethyl-5-phenylbarbituric acid, which was the other drug studied experimentally, has been determined by others\textsuperscript{144}, and is included in Table 5.1.

\begin{center}
\includegraphics[width=0.5\textwidth]{n-methyl-5-ethyl-5-phenylbarbituric-acid.png}
\end{center}

\section*{5.2 Self-association of Substrates}

The three major substrates investigated were 9-ethyladenine, acetanilide and N-phenylurethane. The self-associations of both 9-ethyladenine and N-phenylurethane have been studied previously, but were repeated to test the techniques employed here.

The spectrum of 9-ethyl adenine is shown in Figure 5.3 where the peaks of interest are the symmetric and antisymmetric stretching of the nonbonded NH\textsubscript{2} group at 3410 and 3520 cm\textsuperscript{-1}, and the bonded N - H
Figure 5.3 Infrared Spectrum of 9-Ethyladenine (0.05 M)
stretches for the same group at 3308 and 3480 cm\(^{-1}\). These assignments are based on those made by others\(^{172}\), although the peaks occur at slightly lower frequencies due to the slow scanning speed used here, which minimised mechanical overshooting in the servo system. Three separate determinations of the association constant were made using the concentration dependence of the peak at 3410 cm\(^{-1}\), and in each case a value in the vicinity of 4.3 M\(^{-1}\) was obtained. This is a little higher than the published\(^{172}\) association constant of 3.1 M\(^{-1}\) in deuterochloroform, possibly due to more significant solvent-solute interactions in chloroform solution\(^{192}\). The same effect may be responsible for the value of 13 M\(^{-1}\) which has been observed\(^{188}\) in chloroform containing ethyl alcohol as preservative.

Acetanilide is known to be 90% trans and 10% cis in carbon tetrachloride, and the nonbonded N - H stretching frequencies of both forms have been determined\(^{155}\). They appear at 3434 and 3390 cm\(^{-1}\) respectively in Figure 5.4, where the effect of concentration on the spectrum is illustrated. The broad band at 3330 cm\(^{-1}\) becomes increasingly significant with concentration, and is assigned to a nonbonded N - H stretching mode. If acetanilide self-associates to form a cyclic dimer, which requires the molecule to be in the cis configuration, the nonbonded molecules will reestablish the original equilibrium between
Figure 5.4 Concentration Dependence of Acetanilide Spectrum
cis and trans forms, and the association constant could be determined from the concentration dependence of either nonbonded N - H peak. In fact, both peaks were used, and the calculated association constants were 2.5 M⁻¹ at 3435 cm⁻¹ and 2.2 M⁻¹ at 3390 cm⁻¹, although the latter value has less significance due to the influence of noise levels on the weaker cis peak. These results are comparable to the literature value of 5.4 M⁻¹ in carbon tetrachloride for the related compound, N-methylacetamide, which is also predominantly in the trans configuration. If the extinction coefficients for the nonbonded N - H stretch of the 'pure' cis and trans forms of acetanilide are assumed to be the same, then the extinction coefficients observed here for the equilibrium mixture may be used to estimate the percentage of each form. The calculated values were 143 M⁻¹cm⁻¹ at 3435 cm⁻¹ and 16 M⁻¹cm⁻¹ at 3390 cm⁻¹, indicating 90% trans and 10% cis, as observed in carbon tetrachloride. Since extinction coefficients and association constants are derived from equations (4.7) and (4.8) using the same parameters, this result provides some verification for the calculated association constants. The more reliable value of the two, 2.5 M⁻¹, was used in subsequent calculations.
(Section 5.4).

An equilibrium involving 95% cis and 5% trans occurs for N-phenyl urethane in carbon tetrachloride\(^{155}\), but the latter peak was much too weak for use here. A straight line plot of \(A\) against \(C_t/A\) was obtained for the nonbonded N-H absorbance of the trans form and gave an association constant of 1.2 M\(^{-1}\), which may be compared with the known\(^{195}\) value in carbon tetrachloride, 1.5 M\(^{-1}\). The two values are quite similar, and the overall agreement between the results obtained for the three substrates here and available data from other sources is reasonably good. The present results are summarised in Table 5.2.

### Table 5.2  Self-association of Substrates

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. Range, M</th>
<th>Freq. (cm(^{-1}))</th>
<th>Ext. Coeff. (M(^{-1}) cm(^{-1}))</th>
<th>Assoc. Const. &amp; Range(M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-Ethyladenine</td>
<td>0.01, 0.05</td>
<td>3410</td>
<td>230 ± 5</td>
<td>4.3 (3.8, 5.0)</td>
</tr>
<tr>
<td>Acetanilide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis form</td>
<td>0.02, 0.07</td>
<td>3390</td>
<td>16 ± 2</td>
<td>2.2 (1.1, 9.5)</td>
</tr>
<tr>
<td>trans form</td>
<td>0.02, 0.07</td>
<td>3434</td>
<td>143 ± 2</td>
<td>2.5 (2.2, 2.7)</td>
</tr>
<tr>
<td>N-Phenylurethane</td>
<td>0.02, 0.06</td>
<td>3434</td>
<td>151 ± 3</td>
<td>1.2 (0.9, 1.7)</td>
</tr>
</tbody>
</table>

5.3  Association of Drugs with 9-Ethyladenine

The infrared spectrum of an equimolar mixture of unsubstituted
glutarimide and 9-ethyladenine in chloroform over the range from 2900 to 3800 cm\(^{-1}\) is shown in Figure 5.5, as well as the spectra of the individual compounds. The nonbonded N-H stretching frequencies of 9-ethyladenine at 3410 and 3520 cm\(^{-1}\), and that of glutarimide at 3369 cm\(^{-1}\), are also visible in the spectrum of the mixture. Bonded N-H stretching modes are responsible for the peak at 3480 cm\(^{-1}\), and the broad shoulder in the vicinity of 3330 cm\(^{-1}\).

The effects of hydrogen bonding could be measured using the concentration dependence of the peak at 3369 cm\(^{-1}\) for the drug, with either of the nonbonded N-H bands for 9-ethyladenine. The peak at 3410 cm\(^{-1}\) is least influenced by overlap from adjacent bands and was therefore chosen, although there is also a good deal of overlap between the peaks at 3369 and 3410 cm\(^{-1}\), and from the nonbonded N-H shoulder at 3330 cm\(^{-1}\). The interference from the bonded absorption can be reduced by using lower concentrations, but errors due to noise levels become more significant as measured intensities decrease. On the other hand, the increased importance of the bonded peaks indicates that more association is occurring, so that the decrease in accuracy may be compensated for by an increase in the magnitude of the effect being measured. The concentration range chosen was from 0.002 to 0.012 M, and plots of A against \(C_t/A\) for the peaks at 3369 and 3410 cm\(^{-1}\) are
Figure 5.5  Glutarimide and 9-Ethyladenine Spectra

- - - - 0.1 M glutarimide
- - - - - - 0.05 M 9-ethyladenine
- - - - - - - 0.025 M glutarimide/9-ethyladenine mixture
shown in Figure 5.6. In both cases the experimental data is adequately accounted for by a straight line, and the association constants calculated from their slopes and intercepts using equations (4.13) and (4.16) were 19 M\(^{-1}\) at 3369 cm\(^{-1}\), and 24 M\(^{-1}\) at 3410 cm\(^{-1}\). The extinction coefficients calculated from the same parameters were 147 M\(^{-1}\)cm\(^{-1}\) at 3369 cm\(^{-1}\) and 228 M\(^{-1}\)cm\(^{-1}\) at 3410 cm\(^{-1}\), which compare well with values for the individual compounds, 142 and 230 M\(^{-1}\)cm\(^{-1}\) respectively. This agreement adds weight to the results, and the difference between the two calculated association constants is accounted for by the appropriate error ranges (Table 5.3), which were derived from experimental scatter using the method described in Chapter 4. Similar results were obtained for equimolar mixtures of five \(\beta\)-substituted glutarimides with 9-ethyl-adenine, and these are summarised in Table 5.3. In every case straight lines were obtained for plots of A against \(C_t/A\) for both components of the mixture, and the extinction coefficients were in satisfactory agreement with those calculated earlier. In addition, the two association constants calculated for each mixture were always of similar magnitude. All of these facts point to the formation of complexes involving two hydrogen bonds between the drugs and 9-ethyladenine, which could take either of the forms shown in Figure 5.7.

It is also apparent from Table 5.3 that the association constants
Figure 5.6 Glutarimide/9-Ethyladenine Association

(a) 3410 cm$^{-1}$

(b) 3369 cm$^{-1}$
### Table 5.3 9-Ethyladenine Complexes with β-Glutarimides

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. Range, M</th>
<th>9-ethyladenine, 3410 cm⁻¹</th>
<th>Drug Peak</th>
<th>9-ethyladenine, 3410 cm⁻¹</th>
<th>Drug Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutarimide</td>
<td>0.002, 0.012</td>
<td>228 ± 8</td>
<td>24 (17, 34)</td>
<td>3369</td>
<td>147 ± 7</td>
</tr>
<tr>
<td>β,β-Dimethyl glutarimide</td>
<td>0.002, 0.010</td>
<td>223 ± 4</td>
<td>17 (13, 22)</td>
<td>3371</td>
<td>151 ± 8</td>
</tr>
<tr>
<td>β-Methyl-β-ethyl glutarimide</td>
<td>0.002, 0.012</td>
<td>225 ± 5</td>
<td>22 (18, 27)</td>
<td>3371</td>
<td>152 ± 6</td>
</tr>
<tr>
<td>β-Methyl-β-n-propyl glutarimide</td>
<td>0.002, 0.011</td>
<td>221 ± 4</td>
<td>22 (18, 25)</td>
<td>3372</td>
<td>152 ± 6</td>
</tr>
<tr>
<td>β-Methyl-β-n-butyl glutarimide</td>
<td>0.002, 0.011</td>
<td>225 ± 4</td>
<td>19 (14, 27)</td>
<td>3372</td>
<td>148 ± 6</td>
</tr>
<tr>
<td>β-Methyl-β-n-pentyl glutarimide</td>
<td>0.002, 0.013</td>
<td>216 ± 5</td>
<td>15 (11, 20)</td>
<td>3372</td>
<td>151 ± 7</td>
</tr>
</tbody>
</table>
Figure 5.7  Possible Complexes Between 9-Ethyladenine and β-Glutaramides

for the complexes are effectively independent of the substituent groups, being in the vicinity of 20 M⁻¹ for each of the drugs studied. This may be compared with the association constant for the self-association of 9-ethyladenine, which was 4.3 M⁻¹, and for the drugs, which were all approximately 2 M⁻¹. The formation of complexes between the drugs and 9-ethyladenine is therefore favoured over dimerization by either species. For example, in a 9-ethyladenine/glutarimide mixture containing a total concentration of 0.01 M of each species, the association constants above give equilibrium concentrations of 0.0081 M 9-ethyladenine, 0.0003 M 9-ethyladenine dimer, 0.0083 M glutaramide, 0.0002 M glutaramide dimer
and 0.0013 M 9-ethyladenine/glutarimide complex.

The drug-substrate complex is more strongly favoured over the individual dimers when 9-ethyladenine and N-methyl-5-ethyl-5-phenyl barbituric acid are mixed, and the increased intensity of the bonded peaks at 3340 and 3480 cm\(^{-1}\) is apparent in Figure 5.8. In this case the association constant is known to be approximately 200 M\(^{-1}\), and rises to 1200 M\(^{-1}\) for the N-demethylated compound, 5-ethyl-5-phenylbarbituric acid.\(^{144}\)

As a result of these experiments, it is clear that the glutarimides, like the barbiturates, prefer to form hydrogen bonded complexes with 9-ethyladenine rather than self-associations, and that the complexes are strong enough to be of potential biological significance if the drugs are applied in reasonably high concentrations. This result, taken in conjunction with evidence for the direct action of both the barbiturates\(^{15}\) and the glutarimides\(^{18}\) on the respiratory chain in the vicinity of the adenine-containing coenzymes NAD and FAD, suggests that the adenine component of either or both of these coenzymes could provide the substrate for the drugs in the respiratory chain.

By contrast, the bonding to 9-ethyladenine does not elucidate the convulsant or anticonvulsant action of the drugs, since association
Figure 5.8  N-Methyl-5-ethyl-5-phenylbarbituric Acid and 9-Ethyladenine Spectra

--- 0.1 M N-methyl-5-ethyl-5-phenylbarbituric acid
----- 0.05 M 9-ethyladenine
----- 0.025 M N-methyl-5-ethyl-5-phenylbarbituric acid/9-ethyladenine mixture
constants of similar magnitude were obtained for all of the β-glutarimides, despite the qualitative differences in their activities. The strength of binding with 9-ethyladenine is therefore unlikely to be of direct relevance to the type of activity exhibited by the drugs, and the results provide no reason to suppose that an adenine derivative is the operative substrate for convulsant or anticonvulsant activity. Nevertheless, this possibility cannot be entirely disregarded, since a combination of bonding and conformational effects could conceivably be involved.

In summary, the preceding results indicate that a molecule containing adenine could be the substrate for the direct inhibitory action of high concentrations of the glutarimides on the respiratory chain, but does not show whether or not similar substrates are likely to be involved in their convulsant or anticonvulsant activity. For the latter classes of activity the components of cell membranes are logically more likely substrates, and a preliminary study in this area is described in the next section.

5.4 Association of Drugs with Amides

Figure 5.9 shows the infrared spectra of N-phenylurethane, β-methyl-β-ethylglutarimide and an equimolar mixture of the two species. The concentrations employed were the same as those in earlier figures,
Figure 5.9  \(\beta\)-Methyl-\(\beta\)-ethylglutarimide and N-Phenylurethane Spectra

\[
\begin{align*}
\text{Transmittance (\%)} & \quad \text{Frequency (cm}^{-1}\text{)} \\
0 & \quad 2900 \quad 3300 \quad 3700 \\
\hline
0 & \quad \hline
0.1 \text{ M } \beta\text{-methyl-}\beta\text{-ethylglutarimide} & \quad 2900 \quad 3300 \quad 3700 \\
0.1 \text{ M } N\text{-phenylurethane} & \quad \hline
0.025 \text{ M } \beta\text{-methyl-}\beta\text{-ethylglutarimide}/N\text{-phenylurethane mixture} & \quad \hline
\end{align*}
\]
but no peak due to hydrogen bonding can be discerned in the spectrum of the mixture, so that calculated association constants are expected to be considerably smaller. This expectation was confirmed by the concentration dependence of the nonbonded N-H peaks over the range 0.02 to 0.20 M, for which association constants of approximately 4 M\(^{-1}\) were obtained in both cases. Association constants of the same order were observed for equimolar mixtures of the predominantly trans amide, acetonilide, with both \(\beta\)-methyl-\(\beta\)-ethyl glutarimide and N-methyl-5-ethyl-5-phenylbarbituric acid. However the experimental scatter was too great to allow fruitful comparison of the association constants determined, and in the case of the less soluble protected dipeptide, N-carbobenzyloxy-L-alanyl-L-tyrosine ethyl ester, the existence or
otherwise of an association could not be proved.

The most obvious reason for the apparent loss of accuracy in these measurements is the decrease in size of the association constants, since the uncertainty in transmission intensities becomes increasingly important as the magnitude of the effect being measured decreases. The problem could be overcome by using higher concentrations, but this was not possible for the dipeptide. A better alternative is the use of increased path lengths, which enable higher absorption levels without increase in concentration. However another cause of inaccuracy arises in these systems which did not occur earlier. For associations between species which are fixed in a planar configuration, like 9-ethyladenine and the bonding group of the glutarimides, the change in association constant due to small fluctuations in temperature is comparatively unimportant. By contrast, the nonbonded forms of both acetanilide and N-phenylurethane, and probably the dipeptide, are in equilibrium between the cis and trans configurations. Rapid rotation from one form to the other is incompatible with the formation of hydrogen bonds, so that the temperature dependence of both processes becomes important. The rate of rotation will be strongly temperature dependent, and the slight variations in temperature encountered here (see Chapter 4) might therefore be expected to effect the associations significantly. Further
studies of these systems are planned for the coming year, when improved transmission measurements and temperature controls will be sought, in the hope of gaining a detailed understanding of the behaviour and bonding of the secondary amides. If possible, the work will later be extended to more complex peptides.

For the present, the results indicate that the convulsant drug, $\beta$-methyl-$\beta$-ethylglutarimide, and the anticonvulsant, N-methyl-5-ethyl-5-phenylbarbituric acid, are both capable of forming hydrogen bonds to secondary amides. The association constants for these complexes are comparatively small, but may nevertheless be biologically important. Whether or not the drugs preferentially stabilize the cis form of the peptide bond, which is potentially of great significance, cannot be determined with certainty from these results.
CHAPTER SIX

6.1 Conclusions concerning Drug Activity

The primary aim of this work was to use molecular orbital calculations to seek relationships between the activity of a series of drugs and the atomic charges at either the common bonding group (CBG) or 'biologically active centre' (BAC).

It has been claimed that the charge at the BAC provides a means of distinguishing between convulsant and anticonvulsant activity, and of assessing the potential usefulness of new compounds as anticonvulsants. The calculations presented here clearly refute this claim, since the observed variations in BAC charge were effectively unrelated to anticonvulsant strength determined by either of two experimental techniques, while the charge remained almost constant in all the $\beta,\beta'$-disubstituted glutarimides despite qualitative changes in activity.

In the light of these results, further consideration should be given to the many other applications of the BAC hypothesis, which all rest on the same basic assumptions.

Atomic charges in the CBG were found to be almost unchanged throughout the group of drugs studied, and therefore cannot be related to observed differences in activity. The results suggest that the ability
of all the drugs to form hydrogen bonds through the CBG should be similar, although variations in bond strength to different substrates will naturally arise due to bonding by other components of the drug molecules. It is possible that bonding through the CBG is fundamental to both convulsant or anticonvulsant activity, with the bonding or conformational influence of other groups determining the type or extent of activity displayed. Evidence for this possibility was sought by using infrared spectroscopy to investigate the interactions of the drugs with model peptide systems, but, although some bonding undoubtedly occurs, the results were not sufficiently accurate to warrant firm conclusions at this stage.

On the other hand, the infrared studies did provide definite evidence for a specific association between the $\beta,\beta'$-disubstituted glutarimides and 9-ethyladenine, similar to that observed$^{144}$ between the latter compound and several barbiturates. These results indicate that the adenine moiety of the coenzymes NAD and FAD may be the operative substrate for the inhibition of the respiratory chain caused by high concentrations of the drugs, but provide no evidence concerning their convulsant or anticonvulsant activity.

6.2 Other Conclusions

Some interesting conclusions regarding the application of molecular
orbital theory to biological problems arise from this work, especially since two different all-electron methods have been compared over a rather large range of molecules.

In particular, it was found that both the EHT and CNDO/2 methods give a good qualitative account of the variations in charge on atoms with different nearest neighbours, as exhibited by their ability to predict trends in dipole moments. Either of the two methods is therefore suitable for studies involving atoms in essentially different environments. The CNDO/2 method also proved quantitatively accurate, while EHT invariably exaggerated atomic charges.

In the case of atoms with the same nearest neighbours, no correlation was found between the atomic charges calculated by the two methods, so that neither technique can be safely assumed suitable for determining variations in charge on atoms with similar environments. This conclusion is of particular significance, since many studies of biological problems have assumed that such variations are adequately accounted for by less sophisticated molecular orbital methods.

A further observation of some interest resulted from the EHT calculations on 5-ethylhydantoin and 5-phenylhydantoin. They showed that the atomic charges in the hydantoin ring are insensitive to conformational changes in the substituents.

Finally, CNDO/2 and INDO calculations provided a detailed picture
of the structure and properties of the \( \text{CH}_2\text{NO}^\cdot \) radical, which had been the subject of recent debate in the literature. The calculations were in good agreement with available experimental evidence, and showed the radical to be in the amino form, to which it is constrained by large energy barriers.
REFERENCES


52. E. Hückel, Z. Physik., 70, 204 (1931).


