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CONFORMATIONAL STUDIES ON SOME
CENTRALLY ACTING MOLECULES

by

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A Thesis submitted for the Degree of Doctor of
Philosophy of The City University, London.

Physics Department.

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ABSTRACT

A series of conformational studies on γ -aminobutyric acid (GABA) and its agonists β -guanidino propionic acid (β GP) and muscimol are reported.

The development of a library of computer programs for carrying out the necessary calculations is described.

The crystal structures of GABA.HCl, GABA and β GP are reported. GABA is found to be partially folded in the pure crystal form, but fully-extended in the crystalline hydrochloride. β GP is found to be folded in the crystalline state.

The results of CNDO/2 calculations on GABA, β GP and muscimol are given. Fully-folded conformations are predicted to be strongly favoured for the isolated zwitterions of GABA and β GP, while the muscimol molecule is found to prefer a planar conformation. CNDO/2 calculations on bicuculline, a specific competitive antagonist of GABA, are also reported. Similarities in the electronic structures of bicuculline and the three agonists are noted.

A series of classical energy calculations on GABA and β GP are described. These predict fully-folded conformers as the lowest energy rotamers for the isolated zwitterions of the two molecules.

The various sets of conformational data available on GABA, β GP, and muscimol are discussed. In particular, isolated forms of GABA are found to favour fully-folded conformations, but it is noted that the effect of the environment is significant: a more-extended conformer of GABA is found in the solid state. Extended conformers

of GABA are also likely to exist in solution.

The results from the crystal structure studies and the energy calculations, together with other published data, are used in a discussion of the pharmacological activity of GABA, β GP, muscimol and bicuculline. Possible similarities in charge distributions are elucidated.

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PRINCIPAL ABBREVIATIONS, SYMBOLS AND CONVENTIONS USED

$a, b, c, \alpha, \beta, \gamma$	Crystallographic unit cell parameters
$a^*, b^*, c^*, \alpha^*, \beta^*, \gamma^*$	Reciprocal unit cell parameters
ATA	4-aminotetrolic acid
BDF	X-RAY 70 binary data file
BIC	bicuculline
CNS	Central Nervous System
CPU	Central Processor Unit
c	LCAO coefficient
CNDO	Complete Neglect of Differential Overlap
E_{hkl}	Normalised Structure Factor
EHT	Extended Huckel Theory
f_j	scattering power of an atom
F	Fock matrix
F_{hkl}	structure Factor
F_{calc}	calculated structure factor
F_{obs}	observed structure factor
GABA	γ -aminobutyric acid
GABA HCL	γ -aminobutyric acid hydrochloride
h, k, l	Miller indices
HBIC	protonated bicuculline
INDO	Intermediate Neglect of Differential Overlap
LCAO	Linear Combination of Atomic Orbitals
LCM	Large Core Memory
NAP	Net Atomic Population
NDDO	Neglect of Differential Diatomic Overlap
P	density matrix
$P(u, v, w)$	Patterson function

PCILO	Perturbative Configuration Interaction of Localised Orbitals
PNS	Peripheral Nervous System
QCPE	Quantum Chemistry Program Exchange
R	Residual factor
SCF	Self Consistent Field
T_n	Conformational angle (see Appendix A)
U	Temperature factor
ULCC	University of London Computer Centre
β GP	β - guanidino propionic acid
$\rho(x, y, z)$	electron density

Note on Conformational Energy Maps

Energy surfaces are drawn with contours marked in kcal/mol at intervals best suited to the particular map. On all maps global minima are marked X, while local minima are marked +.

Contours are drawn around the minimum energy positions: hence high energy regions are not detailed.

CHAPTER 1

Introduction. Chemical Transmission of the
Nervous Impulse at Synapses in the Central
Nervous System.

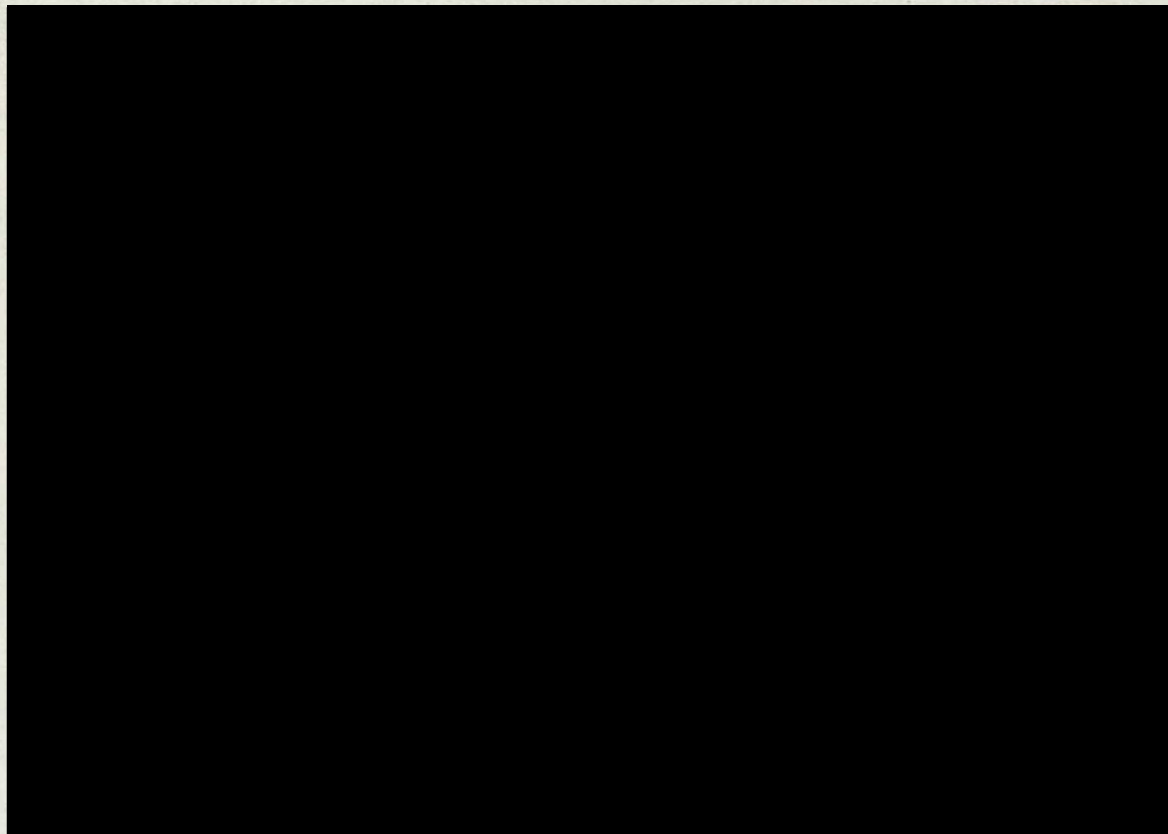
1.1 INTRODUCTION

The nervous system in vertebrates is normally considered as consisting of two parts: the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS is made up of the brain and spinal cord, while the PNS constitutes the link between the other organs of the body and the CNS.

The basic unit of the nervous system is the individual neuron or nerve cell. Interaction between neurons, or between neurons and other cells, takes place at junctions known as synapses. The studies reported here were made in an attempt to further elucidate, at the molecular level, the way in which certain molecules mediate the passage of the nervous impulse from neuron to neuron across the synapse. The particular synaptic junctions of interest here are those involved in inhibitory pathways in the mammalian brain, though the processes involved appear to be similar to those occurring in certain parts of the PNS of crustacea. Since the latter are more amenable to study, they have been used in the collection of much of the experimental pharmacological evidence available.

A general view of a synapse and, in particular, the physical gap between two interacting neurons, the synaptic cleft, is shown in Fig.1.1. The synaptic cleft is normally $\sim 300\text{\AA}$ in width, but may be considerably larger (as much as $1000\text{\AA}$ at the vertebrate neuromuscular junction) or, occasionally, much narrower. A gap of $300\text{\AA}$ is sufficient to effectively block any direct electrical contact between nerve cells across a synapse. Since even electron microscopic studies have failed to reveal any connecting tissue which might act as an electrical bridge across the synaptic cleft, the possibility of

[REDACTED] [REDACTED]
[REDACTED] [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]



electrical transmission of the nervous impulse seems, for the general case, remote; it may, however, play an important role in some "narrow" synapses.

More commonly, when an impulse reaches a synapse a transmitter substance is secreted by the presynaptic neuron. The transmitter molecules diffuse across the synaptic cleft and interact with the post-synaptic membrane. The changes produced by this interaction then result in an "action potential" being propagated along the post-synaptic neuron.

Chemical transmission at a synapse was first demonstrated by Loewi (1921), for the case of the heart neuromuscular junction. Since then convincing evidence has accumulated that this mode of transmission does, indeed, take place at many synapses (Katz, 1966).

The identification of the actual molecule or molecules involved in transmission at a particular synapse has sometimes proved difficult. The criteria for the acceptance of a chemical substance as the naturally occurring transmitter have been summarized by Phillis (1970) in the form of seven conditions to be fulfilled:

- (1) The substance must be present in those neurons from which the synaptic transmitter is released.
- (2) The neuron must possess enzymes for the manufacture and metabolism of the transmitter.
- (3) The presence of the precursors and intermediaries in the synthetic pathway should be demonstrable.
- (4) There may be systems for the inactivation of the transmitter. These could be enzymic or could be a specific uptake mechanism for the reabsorption of the

transmitter into the pre- or post-synaptic tissue.

- (5) During stimulation the substance may be detectable in extracellular fluid collected from the region of activated synapses.
- (6) When applied to the post-synaptic structure, the substance should mimic the action of the synaptically released transmitter. Equilibrium and reversal potentials should be the same.
- (7) Pharmacological agents which interact with the synaptically released transmitter should interact with the suspected transmitter in an identical manner.

In the systems of interest here, inhibitory synapses in the CNS of vertebrates, γ -aminobutyric acid (GABA, $C_4H_9NO_2$, shown in Fig.1.2) is now thought to be the natural transmitter. Convincing evidence of this has, however, only recently become available. Thus in a review published in 1965 (Curtis and Watkins, 1965) the authors could only say: "Whether or not GABA and L-glutamic acid ultimately prove to be synaptically released transmitters in the mammalian nervous system" but by 1974 the situation could be summarized by (Curtis and Johnston, 1974): "the most fully documented case for an amino acid transmitter, and, indeed, for any central transmitter, is that for GABA". A complete list of references to the evidence implicating GABA as the natural inhibitory transmitter in the mammalian brain will not be given here, since they are documented by Curtis and Johnston (1974). However, the work of Krnjevic and Schwartz (1967) should be mentioned as significant in demonstrating the likely role of GABA in the CNS.

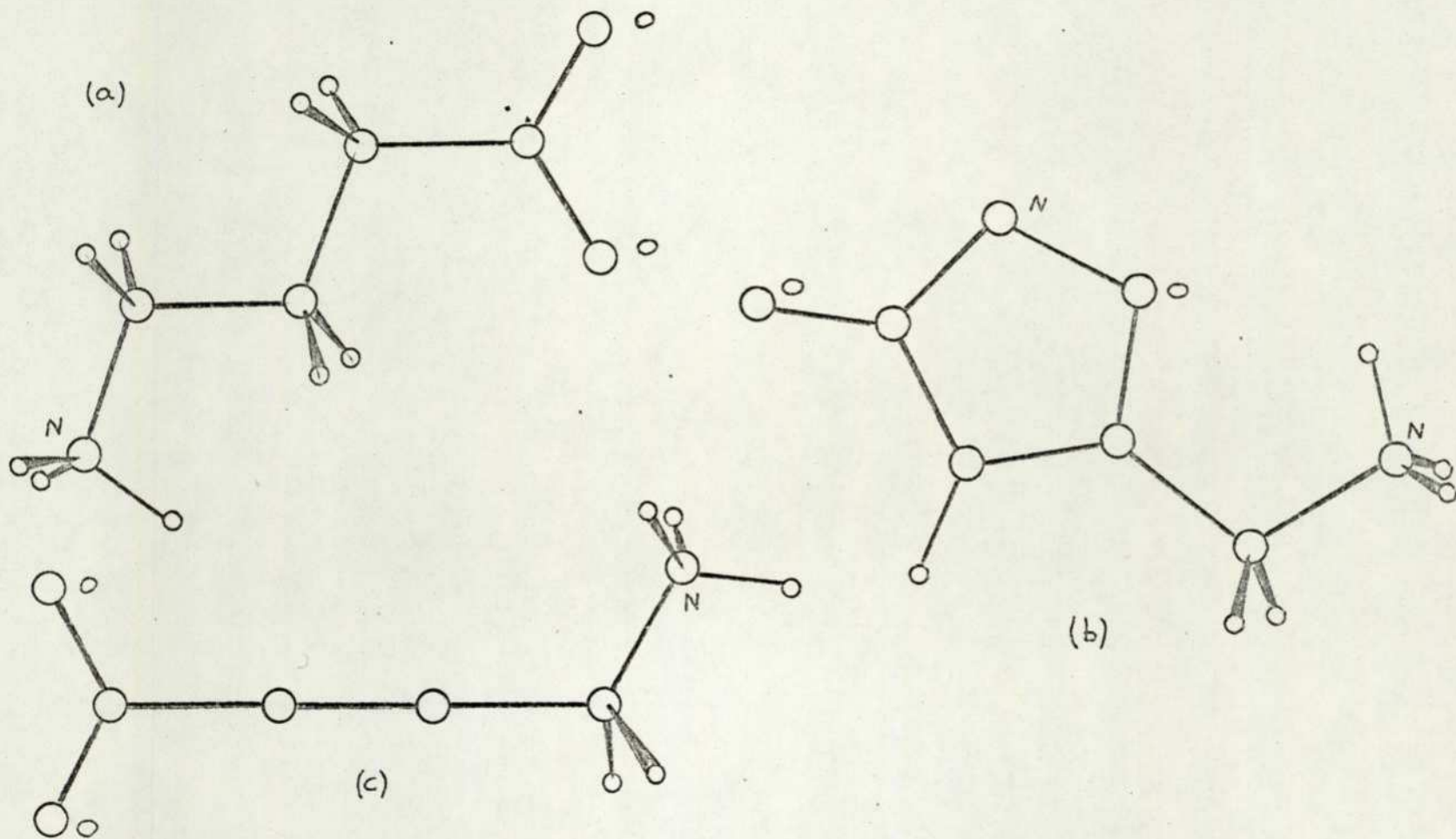


Fig. 1.2. (a) GABA, (b) muscimol
(c) and ATA molecules in the zwitterionic form

1.2 THE MODE OF ACTION OF GABA AT THE MOLECULAR LEVEL

The critical problem in the study of synaptic transmission is to discover the manner in which the transmitter molecule mediates a response at the post-synaptic membrane. The transmitter is generally supposed to interact with responsive areas of the post-synaptic membrane termed "receptors". Techniques for studying directly the mechanism of this interaction, at the molecular level, are not yet available: instead indirect methods must be employed. The approach used has been to examine the structures and relative activities of molecules which can produce, or, equally important, block the response at the post-synaptic membrane. Following conventional usage, such agonists and antagonists will be referred to collectively as "drugs", regardless of whether the molecules are endogenous or exogenous to the CNS. It is hoped that by studying features common to these drugs a model for the receptor, and hence the receptor-transmitter interaction, may be deduced. Ultimately, it will, of course, be necessary to make some form of direct "observation" of the actual interaction. It seems almost certain that developments in crystallographic and electron microscopic studies will one day make this possible. For the time being, however, methods of a rather more indirect nature must be employed.

Several series of compounds which act as GABA agonists have been found, and a putative prerequisite for GABA-like activity has been put forward by Kier and Truitt (1970): they suggested two oppositely charged regions separated by 5-6Å.

It should, however, be noted that some GABA agonists are highly flexible molecules (e.g. the α - ω -amino acids, and α -guanidino

carboxylic acids), while most have at least some degree of flexibility. Kier's suggestion was based on somewhat limited conformational data: until recently only X-ray crystal structure determinations of, and simple theoretical calculations on, the conformations of these molecules have been available. The conformations which such molecules may assume in the biophase are, in general, not known. Ascertaining the optimum charge distribution for action as a GABA agonist is therefore not straightforward. Useful data may be obtained by considering relatively inflexible agonists such as muscimol ($C_4H_6N_2O_2$) and 4-aminotetrolic acid (ATA, $C_4H_5NO_2$). These two molecules are depicted in Fig.1,2.

The flexibility of drug molecules may be important in two possible ways: (i) it may allow stereochemical features of a molecule to attain the correct spatial arrangement for action as an agonist and (ii) the ability to undergo a change in conformation may, in itself, be essential for mediating a response at the receptor.

The mechanism of antagonism is also of interest and might, at first sight, appear to offer the possibility of an immediate insight into drug action. An agonist molecule is presumably active because it can satisfy all the conditions necessary to mediate a response at the post-synaptic membrane. In contrast, however, an antagonist may act to block the response in many ways. Only those antagonists which act by competing with the transmitter molecule at the receptor site can provide evidence concerning the drug-receptor interaction. These specific competitive antagonists are important, however, since such molecules must be assumed to possess all the required attributes to occupy a receptor, and yet do not produce a response. The best-

attested specific antagonist of GABA is bicuculline (Curtis, Duggan, Felix and Johnston, 1970). Although other compounds, such as benzyl penicillin and picrotoxin, have been reported to block GABA, evidence for specific competitive antagonism is, as yet, not conclusive in these cases: for example, the action of benzyl penicillin has not been shown to be competitive, while picrotoxin fails to antagonise GABA in certain preparations.

Some attempts to explain the action of GABA, its agonists and antagonists in terms of their flexibility have been reported (Warner, Player and Steward, 1973; Steward and Clarke, 1975); these have of necessity made use of simple theoretical and crystal conformation data - until recently this was all that was available.

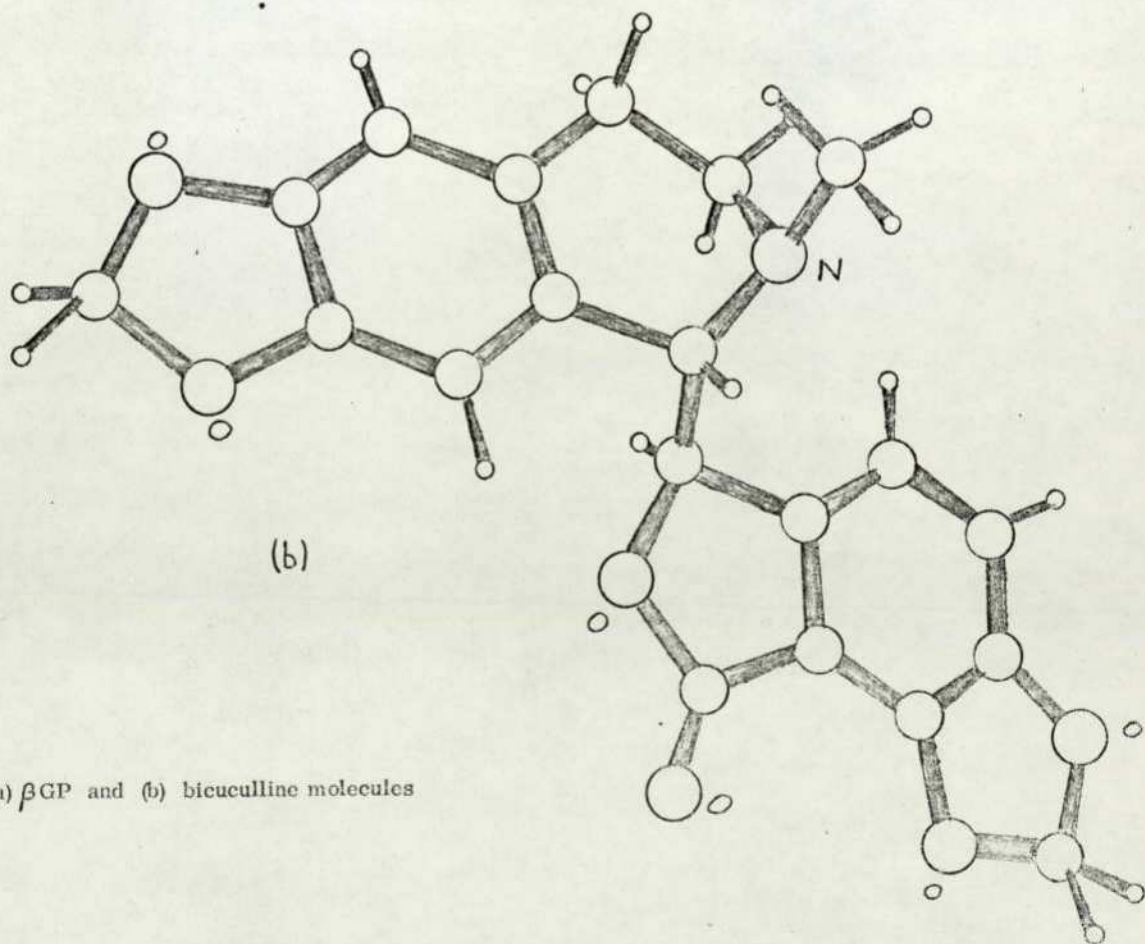
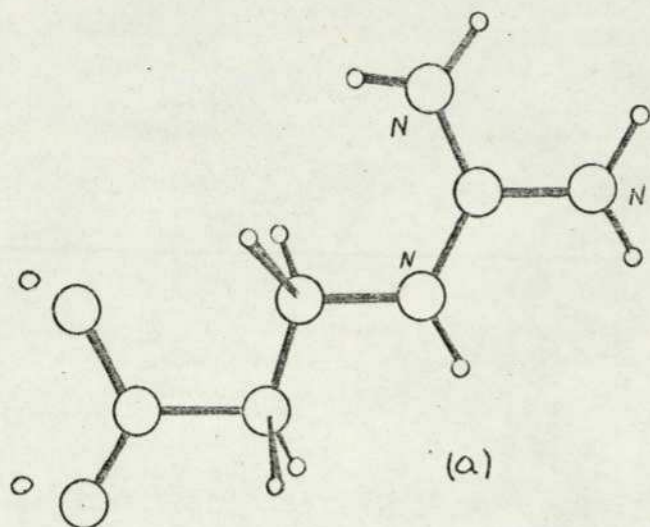


Fig. 1.3. (a) β GP and (b) bicuculline molecules

1.3 CONFORMATIONAL STUDIES OF FLEXIBLE MOLECULES

Various procedures may be used to determine the likely conformations of flexible molecules:

- (i) X-ray diffraction studies provide precise data on molecules in the crystalline state;
- (ii) theoretical molecular orbital calculations can give information on the likely conformations of isolated molecules, of small groupings of molecules, or, if combined with some empirical computations, of molecules in solution;
- (iii) estimates of preferred conformations may be made purely on the grounds of classical empirical energy calculations;
- (iv) the use of nuclear magnetic resonance (NMR) can, in some cases, give evidence as to which conformers of a molecule are present in solution.

The last-mentioned method, NMR spectroscopy, is the only one to give experimental data on the conformational modes present in solution. Until very recently only one set of NMR data was available on GABA (Parry Jones, Roberts and Ahmed, 1971), but two further studies have now been reported. One of these (Ham, 1974) gave a conformational analysis; the other (Richards and Thomas, 1974) did not. The conformational results from NMR will be considered further in Chapter 6.

Although NMR is perhaps the most suitable experimental method for making conformational analyses in the liquid phase, other approaches offer certain features which are useful in the present context. Thus the crystal conformation, as determined by X-ray structure analysis, may be not unrelated to that attained by the

transmitter molecule in the drug-receptor complex. Additionally, X-ray studies give very precise values for bond-lengths and bond-angles and do give information on a minimum energy conformation in a situation where inter-molecular bonding is present.

Theoretical methods of predicting preferred conformations can provide an insight into the intrinsic flexibility of a molecule; also quantum mechanical methods may produce some indications of the electronic structure of the molecule. The weakness of these theoretical methods lies in the approximations which must be made to make the calculations manageable. Thus, it is difficult to explicitly include the effects of the environment: calculations are normally made on the conservative molecule and thus give results on a situation remote from the reality of the biophase. Further, simplifications must be made in the actual methods of computation: the resulting procedures range in sophistication from simple empirical to rigorous *ab initio* methods. Since increased sophistication usually leads to increased computational difficulties and hence decreased applicability, all the methods are useful in some respect or other.

From the above it can be seen that, in conformational analysis, it is essential to obtain results from a variety of approaches. The studies reported here were made with the intention of:

- (i) investigating the applicability of X-ray diffraction and theoretical methods in conformational studies of GABA and other centrally active molecules;
- (ii) implementing a library of computer programs to carry out the required calculations;

- (iii) making crystallographic and theoretical studies on GABA and a representative selection of its agonists and antagonists: β -guanidino propionic acid (β GP, $C_4H_9N_3O_2$), an open-chain GABA agonist (Fig.1.3); muscimol, which has a ring structure (Fig.1.2); and bicuculline (BIC, $C_{20}H_{17}NO_6$), a specific competitive GABA antagonist (Fig.1.3);
- (iv) to compare these results with others available and to consider the possible pharmacological implications in view of the reported electrophysiological data.

The nature of the work which could be done was greatly influenced by the computational facilities which were available: a description of the basic computational work is given in the next chapter. Conformational studies, using X-ray diffraction, the CNDO/2 molecular orbital method, and classical potential-energy calculations, are reported in Chapters 3, 4 and 5. In Chapter 6 these results, together with other published data, are surveyed and used in a discussion of the effect of the environment on the conformations of the molecules studied. Chapter 7 consists of a discussion of the pharmacological implications of these results.

Throughout this report, the conformations of molecules will be specified by conformational angles. The convention adopted, and the nomenclature used for the conformational angles of the molecules of interest, are defined in Appendix A.

CHAPTER 2

Preparatory Computational Work

2.1 INTRODUCTION

To make the calculations necessary for the work described later, very large amounts of computing power were required. In particular, in the case of the lengthy semiempirical molecular orbital calculations (Chapter 4) the computational facilities available were an important factor in deciding the nature of the work done. The X-ray structure determinations described in Chapter 3 also required the use of a large computer installation. In this chapter a description of the computing hardware and software is given and the basic form of the program library which was built up is described. The use of the software and hardware to the best effect is discussed.

Two major computer configurations were available. These were the facilities (i) at The City University Computer Centre and (ii) at the University of London Computer Centre (ULCC). The main machine of The City University centre is an ICL 1905E, which has 64K of central core store. The machine is connected to a series of on-line terminals which run under the MAXIMOP system originally developed at Queen Mary College, London. As well as having only a comparatively small amount of central core store, the central processor of the ICL 1905E is relatively slow: the average number of instructions which can be executed per second is $\sim 2 \times 10^5$. The system was therefore unsuitable for use in the X-ray structure analyses or in the semiempirical molecular orbital studies. In spite of this, The City University equipment was useful for certain ancillary work. In the development of some programs it was convenient to carry out the initial design and debugging operations with the MAXIMOP on-line system before transferring to the more powerful ULCC machines. The ICL 1905E

also proved useful for calculations concerning molecular geometries, as these did not generally require large amounts of computing power.

The majority of work was done with the machines at ULCC. During the duration of this study the hardware configuration at ULCC was centred around three mainframe computers: a CDC 6600, a CDC 6400 and a CDC 7600; the CDC 6400 is normally used as an input station to the 7600. Only very limited access to the 6600 was possible, and hence the 6400-7600 complex was most commonly employed. The following description is therefore restricted to this latter part of the installation.

The CDC 7600 is a very fast computer designed for scientific work. The actual system in use at ULCC consists essentially of: 1 central processor unit (CPU) with 60-bit words and ~100 times faster than the ICL 1905E; 64K small core memory (SCM); 256K of large core memory (LCM); extensive disc storage and magnetic tape facilities. The CDC 6400 and several small peripheral processors are linked with the central machine. In addition, several terminals at remote sites feed into the main installation: one of these terminals is located at The City University.

The amount and disposition of the central core storage is of importance because it affects the design of programs to be run on the system. 'Small Core Memory' is that which may be accessed directly by the CPU and is thus available for storing instructions as well as data. In contrast, instructions held in 'Large Core Memory' must first be transferred to SCM before being executed. In FORTRAN, assignment of data to SCM or LCM is conveniently achieved by use of LEVEL 1 (for SCM) or LEVEL 2 (for LCM) statements. However, execution time

for operations involving LCM may be three or more times greater than equivalent operations using SCM: therefore for maximum program efficiency it is desirable to make as much use as possible of SCM. A further complication arises because of the large core-storage requirements of the SCOPE 2.0 operating system. This basic software normally requires a minimum of 12K SCM and perhaps 130K LCM. Also the CDC 7600 is operated in a multi-programming mode i.e. several programs may be core-resident simultaneously. To make this possible the operating system sometimes requires to 'swap' a program from SCM to LCM and for this operation it must reserve an area in LCM equal in size to SCM. The net results of these demands is that the amount of core store which can be guaranteed to be available to the application program is 24K SCM and 70K LCM. This LCM allocation must include buffer areas for use in the transfer of data to and from disc backing store. Input/output to or from sequential files requires approximately 2.5K of buffer area per file; peripheral activity employing random access files requires a larger amount of LCM. Hence there is a conflict between using sequential files, which may be less efficient as far as run-time is concerned, and random access files for which more core storage is necessary.

To summarise the above points: to make the most efficient use of the CDC 7600 a program should be coded such that

- (i) the maximum SCM used is $< 24K$
- (ii) the maximum LCM used is $< 70K$
- (iii) SCM is used in preference to LCM
- (iv) careful consideration is given to the relative merits of using sequential or random access disc files: where

possible programs should not use temporary storage areas on disc at all.

The requirements detailed above necessitated considerable changes in programs originally written for other computers: these changes are considered in some detail in the chapters concerned with the implementation and use of the programs (molecular orbital calculations in Chapter 4 and classical energy calculations in Chapter 5).

The use of The City University link to the CDC 7600 introduced some further restrictions. The terminal constituting the link has only very slow input/output peripherals. It was therefore necessary to keep input and output at the terminal to an absolute minimum to obtain the fastest possible job turn-round. This was done by creating and maintaining a program library on disc at the central computer, thus reducing punched card input, and by only printing essential output at the remote terminal (where necessary, larger amounts of printout were output on the faster lineprinters at ULCC).

2.2 PROGRAM DEVELOPMENT

At the start of this work no programs suitable for the investigation of the conformations of molecules were available at The City University. It was a prerequisite of the work that suitable means for the processing of X-ray diffraction data, and making quantum-mechanical and classical energy calculations be available. The programs used for these purposes are fully described in Chapters 4 and 5 and Appendices B and G. It was also necessary to have available ancillary programs for tasks such as: producing atomic coordinates from bond-lengths and bond-angle data, manipulating molecular geometry as a function of dihedral angles, and producing convenient graphic representations of molecules.

To produce atomic coordinates from bond-lengths and bond-angles use was made of Dewar's program 136 from the Quantum Chemistry Program Exchange (QCPE). The version obtained was almost completely compatible with the CDC 7600 and no major modifications were made.

In the conformational studies it was found convenient to be able to vary the molecular geometry under consideration as a function of specified dihedral angles. A program was written to perform the required operations by the translation and rotation of the coordinate axes. The program, MOROT, was originally developed on the ICL 1905E and then modified to run on the CDC 7600. Later, MOROT was incorporated into the semiempirical molecular orbital program (Chapter 4). More recently, both MOROT and QCPE 136 have been used as integral parts of a comprehensive package of programs, developed in this laboratory, relevant to studies of the geometries of molecules (P. W. Borthwick, D. Warner, 1974). This package, the

MOGEO system, can be employed in a fully interactive mode via the MAXIMOP system and is rapid and convenient in use.

To obtain suitable pictorial representations of molecules use has been made of the MOPLLOT program written at Birkbeck College, London. MOPLLOT, which creates photographic images of molecules on microfilm from atomic coordinates as input, was written for the CDC 6600 computer and has only been used on that machine.

The programs MOROT and QCPE 136, together with programs for making energy calculations, formed the basis of a program library. The library was stored and maintained using the CDC UPDATE utility system. This facility provides a convenient means of editing and using such a program library. For security reasons, two master copies of the library were held on magnetic tape. These were used to create the version of the library held on disc which was actually accessed by the computer.

The program library described above was used extensively in the work which is reported in the following chapters.

CHAPTER 3

X-ray Crystallographic Studies of
some Centrally Acting Molecules

3.1 BACKGROUND TO THE DETERMINATION OF CRYSTAL STRUCTURES BY X-RAY DIFFRACTION

3.1.1 Introduction

The study of single crystals by X-ray diffraction can provide a complete structural analysis of molecules in the solid state. The results obtained may be expressed within well-defined limits of confidence and with a high degree of precision. The price which must be paid for this accuracy is the "freezing" of the molecule in the crystalline state. The environment of the molecule is then different from that which might be expected in the biophase, and the effect of this difference on the conformation of the molecule will depend on the magnitude of the crystal packing forces as compared with solvent effects, and how susceptible the molecule is to this difference. X-ray structure analyses may give useful information on the likely conformations of molecules in bound states: for example, the possibility of the formation of hydrogen-bonds is often revealed and crystal structure conformations are actual minimum energy conformations for the particular circumstances of inter-molecular bonding in the crystalline state.

The crystal structures of three molecules have been determined during the course of the investigations reported here, viz GABA, GABA hydrochloride (GABAHCl) (a heavy-atom derivative of GABA) and BGP, a GABA agonist. Since the process of structure determination follows a similar course in each case, a general outline of the procedure is given first.

The electron density, $\rho(x,y,z)$, at any point in a crystallographic unit cell of volume V , may be expressed as:-

$$\rho(x,y,z) = \frac{1}{V} \sum_{h=-\infty}^{+\infty} \sum_{k=-\infty}^{+\infty} \sum_{\ell=-\infty}^{+\infty} F_{hkl} e^{-2\pi i(hx+ky+\ell z)} \quad \dots (3.1)$$

where F_{hkl} is the (complex) structure factor of a set of reflecting planes with Miller indices hkl . The structure factor itself, which is the resultant of the N waves scattered in the direction of the hkl reflection by the N atoms in the unit cell, may be written:-

$$F_{hkl} = \sum_{j=1}^N f_j e^{2\pi i(hx_j+ky_j+\ell z_j)}, \quad \dots (3.2)$$

where f_j represents the scattering power of the j th atom in the required direction.

Clearly, from equation 3.1, once the set of structure factors is known the electron density at any point may be computed and the atomic positions determined. Equation 3.2, however, shows that F_{hkl} cannot be directly calculated without a knowledge of the phase of the structure factor. Generally, this can neither be measured experimentally, nor computed in a straightforward way without a previous knowledge of the atomic positions, which appear in the term $e^{2\pi i(hx_j+ky_j+\ell z_j)}$. In contrast, the magnitude of the structure factor is directly related to the measured intensity, I , of the diffracted X-rays:

$$\sqrt{I_{hkl}} \propto |F_{hkl}|$$

i.e. $\sqrt{I_{hkl}} = C |F_{hkl}|. \quad \dots (3.3)$

The process of structure solution may therefore be thought of as having three aspects: the measurement of the I_{hkl} 's in equation 3.3; the assignment of a suitable value to C in equation 3.3; and the determination of the phase of each structure factor. The collection of the diffraction data is a lengthy, though reasonably straightforward matter. It will not be discussed in detail here.

3.1.2 Data Reduction

Some factors contributing to C (equation 3.3) may be dealt with immediately. Rewriting 3.3:

$$|F_{hkl}| = \sqrt{\left[\frac{KI_{hkl}}{Lp} \right]} \quad \dots (3.4)$$

where p, the polarisation factor, is given by

$$p = \frac{1 + \cos^2 2\theta}{2}$$

and appears because of the unpolarised and polarised natures of the incident and reflected X-ray beams respectively. Here, p depends only on the Bragg angle θ and may easily be calculated for each $|F_{hkl}|$.

The term L in equation 3.4 is the so-called (monochromatic) Lorentz factor and takes account of the differing times during which various sets of planes are in a reflecting position. The mathematical form of this correction depends on the geometry of the method of data collection. For the equi-inclination

Weissenberg method it is given by:-

$$L = \frac{\sin \theta}{\sin 2\theta \sqrt{\sin^2 \theta - \sin^2 \mu}},$$

where μ is the equi-inclination setting angle.

The remaining factor in equation 3.4, K , depends on the basic physical conditions of data collection, such as the size of crystal used and the intensity of the X-ray beam. Several methods are available for determining approximate values for this scaling term and at the same time providing a convenient way of obtaining a first estimate of the effect of the thermal motions of the atoms on the diffracted X-ray intensities. The scattering power of an atom (f_j in 3.2) depends on the Bragg angle through:-

$$f_j = f_{oj} e^{-B(\sin^2 \theta)/\lambda^2}, \quad \dots (3.5)$$

where λ is the wavelength of the radiation and f_{oj} the scattering factor of an ideal stationary atom. The overall temperature factor, B , is related to the mean-square amplitude of atomic vibration by

$$B = 8\pi^2 \overline{u^2}.$$

Clearly, the structure factors, which depend on f_j , will show a marked dependence on $\sin \theta$. Statistical calculations with the scattering data are more easily made if this dependence on θ is removed and it is therefore convenient to make use of a normalised structure factor defined by

$$E_{hkl} = \frac{F_{hkl}}{\epsilon \sum_{j=1}^N f_j^2} \dots (3.6)$$

ϵ is an integer introduced to facilitate the comparison of E 's for different classes of reflections and is dependent on the space-group symmetry. From (3.5) and (3.6)

$$E_{hkl} = \frac{F_{hkl}}{\epsilon \sum_j^N f_{oj} e^{-B(\sin^2 \theta)/\lambda^2}},$$

and

$$|E_{hkl}| = \frac{|F_{hkl}|}{\epsilon \sum_j^N f_{oj} e^{-B(\sin^2 \theta)/\lambda^2}},$$

and hence from (3.4)

$$|E_{hkl}| = \left[\frac{[KI_{hkl}]}{[Lp]} \right] / \epsilon \sum_j^N f_{oj} e^{-B \sin^2 \theta / \lambda^2} \dots (3.7)$$

To calculate $|E_{hkl}|$ it remains only to find suitable values for K and B : the normalised structure factors have certain statistical properties which are useful in this respect. Thus the E 's should show no trend with increasing $\sin \theta$: this constraint may be used to estimate a suitable value for B . It may be shown that theoretically

$$\overline{|E_{hkl}|^2} = 1.00 \dots (3.8)$$

whatever the contents of the unit cell. Thus the value of K may be fixed so that (3.8) holds.

The above methods for determining B and K were used in the structure determinations described below. In practice, initial values determined in this way are found only to be moderately accurate; since both temperature and scale factors are refined later by least-squares methods, this is not important.

Returning to equation 3.7, the values of the $|E_{hkl}|$'s and thus the $|F_{hkl}|$'s may now be calculated from the observed I_{hkl} 's

3.1.3 The Determination of the Phases of the Structure Factors

To proceed to the solution of the structure via (3.1) it is now necessary to obtain the phase of each structure factor: no general solution to this problem is currently available. The best that can be done is to produce a tentative set of phases by some means or other and use these to find an initial model for the crystal structure. The structure factors computed using this model (F_{calc} 's) may then be compared with the observed values (F_{obs} 's). The correctness of the trial model is judged by its residual factor, R , which is calculated from

$$R = \frac{\left\{ \sum \left| |F_{obs}| - |F_{calc}| \right| \right\}}{\sum F_{obs}}$$

where the sums are taken over all reflections. A process of

refinement of the model to obtain a low R-factor should then lead to the correct structure.

Two methods which have proved particularly successful for obtaining an initial model are (i) use of the Patterson function and (ii) direct probability methods.

The Patterson function,

$$P(u,v,w) = \frac{1}{V} \sum_h \sum_k \sum_l |F_{hkl}|^2 \cos 2\pi(hu + kv + lw)$$

i.e. essentially a Fourier synthesis using only the magnitude of the F_{hkl} 's squared, has peaks at positions corresponding to interatomic vectors. For those molecules containing a small number of atoms, an easily recognisable molecular fragment (e.g. a ring structure), or a heavy atom, it is sometimes possible to manually solve the Patterson function for the atomic positions. However, because of the large number of peaks (N atoms in a unit cell produce $N(N-1)$ peaks on a Patterson map), many of which are of similar height, direct solution in this way is often difficult.

A more successful method for dealing with crystal structures of moderate complexity, particularly in the centrosymmetric case, is the symbolic addition procedure of Karle and Karle (1963). This is a direct method in that it makes use of information contained in the magnitudes of the F_{hkl} 's to produce estimates of the likely phases of the structure factors. The approach is based upon a result obtained by Sayre (1952) which may be summarised as: for F_{hkl} , $F_{h'k'l'}$,

$F_{h-h'k-k'\ell-\ell'}$ large then

$$S\left(F_{hkl}\right) \cdot S\left(F_{h'k'\ell'}\right) \cdot S\left(F_{h-h'k-k'\ell-\ell'}\right) \sim +1 \quad \dots (3.9)$$

which is to be read as: 'the sign of F_{hkl} times the sign of $F_{h'k'\ell'}$ times the sign of $F_{h-h'k-k'\ell-\ell'}$ is probably equal to +1'. Since the phase of E_{hkl} is the same as that of F_{hkl} , the normalised structure factors may equally well be used in (3.9). Indeed, as the E values include allowances for special classes of reflections (through the value of ϵ in equation 3.6) and do not decrease rapidly as a function of $\sin \theta$, they are more commonly used.

An expression which has been used to assign a quantitative value to the probability, P, of (3.9) being true is:

$$P = \frac{1}{2} + \frac{1}{2} \tanh \left\{ \frac{1}{\sqrt{N}} \left| E_{hkl} E_{h'k'\ell'} E_{h-h'k-k'\ell-\ell'} \right| \right\}.$$

To use expression 3.9 in practice requires sorting through the reflections with large E-values and finding those triplets whose miller indices satisfy:

$$\begin{aligned} h_1 - h_2 &= h_3 \\ k_1 - k_2 &= k_3 \\ \ell_1 - \ell_2 &= \ell_3. \end{aligned}$$

If a large enough number of such relationships can be found, the problem then reduces to solving a set of simultaneous probability expressions for the phases. Normally, a small

number of certain reflections (three or fewer) may be arbitrarily assigned phases. This is merely equivalent to fixing the origin of the unit cell at one of the centres of symmetry. The phases so-defined may then be used to start the generation of others, using expression 3.9.

The method outlined above can only provide reliable values for the phases of large E's. As the magnitudes of the E's decrease, so does the probability that the suggested phase is correct. It is usually possible, however, to determine sufficient phases with high levels of confidence to make possible the calculation of an initial Fourier map. Using the map, a trial model of the crystal structure may be deduced, and the R-factor calculated.

3.1.4 Refining the structure

The next step is to produce an improved model of the structure, perhaps by locating atoms not apparent from the original Fourier map or by obtaining better agreement between the F_{obs} 's and F_{calc} 's by adjusting the atomic positions and modifying the temperature and scaling parameters. Missing atoms are most easily found by computing a difference Fourier synthesis from

$$\Delta\rho(x,y,z) = \frac{1}{V} \sum_h \sum_k \sum_l \Delta F e^{-2\pi i(hx+ky+lz)}$$

where $\Delta F = [|F_{\text{obs}}| - |F_{\text{calc}}|] e^{i\alpha}$ and α is the phase of the calculated structure factor. The ΔF map shows peaks and valleys

where the model is not in agreement with the observed data and atoms missing from the trial structure may then readily be positioned. The same map may be used to correct the scale and thermal parameters, but a better approach, and the one employed here, is to apply the method of least-squares.

The function most commonly minimised is:

$$M = \sum w_{hkl} (|F_{obs}| - |K F_{calc}|)^2, \quad \dots (3.10)$$

where w_{hkl} is a weighting factor indicating the expected reliability of each F_{obs} , and the summation is carried over all the reflections. The F_{calc} 's are treated as functions of the j atomic positions, the scale factors, and the overall temperature factor. It is also simple to take better account of the thermal motion of the atoms by introducing isotropic or anisotropic temperature factors.

The normal equations produced by attempting to minimise the function M in (3.10) are non-linear, so cannot be solved directly for the correct values of the parameters. They can, however, be modified to give estimates of the corrections which should be applied to the initial values of the parameters. The process may then be repeated, using the newly-corrected parameters as the starting point, to calculate the next adjustment. In well-behaved cases the calculated corrections decrease as consecutive cycles of least-squares refinement are carried out. At the same time the R-factor should show a steady improvement. Nonetheless, repeated cycles of least-squares refinement need

not, necessarily, produce a physically correct crystal structure. In practice, any false structures which appear can usually be discounted by reference to chemical evidence (such as bond-lengths and bond-angles).

The process of structure analysis is completed by producing a final Fourier map, which should show the molecule clearly, and a difference Fourier map to check that no large discrepancies remain between the calculated and observed structure factors. With the structure fully refined, accurate values may be obtained for the bond-lengths, bond-angles and dihedral angles of the molecule. Deductions may also be made concerning such matters as hydrogen-bonding.

The procedures outlined above give a brief theoretical background to structure determination by X-ray diffraction studies. The following section gives practical details of the solution of the structures of GABA $\cdot$ HCl, GABA and BGP.

3.2 EXPERIMENTAL DETAILS OF CRYSTAL STRUCTURE DETERMINATIONS

3.2.1 Preliminary work on the crystal structures of GABA, GABAHC ℓ and BGP

Diffraction data had been collected on the three molecules of interest using a tape-controlled automated Stoe-Güttinger diffractometer employing Ni-filtered Cu K α radiation (1.5418Å). The determination of the space groups and unit cell dimensions were made from Weissenberg photographs. The equi-inclination Weissenberg geometry was also used in the collection of intensity data. A summary of the information gleaned from the preliminary investigations and the number of reflections measured for each compound is given in Table 3.1.

Data processing and structure solution was undertaken using the X-RAY 70 suite of programs. A description of the use of this system of programs is given in Appendix B.

3.2.2 A refinement of the crystal structure of GABAHC ℓ

The crystal structure of γ -aminobutyric acid hydrochloride was first determined by Tomita (1965), but his results were only of moderate accuracy ($R = 0.22$ and $R = 0.38$ for the two projections he considered) and for the research in progress in the laboratory it was necessary to obtain a more precise solution for the structure. The problem provided a useful opportunity to become familiar with the use of the X-RAY 70 system.

Having applied the normal Lorentz and polarisation corrections in the data reduction procedure, initial estimates were obtained for scale factors for the six groups of data, and

	GABA·HCl (C ₄ H ₉ NO ₂ ·HCl)	GABA C ₄ H ₉ NO ₂	BGP C ₄ H ₉ N ₃ O ₂
no. of measured reflections	417	890	1131
no. of 'observed' reflections	367	755	940
Space group	P2 ₁	P2 ₁ /a	P2 ₁ /a
unit cell constants	$\left. \begin{array}{l} a(\text{\AA}) \\ b(\text{\AA}) \\ c(\text{\AA}) \\ \beta(^{\circ}) \end{array} \right\} \begin{array}{l} 9.040(3) \\ 6.528(2) \\ 5.900(3) \\ 99.82(3) \end{array}$	$\begin{array}{l} 8.228(5) \\ 10.036(9) \\ 7.210(4) \\ 110.63(5) \end{array}$	$\begin{array}{l} 11.818(4) \\ 7.247(4) \\ 7.311(4) \\ 98.22(4) \end{array}$
no. of molecules per unit cell	2	4	4
$\rho_{\text{meas.}} (\text{gcm}^{-3})$	1.33	1.21	1.403
$\rho_{\text{calc}} (\text{gcm}^{-3})$	1.332	1.201	1.403

TABLE 3.1 Preliminary Crystal data

also for the overall temperature factor. A b -axis Patterson synthesis was then carried out (Fig.3.1). This confirmed the general form of the structure as determined by Tomita. The refinement of the structure was therefore started using the final coordinates reported by him (Table 3.2) and the scale and temperature factors previously determined. This removed the necessity for obtaining an initial set of phases by another method. The weighting scheme adopted during the least-squares refinement was of the form

$$w = X \cdot Y \quad \dots (3.11)$$

where if $\sin \theta > A$, $X = 1$, else $X = \sin \theta / A$
 if $F_{\text{obs}} < B$, $Y = 1$, else $Y = B / F_{\text{obs}}$.

A scheme of this type assigns lower weights to reflections of small $\sin \theta$ and high F_{obs} . In this way some allowance is made for the effects of extinction on the diffracted intensities. The final values adopted for the constants were:-

$$A = 0.4$$

$$B = 9.0.$$

These proved to give a reasonably good set of weights, as judged by dividing the reflections into groups according to $\sin \theta$ or F_{obs} value: the function $\sum w |F_{\text{obs}} - F_{\text{calc}}|^2$, summed for each group, gave fairly constant values.

It was noticed that the largest measured reflection, $0\ 2\ 0$, gave an F_{calc} value much lower than its F_{obs} : it was considered to be suffering from extinction and was assigned zero weight.

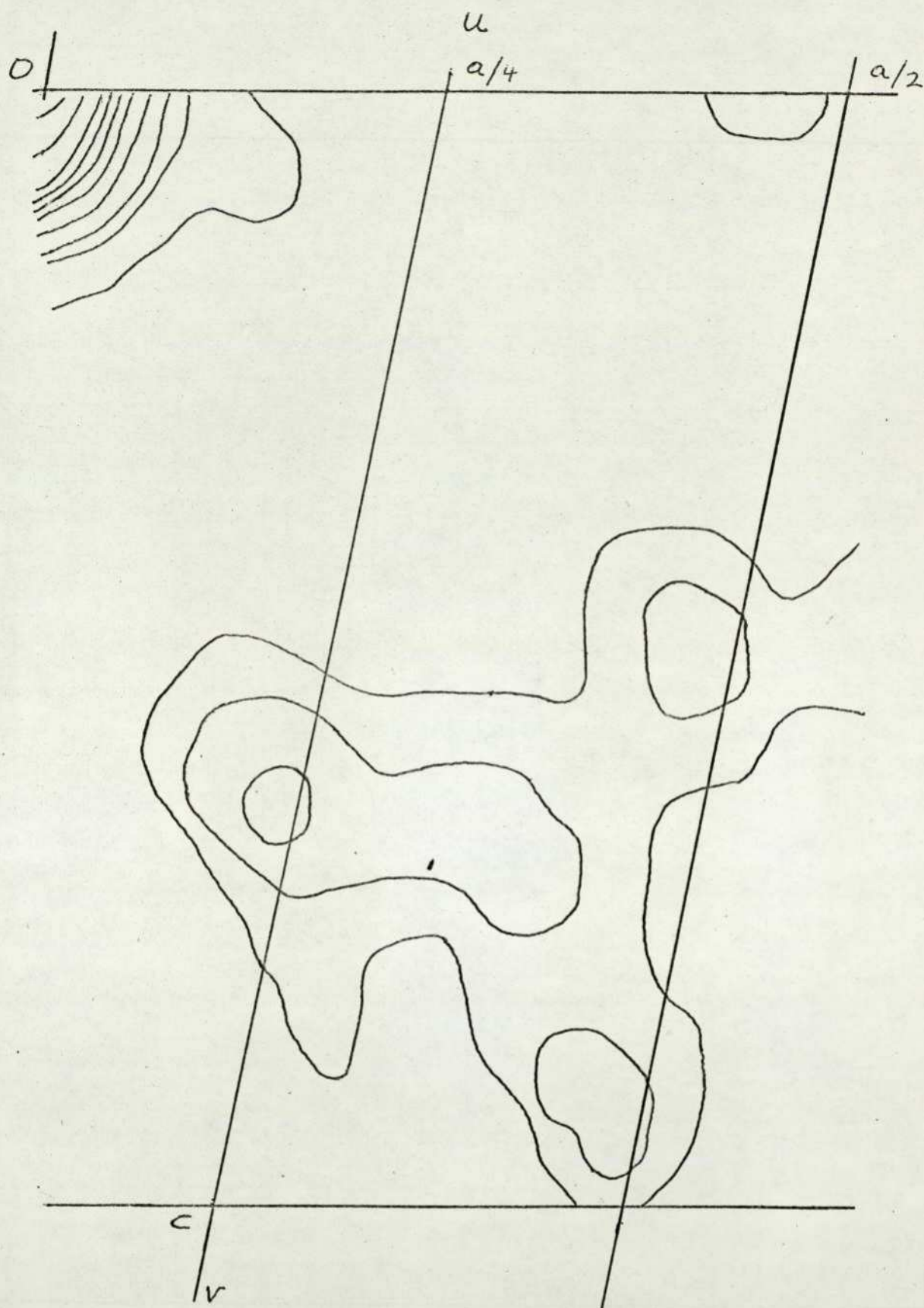


Fig. 3.1 Patterson Map for GABA HCl (b-axis projection).
The structure was solved from a similar map by
Tomita (1965)

	x	y	z
C1	0.062	0.25	0.215
C1	0.247	0.745	0.422
C2	0.324	0.753	0.207
C3	0.496	0.766	0.286
C4	0.578	0.734	0.076
O6	0.520	0.691	-0.113
O5	0.725	0.706	0.145
N7	0.080	0.745	0.336

TABLE 3.2 Initial fractional atomic coordinates for the GABAHC1 structure refinement

	x	y	z	$U \times 100 \text{ \AA}^2$
C1	0.0620(3)	0.25	0.2138(5)	3.71(1)
C1	0.246(1)	0.765(7)	0.420(2)	3.9(3)
C2	0.329(1)	0.733(7)	0.210(2)	3.7(3)
C3	0.497(1)	0.745(6)	0.289(2)	3.6(3)
C4	0.580(1)	0.755(7)	0.085(2)	3.4(3)
O5	0.726(1)	0.726(3)	0.148(1)	4.2(3)
O6	0.518(1)	0.737(4)	-0.115(2)	4.5(3)
N7	0.082(1)	0.744(6)	0.332(2)	4.0(3)

TABLE 3.3: Final fractional atomic coordinates and isotropic temperature factors for GABAHC1

Three cycles of full-matrix least-squares refinement were carried out in the isotropic temperature factor mode, reducing the R-factor from 0.179 to 0.115 for all measured reflections. The R-factor obtained for the "observed" reflections (i.e. those greater than a counting threshold taken in these studies to be 100 counts - those below this threshold are termed "unobserved") was 0.105. An R-factor of this magnitude normally indicates that the structure has been solved correctly and has been reasonably well refined. In view of the small number of reflections measured the result was satisfactory. Even so, the achievement of this low residual factor is due partly to the presence of the chlorine atom, which dominates the diffraction by the GABAHC ℓ unit cell: reasonable positioning of this atom immediately produces fair agreement between the F_{obs} 's and F_{calc} 's. This is emphasised by considering the final values of the atomic coordinates given in Table 3.3 - the uncertainty in the position of the chlorine atom is 1/3 that of the other atoms.

An attempt was made to refine the structure further by introducing anisotropic temperature factors. The results were not satisfactory, suggesting strongly that the amount and quality of the data collected did not warrant the addition of further parameters to the structural model.

Using the final atomic coordinates from the isotropic least-squares refinement F_{obs} and ΔF Fourier syntheses were computed (Figs.3.2 and 3.3). The F_{obs} map clearly shows the form of the molecule, while no significant discrepancies appear on the ΔF map. Final computed bond-lengths, bond-angles and

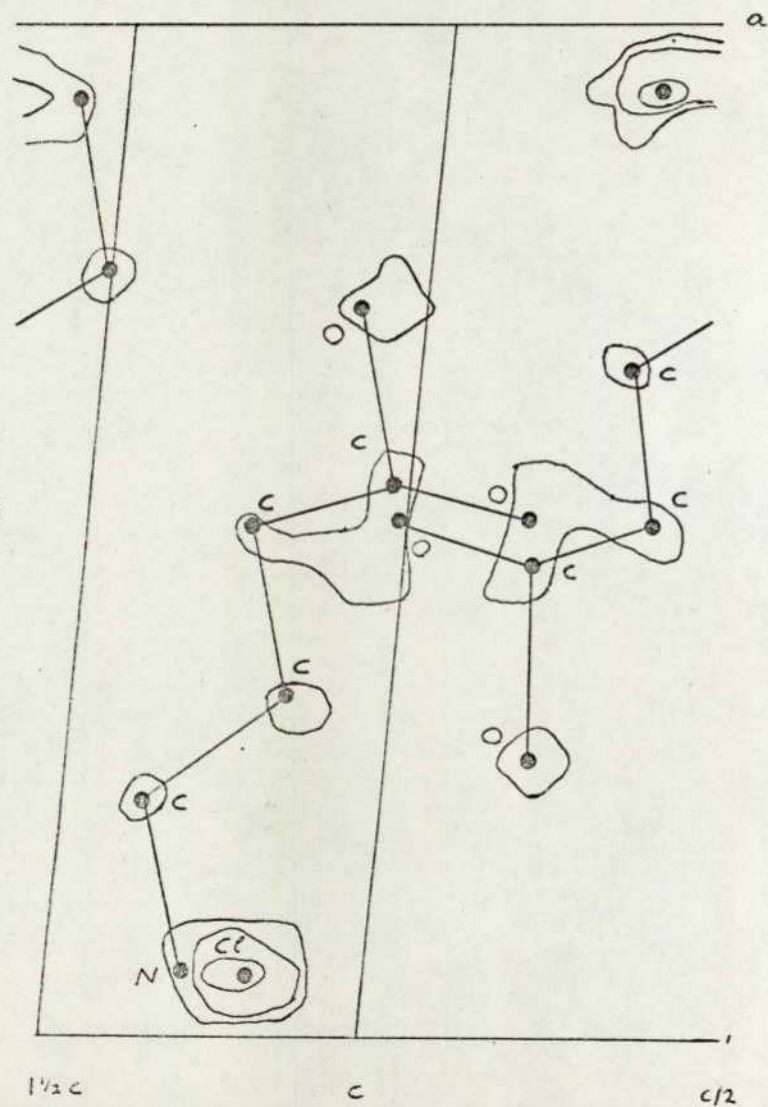


Fig. 3.2 Obs Fourier Map for GABAHI
(Δ -axis projection). Contours marked at 3, 6 and $9 \text{ e}/\text{\AA}^2$

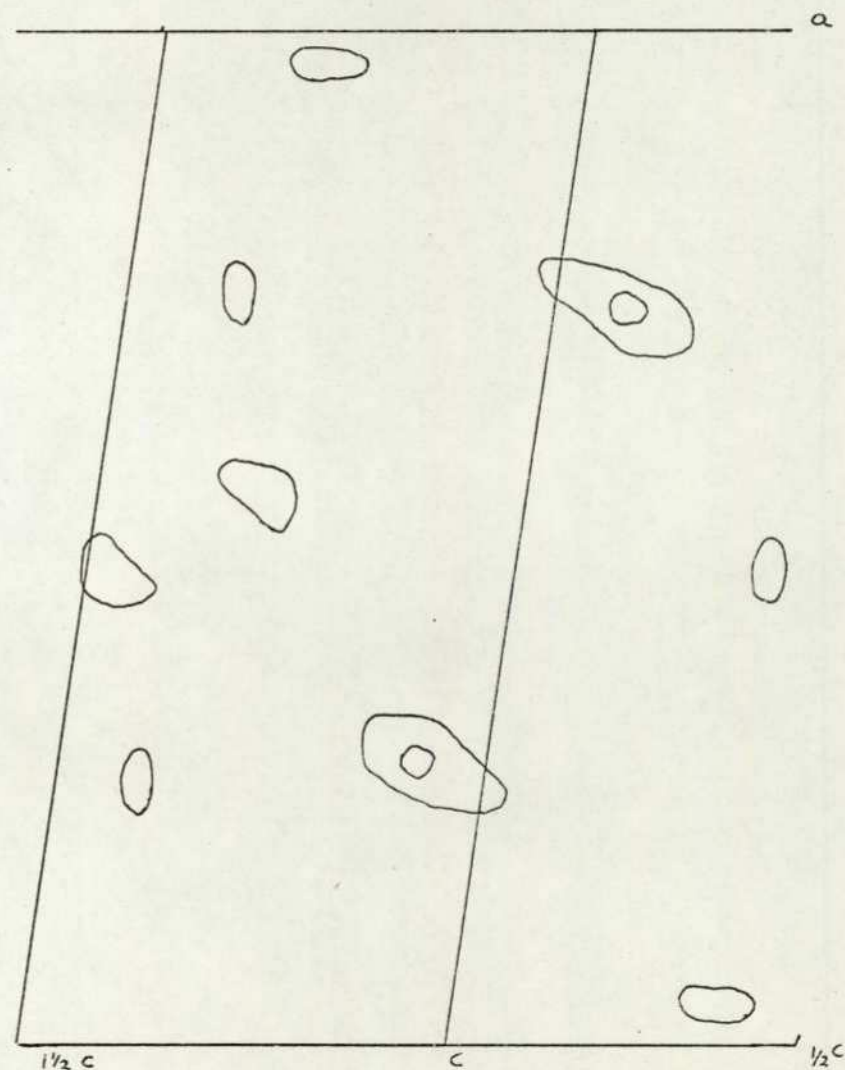
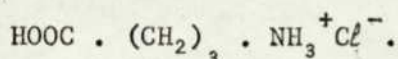


Fig. 3.3 ΔF Fourier Map for GABAHI
(δ -axis projection). Contours marked at 0.3 and $0.45 \text{ e}/\text{\AA}^2$

torsional angles are given in Tables 3.4 and 3.5. Fig.3.4 indicates the arrangement of molecules within the unit cell and a complete listing of F_{obs} and final F_{calc} values is given in Appendix C.

3.2.3 Discussion of the crystal structure of GABAHCℓ

The C-C bond-lengths (Table 3.4) of the final structure are, within experimental error, equal to the standard value (1.54Å), while the N7-Cl distance (1.49Å) is similarly close to the expected value. The two carbon-oxygen distances (1.32Å and 1.22Å) are typical of single and double bonds respectively, thus indicating the presence of an uncharged carboxyl group. Thus it would seem that GABAHCℓ exists in the crystalline state as:-



The hydrogen-bonding scheme is interesting. There are three amino and one carboxyl hydrogen atoms available for hydrogen bonding. However, this structure refinement reveals that in addition to the carboxyl hydrogen that appears to be suitably located for hydrogen bonding, there are four N-Cl distances consistent with hydrogen bond formation. Of these Cl⁽ⁱ⁾ and Cl⁽ⁱⁱⁱ⁾ (Fig.3.4) are arranged tetrahedrally (within 10°) about the amino group. If hydrogen atoms are placed along these N-Cl bonds at a distance of 1.05Å from the nitrogen atom, the position of the third amino hydrogen may be calculated, assuming an approximately tetrahedral configuration for the amino group. The distance of this hydrogen from both Cl⁽ⁱⁱ⁾ and Cl^(iv)

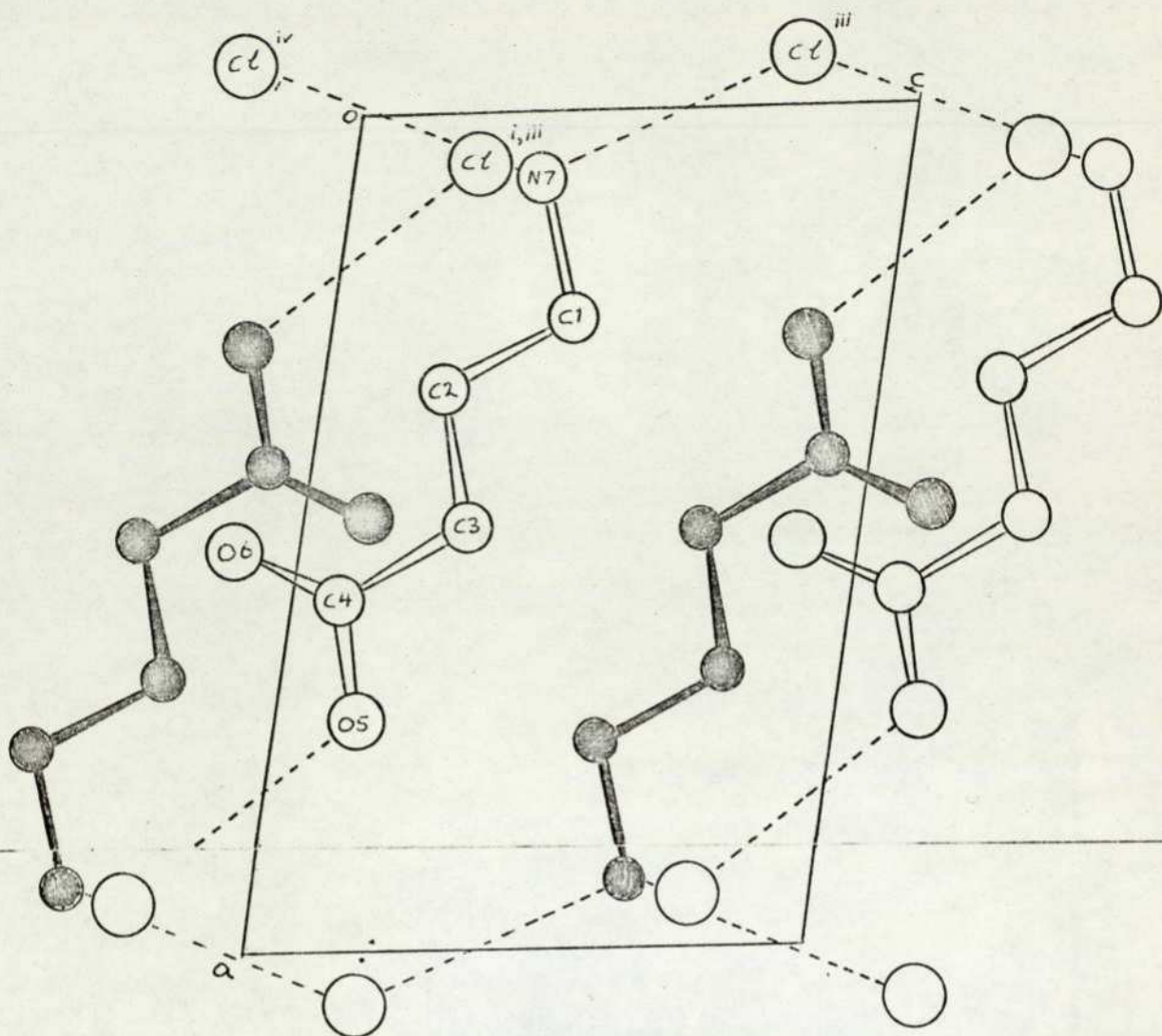


Fig. 3.4 The arrangement of the GABA-HCl molecules in the unit cell (viewed along the b-axis)

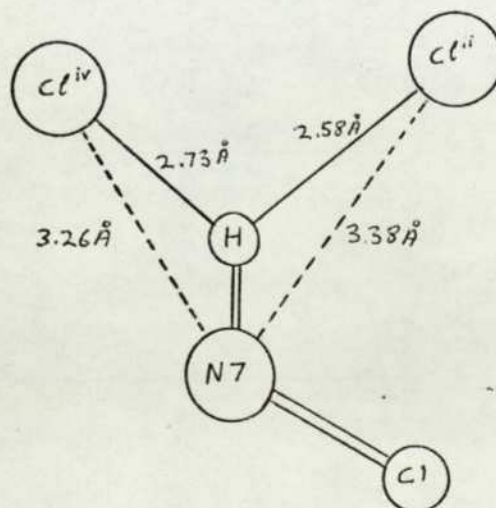


Fig. 3.5 The bifurcated hydrogen bond

Bond lengths:

O5 - C4	1.32 (2)
O6 - C4	1.22 (2)
C4 - C3	1.53 (2)
C3 - C2	1.52 (2)
C2 - C1	1.56 (2)
C1 - N7	1.49 (2)

possible hydrogen-bonds:

N7 - C1 ⁽ⁱ⁾	3.29 (4)	N7 - C1 ⁽ⁱⁱ⁾	3.38 (4)
N7 - C1 ⁽ⁱⁱⁱ⁾	3.18 (1)	N7 - C1 ^(iv)	3.26 (1)
O5 - C1	3.11 (1)		

bond-angles:

O5 - C4 - O6	122 (1)
O5 - C4 - C3	112 (1)
O6 - C4 - C3	124 (1)
C4 - C3 - C2	111 (1)
C3 - C2 - C1	110 (1)
C2 - C1 - N7	107 (1)

angles for the possible hydrogen bonds:

C1 - N7 - C1 ⁽ⁱ⁾	100 (2)	C1 - N7 - C1 ⁽ⁱⁱ⁾	91 (2)
C1 - N7 - C1 ⁽ⁱⁱⁱ⁾	103 (1)	C1 - N7 - C1 ^(iv)	123 (1)
C4 - O5 - C1	120 (1)		

TABLE 3.4 Bond lengths (Å) and bond-angles (°) for GABAHCL

T_1	180*
T_2	+192
T_3	+191
T_4	+182

*calculated indirectly

TABLE 3.5 Conformational angles (°) for GABAHL

is within the sum of the Van der Waals radii of $3.0\text{\AA}$ (Pauling, 1939) and the presence of a bifurcated hydrogen bond may be deduced (Donohue, 1968): the situation is illustrated in Fig.3.5. The bifurcated hydrogen bond, which is a comparatively rare structural feature, was not noted by Tomita.

The conformational angles calculated from the final structure are given in Table 3.5 and show that the molecule is essentially in the "all-*trans*" conformation. Because the hydrogen atoms could not be located directly by this study, the T_1 conformational angle giving the orientation of the amino group cannot be determined immediately. However, C1, C2, N7 and Cl⁽ⁱⁱⁱ⁾ (Fig.3.4) are approximately coplanar and a normal hydrogen bond links Cl⁽ⁱⁱⁱ⁾ and N7. The molecule must therefore be in the *trans* conformation about C1-N7.

As might be expected, this structure refinement confirms that the chlorine atom of GABAHC^l plays a central role in the hydrogen bonding scheme of the molecule in the crystalline state. It would therefore seem possible that the conformation of the flexible GABA molecule is affected by the presence of the heavy atom and to examine this point further a structure determination of pure GABA was undertaken.

3.2.4 The determination of the crystal structure of GABA

The diffraction data for GABA was collected at low temperature. Liquid nitrogen was used as the coolant, producing an estimated temperature at the crystal of $\sim -135^\circ\text{C}$. It was hoped that, from the intensity measurements made under these

conditions, it would be possible to resolve the structure in fine detail, and to define the locations of the hydrogen atoms.

The data had been collected in nine groups and, after data reduction, approximate scaling factors were obtained to place the $|F|$ values on an absolute scale. An initial value for the overall temperature factor was also estimated.

Using the symbolic addition procedure of Karle and Karle a set of 152 phases was obtained. The calculated probability that the assigned phase was correct was high for each reflection (> 0.98) and an F_{obs} Fourier synthesis employing the phased reflections immediately revealed the positions of the non-hydrogen atoms of the GABA molecule. The model deduced from this map gave a residual factor of $R = 0.34$, for all the observed reflections. Three cycles of full-matrix least-squares refinement, with an overall temperature factor and unit weights for all reflections, reduced R to 0.165.

Anisotropic temperature factors and a weighting scheme were then introduced. The weighting scheme was of the same form as equation 3.11, but in this case values of $A = 0.35$ and $B = 10.0$ were found to be more satisfactory for the constants. At this stage it was again noted that although the overall agreement between the F_{obs} 's and F_{calc} 's was quite good, the largest measured reflection, 1 2 0, was observed to have a much lower intensity than that calculated. This was thought to be due to extinction and the reflection was given zero weight. Two further cycles of refinement then produced $R = 0.11$. A difference Fourier synthesis revealed the positions of the

hydrogen atoms clearly, and these were included in the next stage of refinement. The data was found to be insufficient, however, to allow the determination of isotropic temperature factors for the hydrogen atoms: attempts to do so produced large calculated shifts in the parameters and large values of shift/calculated error. This latter is a useful guide as to the state of completion of a least-squares refinement: a low value, maintained over successive cycles, indicates that the structure has refined to a settled form. To continue the analysis of the GABA structure, the overall temperature factor for the molecule was assigned to the hydrogen atoms. This parameter was not refined further. Two further cycles of least-squares refinement were then carried out and a final value of $R = 0.068$ achieved for the observed reflections. Including all measured reflections gave $R = 0.071$. Two final Fourier synthesis (using F_{obs} and ΔF as coefficients) were produced and are shown in Fig.3.6 and Fig.3.7.

Details of the final atomic parameters are given in Table 3.6 and Table 3.7, with bond-lengths and bond-angles for the heavy atoms in Table 3.8. Table 3.9 gives the conformational angles for the GABA molecule and Fig.3.8 shows the arrangement of the four molecules in the unit cell. Final values for the F_{obs} 's and F_{calc} 's are listed in Appendix D.

3.2.5 Discussion of the crystal structure of GABA

The structure shown in Fig.3.8 and the C-C bond-lengths appear consistent with the general pattern for amino acids (Marsh and Donohue, 1967), but the C1-N7 bond-length is slightly

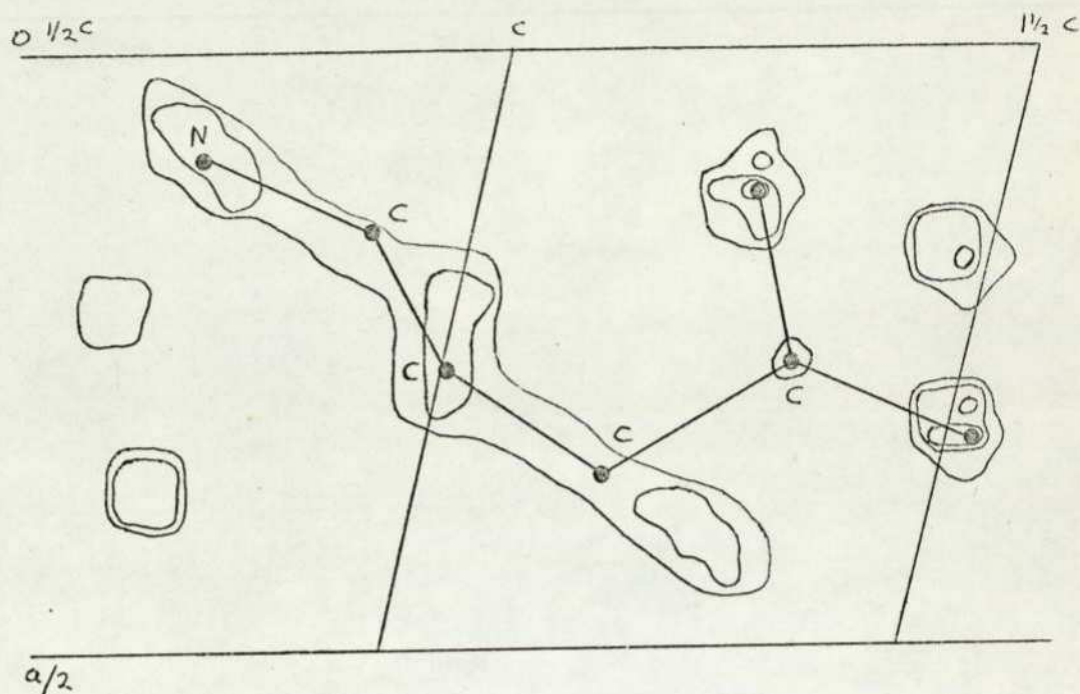


Fig. 3.6 F_{obs} Fourier Map for GABA (b -axis projection).
Contours marked at 4, 5 and 6 $e/\text{\AA}^2$.

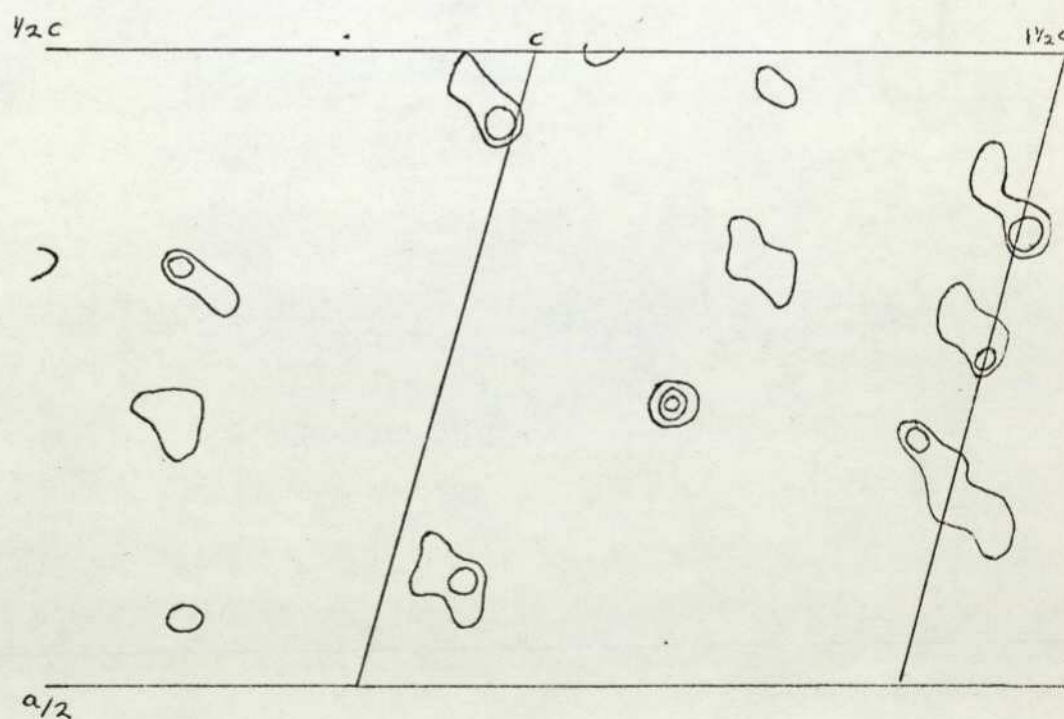


Fig. 3.7 ΔF Fourier Map for GABA (b -axis projection).
Contours marked at 0.2, 0.25 and 0.3 $e/\text{\AA}^2$.

	x	y	z
C1	0.1652(4)	0.8010(4)	-0.1086(5)
C2	0.2942(4)	0.9086(4)	-0.0019(5)
C3	0.3836(4)	0.8773(4)	0.2181(5)
C4	0.2682(4)	0.8948(3)	0.3414(5)
O5	0.1225(3)	0.9481(3)	0.2652(3)
O6	0.3282(3)	0.8525(3)	0.5184(3)
N7	0.0701(3)	0.8412(3)	-0.3190(4)
H8	0.381(6)	0.921(5)	-0.066(7)
H9	0.490(6)	0.938(5)	0.285(7)
H10	0.439(6)	0.786(5)	0.236(7)
H11	0.232(6)	0.712(5)	-0.104(7)
H12	0.075(6)	0.785(5)	-0.042(7)
H13	0.233(6)	0.998(5)	-0.004(8)
H14	0.154(6)	0.857(5)	-0.382(7)
H15	0.002(6)	0.920(5)	-0.316(7)
H16	-0.004(6)	0.778(5)	-0.380(7)

TABLE 3.6 Final Fractional Atomic Coordinates for GABA

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C1	2.3(1)	2.2(2)	1.3(2)	-0.3(1)	0.6(1)	0.0(2)
C2	2.4(1)	2.7(2)	1.5(1)	-0.6(1)	0.6(1)	0.1(2)
C3	2.0(1)	3.1(2)	1.4(2)	0.1(1)	0.8(1)	0.0(2)
C4	1.9(1)	2.0(2)	1.5(1)	0.2(1)	0.5(1)	-0.2(2)
O5	3.2(1)	5.1(2)	1.8(1)	0.4(1)	0.7(1)	0.3(1)
O6	2.3(1)	3.7(2)	1.3(1)	0.5(1)	0.7(1)	0.5(1)
N7	1.8(1)	2.5(1)	1.4(1)	-0.1(1)	0.6(1)	-0.3(1)

The anisotropic temperature factor, T , is of the form:-

$$T = \exp(-2\pi^2 [(ha^*)^2 U_{11} + (kb^*)^2 U_{22} + (lc^*)^2 U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23}])$$

where a^* , b^* , c^* are the reciprocal cell parameters. The overall temperature factor for the hydrogen atoms, $U_{\text{Hyd}} = 2.18$

TABLE 3.7 Final Thermal Parameters $\times 100 \text{ \AA}^2$ for GABA

N7 - C1	1.497 (4)
C1 - C2	1.520 (5)
C2 - C3	1.528 (5)
C3 - C4	1.522 (6)
C4 - O5	1.249 (4)
C4 - O6	1.268 (4)
N7 - C1 - C2	110.1 (3)
C1 - C2 - C3	112.0 (3)
C2 - C3 - C4	114.3 (3)
C3 - C4 - O5	119.5 (3)
C3 - C4 - O6	116.6 (3)
O5 - C4 - O6	124.0 (4)

TABLE 3.8 Bond-lengths ($\text{\AA}$) and bond-angles ($^{\circ}$) for GABA

T_1	58
T_2	175
T_3	-73
T_4	-9

TABLE 3.9 Conformational Angles ($^{\circ}$) for GABA

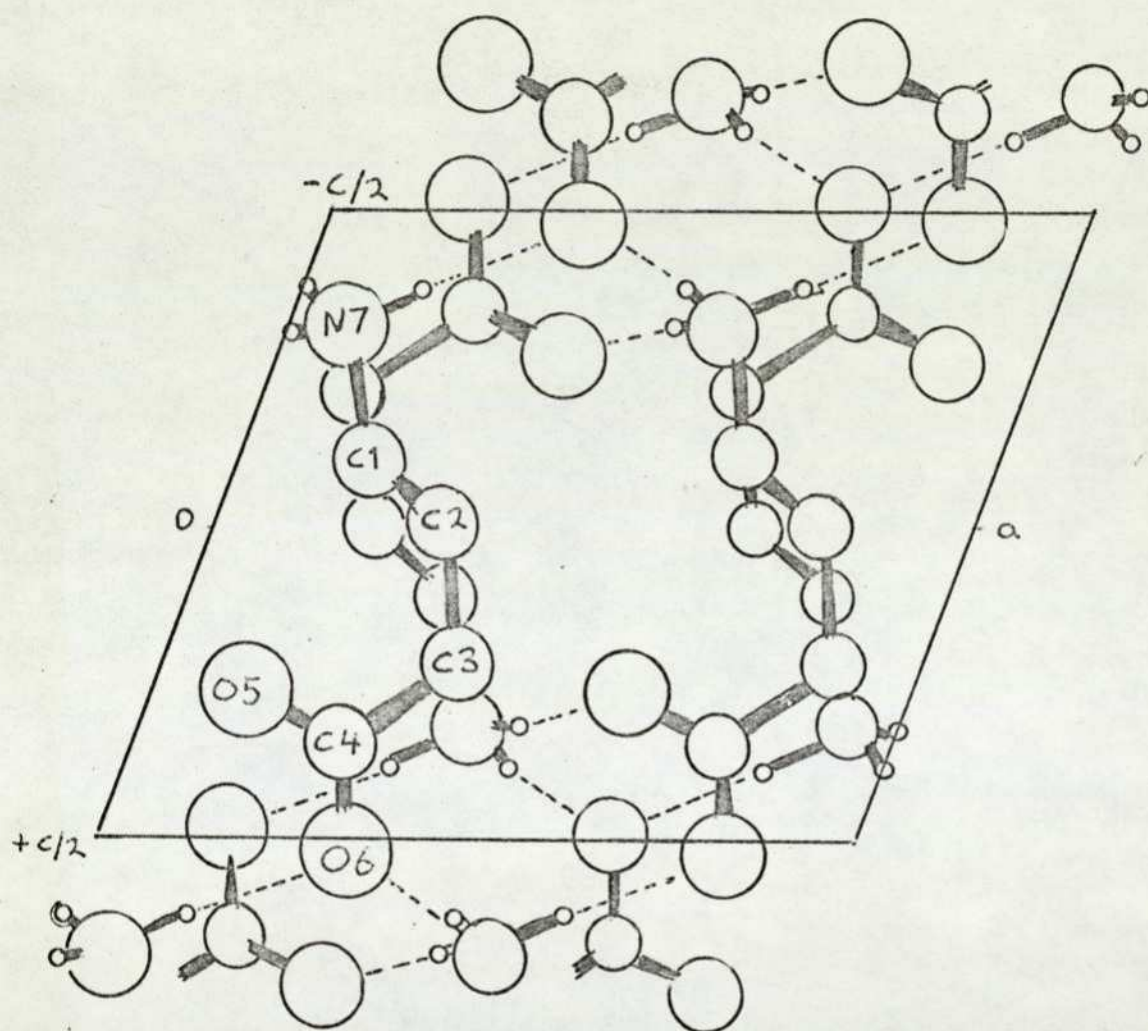


Fig. 3.8 The arrangement of the GABA molecules in the unit cell, viewed along the b-axis. For clarity, only amino hydrogen atoms are shown

greater than the standard value.

The two C-O bond-lengths are essentially equal, indicating the presence of a resonating double bond in the carboxyl group. Also three hydrogen atoms are found to be tetrahedrally bonded to the nitrogen atom, thus confirming the presence of the zwitterionic form of the molecule. These hydrogen atoms are suitably positioned to form intermolecular hydrogen bonds with oxygen atoms in neighbouring molecules. One of the hydrogen bonds links the molecules in chains in the *c*-direction. These chains are cross-linked to form a three-dimensional structure by the remaining two hydrogen bonds. It is possible that the presence of the N-H...O bonds (which have lengths of 2.767(5), 2.751(4) and 2.733(4) Å) may in part explain the extension of the N7-C1 bond.

The molecule is found to be in the *gauche* form about the C2-C3 bond (Table 3.9). This would seem to confirm that the conformation of the GABA molecule, as determined in the hydrochloride form, was influenced by the presence of the chlorine atom.

3.2.6 The determination of the crystal structure of β GP

Data reduction and the estimation of scaling factors for the eight groups of reflections which constituted the diffraction data for β GP was carried out without difficulty in the normal manner. The production of an initial structural model proved more difficult. Three reflections, -1 6 3, 9 -3 -1 and 13 3 0, were allotted positive phases and served to fix the

origin of the unit cell. Using direct methods, it was found possible to determine only 42 phases with a probability of 0.96 or better of being correct. A Fourier synthesis employing this small number of reflections was undertaken but, as was to be expected, did not reveal a discernible model for the structure.

It was noticed that the phases of a large number of reflections were dependent on that of the $-9 -3 -1$ reflection. Assigning a positive phase to this reflection did allow the determination of 163 phases, but once again the Fourier map did not suggest a reasonable structure. With the phase of the $-9 -3 -1$ reflection taken as negative, however, the situation was somewhat improved. It was once again possible to solve for 163 phases, but in this case a Fourier synthesis, using normalised structure factors as coefficients, showed some promise. This E-map did reveal the general form of the β GP molecule (i.e. the non-hydrogen atoms), but was not sufficiently detailed to permit the identification of different atoms. Hence, it was impossible to distinguish between the carboxyl and guanidino ends of the molecule and the only course open was to attempt to proceed with the refinement of both likely models.

The first trial produced an initial R of 0.43. This was considered large but, nevertheless, three cycles of least-squares refinement did give $R = 0.24$. An R-factor of this magnitude generally can be taken as suggesting that some features of the putative structure are correct, though certain changes may be necessary. In this case, some bond-lengths were inconsistent with the assumed structure: for example a C-C

bond-length was found to be $1.47\text{\AA}$, while a C-N bond of $1.56\text{\AA}$ was also present. Although at this stage of refinement no great accuracy was to be expected, it did seem worthwhile to interchange the carboxyl and guanidino ends of the molecule and note the effect. Doing so produced a model with $R = 0.41$. Following three cycles of refinement this was reduced to $R = 0.17$. Though the improvement over the R -values of the first trial model were, perhaps, not great, the second attempt did also produce bond-lengths in good agreement with those to be expected from the assumed chemical structure. The second model was therefore adopted as the correct alternative.

Proceeding with the refinement in the isotropic temperature factor mode gave $R = 0.163$ after two cycles. At this point a weighting scheme of the type given in equation 3.11 with $A = 0.45$ and $B = 6$ was introduced and the refinement continued using anisotropic temperature factors. An R factor of 0.12 was achieved after three cycles. A difference Fourier map (employing the observed reflections) was then prepared and showed, though not very plainly, the positions of eight of the hydrogen atoms. The ninth hydrogen atom, however, could not be discerned. Examination of the observed and calculated structure factors (computed using non-hydrogen atoms only) showed considerable disagreement for one particular group of reflections - the $hk3$ layer. Upon further investigation the residual factor for this layer alone was found to be 0.362 , as compared with the overall R of 0.12 ; also the calculated scale factor for this set of data was considerably different from those of the other seven. It

seemed likely that an error had occurred at the data collection stage, and a detailed examination of the original data tape from the diffractometer showed this to be the case. Accordingly this group of data (55 observed and 34 unobserved reflections) was discarded. The overall R-factor then became 0.089. A ΔF map now clearly revealed the positions of the nine hydrogen atoms. At this stage it was noted that eleven of the strongest reflections (all with $\sin \theta/\lambda < 0.2$) had F_{obs} considerably less than F_{calc} . These reflections were thought to be affected by extinction and were given zero weight. An overall temperature factor was then assigned to the hydrogen atoms and all positional, thermal and scale parameters further refined. The refinement was completed after four cycles, the final residual factors being $R = 0.061$ for the observed reflections and $R = 0.064$ for all measured reflections. The average shift/error of the parameters in the final cycle of least-squares was < 0.1 . Final F_{obs} and ΔF Fourier maps were prepared (Figs.3.9 and 3.10): these did not reveal any significant anomalies in the refined structure.

Final positional coordinates are given in Table 3.10, temperature factors in Table 3.11, bond-lengths in Table 3.12 and bond-angles in Table 3.13. Table 3.14 gives the important conformational angles for the molecule. Final values of F_{calc} and F_{obs} are tabulated in Appendix E. Figure 3.11 shows the arrangement of the four β GP molecules in the unit cell, and the yz-plane hydrogen bonding scheme is depicted in Fig.3.12.

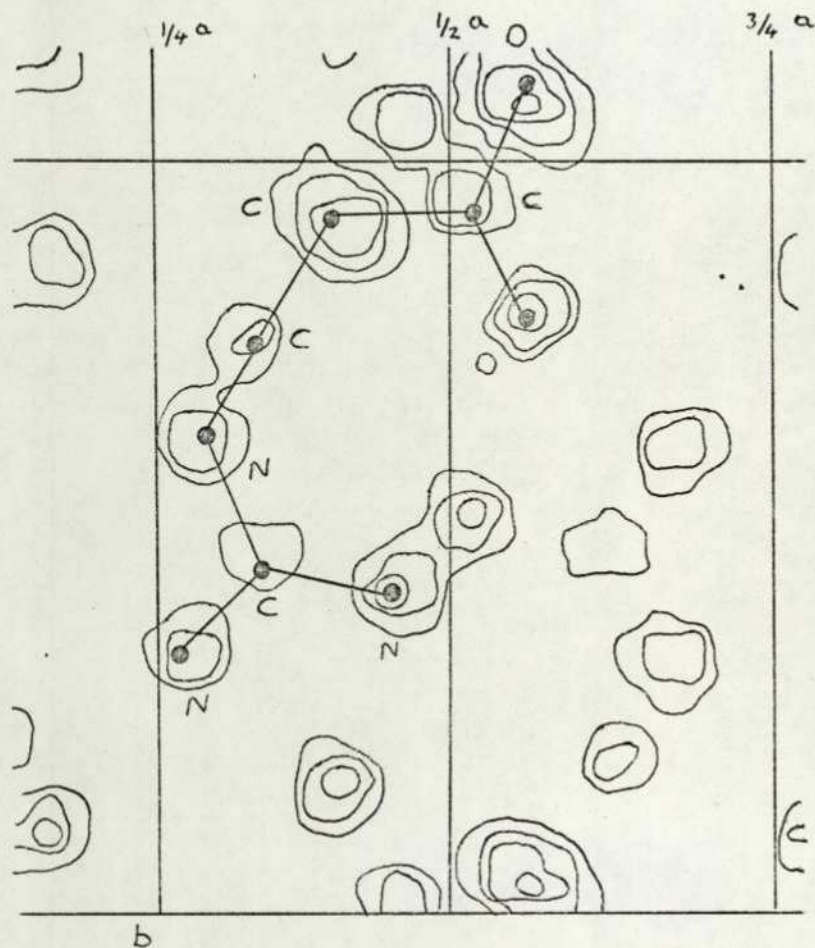


Fig. 3.9 F_{obs} Fourier Map for β GP. (c-axis projection).
Contours marked at 2.5, 4.0, 5.5 and $7.0 \text{ e}/\text{\AA}^2$

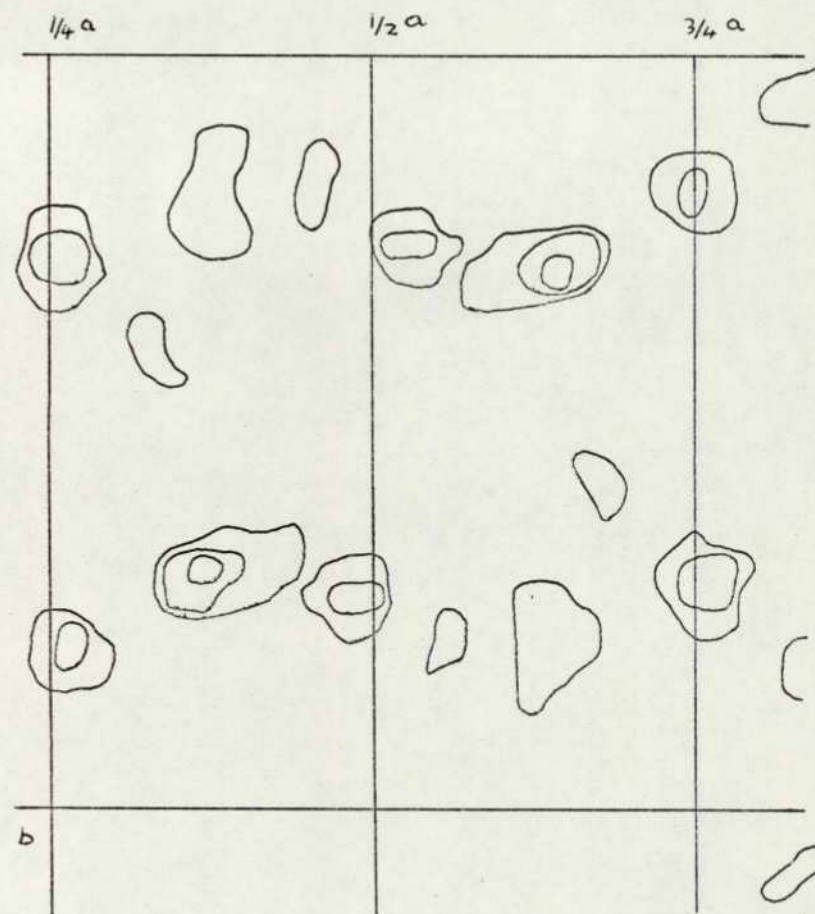


Fig. 3.10 ΔF Fourier Map for β GP (c-axis projection).
Contours marked at 0.1, 0.18 and $0.25 \text{ e}/\text{\AA}^2$

	<i>x</i>	<i>y</i>	<i>z</i>
C1	0.5226(2)	0.0511(5)	0.7714(4)
C2	0.3982(2)	0.0492(5)	0.6783(5)
C3	0.3506(2)	0.2297(5)	0.5955(4)
N4	0.3100(2)	0.3563(4)	0.7267(4)
C5	0.3626(2)	0.5083(5)	0.7935(4)
N6	0.3054(2)	0.6286(5)	0.8834(4)
N7	0.4696(2)	0.5431(5)	0.7711(5)
O8	0.5628(2)	-0.0982(4)	0.8367(4)
O9	0.5789(2)	0.1987(3)	0.7718(4)
H10	0.235(3)	0.337(6)	0.748(5)
H11	0.510(4)	0.453(7)	0.740(6)
H12	0.491(3)	0.656(7)	0.793(6)
H13	0.223(3)	0.604(6)	0.877(5)
H14	0.346(3)	0.711(6)	0.953(6)
H15	0.281(3)	0.202(6)	0.502(5)
H16	0.411(3)	0.294(6)	0.534(5)
H17	0.352(4)	0.002(6)	0.755(5)
H18	0.390(3)	-0.049(7)	0.585(6)

TABLE 3.10 Final Fractional Atomic Coordinates for BGP

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C1	2.4(4)	2.1(4)	3.6(4)	-0.1(1)	0.2(1)	-0.5(2)
C2	2.7(4)	2.6(4)	4.6(4)	-0.3(1)	-0.5(1)	-0.8(2)
C3	3.0(4)	3.3(4)	3.4(4)	-0.3(1)	-0.5(1)	-0.5(2)
N4	2.4(3)	2.5(4)	3.9(4)	-0.2(1)	0.0(1)	-0.8(2)
C5	2.7(4)	2.3(4)	2.9(4)	0.1(1)	-0.5(1)	0.2(2)
N6	3.0(4)	3.2(4)	4.8(4)	-0.2(1)	-0.2(1)	-1.0(2)
N7	2.6(4)	2.5(4)	6.0(4)	-0.3(1)	0.2(1)	-0.2(2)
O8	3.1(3)	2.6(4)	6.1(4)	0.2(1)	-0.4(1)	0.4(2)
O9	2.4(3)	2.5(4)	6.1(4)	-0.3(1)	-0.1(1)	-0.2(1)

The anisotropic temperature factor is of the form:-

$$T = \exp(-2\pi^2 [(ha^*)^2 U_{11} + (kb^*)^2 U_{22} + (lc^*)^2 U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23}])$$

where a^* , b^* , c^* are the parameters of the reciprocal cell.

The overall temperature factor for the hydrogens, $U_{Hyd} = 1.5(3)$

TABLE 3.11 Final Thermal Parameters $\times 100\text{\AA}^2$ for BGP

3.2.7. Discussion of the crystal structure of β GP

In the crystal the molecule is found to exist as a folded zwitterion, each terminal nitrogen atom being bonded to two hydrogen atoms. The five guanidyl hydrogen atoms are suitably placed for the formation of hydrogen bonds (Table 3.12). Three of these hold the molecules in sheets perpendicular to the c -direction (Fig.3.11); the fourth provides a link between pairs of adjacent sheets forming bilayers which are connected to each other purely by non-bonded interactions (Fig.3.12). The fifth is an intramolecular bond between N7 and O8 (Fig.3.11). This heterodesmic hydrogen bonding pattern may, in part, explain the range in melting point which is observed.

The bondlengths obtained (Table 3.12) are in good agreement with the usual values. The C1-O8 and C1-O9 distances are effectively equal, as are the C-N bond-lengths in the guanidyl group. This is as expected for the zwitterion. The bond-angles about C2 and C3 (Table 3.13) deviate from the tetrahedral by about 6° , but the remaining angles are not remarkable.

As is clear from Fig.3.11, β GP is found in a folded conformation in the crystal state: this conformation, which is unlike that adopted by GABA, is stabilised by the formation of an intramolecular hydrogen bond.

	O8	O9	C2		H10	
C1	1.249(4)	1.259(4)	1.529(3)	N4	0.93(4)	
	C3	H18	H17		H13	H14
C2	1.515(5)	0.98(5)	0.95(4)	N6	0.98(4)	0.88(4)
	N4	H15	H16		H11	H12
C3	1.457(4)	1.01(3)	1.01(4)	N7	0.86(5)	0.87(5)
	N4	N6	N7			
C5	1.323(4)	1.333(5)	1.322(4)			

Hydrogen-bonds

N4	—	H10	----	O9	2.826(4)	H10	----	O9	1.89(4)
		H13	----	O8	2.847(3)	H13	----	O8	1.88(4)
N6	/								
		H14	----	O9	2.967(4)	H14	----	O9	2.18(4)
		H12	----	O8	2.837(5)	H12	----	O8	1.98(5)
N7	/								
		H11	----	O9	2.810(4)	H11	----	O9	2.02(5)

TABLE 3.12 Bond-lengths (Å) and Hydrogen-bond lengths (Å) for BGP

O8	—	C1	—	O9	124.3(2)	N4	—	C5	—	N6	118.3(3)
O8	—	C1	—	C2	116.8(3)	N4	—	C5	—	N7	121.3(3)
O9	—	C1	—	C2	118.8(3)	N6	—	C5	—	N7	119.8(3)
C1	—	C2	—	C3	116.6(3)	C3	—	N4	—	C5	125.8(3)
C2	—	C3	—	N4	114.8(3)						

TABLE 3.13 Bond-angles (°) for BGP

T ₁	+181
T ₂	+ 83
T ₃	+254
T ₄	+192

TABLE 3.14 Conformational angles (°) for BGP

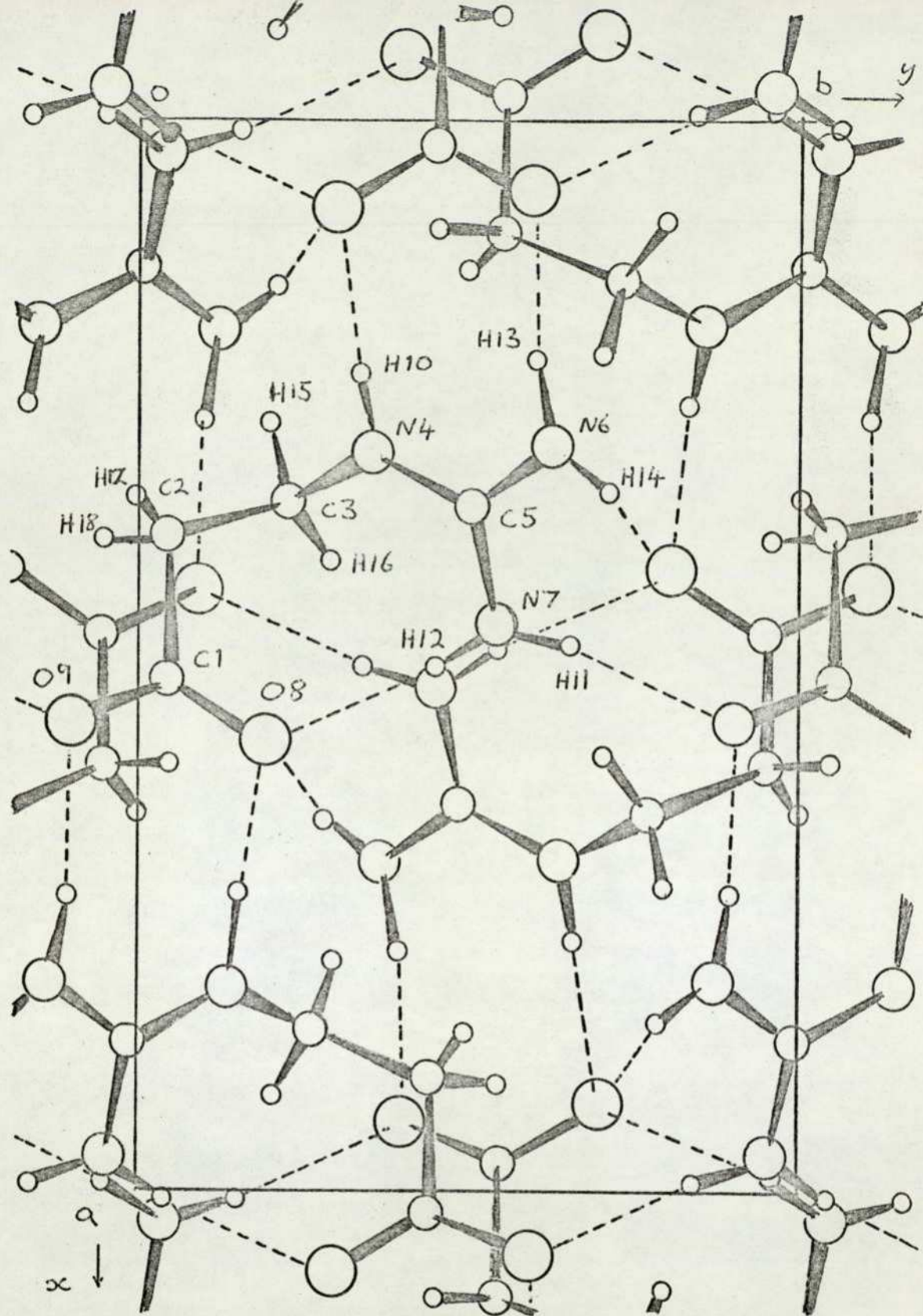


Fig. 3.11 The arrangement of the β GP molecules in the unit cell, viewed along the C -axis.

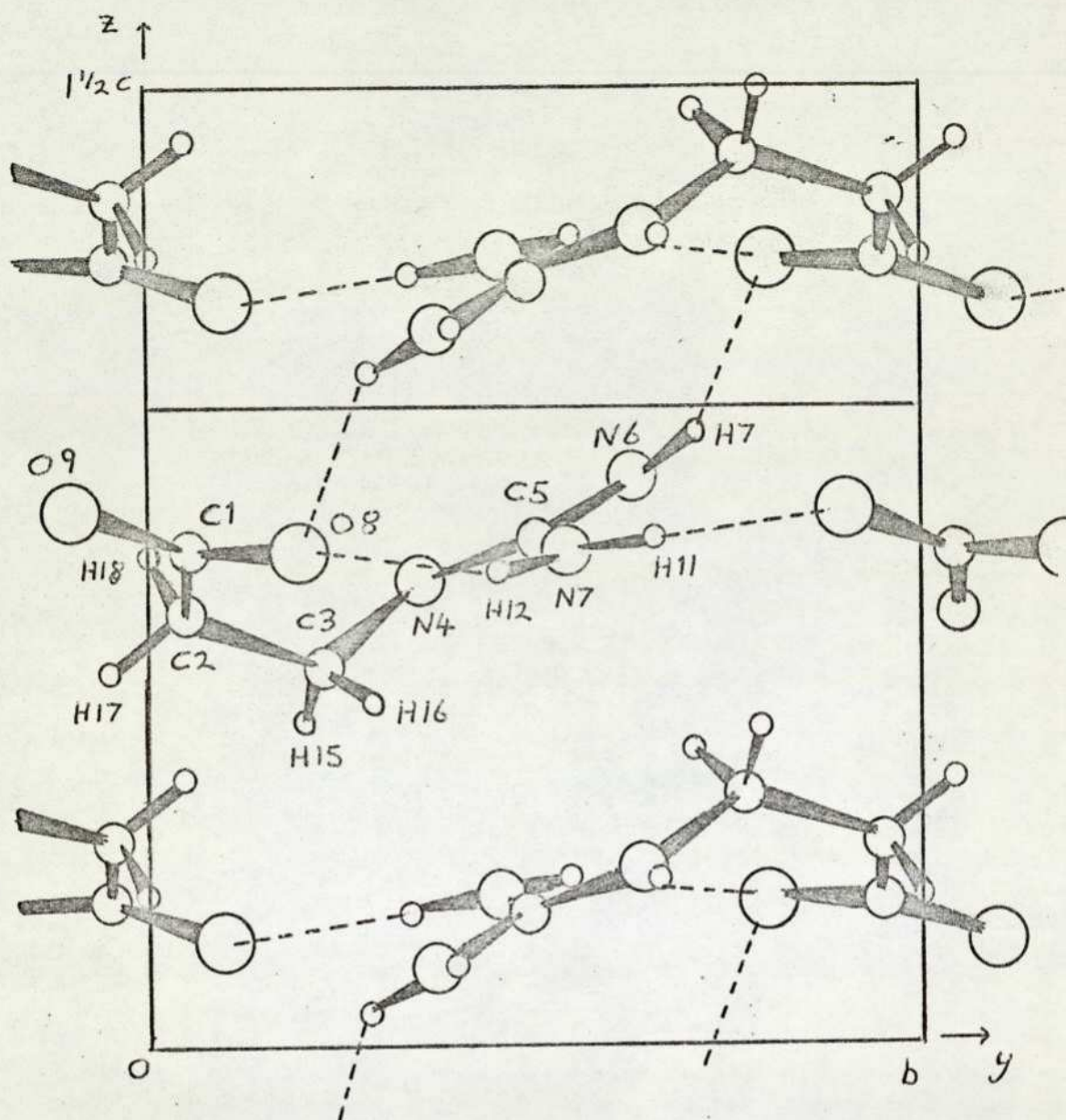


Fig. 3.12 Hydrogen bonding in β GP.
The unit cell viewed along the a -axis.

CHAPTER 4

CNDO/2 Molecular Orbital Calculations
On Some Centrally Acting Molecules.

4.1 INTRODUCTION

The preferred conformation of molecules in solution is determined by two factors: (i) their basic internal structure and (ii) the influence of the environment. In principle, the complete characterisation of both these contributions should be possible by the solution of the Schrödinger equation for the system under consideration. In practice such complete solutions are impossible for other than the simplest two-body system, and approximations must be made in an attempt to make the problem tractable.

The approach usually followed has been to use approximate quantum-mechanical methods to study factor (i) above and perhaps try to represent the effects of (ii) in simple semiempirical or quantum-mechanical terms. Currently no method for simulating the effects of solvent forces can be regarded as completely rigorous or general. The question of the effect of the environment on the molecules of interest in this study will be discussed further in Chapter 6.

A particularly successful approach to making calculations concerned with the internal structure of molecules has been that of molecular orbitals. Described in this chapter are calculations made using a particular semiempirical molecular orbital method - the CNDO/2 (Complete Neglect of Differential Overlap, second parameterisation) method of Pople*. These calculations were carried out to develop the computational techniques necessary to provide some indication of the usefulness of this method in the context of studies of the conformational and electronic structures of GABA, its agonists and antagonists.

A brief theoretical background to the CNDO/2 method is given,

* Pople, Santry and Segal (1965)

together with some comments on its usefulness, advantages and limitations as compared with other widely used procedures. A description of the implementation of a CNDO/2 computer program is included and results of calculations made on four molecules (GABA, β GP, muscimol and bicuculline) are reported.

4.2 THEORETICAL BACKGROUND TO THE CNDO/2 METHOD

The development of the semiempirical self-consistent field molecular orbital methods, stating the approximations made, is described below. A complete mathematical derivation is not given.

The Schrödinger equation for any molecule may be written:

$$H\chi_i = E_i\chi_i, \quad \dots (4.1)$$

where χ_i is the wavefunction corresponding to an energy E_i of the system and H is the Hamiltonian of the system. To simplify the Hamiltonian two assumptions may be made immediately:

- (i) The Born Oppenheimer approximation, which states that, as far as studies of electronic structure are concerned, the nuclei of the atoms may be considered as being at rest.
- (ii) Contributions due to magnetic effects or external fields are neglected.

Then, using atomic units the Hamiltonian may be expressed in terms of atomic numbers, Z 's, and distances, r 's and R 's as:

$$H = \sum_i -\frac{1}{2}\nabla_i^2 + \sum_i \sum_k^M -\frac{Z_k}{r_{ik}} + \sum_{i<j}^n \frac{1}{r_{ij}} + \sum_{k\neq l}^M \frac{Z_k Z_l}{R_{kl}} \quad \dots (4.2)$$

where i and j label the n electrons and k and l label the M nuclei.

A most fruitful approach to solving equation 4.1 for molecules has been to make use of the orbital approximation i.e. to attempt to represent the many-electron problem of (4.1) by a combination of one-electron functions or orbitals, each depending on the coordinates of

one electron. Such a model must neglect electron correlation due to mutual repulsion, though some account is taken of the modified repulsion between electrons of parallel spin.

Using this orbital model it is then possible (eg Pople and Beveridge, 1970) to express the total electronic energy, E , of a closed-shell molecule by:

$$E = 2 \sum_i^n H_{ii} + \sum_i^n \sum_j^n (2J_{ij} - K_{ij}) \quad \dots (4.3)$$

where H_{ii} represents the energy of an electron moving in the field of the nuclei, and J_{ij} represents the coulomb repulsion between two electrons, modified by the K_{ij} term which reflects the effect of electron spin. The terms H_{ii} , J_{ij} and K_{ij} are all of integral form: J_{ij} and K_{ij} are therefore known as coulomb and repulsion integrals respectively. Making use of previous work by Hartree (1928), Fock (1930) proposed a method of determining the differential equations required to specify the orbitals necessary to compute E from (4.3). The application of the Hartree-Fock method to molecules is not, however, straightforward, since it involves solving the Schrödinger equation for an electron moving in the potential field due to all the constituent nuclei i.e. for M nuclei it is an $M+1$ body problem.

A way round the difficulty was shown by Roothaan (1951). He expressed each molecular orbital (ψ_i) as a linear combination of atomic orbitals (LCAO) i.e.

$$\psi_i = \sum_v c_{vi} \phi_v,$$

where c_{vi} is a multiplying coefficient for each atomic orbital, ϕ_v .

The quality of this approximation depends on the number and form of the basis functions, the ϕ_{vi} 's. In principle, the approximation can be made as exact as desired or computationally convenient. Each of the terms in equation 4.3 may be expressed in terms of atomic orbitals and it is then necessary to choose the 'best' values of the coefficients i.e. those which give the closest approximation to the actual wavefunction. According to the variational principle this is done by varying the c's until the calculated energy is a minimum. Applying this constraint leads to the Roothaan equations of the form:-

$$\sum_v (F_{\mu v} - S_{\mu v} \epsilon_i) c_{vi} = 0 \quad \dots (4.4)$$

where ϵ_i is the orbital energy for the molecular orbital ψ_i . The elements of the Fock-matrix $F_{\mu v}$ are given by:

$$F_{\mu v} = H_{\mu v} + G_{\mu v}$$

where $H_{\mu v}$ is the matrix element of the one-electron Hamiltonian and $G_{\mu v}$ represents the electron-electron interactions in terms of atomic orbitals. $S_{\mu v}$ is the overlap matrix defined by:

$$S_{\mu v} = \int \phi_{\mu} \phi_{\nu} d\tau$$

the integral being taken over all space. The $G_{\mu v}$ elements are functions of products of the coefficients and equation 4.4 is therefore cubic in the c's. Appropriate values for the coefficients must therefore be determined by an iterative process; an initial guess is made at a set of c's and these are used to calculate the elements of F. From (4.4) another estimate of the values of the

coefficients can then be obtained; these may be used as the input to a new cycle of calculations. The process is continued until the input and output are the same, to within a satisfactory tolerance. The whole procedure is thus known as a self-consistent field (SCF) method.

The chief difficulty in using the Roothaan equations directly is the large number of electron-electron repulsion integrals which must be handled. Pople produced an approximate semiempirical SCF framework by: considering only valence electrons explicitly; regarding the remaining electrons and nuclei as forming charged cores; and systematically neglecting certain of the remaining interactions. The basic approximation was the neglect of differential overlap i.e. all two-electron integrals which depend on the overlap of charge densities of different basis orbitals are neglected. Other approximations were made to ensure that the calculations were independent of the choice of coordinate axes and the computations were further simplified by approximating many of the remaining integrals by empirical values obtained from atomic data. The resulting expressions for the elements of the Fock-matrix are:

$$F_{\mu\mu} = -\frac{1}{2}(I_{\mu} + A_{\mu}) + [(P_{AA} - Z_A) - \frac{1}{2}(P_{\mu\mu} - 1)]\gamma_{AA} + \sum_{B(\neq A)} (P_{BB} - Z_B)\gamma_{AB}$$

$$\text{and } F_{\mu\nu} = \beta_{AB} S_{\mu\nu} - \frac{1}{2} P_{\mu\nu} \gamma_{AB},$$

where β_{AB} , I_{μ} , A_{μ} are determined empirically, Z_A is the charge on the core of atom A; γ_{AB} represents the interaction between an electron in a valence atomic orbital centred on atom A and another

centred on atom B; and the density matrix (P) is defined by:

$$P_{\mu\nu} = 2 \sum_i^{\text{occ}} c_{i\mu} c_{i\nu}$$

with P_{AA} , the total charge on atom A, being given by

$$P_{AA} = \sum_{\mu} P_{\mu\mu}$$

summed for all orbitals on A.

The resulting equations are solved in an iterative manner as in the original Roothaan method. This completes the specification of the CNDO/2 method as a LCAO SCF MO method. A summary of the main points leading to this pedigree is given below:

- (i) Born-Oppenheimer approximation
- (ii) Orbital approximation
- (iii) LCAO approximation leading to the Roothaan equations
- (iv) Pople's semiempirical approximations to the Roothaan equations.

The resulting method is reasonably rapid computationally and requires as input only the atomic coordinates and atomic numbers of atoms making up the molecule, together with the net charge on the molecule. The output available includes the total energy of the molecule and net atomic populations (NAP's) as determined by the density matrix.

4.3 SOME POINTS OF COMPARISON BETWEEN THE CNDO/2 AND OTHER QUANTUM-MECHANICAL METHODS

First, some of the distinguishing features of other widely-used quantum mechanical methods may be noted:

- (i) *Ab Initio* Methods - seek to deal with all the electronic integrals in Roothaan's equations theoretically, without resorting to empirical approximations.
- (ii) Intermediate Neglect of Differential Overlap (INDO) - a semiempirical scheme developed by Pople, which is similar to CNDO, but retains certain one-centre integrals which are neglected in the simpler method.
- (iii) Neglect of Diatomic Differential Overlap (NDDO) - a further Pople semiempirical method which includes all differential overlap for orbitals centred on the same atom.
- (iv) Extended Hückel Theory (EHT) - an independent electron method developed by Hoffmann (1963). Electron repulsion is not explicitly included in the calculations.
- (v) Perturbative Configuration Interaction of Localised Orbitals (PCILO) - a non-SCF method (Diner, Malrieu and Claverie, 1969) which uses the chemical structure of the molecule as a basis for building a set of localised orbitals. The method deals with all valence electrons.

To facilitate a comparison of CNDO/2 with the other methods mentioned above, it is worthwhile surveying some of the successes and shortcomings of the simple Pople method. Since Pople's original papers of 1965-66, the procedures he outlined have found wide application; the few examples given here are meant only to illustrate this diversity.

Using net atomic populations calculated as suggested by Mulliken (1955), the CNDO/2 method has been found to yield dipole moments in fair agreement with experimental measurements for many molecules (Pople and Gordon, 1967). Although it may not be correct simply to interpret the Mulliken population analysis as giving the electronic charges located at the atomic positions, the values obtained do seem to be a useful indication of the electronic distribution in the molecule. In spite of the fact that other schemes have been suggested (e.g. Roby, 1974 and Jug, 1973), the original method is still widely used; Schroeder and Makino (1973) have given a summary of the justifications for using CNDO/2 calculated charge distributions in this way.

The CNDO/2 method has also been used to predict bond-lengths, bond-angles and conformational angles: good values have been obtained for bond-angles (Klopman and O'Leary, 1970), though calculated bond-lengths are not so satisfactory (Davidson, Jorgensen and Allen, 1970). The major failing of CNDO/2 has been found to be its inability to predict the correct preferred conformations of conjugated molecules (Gropen and Seip, 1971); apart from this the method has been used with some success in the study of rotations about single bonds (Davidson, Jorgensen and Allen, 1970). Other examples of the use

of the CNDO/2 method in conformational problems include a study of Glycine by Oegerle and Sabin (1973), and work on acetylcholine (Saran and Govil, 1972).

To provide a useful tool for investigating the possible conformational modes of several molecules, any method employed must be reasonably fast computationally: for work on the molecules of interest (which have >7 non-hydrogen atoms) this effectively rules out, at the moment, standard *ab initio* methods. The fastest *ab initio* program available is reported to be some twenty times slower than CNDO/2. Of the other methods suggested by Pople, NDDO calculations would also appear to be too lengthy, while results from INDO are expected to be similar to those from CNDO/2 in conformational studies (e.g. Pullman and Courriere, 1973). The non-SCF PCILO (Perturbative Configuration Interaction of Localised Orbitals) method developed by Pullman and co-workers has been found to be very useful for rapidly plotting conformational energy surfaces. The method is also said to be quite useful for determining charge distributions (Pullman, 1973). It is worth mentioning that partial double bonds, as between the carbon and oxygen atoms of carboxyl groups in zwitterions, cannot be represented within the PCILO framework. Instead, the double bond must be localised in one position (Pullman, 1974).

Another quantum-mechanical method which has frequently been employed in conformational studies is EHT. The method has been extensively used by Kier (1971) in studying drug molecules; he comments that good values for charge distributions are not obtained, and that results for polar molecules are less reliable (Kier, 1971). Because of its simplicity, EHT is convenient computationally but

this probably does not outweigh its other failings.

In summary, it may be said that the CNDO/2 method should be useful at least for giving some initial indication of the shape and electronic structure of molecules of a reasonable size. The fact that it is a semiempirical method, incorporating several approximations, should be borne in mind and comparisons with experimental results and other theoretical methods, such as PCILO, should be made wherever possible.

4.4 IMPLEMENTATION OF THE CNDO/2 METHOD

4.4.1 Modification of program QCPE 91

A copy of the QCPE 91 CNDO/2 program written in FORTRAN by Segal (1966) was available. In its original form this program was unsuitable for use in conformational studies and very extensive modifications have been made to it. The major improvements made to the program are listed below, and a complete description of all the changes is given in Appendix F.

Important alterations to QCPE 91 were:

- (i) The addition of molecular geometry routines.
- (ii) Facilities for dealing with non-converging SCF processes were added.
- (iii) The enlargement of the program to deal with molecules of up to 50 atoms and 150 basis functions.
- (iv) Facilities for the efficient computation of conformational energy surfaces were included.
- (v) An extrapolation routine for decreasing the number of necessary SCF iterations was developed

4.4.2 Testing the modified CNDO/2 program

It is true to say that no complex computer program can ever be said to be "fully" tested. This is because of the enormous number of possible combinations of values for input data and the large number of possible logical paths through a complicated program: any set of tests can only examine a few of these combinations and there is always the possibility that, when the program is in use, a previously untried situation will

produce an incorrect result. The program QCPE 91 has been in use for a number of years and may therefore be regarded as "well tested". The only weakness in the program appears to be that the matrix diagonalisation routine is, in some cases, found to be unsatisfactory. It was still felt advisable, however, to run a number of calculations as tests. These runs could then be regarded both as trials of QCPE 91 and of the extensive modifications made to it.

The original program was supplied with a standard test deck (for β -alanine) and a sample print-out. The modified program reproduced these results exactly. Thus it appeared that the program was working as originally designed. A more complete test was to compare a set of published results produced by an entirely independent program with a QCPE 91 calculation. It was not entirely straightforward to do this because many authors fail either to define precisely the molecular geometry employed in their work, or whether any changes have been made in the method of calculation. A paper by Schroeder, Makino and Tolles (1973) was found to include all relevant details of a set of calculations made with a program written by Tolles. The modified QCPE 91 program reproduced their results on 1H-5-amino-tetrazole precisely. This was excellent evidence that the program was working satisfactorily. It was also found possible to reproduce results quoted by Pople * on hydrogen fluoride. Results on Glycine, reported by Oegerle and Sabin (1973), could not be duplicated precisely; whether the discrepancy lay in the two programs or in the input data, was impossible to say. The discrepancy was, however, small and, in view of the satisfactory

* Pople and Beveridge, 1970

behaviour of QCPE 91 in earlier tests, was not thought significant. One further difficulty was found in repeating the calculations reported by Vega, Fang and Hayes (1969) on ethane. This was the problem concerned with matrix diagonalisation mentioned earlier. In this case, a minor change in input data gave satisfactory results.

Taken overall, the tests listed above indicated that the modified CNDO/2 program was operating as intended and could be applied to the problems of interest with some confidence.

4.5 PRACTICAL DETAILS OF THE CNDO/2 CALCULATIONS ON GABA, β GP, MUSCIMOL AND BICUCULLINE

4.5.1 General procedure for the computation of conformational energy surfaces and net atomic populations

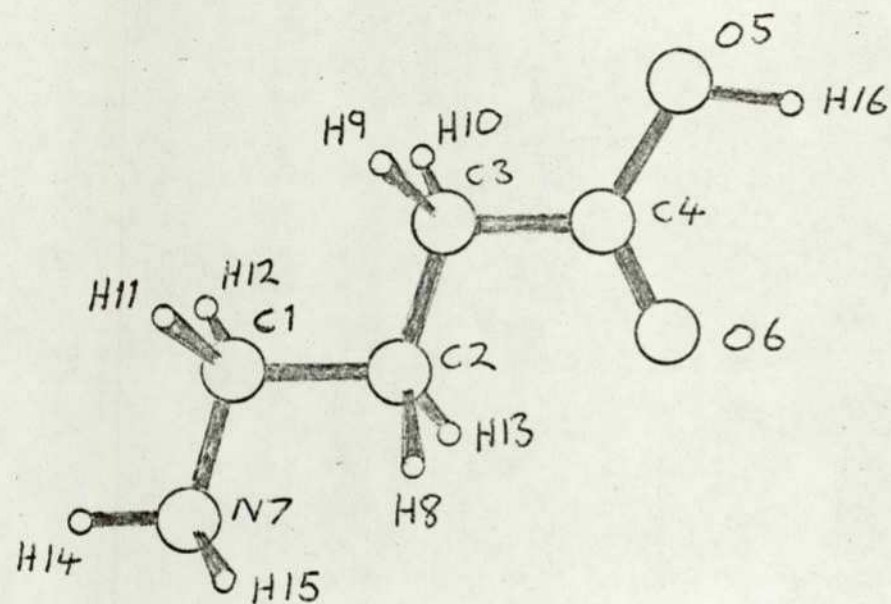
The CNDO/2 program required as input the atomic coordinates of the atoms making up the molecule under study. It was thus necessary to have some molecular model available: this data could be obtained from experimental studies (e.g. X-ray crystallographic studies) or by constructing a model using standard bond-lengths and angles. Another possibility is to carry out a complete minimisation of energy as a function of every independent geometrical parameter, rather than just varying the conformational angles of interest. This latter method could be extremely time consuming for large molecules; neither would it necessarily provide good results since, as has been noted previously, equilibrium bond-lengths determined by the CNDO/2 method are not always satisfactory. The use of standard parameter values is advantageous in that small differences in electronic populations may be studied more easily. For example, with equal C-H bond-lengths fine details in electronic structure may be noted which would, perhaps, be hidden when using experimentally determined values. However, molecules containing strained-ring structures cannot be easily modelled with standard parameters. Experimentally determined structures have the intrinsic advantage that it is known that they represent physically real structures. In addition, the bond-lengths in the most commonly used set of standard parameters (Pople and Beveridge, 1970) were determined from experimental values. In

the calculations made here, therefore, experimental structures have been used where possible; otherwise Pople standard values have been employed.

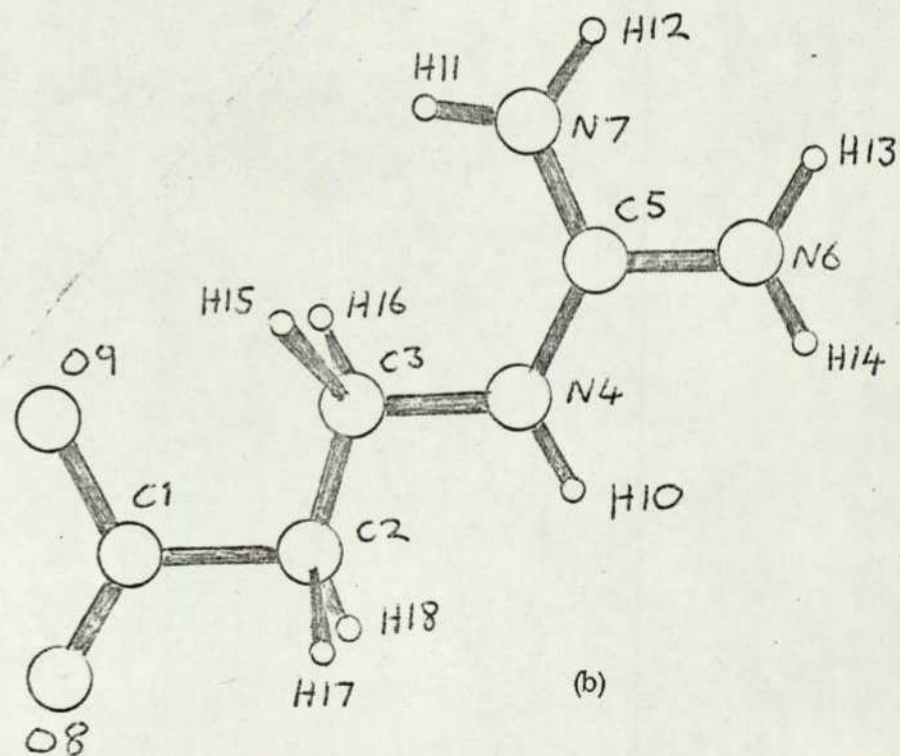
In the molecules studied, given a structural model, several dihedral angles must also be defined to completely characterise the structure. Determining the variation of the molecular energy as a function of these angles formed the most important part of the CNDO/2 calculations. For molecules such as GABA or β GP, four conformational angles (T_1 , T_2 , T_3 , T_4 as specified in Appendix A) were required to define any conformation. A search involving every possible combination of these four angles, even employing a relatively coarse grid, would have needed a prohibitive number of calculations. Instead it was noted that the shape of these chain-like molecules was largely determined by the values of the two central conformational angles, T_2 and T_3 , and the effect of varying these was studied first. Further calculations were then made to examine the effect of changing T_1 and T_4 .

To obtain a true picture of the significance of the two central conformational angles, it was necessary to plot a complete energy surface. In general, this was constructed using a 20° grid with a grid of 5° being employed in the regions of minimum energy. For these calculations T_1 and T_4 were held in the *trans* position. With the minima on the T_2 - T_3 energy surface located, the effect of varying T_1 and T_4 independently was also studied.

In principle it would have been possible to use a



(a)



(b)

Fig. 4.1 (a) GABA non-zwitterion and
(b) β GP zwitterion

geometry-optimisation scheme (McIver and Komornicki, 1971) to reduce the number of energy calculations necessary to determine the minimum energy conformations. Such a method was not employed because values of the molecular energy were required at points across the whole energy surface rather than just at the minima - it is hoped to use this data in future studies of solvent effects.

In these studies the criterion for SCF convergence was taken as .0001 a.u. in the electronic energy. Results have been quoted to four decimal places, so there is some uncertainty in the final figure (~ 0.1 kcal/mol). At this level of precision, it is common practice to quote calculated net atomic populations to .001 units of electronic charge. To discover whether this was justified some exploratory calculations on β GP were made. The results, displayed in Table 4.1, show some considerable variation in the final decimal place of the calculated NAP's as convergence is approached. NAP's are therefore only quoted to .01 units of electronic charge in this report.

4.5.2 CNDO/2 calculations on GABA and its zwitterion

The non-zwitterionic form of GABA, with conformational angles indicated is shown in an extended conformation in Fig.4.1. The conformational angles are as defined in Appendix A. In the zwitterionic form of GABA, which is thought to be present in solution at biological pH, the $-\text{NH}_2$ and $-\text{COOH}$ groups in Fig.4.1 become $-\text{NH}_3^+$ and $-\text{COO}^-$ groups respectively.

Calculations were made on both the zwitterionic and

ITERATION	TOTAL ENERGY (a.u.)	Net atomic populations		
		C1	N4	O8
6	-106.96732	5.602	7.221	8.267
7	-106.96750	5.613	7.226	8.272
8	-106.96755	5.608	7.223	8.269
9	-106.96757	5.612	7.224	8.270
10	-106.96757	5.610	7.224	8.269
11	-106.96757	5.611	7.224	8.270

TABLE 4.1 The change of NAP's as SCF convergence is approached
(PGP taken as an example)

non-zwitterionic forms of GABA. For the zwitterion the crystal structure, as reported in Chapter 3, was taken as the structural model. Other bond-lengths and angles required for the non-zwitterion were assigned standard values.

The T_2 - T_3 conformational energy surfaces for $0^\circ \leq T_2 \leq 180^\circ$ and $0^\circ \leq T_3 \leq 360^\circ$ were produced, keeping T_1 and T_4 in the *trans* positions. Because the experimental structure was used, this range of conformational angles does not strictly cover the whole unique conformational surface. However, differences in energy due to this departure from exact symmetry were found to be small.

The T_2 - T_3 energy surfaces for GABA and its zwitterion are shown in Fig.4.2 and Fig.4.3 and the minimum energy conformations are listed in Table 4.2 and Table 4.3. Calculated NAP's and dipole moments are given in Table 4.4.

4.5.3 Discussion of the CNDO/2 results on the GABA non-zwitterion

Minimum M4 (Table 4.2), which represents a fully-folded conformation, is the global minimum on the T_2 - T_3 surface, but energy differences between the various minima are relatively small. It is clear from Table 4.4 that the NAP's change but little from minimum to minimum.

It was found that the preferred position for T_1 was *trans*. Usually, barriers to rotation away from this orientation were found to be small (~ 1 kcal/mol). For M4, changes in T_1 were somewhat more hindered; here strongly repulsive inter-atomic forces tend to lock T_1 at one preferred

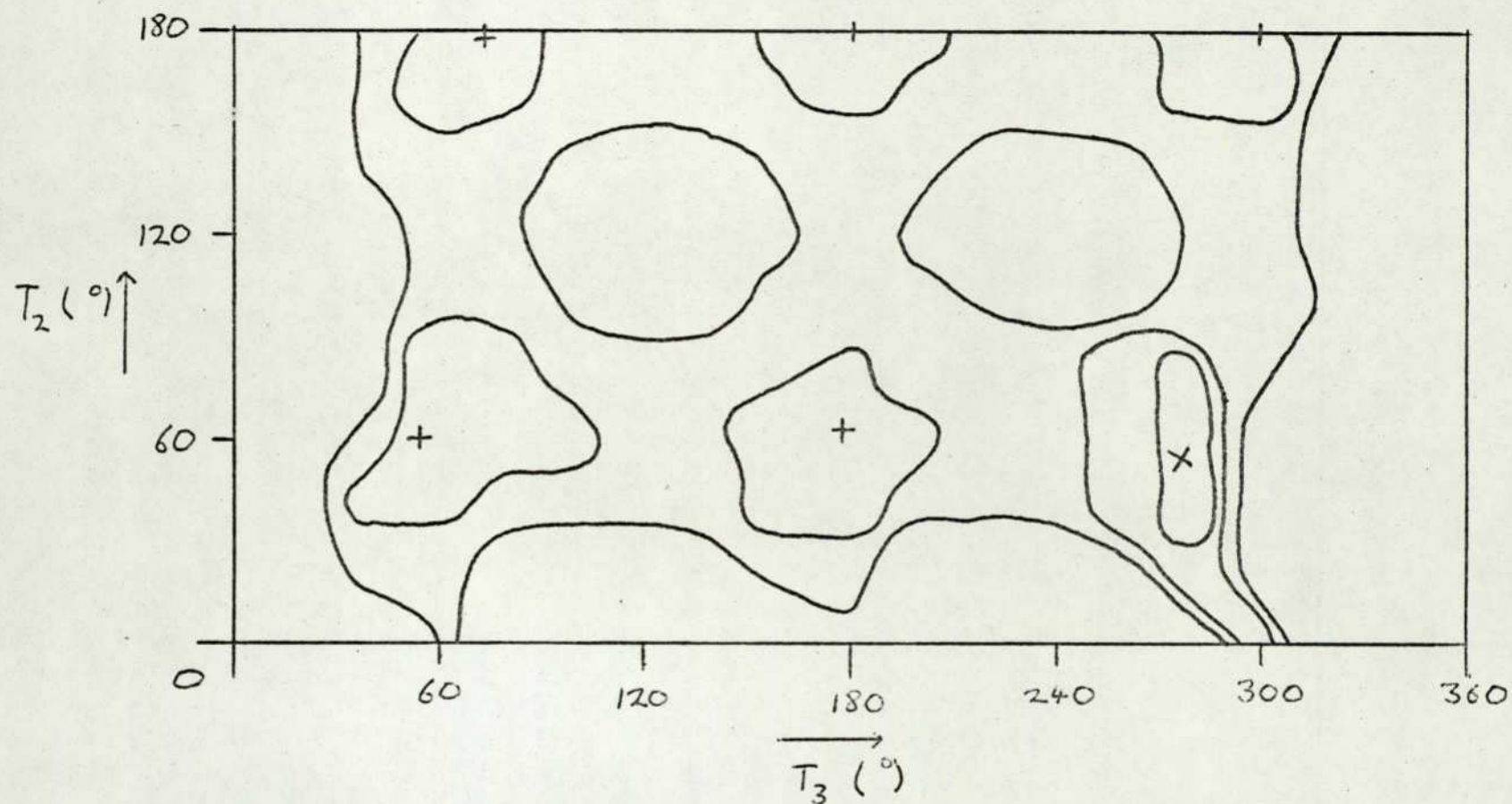


Fig. 4.2 CNDO/2 energy surface for GABA non-zwitterion.
Contours marked at 2 kcal/mol intervals.

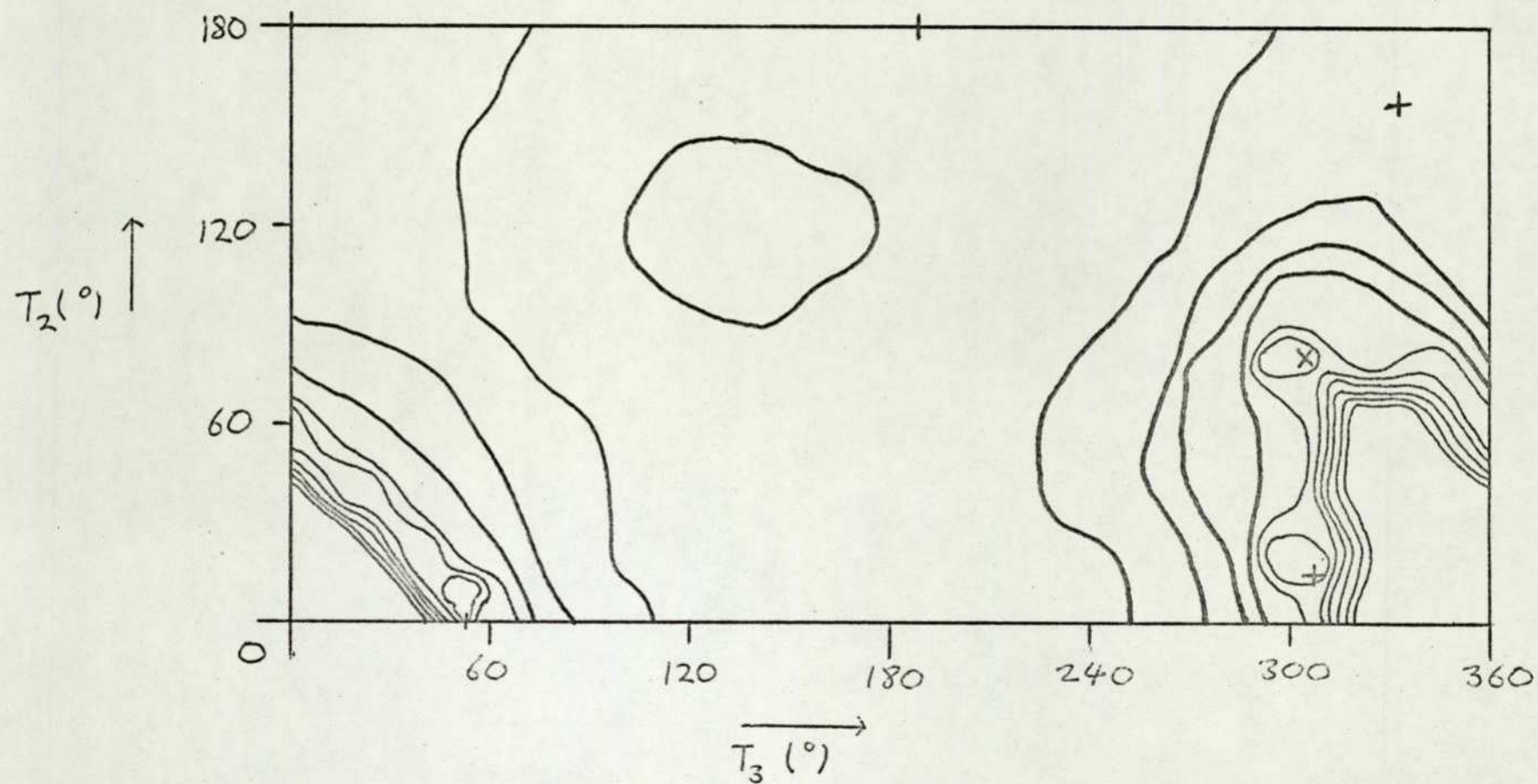


Fig. 4.3 CNDO/2 energy surface for the GABA zwitterion.
Contours marked at 10 kcal/mol intervals.

MINIMUM	T ₂	T ₃	Rel.energy (kcal/mol)	Dipole Moment (Debyes)			
	(°)	(°)		X	Y	Z	Resultant
M1	180	295	2.9	0.56	0.12	0.83	1.07
M2	180	180	3.1	-0.65	0.57	0.03	0.86
M3	175	65	2.8	0.54	0.12	-0.75	1.04
M4	65	280	0	0.53	0.54	0.59	0.96
M5	60	60	2.4	0.62	0.33	-0.87	1.12
M6	60	180	2.8	-0.65	0.57	0.03	0.87

TABLE 4.2 CNDO/2 Minimum energy conformations for GABA (non-zwitterion)

MINIMUM	T ₂	T ₃	Rel.energy (kcal/mol)	Dipole Moment (Debyes)			
	(°)	(°)		X	Y	Z	Resultant
MZ1	180	180	66.2	-21.09	-11.40	-0.03	23.98
MZ2	180	0	51.7	-3.23	-18.26	0.02	18.50
MZ3	155	330	51.0	-4.37	-17.08	2.30	17.76
MZ4	80	305	0	-4.67	-6.82	1.45	8.39
MZ5	10	305	4.9	-4.97	-4.53	4.17	7.91
MZ6	0	50	3.9	-4.53	-4.58	-4.33	7.76

TABLE 4.3 CNDO/2 calculated minimum energy conformations for
GABA (zwitterion)

MINI-MUM	C1	C2	C3	C4	O5	O6	N7	H8	H9	H10	H11	H12	H13	H14	H15	H16*
M1	5.89	5.99	6.05	5.63	8.28	8.30	7.28	0.99	0.98	0.97	1.02	1.01	0.99	0.90	0.90	0.84
M2	5.89	5.99	6.05	5.63	8.28	8.30	7.28	0.98	0.97	0.97	1.02	1.02	0.99	0.90	0.90	0.84
M3	5.89	5.99	6.05	5.63	8.23	8.30	7.28	0.98	0.97	0.97	1.00	1.02	1.00	0.90	0.90	0.84
M4	5.88	5.99	6.04	5.63	8.28	8.30	7.31	0.99	0.98	0.97	1.02	1.02	0.99	0.85	0.91	0.84
M5	5.89	5.99	6.05	5.63	8.28	8.30	7.28	0.99	0.97	0.93	1.02	1.00	1.00	0.90	0.90	0.84
M6	5.89	5.99	6.05	5.63	8.28	8.30	7.28	0.98	0.97	0.97	1.02	1.02	0.99	0.90	0.90	0.84
MZ1	5.93	5.99	6.09	5.62	8.53	8.57	7.02	0.98	1.00	1.00	0.95	0.96	0.98	0.79	0.79	0.81
MZ2	5.93	5.99	6.09	5.62	8.58	8.52	7.03	1.01	0.99	0.98	0.91	0.91	1.01	0.80	0.80	0.81
MZ3	6.00	5.98	6.10	5.62	8.58	8.51	7.01	1.01	0.98	0.98	0.97	0.83	1.01	0.81	0.79	0.82
MZ4	5.90	6.00	6.10	5.60	8.53	8.46	7.16	0.99	0.98	0.98	1.00	0.98	0.99	0.83	0.67	0.86
MZ5	5.89	6.00	6.10	5.63	8.53	8.46	7.15	0.99	0.97	1.00	0.97	0.99	0.98	0.68	0.82	0.85
MZ6	5.89	6.00	6.10	5.63	8.52	8.46	7.15	0.97	1.00	0.97	0.99	0.97	1.00	0.83	0.67	0.85

* In the zwitterion H16 bonded to N7

TABLE 4.4 net atomic populations for GABA(M1-6) and its zwitterion (MZ1-6)

orientation. The preferred positions for T_4 were found to be $\sim 120^\circ$ and $\sim 240^\circ$ for M1, M2, M3, M5 and M6; and *trans* for M4. Predicted barriers to rotation were small (~ 2 kcal/mol).

For some folded conformations, which are predicted on the conformational energy map to have low calculated energies, some non-bonded atoms approach to within the sum of their Van der Waals radii. This is discussed further in section 4.5.5.

4.5.4 Discussion of CNDO/2 results on the GABA zwitterion

The results for the zwitterion are given in Table 4.3 and Table 4.4. A stabilisation of ~ 60 kcal/mol is predicted for some folded conformers. The local minima which occur for the more extended conformations are too shallow to appear on Fig.4.3, but are included in Table 4.3. It is immediately obvious from Fig.4.3 that energy differences between conformations are considerably greater for the zwitterion than for the non-zwitterion. The global minimum, MZ4, is again a fully-folded rotamer. The NAP's for the zwitterion (Table 4.4) show the expected negative nature of the carboxyl oxygen atoms. The net positive character of the amino group is apparently shared among the three hydrogen atoms, though the nitrogen atom is considerably less negative than in the non-zwitterion.

The more pronounced preference for the folded rotamers of the polar zwitterion as compared with the non-zwitterion is to be expected: this aspect is discussed more fully in Chapter 6.

For the conservative zwitterion it was found that the preferred values of T_1 and T_4 corresponded to a *trans*

conformation. Again, the limiting factor for changes in these conformational angles for the folded conformations was the close approach of non-bonded atoms; otherwise barriers to rotation for T_1 and T_4 were relatively small (<2 kcal/mol).

Rotations about T_2 and T_3 in the zwitterion produced some conformations where short atom-atom contacts are involved.

4.5.5 The predicted close approach of non-bonded atoms

It has been noted that low-energy conformations exist for folded conformations of both the zwitterion and non-zwitterion where non-bonded atoms are required to approach to within the sum of their Van der Waals radii. Similar short contacts have previously been noted by, for example, Beveridge and Radna (1971) when using the INDO method; Froimowitz and Gans (1972) have also discussed the subject. Since CNDO/2 and INDO are closely related semiempirical SCF methods, it might be suggested that these results are due to a deficiency common to simpler MO methods. In the calculations reported here, however, those situations in which short contacts occur are just those in which the formation of an intra-molecular hydrogen bond would be possible. The calculated low energies for these rotamers may well be an expression of this possibility and it is not possible, therefore, to comment on whether there may also be an intrinsic failing in the method of calculation.

4.5.6 CNDO/2 calculations on β GP and its zwitterion

The calculations were carried out in a manner exactly analogous to that described for GABA. The β GP molecule is shown

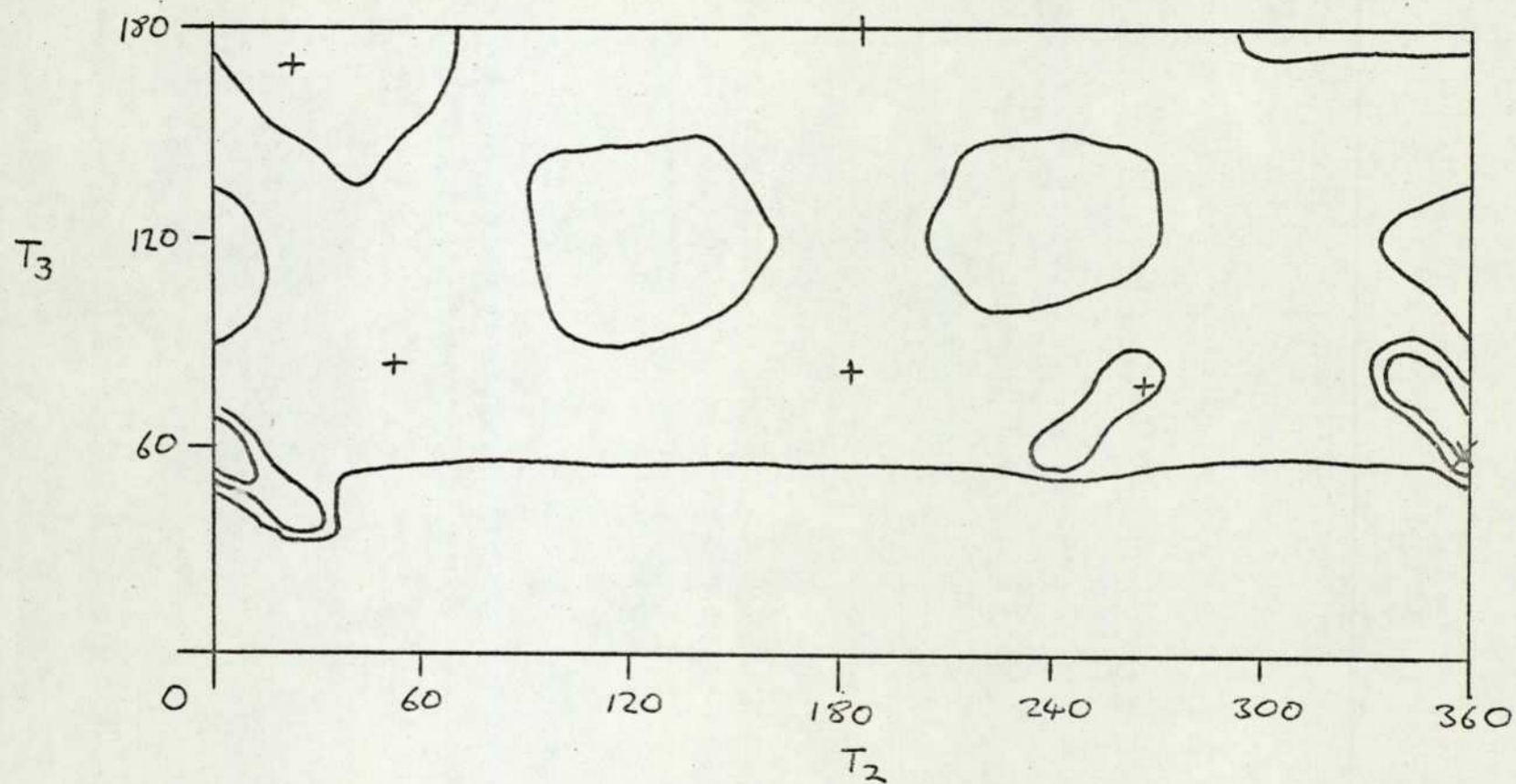


Fig. 4.4 CNDO/2 energy surface for β GP non-zwitterion.
Contours marked at 3 kcal/mol intervals.

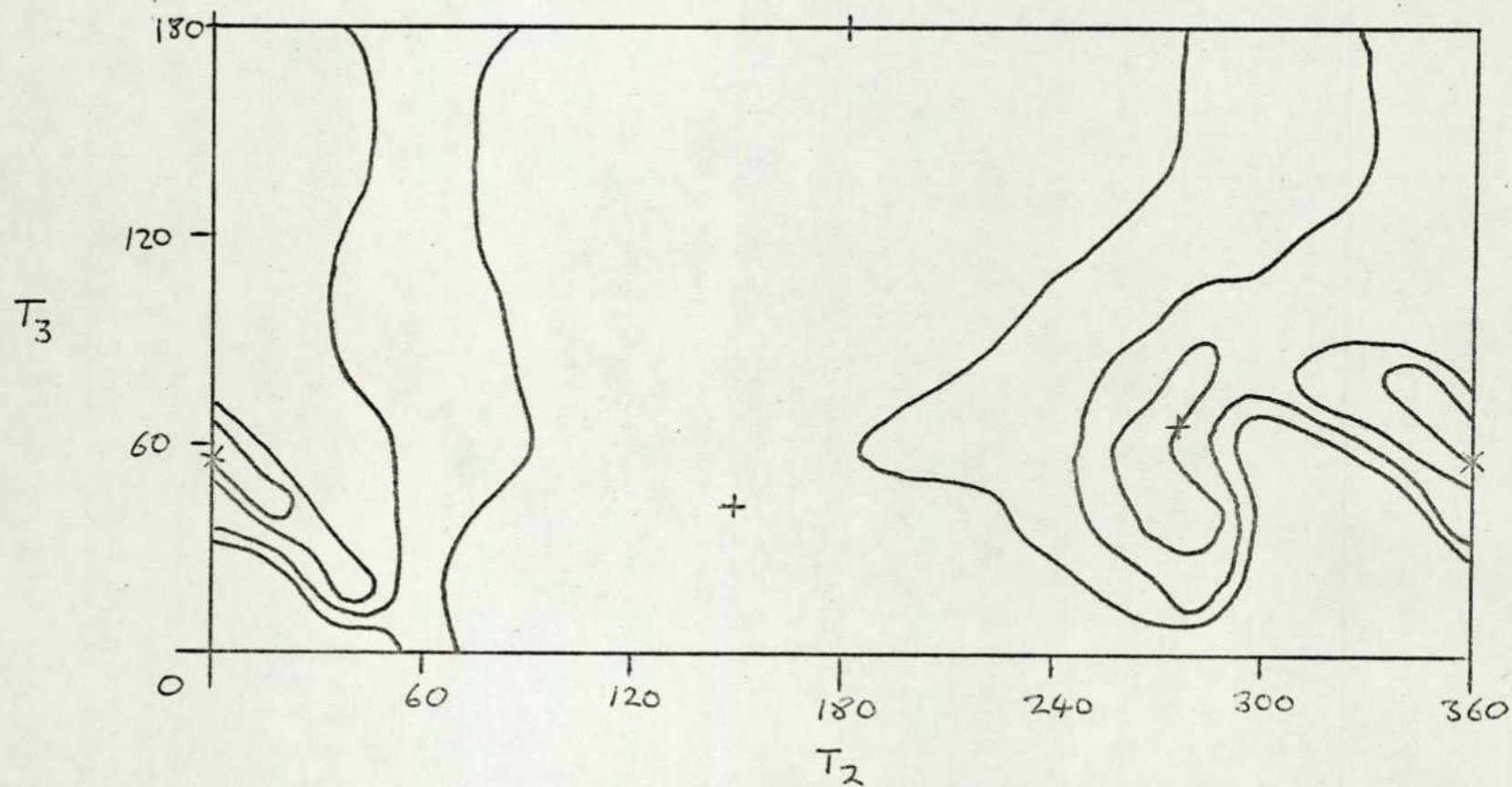


Fig. 4.5 CNDO/2 energy surface for the β GP zwitterion.
Contours marked at 10 kcal/mol intervals.

MINIMUM	T ₂ (°)	T ₃ (°)	Rel.energy (kcal/mol)	Dipole moment (Debyes)			
				X	Y	Z	Resultant
B1	180	180	8.2	-2.98	.50	.0	3.02
B2	25	165	6.6	3.84	-3.22	1.30	5.18
B3	180	75	8.9	-3.00	-1.44	1.45	3.63
B4	255	70	5.7	-2.29	-2.23	-2.96	3.94
B5	60	80	8.2	2.55	-4.01	2.11	5.20
B6	355	55	0	2.26	-4.75	-1.46	5.46

TABLE 4.5 CNDO/2 calculated minimum energy conformations for β GP (non-zwitterion)

MINIMUM	T ₂ (°)	T ₃ (°)	Rel.energy (kcal/mol)	Dipole moment (Debyes)			
				X	Y	Z	Resultant
BZ1	180	180	49.4	22.54	9.53	.00	24.47
BZ2	0	180	24.2	5.20	16.56	0.	17.36
BZ3	0	50	0.	4.36	-4.18	9.56	11.31
BZ4	275	65	12.	11.03	-8.21	4.34	14.39
BZ5	145	40	46.9	20.70	2.04	8.39	22.43

TABLE 4.7 CNDO/2 calculated minimum energy conformations for β GP (zwitterion)

MINIMUM	T_1 (°)	E_1 kcal/mol	T_4 (°)	E_2 kcal/mol
B1	120,140	1	0,180	9
B2	140,320	2	0,180	10
B3	120,240	1	0,180	10
B4	0,160	15	0,180	14
B5	120,240	1	0,180	10
B6	0,200	43	160,340	large

TABLE 4.6 Preferred orientations for T_1 and T_4 in pGP (non-zwitterion). E_1 and E_2 give an indication of the maximum barrier to rotation about T_1 and T_4 respectively.

MINIMUM	T_1 (°)	E_1 kcal/mol	T_4 (°)	E_2 kcal/mol
BZ1	0,180	1	0,180	25
BZ2	0,180	16	0,180	21
BZ3	0,180	27	20,200	large
BZ4	0,180	18	0,180	49
BZ5	200,200	1	0,180	38

TABLE 4.8 Preferred orientations for T_1 and T_4 for pGP (zwitterion). E_1 and E_2 as in Table 4.6

MINI- MUM	C1	C2	C3	N4	C5	N6	N7	O8	O9	H10	H11	H12	H13	H14*	H15	H16	H17	H18
B3	5.61	6.08	5.88	7.28	5.65	7.37	7.27	8.27	8.33	0.87	0.89	0.87	0.95	0.83	1.00	1.00	0.96	0.96
B6	5.61	6.08	5.88	7.23	5.59	7.39	7.28	8.27	8.30	0.87	0.90	0.88	0.95	0.83	1.00	1.01	0.87	0.96
BZ1	5.61	6.12	5.88	7.17	5.55	7.25	7.24	8.54	8.55	0.85	0.80	0.83	0.83	0.82	1.00	1.00	0.99	0.99
BZ4	5.61	6.13	5.88	7.19	5.55	7.26	7.33	8.48	8.55	0.87	0.68	0.86	0.83	0.84	1.00	1.01	0.98	0.96

* In the zwitterion H14 bonded to N6

TABLE 4.9 typical net atomic populations for β GP non-zwitterion, B3 and B6, and zwitterion, BZ1 and BZ4

in Fig.4.1 .

The results for the non-zwitterion are detailed in Tables 4.5, 4.6 and 4.9 and Fig.4.4. Those for the zwitterion are given in Tables 4.7, 4.8 and 4.9 and Fig.4.5.

The global minimum for the non-zwitterion, B6, is found to be a fully-folded conformation. Generally, for the non-zwitterion, the *trans* position was preferred for T_4 ; T_1 was more often found to favour *gauche* positions. The studies on T_1 and T_4 are summarised in Table 4.6.

NAP's did not change greatly with conformation (Table 4.9).

The studies on the zwitterion indicated a strong preference for the folded conformer BZ3 (Table 4.7). The preferred positions for T_1 and T_4 are summarised in Table 4.8. On zwitterion formation the oxygen atoms become more strongly negative in character; the guanidino carbon atom becomes significantly more positive (it is interesting to note that the change in charge on the carbon atom of the C-NH₃ group in GABA is not as great). The hydrogen atoms bonded to the terminal nitrogen atoms in β GP also become slightly more positive. Once again the electronic structure is relatively independent of conformation.

4.5.7 CNDO/2 calculations on muscimol and its zwitterion

The crystal structure of muscimol determined by Brehm^{*} (Fig.4.6) was taken as the molecular model. The bond-lengths and angles for this structure are given in Table 4.10.

* Brehm, Hjeds and Krogsgaard-Larsen (1972)

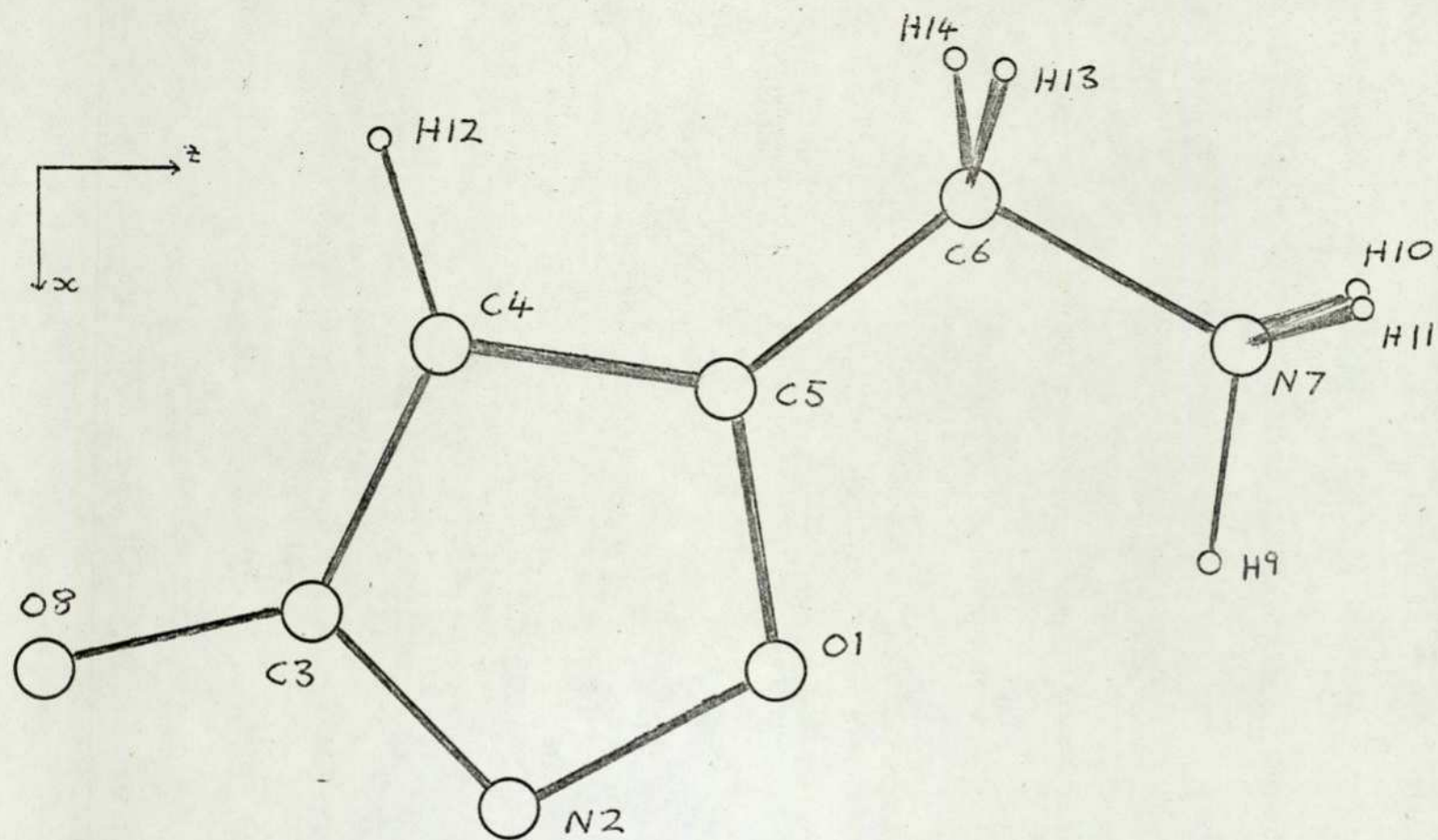


Fig. 4.6 The muscimol zwitterion.

O1 - N2	1.427	C4 - H12	1.04
O1 - C5	1.358	C6 - H13	1.06
N2 - C3	1.330	C6 - H14	1.05
C3 - C4	1.440	N7 - H9	0.96 *
C3 - O8	1.284	N7 - H10	0.98 *
C4 - C5	1.338	N7 - H11	0.96 *
C5 - C6	1.473		
C6 - N7	1.495		

N2 - O1 - C5	108.2	C3 - C4 - H12	135
O1 - C5 - C4	110.0	C5 - C6 - H13	119
O1 - C5 - C6	117.7	C5 - C6 - H14	109
C5 - C4 - C3	106.2	C6 - N7 - H9	110 *
C4 - C3 - N2	109.5	C6 - N7 - H10	107 *
C4 - C3 - O8	127.9	C6 - N7 - H11	108 *
C3 - N2 - O1	106.2		

TABLE 4.10 Bond-lengths ($\text{\AA}$) and Bond-angles ($^{\circ}$) from the crystal structure of muscimol. Parameters marked * were replaced by standard values in the CNDO/2 calculations.

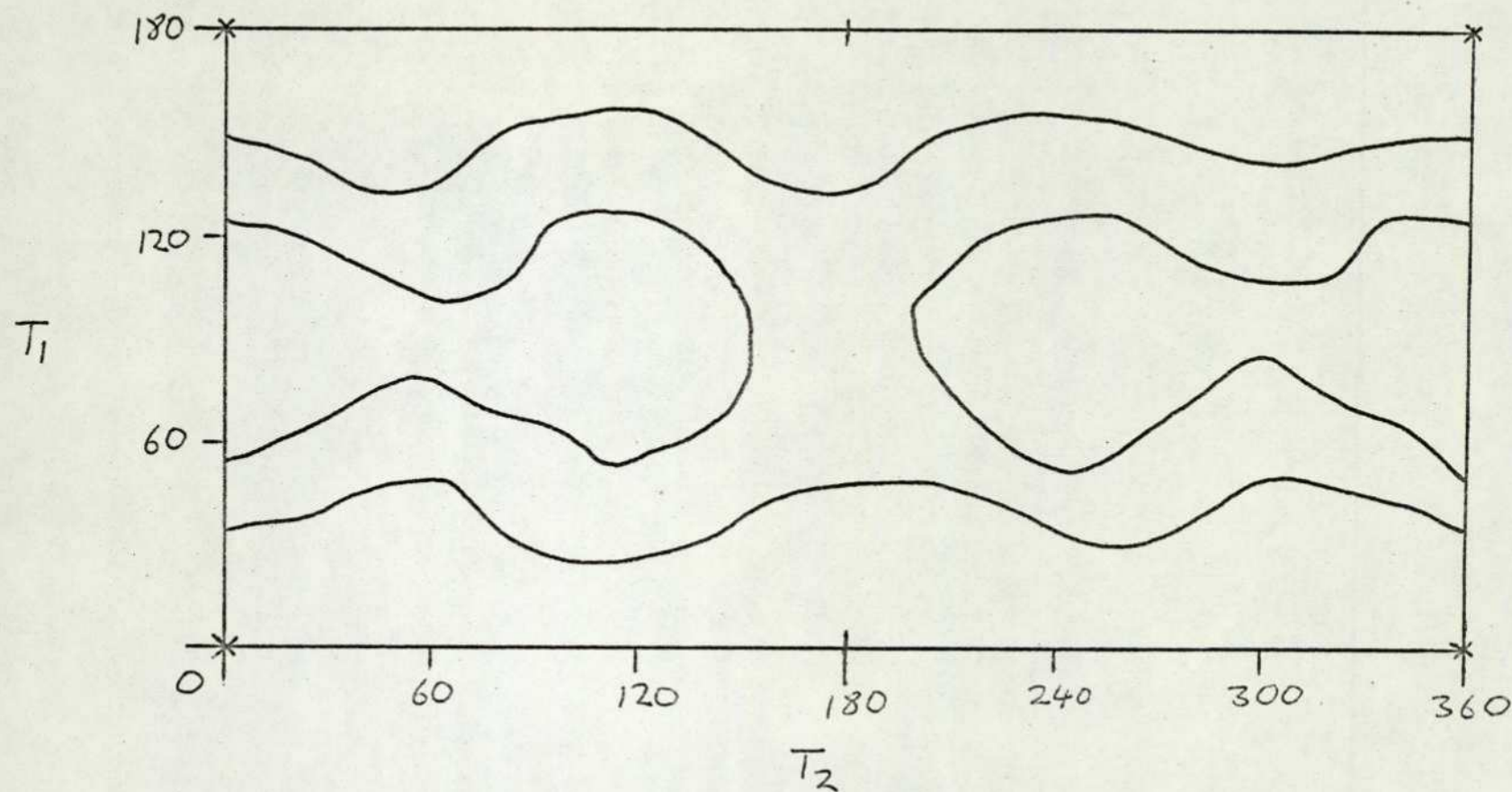


Fig. 4.7 CNDO/2 energy surface for muscimol non-zwitterion.
Contours marked at 1 kcal/mol intervals.

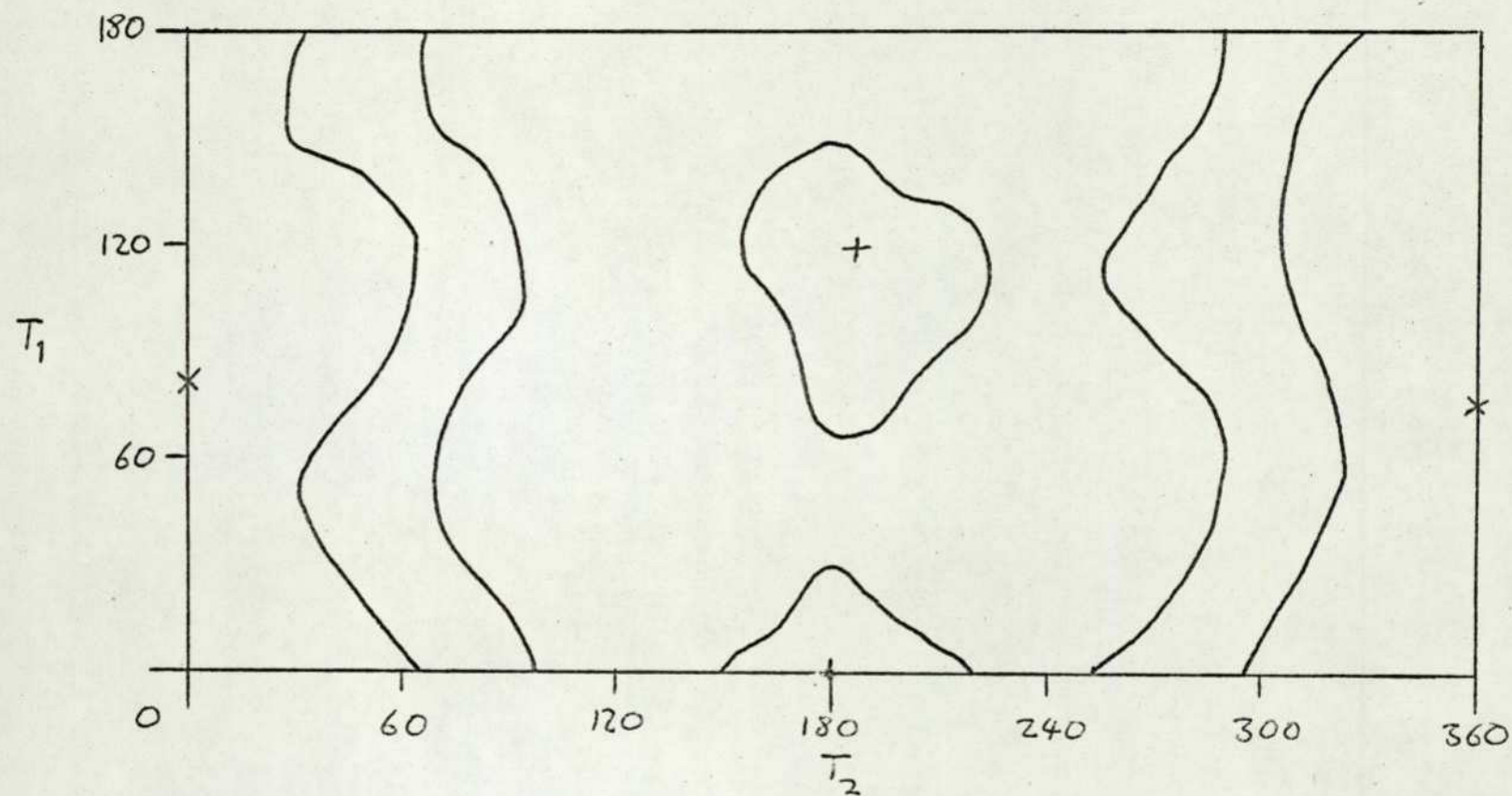


Fig. 4.8 CNDO/2 energy surface for the muscimol zwitterion.
Contours marked at 2 kcal/mol intervals.

MINIMUM	T_1 ($^\circ$)	T_2 ($^\circ$)	Rel. Energy kcal/mol	Dipole Moment (Debyes)			
				x	y	z	Resultant
MU1	0	0	0	-.42	2.02	.14	2.07
MU2	0	180	<0.2	.84	2.37	.14	2.52
MUZ1	0	180	3.5	-18.39	0.67	12.23	22.16
MUZ2	80	0	0	-9.89	-0.02	18.56	21.03

TABLE 4.11 CNDO/2 calculated minimum energy conformations for muscimol.

Non-zwitterion (MU1 and MU2) and zwitterion (MUZ1 and MUZ2)

MINIMUM	O1	N2	C3	C4	C5	C6	N7	O8	H9	H10	H11	H12	H13	H14
MU1	8.16	7.19	5.70	6.16	5.80	5.91	7.27	8.24	0.89	0.90	0.84	0.97	1.00	1.00
MU2	8.16	7.18	5.70	6.15	5.80	5.91	7.27	8.24	0.89	0.90	0.84	0.97	1.00	1.00
MUZ1	8.24	7.29	5.70	6.10	5.90	5.92	7.01	8.52	0.78	0.78	0.81	1.01	0.98	0.97
MUZ2	8.25	7.29	5.69	6.10	5.89	5.92	7.02	8.51	0.80	0.76	0.80	1.01	0.98	0.97

TABLE 4.12 Net atomic populations for muscimol. Non-zwitterion

(MU1 and MU2) and zwitterion (MUZ1 and MUZ2).

As there are only two dihedral angles of interest, the necessary calculations were less extensive than in the case of β GP or GABA.

It was found that the molecular energy of muscimol varied only slightly between conformations and because of this the small differences in the experimentally determined N-H bond-lengths tended to obscure the true nature of the conformational energy surface. To elucidate the true nature of the conformational energy changes the N-H bond-lengths were therefore assigned standard values. The resulting energy surfaces are shown in Fig.4.7 (non-zwitterion) and Fig.4.8 (zwitterion). In each case only two unique minimum energy conformations were predicted (Table 4.11), each with the molecule approximately planar. Because of the relatively small energy differences involved a fine grid was not used to locate the minima more precisely. The NAP's for muscimol and its zwitterion in the minimum energy conformations are listed in Table 4.12.

4.5.8 CNDO/2 calculations on bicuculline

Because of the size of the bicuculline molecule (Fig.4.9) it was not possible to carry out calculations as comprehensive as those previously described for GABA, β GP and muscimol. However, it was still thought worthwhile to make a limited study of bicuculline using the CNDO/2 method and so produce an MO description of the molecule within the same framework as that used to describe GABA and its agonists.

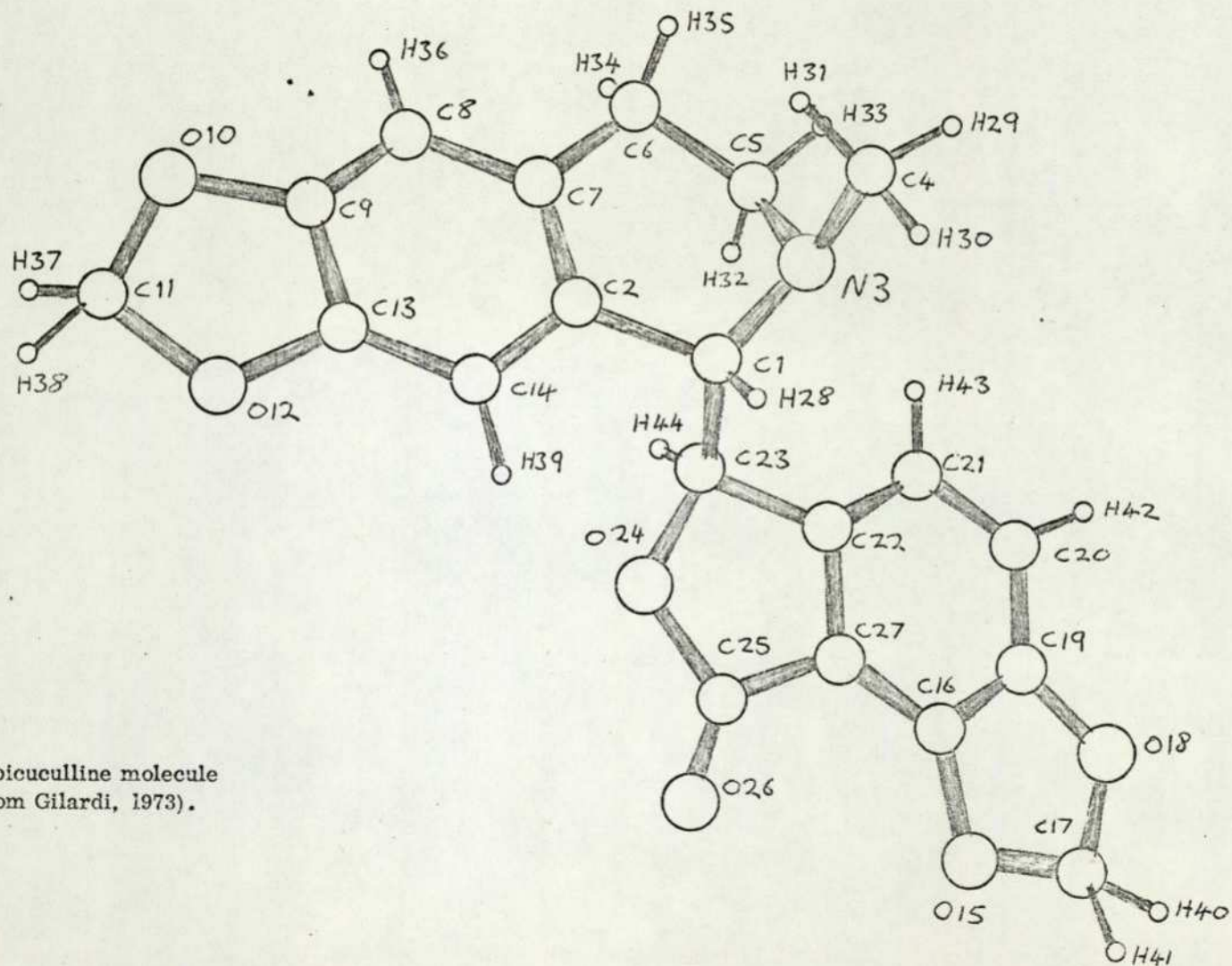


Fig. 4.9 The bicuculline molecule
(from Gilardi, 1973).

The crystal structure as determined by Gorinsky and Moss (1974) was taken as the basic structural model. A single complete CNDO/2 calculation on bicuculline was found to require ~ 1200 seconds of CDC 7600 central processor time. It was impractical to use this amount of CPU time in one computer run: instead, the calculation was segmented, with intermediate results being saved as a scratch file on disc.

The final LCAO coefficients from the initial calculation were used as the starting point for subsequent computations: the time required per run was then reduced to ~ 700 seconds.

Two conformations of bicuculline were considered: (i) that found in the crystal and (ii) with T_1 (Fig.4.9) = 270° . Gilardi (1973), using classical energy calculations, has predicted this latter to be a minimum-energy conformer. The NAP's were not found to change significantly between the two conformations: those for conformer (ii) are listed in Table 4.13. The second conformer was found to be of lower energy, but only by 1 kcal/mol, which agrees with Gilardi's general results.

Gilardi also pointed out that, since bicuculline is usually administered in strongly acidic solution, the protonated cationic form may be the physiologically active agent. He predicted a minimum energy conformation for this molecular species, once again around $T_1 = 270^\circ$. A further CNDO/2 calculation was therefore made on this bicuculline derivative. The proton was assumed to add on to the molecule in the region of the nitrogen atom, with a tetrahedral bond-

C1	5.90	C16	5.83	H31	1.01
C2	6.00	C17	5.71	H32	1.02
N3	7.17	O18	8.23	H33	1.01
C4	5.92	C19	5.89	H34	1.00
C5	5.90	C20	6.00	H35	1.00
C6	6.01	C21	6.02	H36	0.99
C7	5.98	C22	5.98	H37	1.02
O8	6.04	C23	5.87	H38	1.02
C9	5.86	O24	8.26	H39	1.00
O10	8.23	C25	5.60	H40	1.02
C11	5.72	O26	8.30	H41	1.02
O12	8.23	C27	6.10	H42	0.99
C13	5.87	H28	1.02	H43	1.00
C14	6.05	H29	1.01	H44	1.00
O15	8.22	H30	1.01		

Dipole moment (Debyes)

X	Y	Z	Resultant
-3.84	0.80	1.97	4.39

Total Energy = -277.9699 a.u.

TABLE 4.13 CNDO/2 calculated net atomic populations, dipole moment and total energy for bicuculline.

angle. The local molecular geometry around the nitrogen atom, as determined by X-ray crystallography, is, in any case, tetrahedral: the addition of the proton to complete the quaternisation of the nitrogen atom would therefore seem reasonable.

The NAP's for protonated bicuculline are listed in Table 4.14.

C1	5.92	C16	5.83	H31	0.94
C2	6.00	C17	5.71	H32	0.98
N3	6.94	O18	8.22	H33	0.96
C4	5.95	C19	5.87	H34	0.98
C5	5.91	C20	6.00	H35	0.95
C6	6.01	C21	6.01	H36	0.97
C7	5.98	C22	5.98	H37	1.00
C8	6.03	C23	5.87	H38	1.00
C9	5.85	O24	8.26	H39	0.98
O10	8.22	C25	5.60	H40	1.00
C11	5.72	O26	8.28	H41	1.01
O12	8.22	C27	6.08	H42	0.97
C13	5.85	H28	0.99	H43	1.00
C14	6.04	H29	0.95	H44	1.00
O15	8.22	H30	0.95	H45	0.84

Dipole moment (Debyes)

X	Y	Z	Resultant
1.00	30.51	6.07	31.11

Total Energy = - 278.5073 a.u.

TABLE 4.14 CNDO/2 calculated net atomic populations, dipole moment, and Total Energy for protonated bicuculline.

4.6 PRELIMINARY SYNOPSIS OF THE CNDO/2 CALCULATIONS

A brief summary of some salient results from the CNDO/2 calculations will be given here. They will be further discussed in Chapter 6 and Chapter 7 in the broader context of other experimental and theoretical results.

The results on GABA and β GP have some interesting points in common:

- (i) For the non-zwitterionic forms, energy differences between conformers are relatively small and the global minima are folded rotamers.
- (ii) For the zwitterionic forms, a pronounced preference for the folded rotamers is found. Also, the calculated NAPs appear to indicate the expected development of large charges on the molecules.

The electronic structure of the muscimol zwitterion, as represented by the NAPs, shows some similarity to that of the GABA and β GP zwitterions. The NAPs of the N2-C3-O8 group of muscimol (Table 4.12) resemble those of the carboxyl groups of both GABA and β GP: this similarity is particularly striking in view of the different chemical natures of the two groups. As might be expected the $-\text{NH}_3$ groups of the GABA and muscimol zwitterions exhibit similar NAPs. Also, as noted earlier, the guanidino end of the β GP zwitterion shows a considerable positive character, particularly around the carbon atom.

The calculated NAPs for bicuculline and its protonated cation are also interesting. In spite of the fact that atoms C25 and O24

are part of a ring, the NAPs for the O26-C25-O24 group (Fig.4.9 and Tables 4.13 and 4.14), resemble those for the carboxyl groups of β GP and GABA. In uncharged bicuculline the nitrogen atom does not exhibit an unusual NAP (i.e. is close to neutral, Table 4.13). The situation for the cation is markedly different: the nitrogen atom takes on a slightly positive character, while the region of the added proton remains notably positive overall. It must also be pointed out that other groups in the bicuculline molecule do show NAPs which indicate considerable positive or negative characters. In particular, atoms C11 and C17 give NAPs of ~ 5.7 in both the normal and protonated forms.

CHAPTER 5

Classical Potential-Energy Calculations
on some Centrally Acting Molecules

5.1 INTRODUCTION

Classical potential-energy calculations, of an empirical nature, have been used extensively in the past to study molecular properties such as crystal packing, conformational rigidity, and intermolecular forces. The methods used are essentially straightforward: the total energy of the molecule is taken as the sum of several independent contributions - bond-angle bending strain, bond-length distortion energy, electrostatic interactions, barriers to internal rotation, and non-bonded interactions are amongst the factors commonly considered.

Each element is computed as a sum over all the atoms constituting the molecule, with suitable restrictions (for example, if conformational energy is of interest, interactions between atoms whose relative position is to be unchanged need not be considered). The form of the potential function for each constituent of the total energy must be specified, as must the parameters pertinent to each atomic species: because of the empirical nature of the calculations, the assignment of these values must be somewhat tentative. In spite of this, the method has been applied with some success, as for instance, in the work of Scheraga (for example, Warne and Scheraga, 1974), Giglio (e.g. Giglio, 1969) and Liquori (e.g. Liquori, Damiani and Elefante, 1968). As the calculations are simple, they can normally be carried out very rapidly with the aid of a computer. It seemed worthwhile, therefore, to make calculations on GABA and β GP, using this approach, for the purposes of comparison with the experimental determinations and quantum-mechanical computations described in Chapter 3 and Chapter 4.

A computer program for making the calculations was obtained from Busing (1972) and adapted for the present purposes.

5.2 MODIFICATION AND TESTING OF THE BUSING PROGRAM

The Busing program had facilities for making extensive classical potential energy calculations. A description of the structure of the program is given in Appendix G.

The problems encountered in adapting the program to run on the CDC 7600 computer were similar to, though greater in magnitude and difficulty than, those encountered in implementing the CNDO/2 routine. The basic problems were essentially:

- (i) The program was written in IBM FORTRAN and hence made use of non-standard subroutines. Also computations were made to IBM double-precision accuracy.
- (ii) A large amount of central core-storage was necessary to run the program.
- (iii) The program was extremely large and complex. It consisted of some 3000 lines of FORTRAN coding, with CALLS of subroutines nested to a considerable depth (see Appendix G).
- (iv) The program was considered, by Busing, to be experimental.

Point (i) was dealt with by a straightforward, though lengthy, conversion to CDC FORTRAN. The use of CDC single-precision accuracy was adopted since this is comparable with IBM double-precision (fourteen significant figures for CDC, as compared with fifteen for IBM).

As usual, point (ii) was overcome by assigning the numerous large arrays in the program to LEVEL 2 core storage (i.e. LCM). This itself produced considerable difficulties because of the use in the program of a complex hierarchy of subroutines (point (iii)). It was

essential to ensure that, for every CALL of a particular subprogram, the actual parameters of the routine were declared at the same LEVEL as the dummy parameters for the routine. In addition FUNCTION subprograms may not use LEVEL 2 data as actual parameters. These problems necessitated a complete analysis of the program and the design of a consistent scheme of store allocation. It was sometimes not possible to avoid setting up dummy variables in LEVEL 1 core store (SCM) but, in spite of this, the whole program could be accommodated in the 7600.

In testing the program only a few minor errors have been discovered: the description "experimental" (point (iv)), may therefore be somewhat pessimistic.

Several test problems were supplied and the modified program successfully duplicated these example results. The work of Liquori, Damiani and Elefante (1968) on muscarine was used as an independent test. After correcting an obvious error in the published atomic coordinates, it was possible to reproduce well the reported conformational energy map.

It seemed most fruitful to use the program for calculations on GABA and β GP, the two open-chain molecules amongst those on which CNDO/2 calculations were made. Details of the calculations are reported next.

5.3 CLASSICAL ENERGY CALCULATIONS ON GABA AND β GP

5.3.1 Practical details of the calculations on GABA

The X-ray determined structure of GABA (Chapter 3) was used as the molecular model. Four contributions to the total classical energy were included:

- (i) non-bonded repulsive forces,
- (ii) Van der Waals forces,
- (iii) torsional potentials about the two central bonds,
- (iv) electrostatic forces.

As the conformation of the molecule about the two central bonds was of primary interest, parameters other than the above were not considered.

A modified Buckingham-type potential of conventional form was used to represent (i) and (ii):

$$V_v = a \exp(-cr) - br^{-6}. \quad \dots (5.1)$$

Contributions of type (iii) were calculated on the basis of a simple three-fold cosine function:

$$V_T = -\frac{d}{2} (1 - \cos 3\theta). \quad \dots (5.2)$$

Electrostatic interaction energies were obtained from the classical expression:

$$E_c = \frac{Q_1 Q_2}{r} \quad \dots (5.3)$$

The values of the parameters a, b, c and d (listed in Table 5.1) were those adopted by Liquori, Damiani and Elefante (1968): Snook and Spurling (1970) have concluded that this set of parameters are reasonable for use in conformational problems.

An expression of the form of (5.1) is thought to give a reasonable representation of the interatomic potential except for extremely close contacts, where a false maximum occurs. This can cause problems during the automatic calculation of total energies unless precautions are taken. In the results given below the difficulty has been dealt with by imposing a 'cut-off' distance of $1\text{\AA}$: energies resulting from closer approaches than this are not calculated, and the total energy for the conformation is set to ∞ . For the calculation of the coulomb energies from equation 5.3, some estimate of the partial charges resident on the individual atoms is needed. Since no such values are available from classical considerations, the net atomic populations calculated by the CNDO/2 method (Chapter 4) have been used. The NAP's of GABA in the fully-extended conformation were used, rounded to 0.1 units of electronic charge. As has been previously noted, NAPs may not necessarily accurately represent partial charges in a molecule, but using them as such did appear preferable to the alternative of disregarding this contribution entirely; partial charges calculated by semi-empirical quantum mechanical methods have been used, for example, by Froimowitz and Gans (1972), in their work on acetylcholine. For purposes of comparison, in this study, conformational energy maps both including and excluding the classical coulomb energy were computed.

atom pair	a X10 ⁻³ (kcal/mol)	b (kcal Å ⁶ /mol)	c (Å ⁻¹)
C - C	237.0	297.8	4.32
C - O	212.1	244.0	4.44
C - N	212.1	244.0	4.44
C - H	31.4	121.1	4.20
O - O	186.4	200.0	4.55
O - N	186.4	200.0	4.55
O - H	28.1	99.2	4.32
N - N	186.4	200.0	4.55
N - H	28.1	99.2	4.32
H - H	6.6	49.2	4.08

for the torsional potential:

$$d = 2.7 \text{ kcal/mol}$$

TABLE 5.1 Parameter values for the classical energy calculations

The conformational energy surfaces shown in this section all represent the change in molecular energy as a function of the two central conformational angles of GABA: for these calculations the other two conformational angles (those determining the orientations of the carboxyl and amino groups) were held at 180° . A grid of 20° intervals has been used throughout.

The two conformational energy surfaces for the GABA non-zwitterion are shown in Fig.5.1 and Fig.5.2: the first gives the variation of the total molecular energy, while the second excludes the coulomb energy. Similarly Figs.5.3 and 5.4 are the energy maps for the isolated GABA zwitterion.

As classical energy calculations can be made very rapidly, the influence of the environment in the crystalline state may be taken into account. To do this, the energy was summed over all interactions within a single GABA zwitterion, and, additionally, between symmetry related molecules. Sufficient molecules were included in the calculations to include all major contributions. The crystal structure and symmetry reported in Chapter 3 was used as a basis for the calculations and the results are displayed in Fig.5.5 and Fig.5.6, the former representing the total molecular energy variation and the latter the energy excluding coulomb interaction. It should be noted that, by assuming the size and symmetry of the crystallographic unit cell, the possible conformations of the molecule are severely restricted: the results did, however, provide interesting data on the flexibility of the molecule and the effect of coulomb forces in

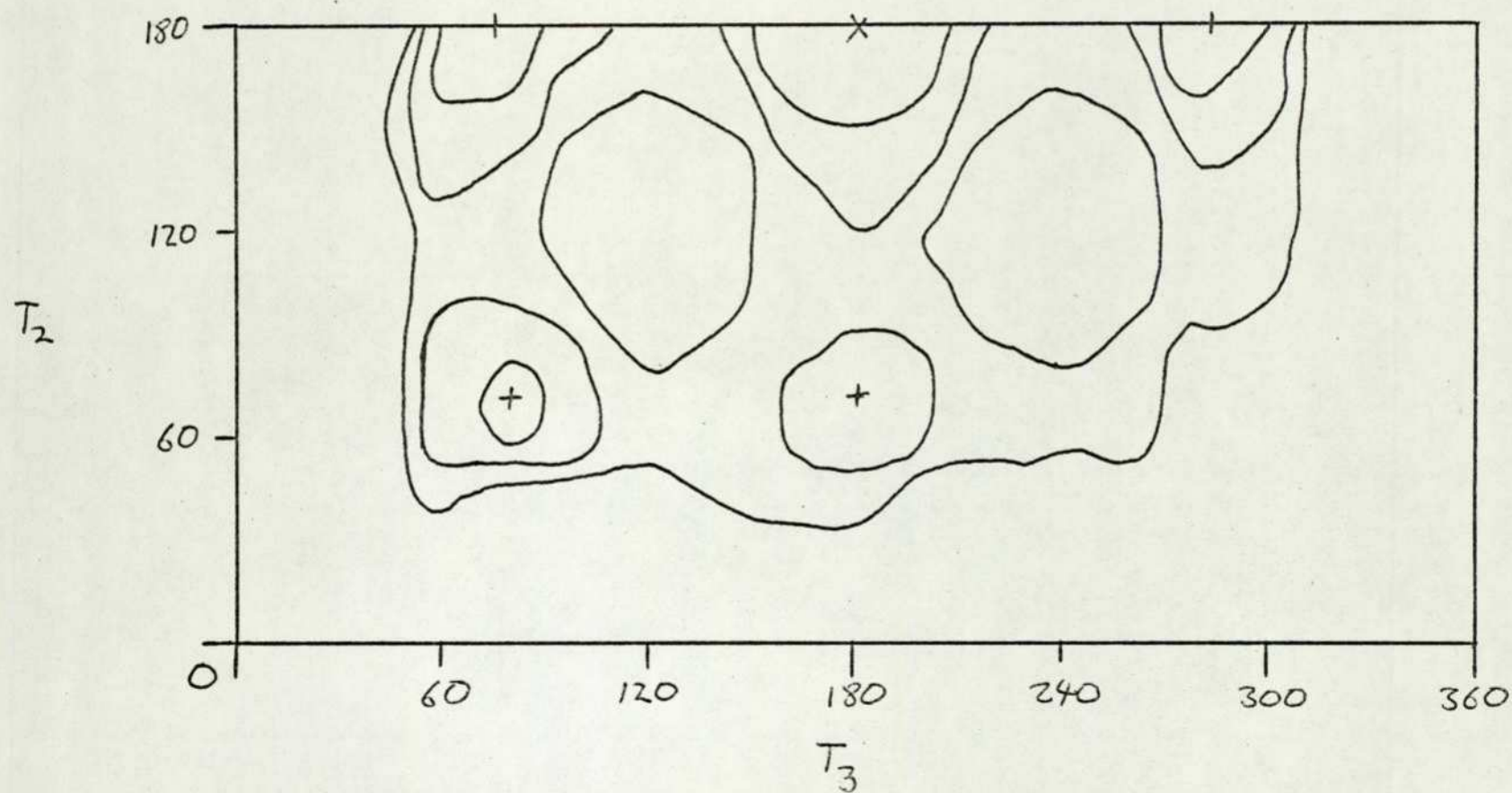


Fig. 5.1 Classical energy surface (total energy) for the GABA non-zwitterion. Contours marked at 2 kcal/mol intervals.

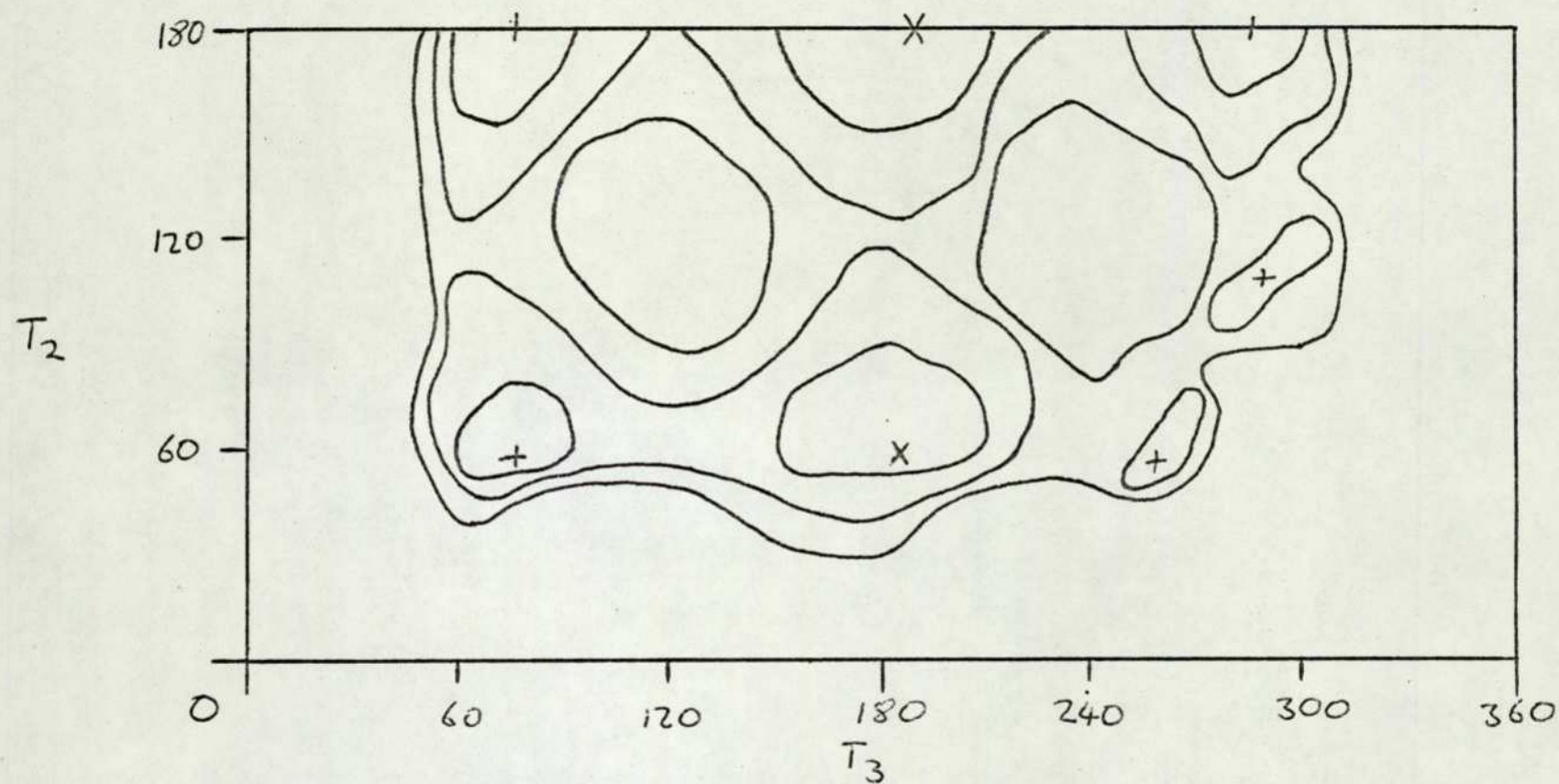


Fig. 5.2 Classical energy surface (excluding coulomb energy) for the GABA non-zwitterion. Contours marked at 2 kcal/mol intervals.

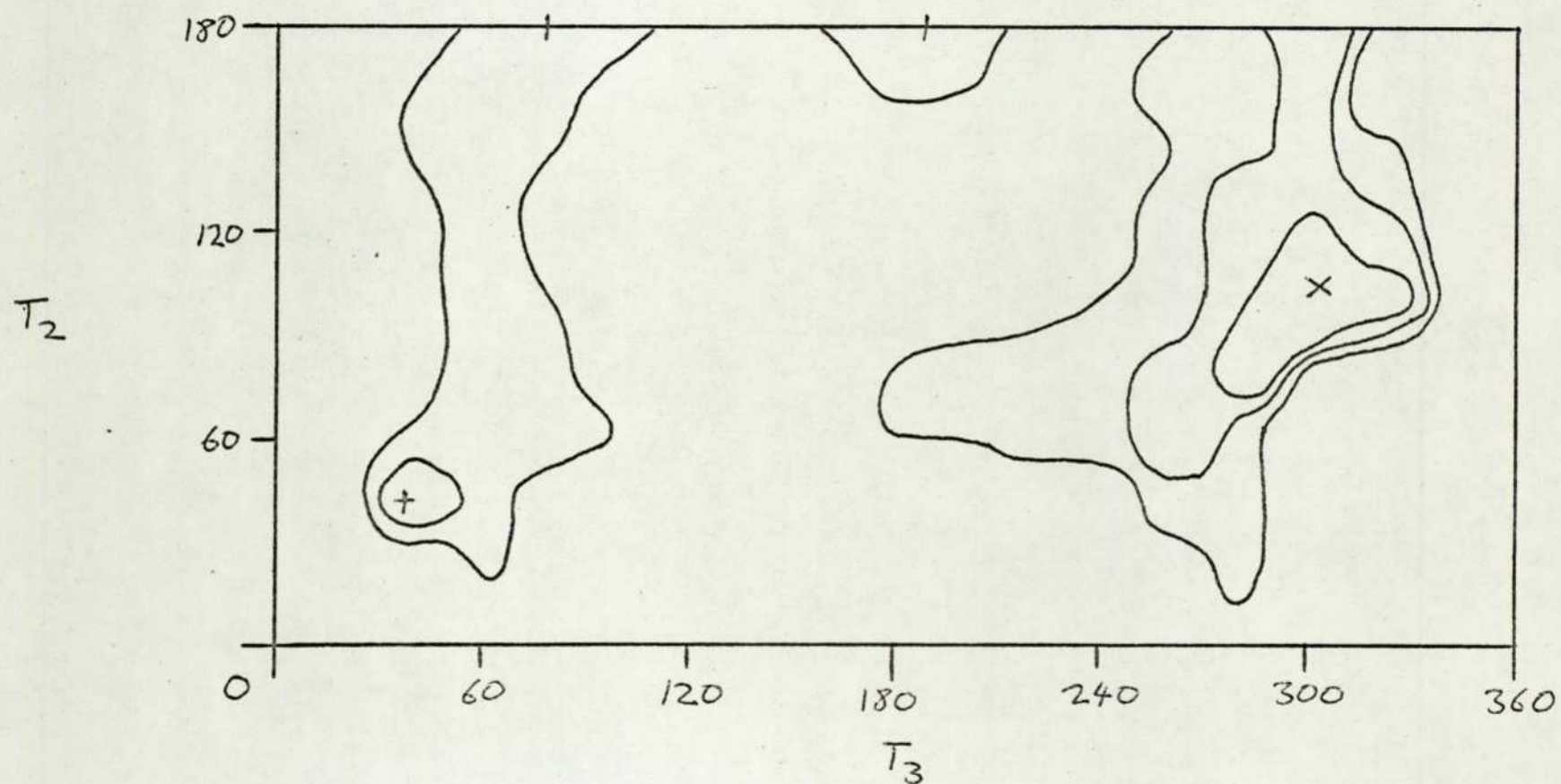


Fig. 5.3 Classical energy surface (total energy) for the conservative GABA zwitterion. Contours marked at 6 kcal/mol intervals.

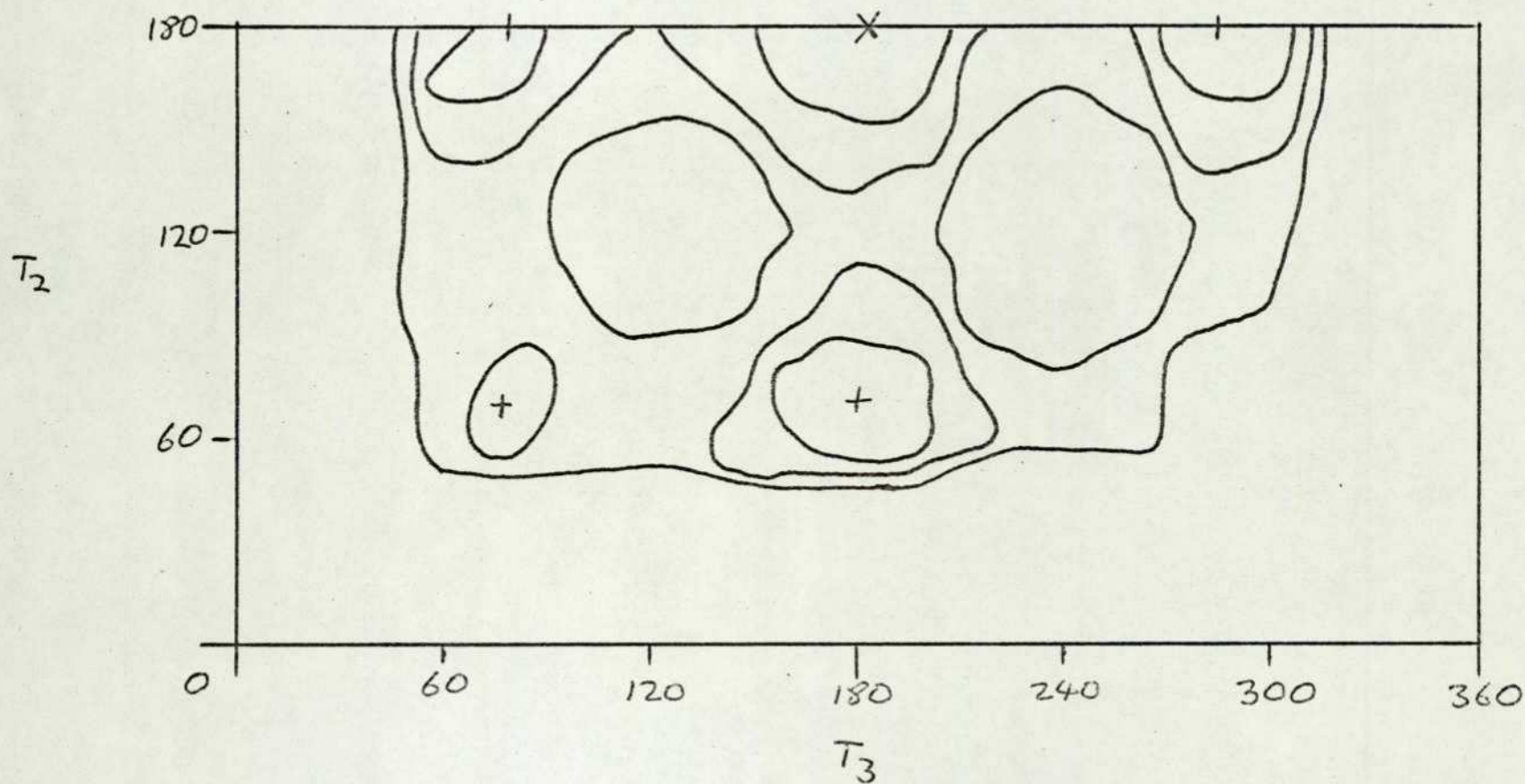


Fig.5.4 Classical energy surface (excluding coulomb energy) for the conservative GABA zwitterion. Contours marked at 2 kcal/mol intervals.

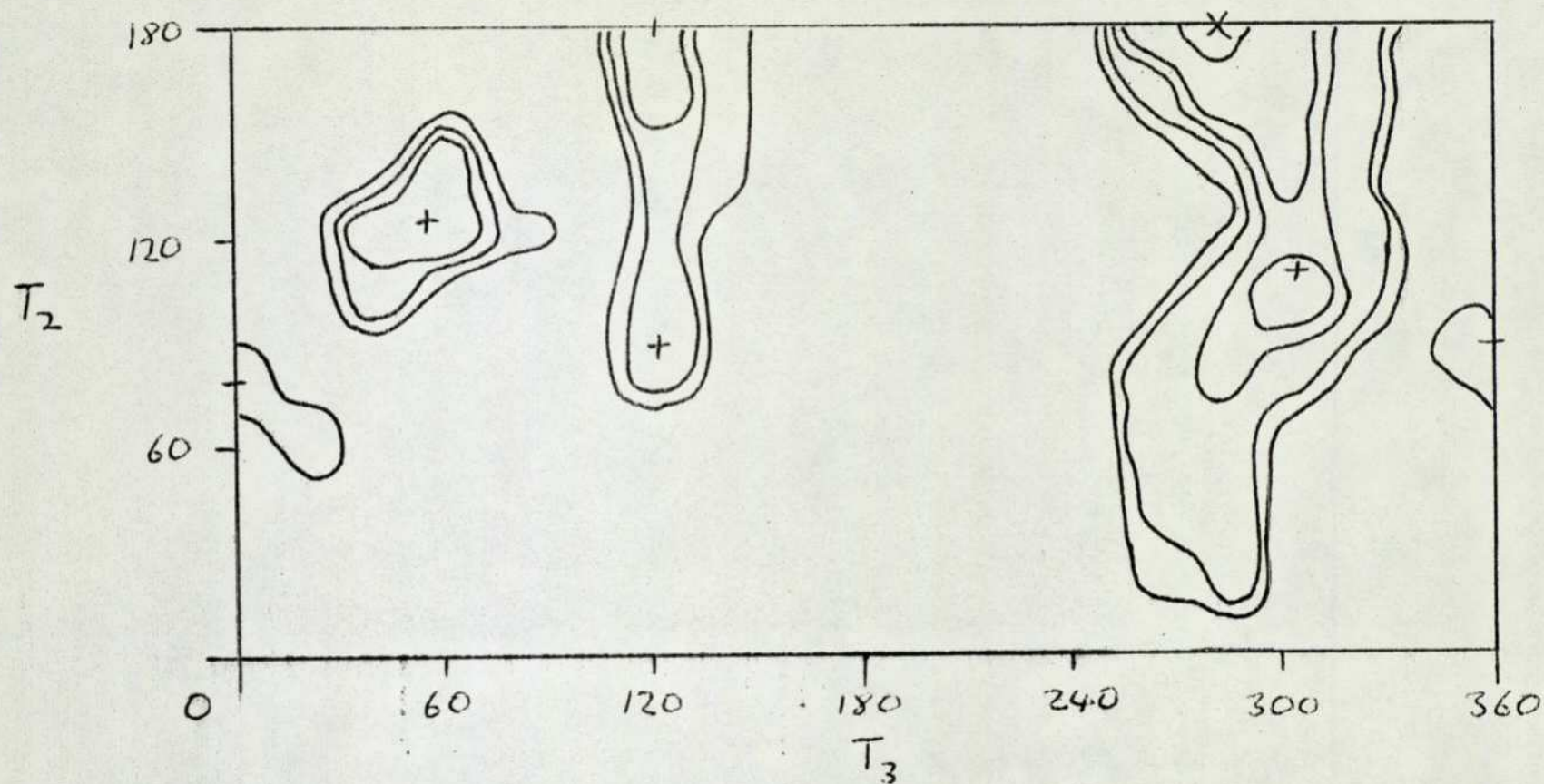


Fig.5.5 Classical energy surface (total energy, including contributions from neighbouring molecules) for the GABA zwitterion. Contours marked at 20 kcal/mol intervals

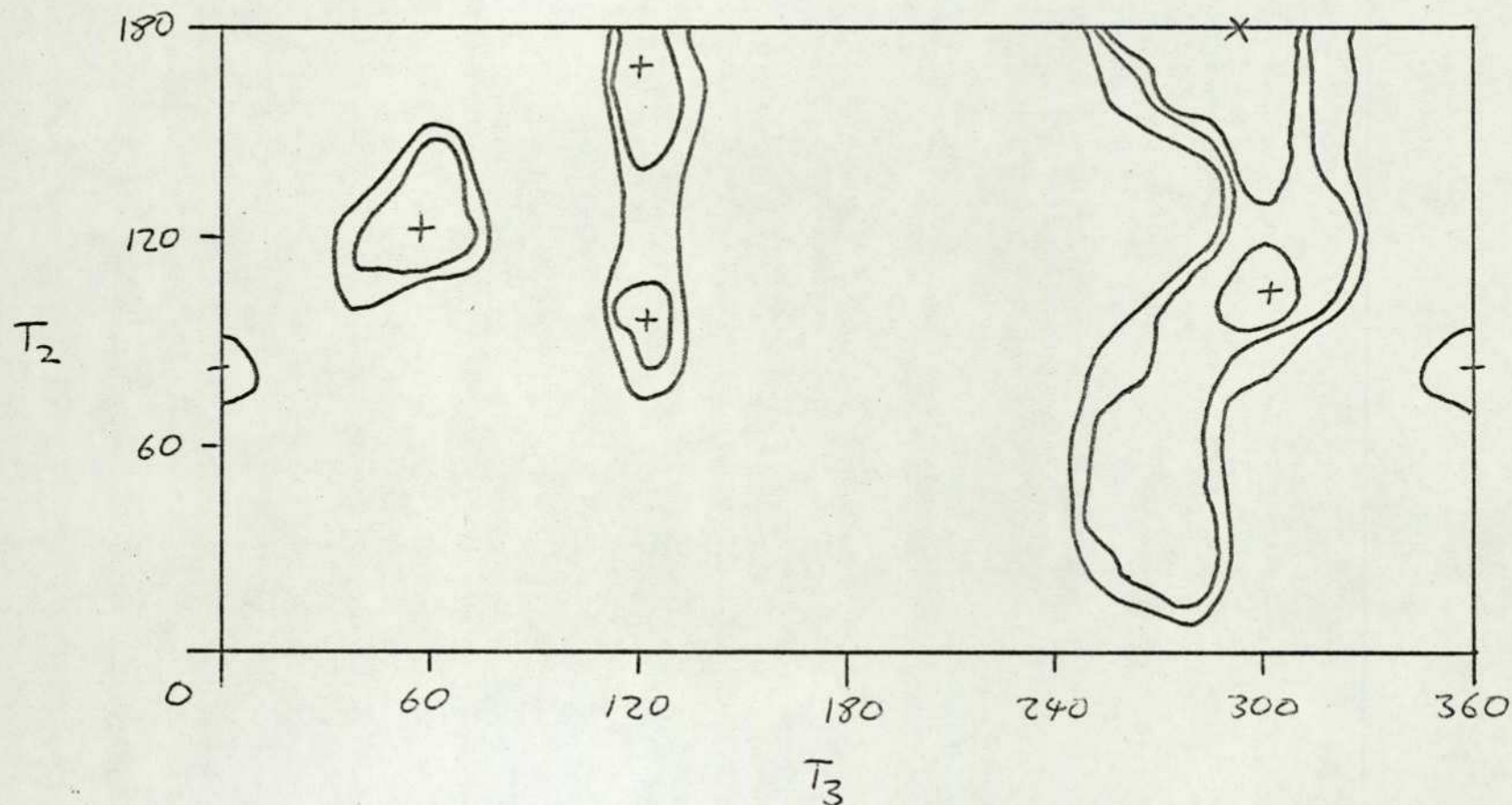


Fig. 5.6 Classical energy surface (without coulomb energy but including other contributions from neighbouring molecules) for the GABA zwitterion. Contours marked at 20 kcal/mol intervals.

MINI- MUM	T ₂ (°)	T ₃ (°)	Rel. Energy (kcal/mol)
CM1	180	80	1
CM2	180	180	0
CM3	180	280	1
CM4	80	80	2
CM5	80	180	1

TABLE 5.2 Classical-energy minima for GABA (non-zwitterion): total energy

MINI- MUM	T ₂ (°)	T ₃ (°)	Rel. Energy (kcal/mol)
CMZ1	180	80	13
CMZ2	180	180	13
CMZ3	100	300	0
CMZ4	40	40	10
CMX1	180	280	0
CMX2	180	120	37
CMX3	120	60	23
CMX4	80	0	68
CMX5	80	120	51
CMX6	100	300	18

TABLE 5.3 Classical-energy minima for GABA zwitterion: total energy
(isolated: CMZ1-4. With intermolecular interactions:
CMX1-6)

the constrained crystal state.

5.3.2 Discussion of the results of the classical energy calculations on GABA

The results for the non-zwitterion are summarised in Table 5.2. The conformational energy maps, with and without calculated coulomb energy, are similar: both show

the all-*trans* conformation being favoured, though energy differences are small.

As might be expected with this mode of calculation, the results for the isolated zwitterion if coulomb forces are not taken into account (Fig.5.4) are similar to those for the non-zwitterion. The rearrangement of the hydrogen atoms merely produces a shift in the position of the most folded minimum energy conformation such that it coalesces with the low-energy region at $T_2 = 60^\circ$, $T_3 = 180^\circ$. Introducing the effect of the partial charges changes the situation markedly. The energy surface (Fig.5.3) shows just four minima, by far the deepest being a fully-folded rotamer. This conformation is predicted to be some 18 kcal/mol lower in energy than the fully-extended conformation (Table 5.3). Taking the deepest minimum on Fig.5.3 as an example, the inclusion of classical coulomb energy lowers the total calculated energy by 34 kcal/mol; for the second minimum ($T_2 = 40^\circ$, $T_3 = 40^\circ$), the total energy is reduced by 97 kcal/mol.

The energy maps produced for the GABA zwitterion in the crystal environment are shown in Fig.5.5 and Fig.5.6 and summarised in Table 5.3. As anticipated, the crystal packing

conditions imposed in the calculations produce large energy differences across the energy surface: in contrast to the results on the conservative molecule, only certain regions are at all accessible to the molecule. The crystal conformation appears, of course, as the global minimum, but a local minimum still occurs for a more folded conformer with $T_2 = 100^\circ$ and $T_3 = 300^\circ$.

It is interesting to note that, in the crystal environment, the inclusion of coulomb forces in the calculations favours the more extended conformers of GABA over the fully-folded rotamers. Thus the energy difference between the global minimum at $T_2 = 180^\circ$, $T_3 = 280^\circ$ and the local minimum at $T_2 = 100^\circ$, $T_3 = 300^\circ$ is increased from 13 kcal/mol to 18 kcal/mol by the inclusion of coulomb forces. This is due to electrostatic interactions between neighbouring molecules.

5.3.3 Classical energy calculations on β GP

β GP is an open-chain molecule, like GABA, but exhibits a more folded form in the crystal state. It was therefore useful to make a parallel set of classical energy calculations for the guanidino acid. The same parameters as were used for GABA were employed and an exactly analogous set of energy surfaces plotted. Again, the structural model previously used in the CNDO/2 calculations, together with the NAPs for the extended conformations of the zwitterion and non-zwitterion, were used. The results are shown in Figs.5.7-12, the minimum energy conformations are summarised in Table 5.4 and Table 5.5.

The results obtained differ from those for GABA in one immediately noticeable aspect: a fully-folded conformation does not appear as a local minimum for the non-zwitterion when coulomb energy is excluded. Apart from this, the situation for the isolated forms of the molecule is similar to that revealed for GABA.

Examination of the results for the β GP zwitterion in the crystalline state (Table 5.5, Fig.5.11 and Fig.5.12) shows, as in the case of GABA, that only limited regions of the conformational energy surface are at all energetically accessible to the molecule. For β GP, the imposed crystal packing considerations exclude the possibility of fully-extended conformers appearing as low-energy modes in the calculations. The crystal conformation ($T_2 = -83^\circ$, $T_3 = 106^\circ$) appears as the global minimum; a second area of comparatively low energy also exists. The inclusion of coulomb energy in the calculations hardly alters the relative energies of the minima, other than to marginally favour the crystal conformation. In particular, there is no preference exhibited for the more extended conformers.

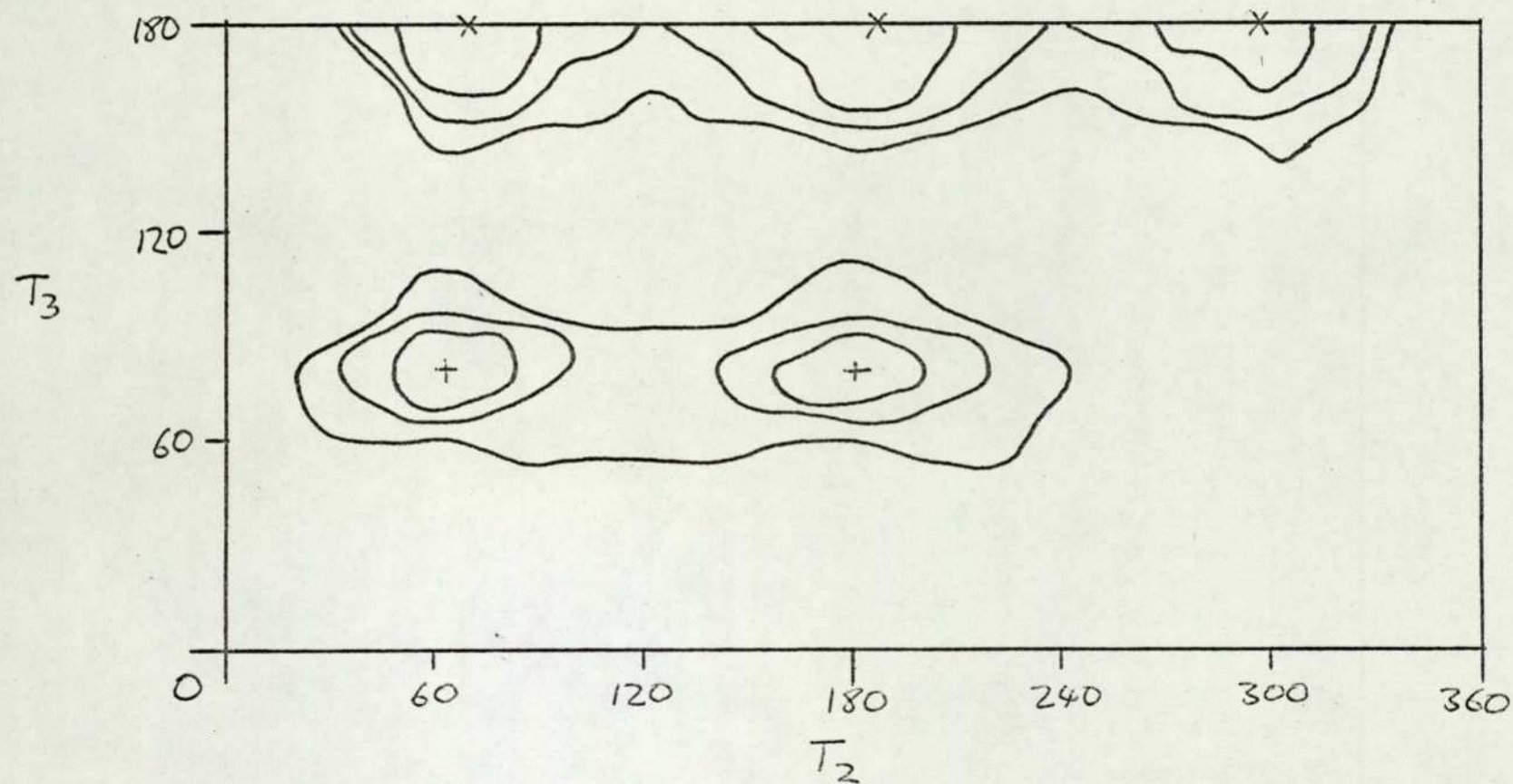


Fig. 5.7 Classical energy surface (total energy) for β GP non-zwitterion. Contours marked at 2 kcal/mol intervals.

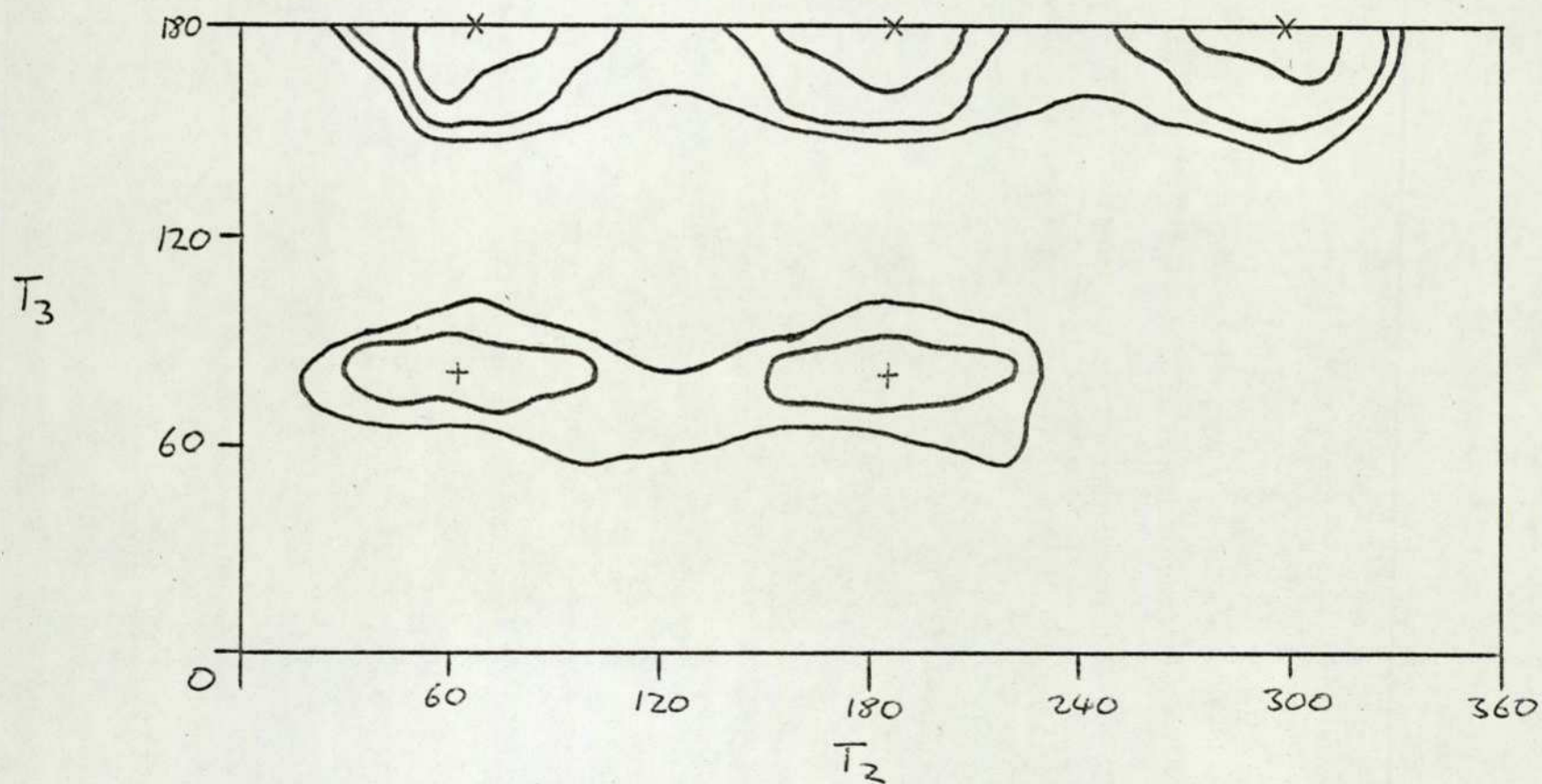


Fig. 5.8 Classical energy surface (excluding coulomb energy) for β GP non-zwitterion. Contours marked at 2 kcal/mol intervals.

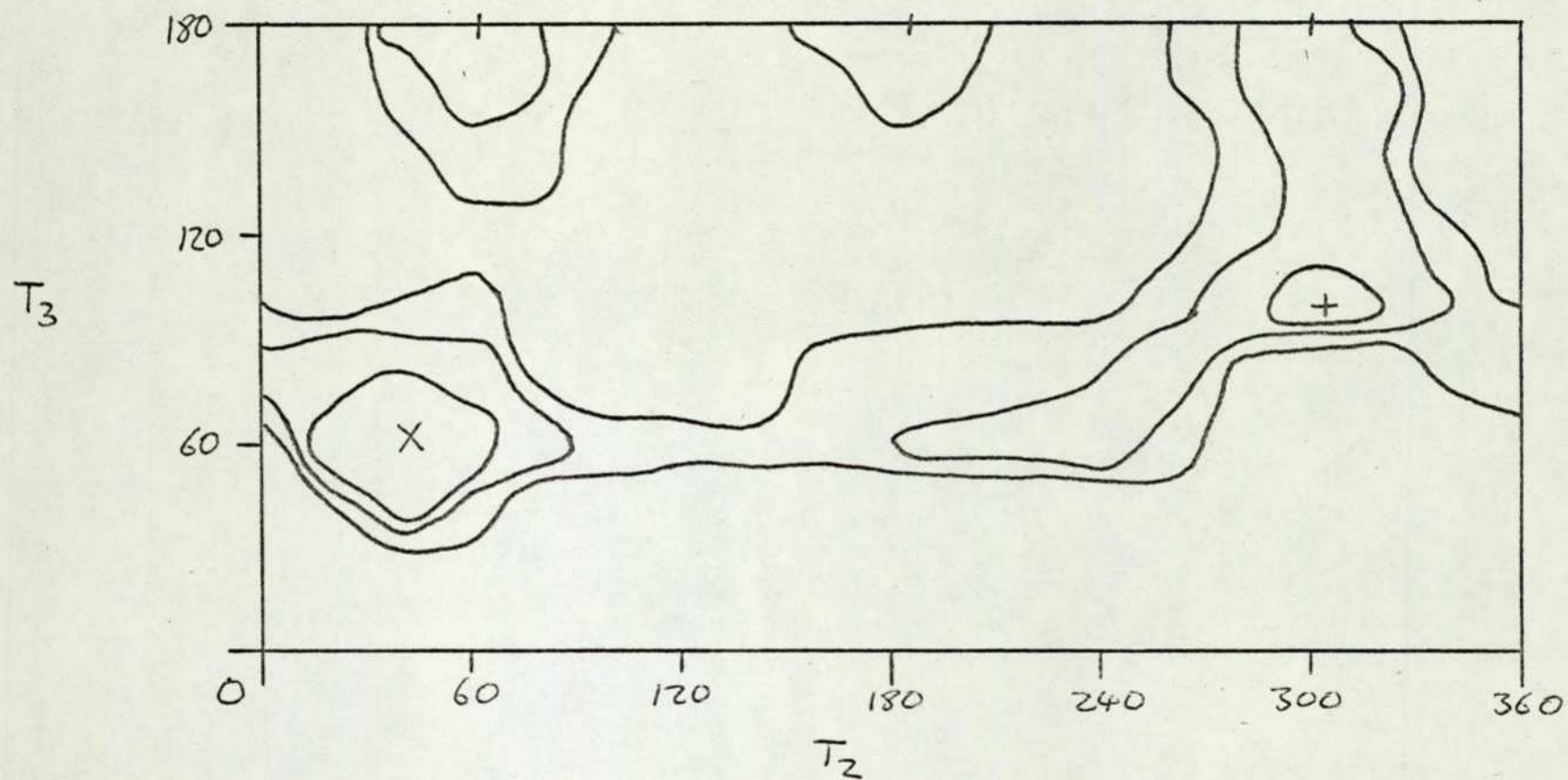


Fig. 5.9 Classical energy surface (total energy) for the conservative β GP zwitterion. Contours marked at 3 kcal/mol intervals.

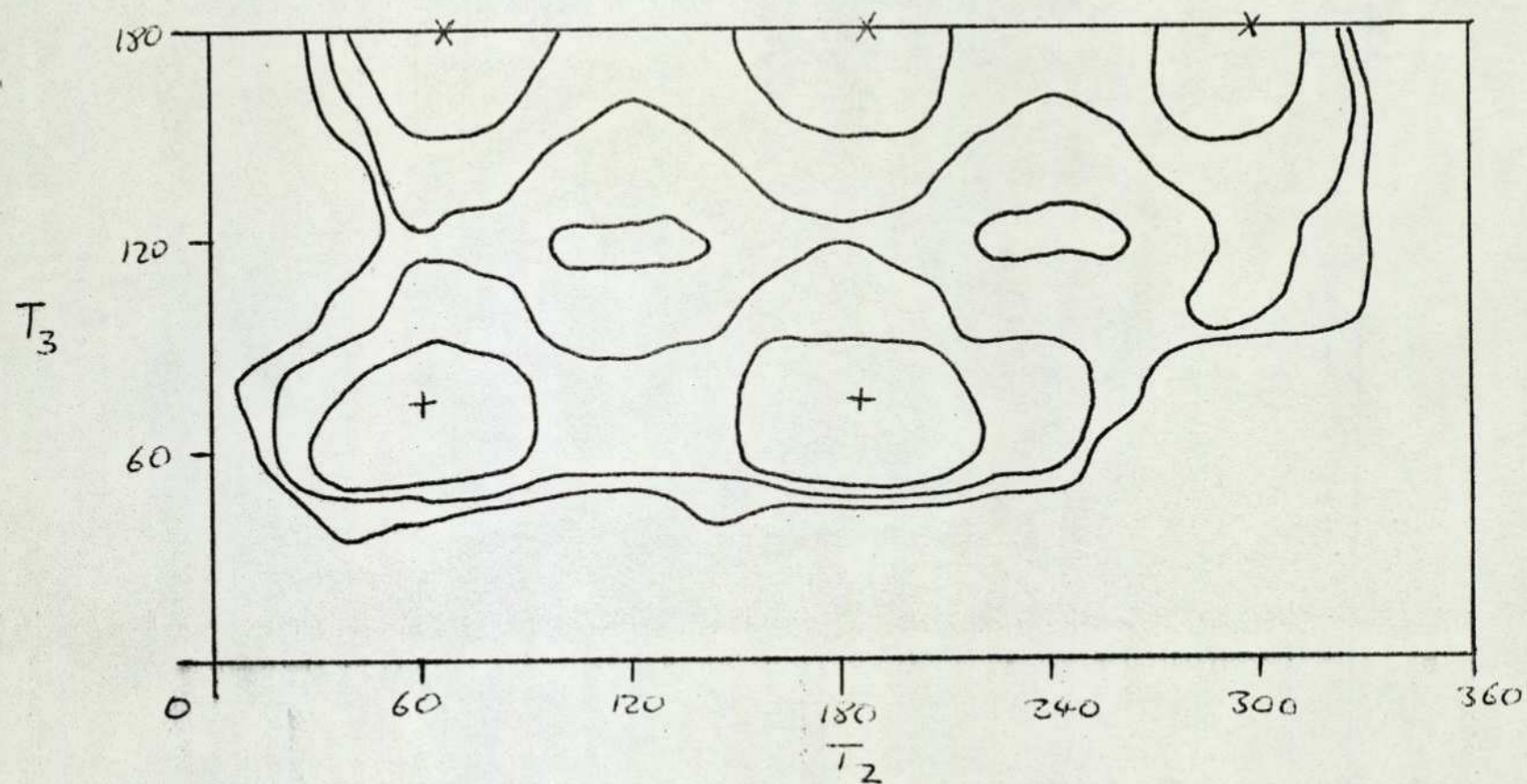


Fig. 5.10 Classical energy surface (excluding coulomb energy) for the conservative β GP zwitterion. Contours marked at 3 kcal/mol intervals.

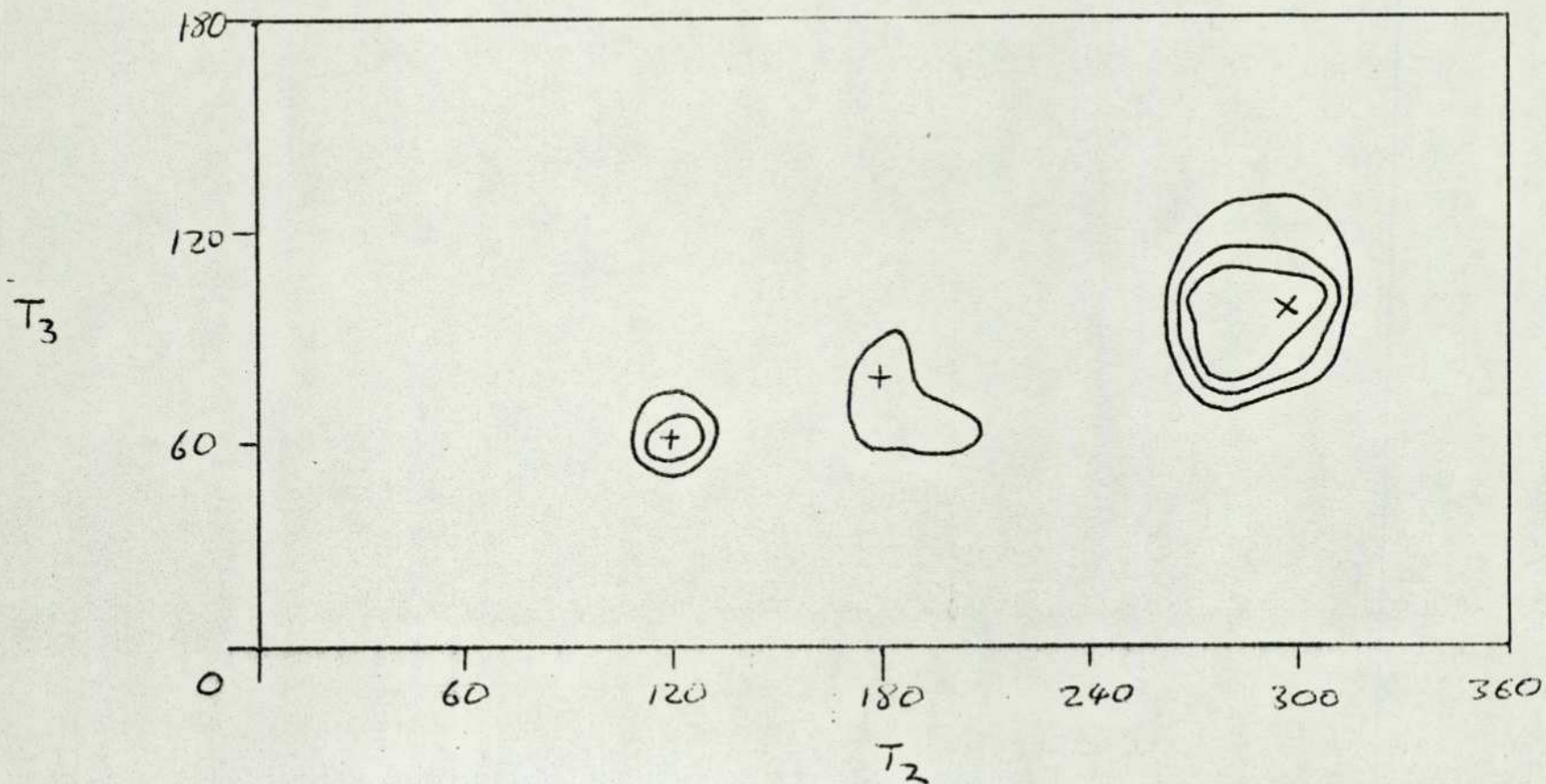


Fig. 5.11 Classical energy surface (total energy, including contributions from neighbouring molecules) for the β GP zwitterion. Contours marked at 20 kcal/mol intervals.

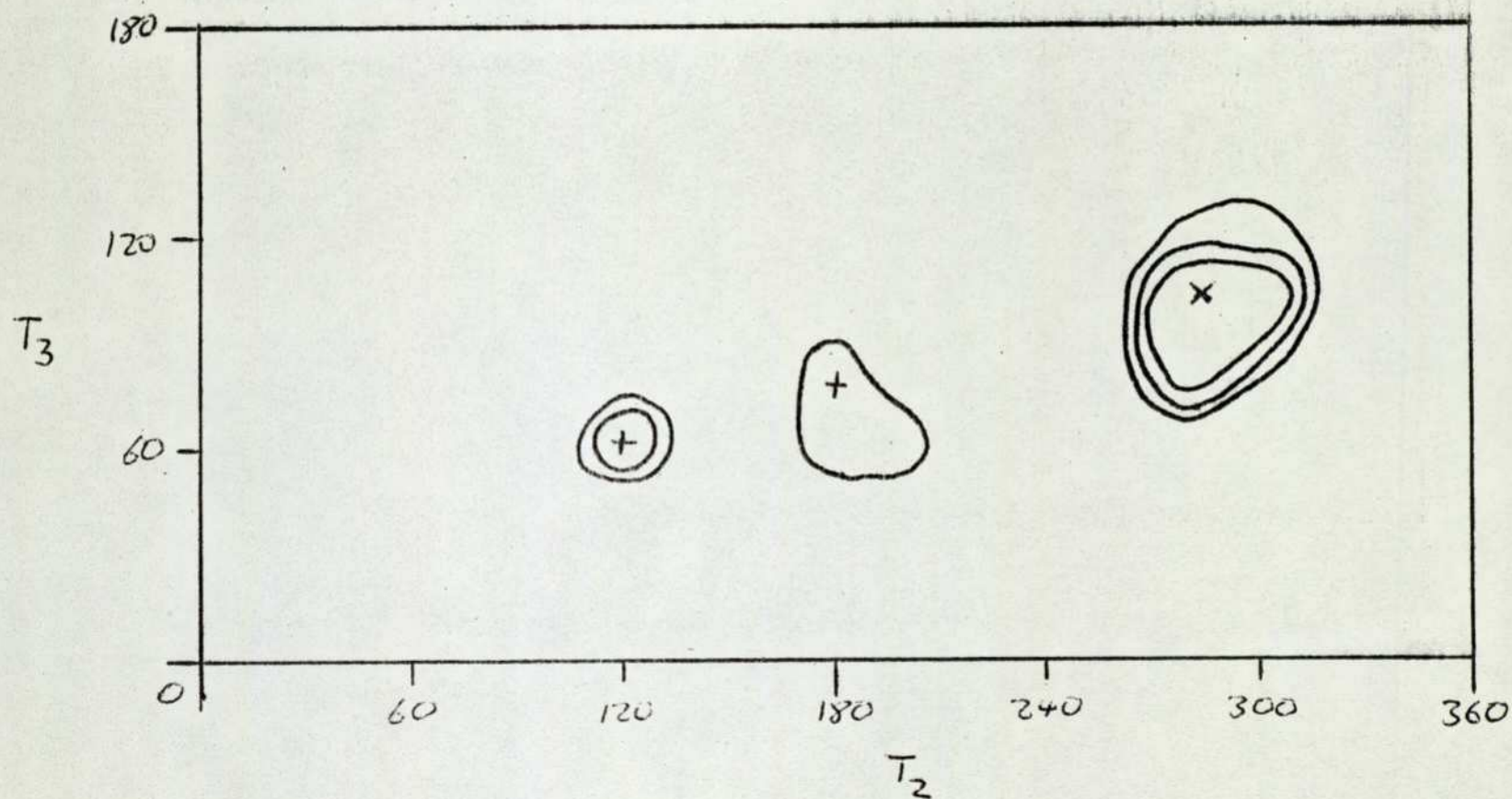


Fig. 5.12 Classical energy surface (excluding coulomb energy, but including other contributions from neighbouring molecules). For the β GP zwitterion. Contours marked at 20 kcal/mol intervals

MINIMUM	T_2 ($\begin{smallmatrix} 2 \\ 0 \end{smallmatrix}$)	T_3 ($\begin{smallmatrix} 3 \\ 0 \end{smallmatrix}$)	Rel. energy kcal/mol
CB1	60	180	0
CB2	180	180	0
CB3	300	180	0
CB4	60	80	1
CB5	180	80	1

TABLE 5.4 Classical minimum energy conformations for the
PGP non zwitterion : total energy

MINIMUM	T_2 ($\begin{smallmatrix} 2 \\ 0 \end{smallmatrix}$)	T_3 ($\begin{smallmatrix} 3 \\ 0 \end{smallmatrix}$)	Rel. energy kcal/mol
CBZ1	60	180	4
CBZ2	180	180	7
CBZ3	300	180	4
CBZ4	40	60	0
CBZ5	300	100	2
CBZX1	300	100	0
CBZX2	180	80	51
CBZX3	120	60	22

TABLE 5.5 Classical minimum-energy conformations for the PGP
zwitterion (CBZ1-5: isolated; CBZX1-3: with
neighbouring molecules) : total energy

5.4 SUMMARY OF THE CLASSICAL ENERGY CALCULATIONS

The potential-energy calculations on GABA and β GP give, in some respects, similar results. Thus energy differences between different conformers of the non-zwitterions are small. Also energy differences between extended and folded conformations of the zwitterionic forms only become large if coulomb forces are included.

Not surprisingly, calculations involving neighbouring molecules, located according to the crystal structures previously determined, give global minima for the crystal conformations - both in the case of GABA and of β GP; the effect of the charged nature of the GABA zwitterion in stabilising extended conformations is significant.

Finally, it may be mentioned that the potential energy calculations (with the inclusion of coulomb energy) on the isolated molecules generally parallel the CNDO/2 results reported in Chapter 4. The major difference is that the barriers to rotation predicted by the quantum mechanical calculations are somewhat larger than those shown on the classically calculated energy surfaces.

CHAPTER 6

Discussion and Comparison of the Conformational Results

6.1 INTRODUCTION

This chapter draws together the work reported in the previous chapters and the results published by other authors. A comparison and discussion of the various data is given. This review is then used as a basis for a discussion of the effects of the environment on the conformational modes adopted by the molecules studied. Also considered is the possibility of intermolecular and intramolecular hydrogen-bonding.

It may be mentioned here that conformational studies should strictly be made in terms of Free Energies. Later in this chapter (6.2.3) the consequences of invoking this function, rather than Internal Energy, are explored for the case of the GABA zwitterion.

The results for each molecule whose conformation has been studied are reported here (viz GABA (6.2), β GP (6.3) and muscimol (6.4)). A review of general conclusions made from the conformational studies concludes the chapter.

In this section, the discussion of conformations is in terms of the conformational angle specifications listed in Appendix A. In addition, consideration here is limited to minimum-energy conformations. Thus, for brevity, 'minimum-energy folded conformations' are referred to as 'folded conformations'.

6.2 THE RESULTS OF CONFORMATIONAL STUDIES ON GABA

6.2.1 The available results

The results of several conformational studies of GABA are now available. Apart from those described in previous chapters, other results which have been reported are: EHT calculations on the isolated zwitterion (Kier and Truitt, 1970); PCILO calculations on the isolated zwitterion (Pullman and Berthod, 1974); dielectric measurements (Edwards, Farrell and Job, 1973); NMR studies (Parry Jones, Roberts and Ahmed, 1971; Ham, 1974; Richards and Thomas, 1974). Additionally, the crystal structures of GABA and GABAHC₂ have also been reported by Tomita (Tomita, Higashi and Fujiwara, 1973; Tomita, 1965): since his results, though less precise, agree essentially with those given in Chapter 3, they will not be discussed further here.

Mention should also be made of preliminary results, obtained in this laboratory, of a set of theoretical calculations on GABA in solution. These calculations (Steward, Clarke and Borthwick, 1974) were made by taking the CNDO/2 energy-surface obtained for the GABA zwitterion (as described in Chapter 4) and applying the method described by Beveridge (Beveridge, Radna, Schnuelle, Kelly, 1974) for the computation of solvent effects. The initial results seem to favour an extended conformation for GABA in solution.

Some of the work on GABA reported in Chapters 3 and 4 has also been published elsewhere. For completeness, the references are given here: The crystal structure of GABA (Steward, Player

and Warner, 1973a); a refinement of the crystal structure of GABAHC_l (Steward, Player and Warner, 1973b); CNDO/2 studies of GABA (Warner and Steward, 1975).

Table 6.1 is a complete summary of the results of the conformational studies, as far as possible in terms of the minimum energy conformations with respect to the T_2 and T_3 conformational angles of GABA. Table 6.2 gives a more qualitative classification of the various minima.

6.2.2 The conformation of the GABA zwitterion - the effect of the environment

Considering first of all the calculations made on the isolated zwitterion, three sets of results (the CNDO/2, PCILO and classical energy calculations) strongly favour folded conformations of the molecule. As remarked in Chapter 4, the precise equilibrium values for T_2 and T_3 for the fully-folded conformers, at least in the CNDO/2 calculations, are fixed by close atom-atom contacts and, as such, are susceptible to the values of T_1 and T_4 : the existence of a fully-folded conformer, whatever its exact specification, as the global minimum is not, however, in doubt. The agreement of three independent methods on this point is significant.

The EHT results of Kier and Truitt (1970) do not predict a fully-folded form as the preferred conformation. As noted by Kier (1971), however, EHT is sometimes unsuccessful with polar molecules, for which unrealistic charge distributions may be predicted. The general agreement amongst the three other methods of calculation would seem to indicate that the GABA

<u>method</u>	<u>minima and relative energies</u> ($T_2, T_3 : \Delta E$ (kcal/mol))					ΔE given where available)
(1) <u>NON-ZWITTERION</u>						
CNDO	65,280:0	60,60 :2	60,180:3	175,65 :3	180,295:3	180,180:3
Classical : total	180,180:0	180,80 :1	180,280:1	80,180:1	80,80 :2	
Classical : total-coulomb	180,180:0	60,180:0	180,80 :1	180,280:1	60,80 :1	60,260:4
(2) <u>ZWITTERION</u>						
(a) <u>Conservative</u>						
CNDO	80,305:0	0,50 :4	10,305:5	155,330:51	180,0 :52	180,180:66
EHT (Kier)	180,180					
PCILO (Pullman)	0,±60:0	90,±60:9	180,±60:41	180,180:54		
Classical: total	100,300:0	40,40 :10	180,80 :13	180,180:17		
Classical: total-coulomb	180,180:0	180,80 :1	180,280:1	80,180:1	80,80 :2	
(b) <u>Non-conservative</u>						
NMR (Ham)*	180,60	60,180	180,180	60,60	60,300	
NMR (Parry Jones)	180,180					
Theoretical(Steward)	65,140					
Crystal conformer	175,287					
Classical: total	180,280:0	100,300:18	120,60 :23	180,120:28	80,0 :68	
Classical: total-coulomb	180,280:0	100,300:13	160,120:22	40,260:26	120,60 :27	100,120:38
dielectric meas. (Edwards)	180,180					
GABA HCL (crystal)	192,191					

Table 6.1 Conformational results for GABA. Full references to the results are given in the text.

*conformers in order of decreasing fractional populations; the final two are predicted to be present in equal amounts.

<u>method</u>	<u>minima</u>
(1) <u>NON-ZWITTERION</u>	
CNDO	gg, gg, gt tg tg tt
classical:total	tt, tg tg gt, gg
classical:total-coulomb	tt gt, tg tg gg, gg
(2) <u>ZWITTERION</u>	
(a) <u>conservative</u>	
CNDO	gg, cg, cg, tg, tc, tt
EHT (Kier)	tt
PCILO (Pullman)	cg cg, gg gg, tg tg, tt
classical:total	gg, gg, tg, tt
classical:total-coulomb	tt, tg tg gt, gg
(b) <u>non-conservative</u>	
NMR (Ham)	tg, gt, tt, gg gg
NMR (Parry Jones)	tt
Theoretical(Steward)	gg
crystal conformer	tg
classical:total	tg, gg, gg, tg, gc
classical:total-coulomb	tg, gg, tg, gg, gg, gg
dielectric meas. (Edwards)	tt
GABA HCL (crystal)	tt

Table 6.2 Summary of the conformational results for GABA. Conformers classified in terms of approximate trans (t), gauche (g) and cis (c) positions about T₂ and T₃. Conformers are listed in order of increasing energy; those not separated by commas are of equal energy.

zwitterion may be a case for which EHT proves to be unreliable.

Those set of results pertinent to GABA in solution (NMR, dielectric measurements, and preliminary theoretical calculations), all predict the extended conformers of the zwitterion to be more stable than the fully-folded rotamers. In addition, the crystal structure studies of GABA and of GABAHC α both show GABA to be in somewhat extended rather than fully-folded conformations. Similarly, empirical energy calculations on GABA, with neighbouring molecules arranged in accordance with the space group of the crystal structure (Chapter 5), confirm the preference for the observed crystal conformation. These results are in marked contrast to those referred to above for the conservative zwitterion. To account for this difference it is necessary to consider the environmental factors involved: this will be attempted in a semiquantitative fashion.

Comments on the data available

First it is worth noting the following points concerning the various data.

- (i) the classical energy calculations did not explicitly include the possibility of the formation of intermolecular or intramolecular hydrogen-bonding. In reality, in the solid state each molecule of GABA takes part in the formation of five intermolecular N-H...O hydrogen-bonds. Had the calculations made allowance for this, the global minimum predicted for the crystal conformation, where nitrogen, hydrogen

and oxygen atoms are well-placed for the formation of hydrogen-bonds (i.e. are in approximately linear arrangements), would have been somewhat deeper. Similarly, for the isolated molecule, at least one intramolecular hydrogen-bond would be expected for some fully-folded conformations.

- (ii) Edwards, Farrell and Job, who demonstrated that the measured dielectric constant of GABA in solution was consistent with an extended conformation of the molecule, did not state whether this was the only feasible deduction from the data.
- (iii) Ham did not state the exact method of analysis used in his NMR study. He did, however, state that the approach he used interpreted "the spectrum in terms of variable populations of staggered rotamers having dihedral angles of 60° and 180° " (Ham, 1974). The same point concerning this type of NMR conformational analysis has been made, though perhaps less explicitly, by Partington, Feeney and Burgen (1972). It is quite possible that this empirical assumption concerning the rotamers present in solution may be unwarranted. The CNDO/2 calculations reported in Chapter 4 indicate minimum-energy conformations noticeably displaced from the precise 180° and 60° positions (the classical *trans* and *gauche* conformers). In the case of a solute molecule, solvent-solute interactions may increase the likelihood of the occurrence of

non-classical values for conformational angles. The solvent-effect calculations currently being made in this laboratory certainly seem to show a shift of the global minimum away from the exact classical conformation (Steward, Clarke and Borthwick, 1974).

- (iv) Of the other two sets of NMR data, Richards and Thomas did not give a conformational analysis and Parry Jones, Roberts and Ahmed suggested that the GABA molecule was in the *trans* conformation with respect to T_2 and T_3 . The authors do not mention the possibility of the existence of other conformers.

Thus there appears to be at least two possible conclusions from NMR results: (a) a spectrum of modes present in solution (Ham) or (b) the fully-extended conformer only is found in solution (Parry Jones, Roberts and Ahmed).

Even with the above reservations in mind, and noting the conflicts between the sets of NMR data, the difference between the predicted conformations of the conservative and non-conservative zwitterions is marked. Comparing on one hand the results for the zwitterion with those for the non-zwitterion and on the other, the classical energy calculations including coulomb energy with those omitting it (Tables 6.1 and 6.2), strongly suggests that a major factor in determining the minimum-energy conformations of the molecule is the charged nature of the zwitterion: this factor is considered next.

The conformational significance of the polarity of the
GABA zwitterion

Both classical and quantum mechanical methods predict only a slight preference for the folded forms as minima for the isolated GABA non-zwitterion (Table 6.1). Similarly potential energy calculations on the conservative zwitterion do not favour the "*gauche-gauche*" conformers unless coulomb interactions are taken into account. Also classical energy calculations on the zwitterion in the solid state (Table 6.1) show that electrostatic interactions tend to stabilise the crystal conformation.

The importance of the charged nature of the zwitterion and the effect of the environment may be further demonstrated by the following simple calculation: in the potential energy calculations on the GABA zwitterion (Chapter 5) the coulomb energy, E_c , was calculated according to:-

$$E_c = \sum_{A>B} \frac{Q_A Q_B}{r_{AB}}$$

where, as usual, Q_A and Q_B are the partial charges on two atoms separated by a distance r_{AB} . It was found that for the "*all-trans*" conformation:

$$E_{c1} = -21 \text{ kcal/mol}$$

while for a fully-folded conformer ($T_2 = 30^\circ$, $T_3 = 300^\circ$) the value obtained was

$$E_{c2} = -52 \text{ kcal/mol.}$$

Therefore, the folded rotamer was stabilised by 31 kcal/mol by the electrostatic interactions. However, this calculation takes no account of the effect of the environment of the molecule. This may be introduced, non-atomistically, through the dielectric constant, ϵ , of the medium. Then, expressing the coulomb energy by

$$E_c = \sum_{A>B} \frac{Q_A Q_B}{\epsilon r_{AB}},$$

the difference in coulomb energy between the extended and folded rotamers considered above, becomes

$$\Delta E_c = E_{c2} - E_{c1} = -31/\epsilon \text{ kcal/mol}$$

i.e. inversely proportional to the dielectric constant. It is not easy to assign an exact value to ϵ appropriate for calculations made at the molecular level: instead reasonable values may be taken as examples.

For an isolated molecule ϵ may be taken as 1 and the magnitude of ΔE_c (= 31 kcal/mol), then goes some way to explaining the predicted predominance of folded over extended conformers for this situation. In fact the

CNDO/2 calculations (Table 6.1) predict a somewhat larger energy difference than this (~ 60 kcal/mol), but a comparison of the semiempirical and the classical energy maps shows, in any case, a general tendency towards higher energy barriers in the former case.

Taking $\epsilon = 80$, the value for water, gives $\Delta E_c \sim 0.4$ kcal/mol. In solution, therefore, the effect of the electrostatic interactions might be expected to be much reduced and there would, presumably, be a greater likelihood of the occurrence of the extended conformers.

Increasing ϵ to 200 (a value which has been used in some protein work (Tredgold, 1973)) would indicate a further shift away from the folded rotamers.

The above discussion shows the importance of the effect of the interaction between the highly-polar zwitterion and its environment, so far as conformational modes are concerned.

Hydrogen-bonding

The formation of hydrogen bonds may be important in solution. The results of the crystal structure studies (Chapter 3) amply demonstrate the capacity of the GABA zwitterion for forming hydrogen-bonds in the solid state; intermolecular hydrogen-bonds are also likely to occur in aqueous solution. It would seem that the hydrogen-bonding of water molecules to GABA would restrict, on purely stereochemical grounds, the range of possible conformations: the effect would be greatest for folded

conformations. In addition, the presence of hydrogen-bonded water molecules might have other, more subtle, effects on the preferred conformation of GABA: these could only be determined by a complete conformational analysis of the 'supermolecule' complex, using, for example, the techniques developed by Pullman (e.g. Pullman and Port, 1974).

6.2.3. Free energy aspects of the conformation of the GABA zwitterion

The discussion so far has been in terms of calculated Internal Energies. As has been pointed out by Farnell, Richards and Gannellin (1974), the prediction of a preferred conformation should strictly make use of Free Energies, otherwise the calculation of relative populations of different rotamers may be in error. Examination of the various conformational energy maps for GABA shows that, particularly for the zwitterion, regions of low energy for extended conformers tend to be broad; those for folded conformers, on the other hand, are much more sharply defined. This is just the situation which would be expected to favour a greater population of the extended modes (i.e. those with wide valleys) than would be expected purely from a comparison of Internal Energies.

To determine whether this effect is significant here an idealised calculation may be made. Two minima, one of area x_1 and of relative depth -4 kcal/mol, and the second of area x_2 and depth -60 kcal/mol will be considered as an approximate model of the CNDO/2 calculated valleys for the fully-extended

and fully-folded conformers of the GABA zwitterion. In general, the area of a minimum is taken as that of the conformational energy surface contained within a particular energy range (e.g. 2 kcal/mol above the local minimum). Here, however, for simplicity, the two minima will be assumed to be of a 'square-well' type i.e. to have sides of infinite slope.

Following Farnell, Richards and Gannellin, a classical Boltzmann distribution partition function may be calculated for each minimum according to

$$Z = \sum_{T_2, T_3} e^{-\{E(T_2, T_3)/R\theta\}} \quad \dots (6.1)$$

where the summation is taken over the whole area of the minimum and $E(T_2, T_3)$ is the calculated Internal Energy at a point defined by the dihedral angles T_2 and T_3 . The partition function is computed by making calculations at grid-points across the minimum. R is Boltzmann's constant and θ the absolute temperature.

The free-energy difference between two minima may then be calculated from

$$\Delta G = -R\theta \log(Z_1/Z_2),$$

where Z_1 and Z_2 are calculated using equation (6.1). A distribution of populations between the conformations is then obtained from

$$\frac{P_1}{P_2} = e^{-\Delta G/R\theta} = \frac{Z_1}{Z_2}.$$

Since for this idealised case, the number of grid points within each minimum is proportional to the area of that minimum, and each grid point within a minimum is at the same energy level then

$$z_1 = C x_1 e^{4/R\theta}$$

$$z_2 = C x_2 e^{60/R\theta}$$

where C is a scaling term.

$$\therefore \frac{z_1}{z_2} = \frac{x_1}{x_2} e^{-56/R\theta}$$

Clearly the magnitude of the exponential term ensures that the deeper minimum remains the preferred conformation, whatever the relative areas of the two regions. This will also be true for the non-idealised (i.e. non-square well type) valleys found on the conformational energy surface for the GABA zwitterion: the energy differences between the fully-folded and fully-extended conformers are so great as to exclude any significant effect due to the shape of the valleys.

Where energy differences are smaller, such as between two extended conformers or if considering the non-zwitterion, the effect of using Free Energies rather than Internal Energies may, however, be important.

6.2.4 The conformation of the GABA non-zwitterion

The discussion so far has centred on the zwitterionic form of GABA. This is the form of the molecule expected to predominate in solution and is thus likely to be the

pharmacologically active agent. For purposes of comparison it is worthwhile, however, briefly noting the data available on the conformation of the non-zwitterion. The data is summarised in Table 6.1 and Table 6.2.

The important features of the results on the non-zwitterion are

- (i) calculated energy differences between local minima (both extended and folded) are relatively small: there is little difference between the results of the CNDO/2 and classical energy methods in this respect.
- (ii) the effect of including coulomb interactions in the classical energy calculations is marginal.
- (iii) in the crystalline state, the GABA non-zwitterionic moiety in GABAHC₇ is in the fully-*trans* conformation. However, the presence of the chlorine atom in the crystal structure is likely to influence this.

6.2.5 Conclusions from the conformational studies of GABA

The results of the conformational studies on GABA lead to the following conclusions.

- (i) The low-energy conformations for the isolated zwitterion are predicted to be fully-folded rotamers. These folded rotamers have considerably lower energies than more extended conformations.
- (ii) Theoretical calculations (Table 6.1) indicate that

minimum-energy extended conformations are approximately of the same energy; also the various minimum energy folded-conformations are within a few kcal/mol of each other.

- (iii) In solution, and in the crystalline state, the predominance of the fully-folded conformers of the zwitterion disappears.
- (iv) The environment of the GABA zwitterion plays an important role in determining the preferred conformation.
- (v) The non-zwitterionic form of GABA does not exhibit any marked preference for a fully-folded conformation.
- (vi) The polarity of the GABA zwitterion is, to a great extent, responsible for the large differences in energy between the fully-folded and the extended conformations.

6.3 THE RESULTS OF THE CONFORMATIONAL STUDIES ON β GP

6.3.1 The results available

The only conformational studies which have been made on β GP are those that have been reported in Chapters 3, 4 and 5; the results from these are tabulated in Table 6.3. (The crystal structure of β GP has also been published elsewhere: Steward, Warner and Clarke, 1974). The absence of results concerning β GP in solution means that any conclusions drawn concerning the preferred conformation of the molecule in the biophase must be tentative.

6.3.2 Summary of the results of conformational studies for β GP

In contrast to the results for GABA, the β GP zwitterion is found as a folded rotamer in the crystalline state. Classical energy calculations including neighbouring molecules (Chapter 5) show that, with the crystal symmetry imposed in the calculations, the more extended conformers are not accessible to the molecule: the inclusion of coulomb energies does not change this situation.

The theoretical studies of the isolated zwitterion do not predict the conformation found in the crystal to be the global minimum on the energy surface. However, there is a local minimum in the region of the crystal conformation: this is calculated to be 2 kcal/mol and 12 kcal/mol above the global minimum on the classical and CNDO/2 energy surfaces respectively. It is interesting that this local minimum for the isolated zwitterion only appears on the classical energy maps if the clearly important coulomb interactions are included.

Method	minima and relative energies. ($T_2, T_3 : \Delta E(\text{kcal/mol})$ ΔE given where available)					
(1) <u>NON-ZWITTERION</u>						
CNDO	355,55 :0	255,70 :6	25,165:7	180,180:8	60,80 :8	180,75 :9
classical : total	60,180:0	180,180:0	300,180:0	60,80 :1	180,80 :1	
classical : total-coulomb	60,180:0	180,180:0	300,180:0	60,80 :2	180,80 :2	
(2) <u>ZWITTERION</u>						
(a) <u>conservative</u>						
CNDO	0,50 :0	275,65 :12	0,180:24	145,40 :47	180,180:49	
classical : total	40,60 :0	300,100:2	300,180:4	60,180:4	180,180:7	
classical:total-coulomb	60,180:0	180,180:0	300,180:0	60,80 :1	180,80 :1	
(b) <u>non-conservative</u>						
classical : total	300,100:0	120,60 :32	180,80 :51			
classical:total-coulomb	300,100:0	120,60 :25	180,80 :47			
crystal conformer	83,254					
(1) <u>NON-ZWITTERION</u>						
CNDO	cg , gg , ct , tt	gg , tg				
classical : total	gt tt	gt , gg	tg			
classical:total-coulomb	gt tt	gt , gg	tg			
(2) <u>ZWITTERION</u>						
(a) <u>conservative</u>						
CNDO	cg , gg , ct , gg , tt					
classical : total	gg , gg , gt	gt , tt				
classical:total-coulomb	gt tt	gt , gg	tg			
(b) <u>non-conservative</u>						
classical : total	gg , gg , tg					
classical:total-coulomb	gg , gg , tg					
crystal conformer	gg					

Table 6.3 Conformational results for β GP. The lower portion of the table gives a qualitative summary of the minima in terms of approximate trans (t), gauche (g), cis (c) positions about T_2 and T_3 . Conformers are listed in order of increasing energy; those of equal energy are not separated by

Table 6.3 Conformational results for β GP. The lower portion of the table gives a qualitative summary of the minima in terms of approximate *trans* (t), *gauche* (g), *cis* (c) positions about T_2 and T_3 . Conformers are listed in order of increasing energy: those of equal energy are not separated by commas.

For the isolated β GP zwitterion, energy differences between extended and folded rotamers are predicted to be considerably less than for GABA. Similarly, the computed magnitude of the classical coulomb energy is smaller, being generally less than 20 kcal/mol for sterically allowed conformers. As with GABA, the effect of the electrostatic interactions would be expected to be reduced in solution, making the appearance of, for example, the "all-*trans*" conformer more likely. The probability of this is increased by the fact that intrinsic energy differences between extended and folded rotamers appear to be less for β GP than for GABA.

The formation of hydrogen bonds must also play an important role in the determination of the solute conformation of β GP: the crystal conformation is certainly stabilised by the presence of one intramolecular, and four intermolecular hydrogen bonds.

Consideration of Free Energies leads to similar conclusions as for GABA: the regions of low energy for folded rotamers of the isolated zwitterion tend to be deeper and steeper than those for the extended conformations. This greater depth is sufficient to ensure that the determination of the preferred conformation in terms of Internal Energies gives a reasonably accurate picture of the true situation, at least as regards 'extended' compared with 'fully-folded' conformers.

6.3.3 Conclusions from the conformational studies of β GP

The salient conclusions that can be drawn concerning the conformation of β GP are:

- (i) All the studies predict folded conformers of the molecule (zwitterion and non-zwitterion, isolated and in the crystalline state) to be of lower energy than the extended forms.
- (ii) The formation of an intramolecular hydrogen bond is likely for some folded conformers.
- (iii) The polarity of the zwitterion is important in producing an energy minimum in the region corresponding to the crystal conformation.

6.4 CONFORMATIONAL RESULTS FOR MUSCIMOL

The three conformational studies of muscimol (Table 6.4) consist of one crystal structure determination (Brehm, Hjeds and Krogsgaard-Larsen, 1972), EHT calculations (Kier and Truitt, 1970), and a set of CNDO/2 results (this work).

For this molecule, there is general agreement between the EHT and CNDO/2 results. Both suggest two minimum energy conformations for the muscimol zwitterion, each with the side chain in the same plane as the ring. Indeed, both agree that the $T_2 = 0^\circ$ minimum is the deeper of the two. Again, both methods suggest only relatively small barriers to rotation away from the minimum energy positions: those predicted by the CNDO/2 method (~ 6 kcal/mol) are somewhat larger than those of the EHT results (< 2 kcal/mol).

In view of the small energy differences between different conformers, it is not surprising that crystal-packing forces can produce a different conformation for the muscimol zwitterion in the solid state from that predicted by the theoretical methods (Table 6.4). Similarly, in the biophase, effects such as the hydrogen-bonding of solvent molecules to the muscimol molecule may well result in a shift away from the minimum energy conformation of the conservative molecule.

Consideration of the CNDO/2 energy surface for the non-zwitterion does not produce any unexpected results. The tendency for a planar arrangement is maintained, though energy differences across the conformational surface are even smaller than for the zwitterion.

<u>method</u>	<u>minima and relative energies</u>	
	(T ₁ , T ₂ : Δ E (kcal/mol). Δ E given where available)	
(1) <u>NON-ZWITTERION</u>		
CNDO	0,0:0	0,180:0
(2) <u>ZWITTERION</u>		
CNDO	80,0:0	0,180:4
EHT	0,180 (value of T ₁ unspecified)	
Crystal conformer (Brhem)	33,59	
	<u>minima</u>	
(1) <u>NON-ZWITTERION</u>		
CNDO	cc,ct	
(2) <u>ZWITTERION</u>		
CNDO	gc,ct	
EHT (Kier)	c,t(value of T ₁ unspecified)	
Crystal conformer (Brhem)	gg	

Table 6.4 Conformational results for muscimol. The lower half of the table gives a qualitative summary of the minima in terms of approximate trans (t), gauche (g) and cis (c) positions of T₁ and T₂. Full references to the results are given in the text

6.5 GENERAL CONCLUSIONS FROM THE CONFORMATIONAL STUDIES

In addition to the detailed discussion of the results for each molecule which has been given above, some points of a more general nature may now be made.

The results reported here illustrate how, in conformational analysis, it is essential to make use of as many different methods of study as is practicable. This is the only way to obtain an overall view of the conformational adaptability of a molecule, and how the conformation of a molecule is affected by the nature of the environment.

Notwithstanding the need for using a variety of methods, computational procedures must be chosen with some care. Thus, for example, since the results on the GABA and β GP isolated zwitterions show that the polarity of the molecules is important in determining their minimum energy conformations, methods which do not deal satisfactorily with polar molecules are not suitable tools for studying these drugs. The EHT method, would in this case, appear to be found lacking by comparison with the CNDO/2 and PCILO procedures.

It is worth reiterating that the results tend to stress the flexibility of the molecules studied. For example, the theoretical studies on GABA predict a local minimum only a few kcal/mol above the global minimum for the conservative zwitterion.

Finally, it has been shown how drastically the 'preferred' conformation may change as a result of outside influences.

CHAPTER 7

Structural Aspects of the Pharmacological Activity of GABA, its Agonists, and competitive Antagonist

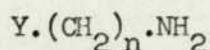
7.1 INTRODUCTION

In this chapter some of the main results reported earlier in this work are discussed in the context of the physical action of drugs at CNS synapses. The activity of GABA, the agonist activity of β GP and muscimol, and the antagonist action of bicuculline (BIC) are examined in the light of their electronic structure and conformational flexibility.

7.2 STRUCTURAL REQUIREMENTS FOR ACTIVITY AS A GABA AGONIST

7.2.1 Early ideas

The attributes of chemical structure necessary for action as a GABA agonist have been set out by Curtis and Watkins (1965). They suggested that the main features of such drugs could be summarised by



where Y is CO_2H , SO_2H or SO_3H and n is between 1 and 5.

Replacement of the $-NH_2$ group by a guanidino group also produces an active molecule, if n is between 1 and 4 inclusive. It must be recognised that since the publication of the Curtis and Watkins (1965) paper further agonists (such as ATA and muscimol) have been found which do not fit into the above scheme. Kier and George (1970) put forward a generalisation in terms of electronic structure, rather than homologous series. They suggested that for agonist action "2 highly-charged regions, of opposite sign, separated by at least $5\text{\AA}$, but more likely approximately $6\text{\AA}$ " were required. They assumed that, for the GABA zwitterion, the charged regions were centred on the oxygen and nitrogen atoms: a more-or-less extended conformer of the molecule would then be required to meet the specification. Their evidence for the existence of such a conformation in the biophase is discussed later in this section.

Curtis and co-workers have also favoured an extended conformation of GABA as the active agent (eg Beart, Curtis and Johnston, 1971; Curtis, Duggan, Felix and Johnston, 1970). A brief summary of some of the important results which led the various research groups to focus attention on the extended conformations of GABA is given below, but it is worth pointing out at this stage that most suggestions as to the form of the

GABA pharmacophore have been in terms of a single inter-charge distance: whether such a simple parameter can adequately account for the drug-receptor interaction, and its specificity must be open to some doubt. This is further discussed in section 7.2.2.

ATA, a potent GABA agonist, is a relatively rigid molecule and the distance between the two charged-regions cannot differ greatly from $6\text{\AA}$. This would appear to give at least some indication of the size of the GABA pharmacophore. Another potent agonist, muscimol, was also studied by Kier and Truitt (1970). They predicted the distance between the onium nitrogen atom and the exocyclic oxygen atom (which was taken to be the negatively charged region of the molecule) in the preferred conformation to be $\sim 5.8\text{\AA}$: this distance could vary with conformation. Kier's EHT calculations on GABA (Kier and George, 1970) predicted a fully-extended conformer to be preferred, with N - O distances satisfactorily in the range $5-6\text{\AA}$. Finally, both Curtis (Curtis, Duggan, Felix and Johnston, 1970) and Kier (Kier and George, 1973) have claimed support for their ideas from studies of bicuculline, the competitive GABA antagonist (Curtis, Duggan, Felix and Johnston, 1970). A further discussion of the action of bicuculline is reserved for later (section 7.3).

The possible importance of a slightly folded conformer of GABA has been pointed out (Warner, Player and Steward, 1973). Van Gelder (1971) has suggested that a much more folded conformer of GABA may be significant in ion transport mechanisms.

7.2.2 Recent suggestions

It is clear that ideas such as those mentioned in section 7.2.1, which are generally expressed in terms of a single parameter characterising the GABA pharmacophore, cannot tell the whole story of drug-receptor interactions, the mechanism of competitive antagonism, and the agonist activity of relatively short-chain molecules such as β -alanine.

In efforts to produce a more sophisticated model, the concept of flexibility has attracted considerable attention. Kier (1972) suggested that a transmitter molecule might approach a receptor in an initial conformation, and then combine with the receptor in a different conformation.

The idea of the possible importance of two conformations has been developed further by Warner, Player and Steward (1973). Examining the conformational data then available they noted that GABA agonists exhibited several possible low-energy conformations and that two possible geometries of charge distributions in particular were common to each agonist (with the exception of ATA). Further, inspection of the molecular model of bicuculline suggested that this competitive antagonist molecule could only match one of these possible geometries. It was therefore surmised that one of these charge distributions could represent a "recognition" mode, in which form a drug molecule would combine with a receptor. The resulting complex would then undergo a conformational change, with the drug molecule taking up its "action" conformation. We thought in terms of the conformational change of the complex initiating the critical change in permeability at the post-synaptic membrane. In this scheme the antagonist activity of bicuculline could be neatly explained by noting that it appeared that this molecule could only match the "recognition" mode of the

agonist molecule (Steward, Player, Quilliam, Brown and Pringle, 1971; Warner, Player and Steward, 1973). Bicuculline might therefore be able to occupy a GABA receptor, but would then restrain the receptor from taking up the necessary "action" conformation. Thus permeability changes would be blocked.

In the above theory no explicit explanation of the activity of short-chain molecules was given, other than to imagine a certain tolerance in the allowable inter-charge distances for "recognition".

A major difficulty with the above two-conformation scheme was the potent agonist activity of ATA, a molecule with essentially only one possible inter-charge distance. Nonetheless, the possibility that a conformational change could be of importance at the receptor remains an enticing concept. The mathematical theories of drug-receptor interactions, such as allosteric theory, usually invoke some form of change of state at the receptor; also the "credentials" of conformational transformations in some biological systems have been well-established, eg the oxygen haemoglobin interaction. Perhaps it is as well to recall here that the ATA molecule is flexible in that it contains single-bonds: it is only the charge separation which remains essentially invariant. It is interesting to speculate that this is an indication that some parameter, more subtle than a crude single inter-charge separation is involved: for the present, however, this must remain conjecture.

Steward and Clarke (1975) have explored the consequences of adopting a model in which the receptor is "flexible" in the sense that molecules with charge separations differing from the ideal can still be "recognised" for complexing, though with a lower

probability of success. The relatively small, though significant, agonist action of, for example, β -alanine, can then be readily accounted for. This hypothesis is a departure from the idea that a GABA agonist must be able to present to the receptor one definite inter-charge separation (and change to a second in the two-conformation theory) to elicit a response.

It is now possible to review some of the ideas discussed above in the light of the results reported earlier.

7.2.3 The electronic structure of GABA and its agonists BGP and muscimol

In this discussion, the CNDO/2 calculated NAP's will be regarded as giving an indication of the electronic structure of the drugs studied. The results reported in Chapter 4 show common features as far as electronic structure is concerned. Thus the (classically) expected negative nature of a -COO^- group is apparent and similarly the -NH_3^+ group has the anticipated positive charge, though it is distributed among the three hydrogen atoms rather than resident on the nitrogen atom. Other CNDO/2 results obtained in this laboratory, but not reported in detail here (on ATA: Warner, Borthwick and Steward, 1974; on β -hydroxy GABA: Borthwick and Steward, 1974; and on α -fluoro GABA: Borthwick, Warner and Steward, 1974) show that this pattern of NAP's on carboxyl and amino groups is quite general. It would seem, therefore, that, for the sake of consistency, the centroid of the three amino hydrogen atoms would be a suitable place from which to make inter-charge measurements (assuming such a simple parameter were significant):

of course, distances so-measured differ but little from those obtained by assuming the nitrogen atom to be the centre of the +ve region.

The NAP's of the $N = C-O$ group of muscimol show a somewhat similar - + - nature to the carboxyl group of GABA (table 7.1). This occurs regardless of the different chemical natures of the two groups.

The CNDO/2 calculations on β GP indicate a positive charge centre for the zwitterion (Chapter 4): here the region with the greatest +ve character is apparently centred on the carbon atom of the guanidino group.

Therefore, the semiempirical quantum mechanical calculations tend to confirm the general attributes expected of the electronic structure of a GABA agonist.

7.2.4 The importance of conformation with respect to activity of GABA agonists

The question now arises as to how the charged regions of drug molecules are arranged in space. In general this will of course be variable and depend upon the conformation adopted by the molecule in its environment. Probably the most important conclusion to be drawn from the results currently to hand (Chapter 6), is that it is impossible to be dogmatic about what is the preferred conformation of the GABA zwitterion in the biophase. The results do not point conclusively to a single preferred conformation, but rather, if anything, tend to suggest that a spectrum of modes is likely. Thus, even for the isolated GABA zwitterion, for which energy differences

between extended and folded conformations are large, there are two other minima within a few kcal/mol of the global minimum energy conformation. It would seem, therefore, an oversimplification to consider just one "preferred" conformation, whether this be fully-extended, extended, or even fully-folded. For example, the so-far available results by no means exclude the possibility of the occurrence of the conformers thought to be important by Van Gelder. Again, CNDO/2 calculations on the isolated zwitterion predict the difference in energy between the "all-trans" and the "crystal" conformers to be only ~ 4 kcal/mol.

This support for a spectrum of conformational modes of GABA being present in the biophase is in accordance with the assumed basis of the calculations of Steward and Clarke (1975).

In the light of Chapter 6, it would seem fortuitous that Kier's EHT work on the isolated GABA zwitterion (which predicted a fully-extended conformation) supported the hypothesis of a pharmacophore of 5-6 $\text{\AA}$ in size. However, as demonstrated in the previous chapter, extended conformers of GABA become likely once the effects of the environment are taken into account. Hence it would seem quite feasible that the GABA molecule in solution could readily attain a conformation with a charge separation at least somewhat similar to that of ATA.

Muscimol

If the N=C-O group of muscimol is considered as the pharmacological equivalent of a carboxyl group (for which the calculated NAP's give some support), then it is logical to

consider the mid-point of the N to O line as a suitable point from which to make measurements. Doing so, and measuring to the centroid of the three amino hydrogen atoms gives a distance $\sim 5.0\text{\AA}$. Because of the geometry of the molecule, this distance is independent of conformation. This, and the relatively small barriers to rotation about the single-bonds of the side chain (Chapter 6) may be important pharmacologically: considered in terms of a simple model, all molecules of muscimol would occur with the same charge separation, and the fairly high potency of the molecule could be accounted for.

β GP

An explanation of the pharmacological activity of β GP cannot be so straightforward as that given for muscimol. Essentially, this is because β GP is a much more flexible molecule than muscimol, and fewer results are available than for GABA: the discussion must therefore be tentative.

A point which should be made immediately is that β GP has a longer chain-length than GABA. It would therefore appear, at first sight, difficult for a fully-extended β GP molecule to effectively match a receptor the size of a fully-extended GABA. Three immediate explanations of the GABA agonist activity of β GP seem possible.

Firstly, the calculated NAP's for the β GP zwitterion indicate that the region of the molecule with the major +ve nature is the carbon atom of the guanidino group. The hydrogen atoms of the $-\text{NH}_2$ groups are also +ve in character, but not as significantly. Perhaps what might be termed the 'pharmacologically

effective length' of β GP is somewhat shorter than the actual molecular chain-length.

Secondly, as noted in Chapter 6, all the available conformational studies of the β GP zwitterion indicate folded conformers of the molecule as global minima. It is possible that a folded conformer of β GP is the active agent in the biophase. However, as clearly demonstrated by the work on GABA, it is hazardous to assume that the results of β GP, none of which relate to the solute phase, can give a reliable picture of the situation in solution.

A third possible explanation is: accepting that the GABA receptor is flexible, a fully-extended β GP molecule might be able to meet the requirements for pharmacological activity, even if the critical interchange distance were greater than for GABA.

It is, of course, conceivable that a combination of the factors discussed above could be relevant: thus, for example, if the +ve nature of the $-\text{NH}_2$ groups is important, a suitable folding of the molecule could present to the receptor an acceptable positive-negative charge separation.

GABA (zwitterion)		MUSCIMOL (zwitterion)	
atom	z - NAP	atom	z - NAP
C4	0.38	C3	0.30
O5	-0.53	O8	-0.52
O6	-0.57	N2	-0.29

TABLE 7.1 Comparison of some NAP's for GABA and Muscimol
(z's are atomic numbers)

GABA (zwitterion)		Steward <u>et al</u> HBIC		Curtis <u>et al</u> HBIC	
atom	z - NAP	atom	z - NAP	atom	z - NAP
C3	-0.09	C27	-0.08	O24	-0.26
C4	0.38	C25	0.40	C25	0.40
O5	-0.53	O24	-0.26	C27	-0.08
O6	-0.57	O26	-0.28	O26	-0.28
N7	-0.02	N3	0.06	N3	0.06
H14	0.21	H45	0.16	H45	0.16
H15	0.21				
H16	0.21				

TABLE 7.2 Comparison of some NAP's for GABA and protonated
bicuculline (HBIC). The data is ordered as
suggested (i) by Steward et al and (ii) by Curtis et al
z's are atomic numbers.

7.3 THE ACTION OF BICUCULLINE AS A GABA ANTAGONIST

The possible physical basis for the activity of bicuculline as a specific competitive antagonist of GABA has been the subject of studies by many workers. It has been suggested (Curtis, Duggan, Felix, Johnston, 1970) that the atoms N3, C27-C25=O26 of bicuculline (with the molecule in a suitable conformation with respect to the central single bond) could correspond, pharmacologically, to the nitrogen atom and carboxyl group of GABA (fig. 7.1). An alternative model (Steward, Player, Quilliam, Brown, Pringle, 1971) was that a different conformer of bicuculline could result in a geometrical match between N3, O26=C25-O24 and the nitrogen atom and carboxyl group of GABA (fig. 7.1). This second idea would seem attractive in that a correspondence of atomic species is obtained. The calculated NAP's (Chapter 4) for the two possibilities are given in table 7.2, together with the relevant NAP's for the GABA zwitterion. For completeness, the NAP's of the added H45 of the bicuculline cation and of the amino hydrogen atoms for GABA are included in table 7.2. It can be seen that the results tend to favour the correspondence suggested by Steward et al rather than that put forward by Curtis et al.

In a recent paper Gilardi (1973) has pointed out that the above two studies were based on molecular models inconsistent with the structure later determined by X-ray analysis (Gilardi, 1973; Gorinsky and Moss, 1973). However, the CNDO/2 studies were made taking the crystal structure as the starting model and showed the correspondence with the NAP's of GABA, in agreement with the ideas of Steward et al. Further, an examination of a molecular model of bicuculline in the

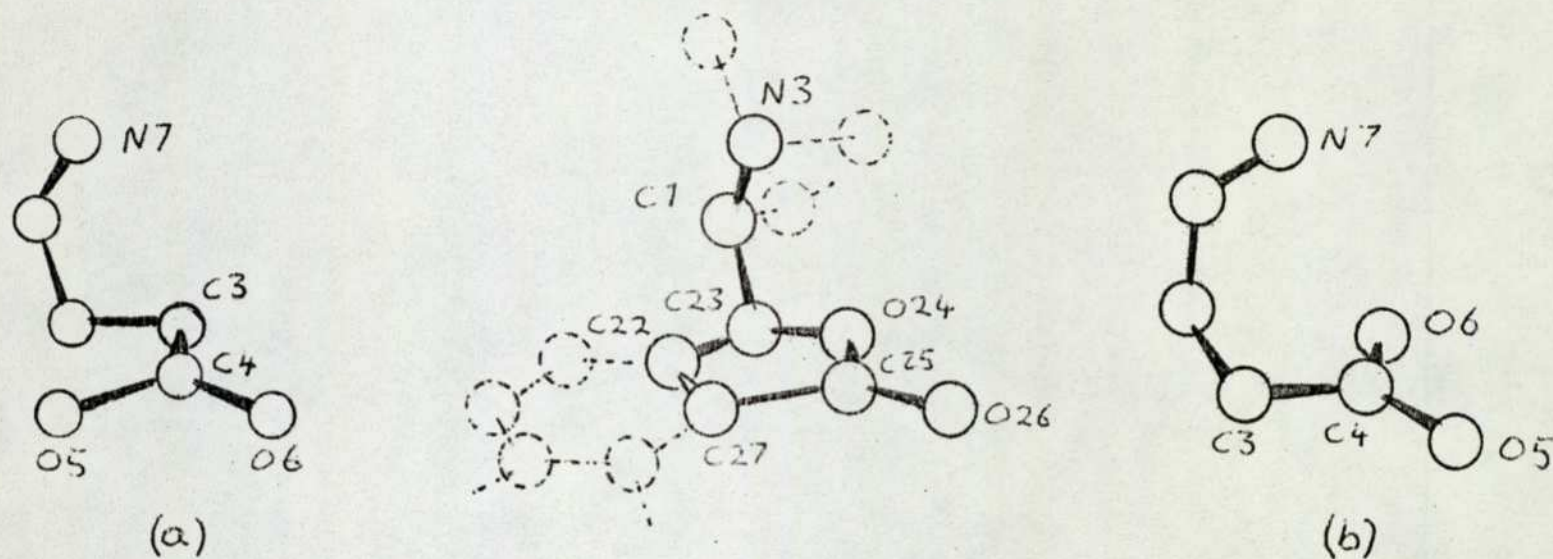


Fig. 7.1 Bicuculline (centre) as compared with GABA
 (a) by Curtis, Duggan, Felix and Johnston, 1970 and
 (b) by Steward, Player, Quilliam, Brown and Pringle, 1971.

configuration found by Gilardi showed that the molecule could still only match one of the inter-charge distances required for the two-conformation theory (section 7.2.2). The previous discussions would therefore still appear to be valid.

Gilardi (1973) has also noted that the protonated cation of bicuculline is probably the pharmacologically active agent. The CNDO/2 calculated NAP's discussed in Chapter 4 certainly suggest that no large +ve character develops in the region of the nitrogen atom unless the added proton is included. Thus, if a complete match of charge characteristics is required for specific antagonism of GABA, it would seem that uncharged bicuculline should be inactive. Further, as deduced by Gilardi, steric hindrances resulting from the addition of the proton on the nitrogen atom would then disallow the crystal conformer of BIC as the active rotamer.

Kier and George (1973) studied protonated bicuculline using the EHT method. They concluded that the preferred conformation was one in which the distance from the ~~onium~~ group to the carboxyl oxygen atom was about 5.6⁸Å.

It is feasible that only certain features of BIC need match the GABA receptor: BIC might then be able to occupy the receptor and render it inactive. The results of Collins and Hill (1974) tend, however, to suggest that the relative positions of structural features in both portions of the bicuculline molecule are important. Collins and Hill produced an isomer of bicuculline by inversion at C23 and C1: it was not active as a GABA antagonist.

The same results would indicate that the large +ve nature of the NAP of C17 (mentioned in Chapter 4) is not significant

pharmacologically, since the position of this atom relative to the O26=C25-O24 groups (assumed pharmacologically equivalent to carboxyl group) is independent of the configuration of the molecule about C1 and C23.

The other atom with a large +ve NAP is C11: this atom is, however, rather distant from the O26=C25-O24 group, and is therefore less likely to be significant in the mechanism of competitive antagonism.

The evidence available therefore suggests that a match of more than one feature between bicuculline and the GABA receptor is necessary, and that a suitable positive region only arises when the protonated bicuculline cation is considered.

7.4 SUMMARY

The main points made in this chapter are listed below:

- (i) A two-conformation theory can explain some aspects of the pharmacological activity of GABA, some of its agonists, and bicuculline (the competitive antagonist). However, some anomalies exist, in particular the agonist action of ATA.
- (ii) The CNDO/2 calculations provide some details of the features of electronic structure that may well prove to be necessary for action as a GABA agonist. The fine details of the results indicate that the charge centres may not be precisely where previously assumed.
- (iii) Some similarities between the calculated NAP's of GABA and its agonists and bicuculline are found. The calculations also tend to favour the comparison of the structures of GABA and bicuculline made by Steward, Player, Quilliam, Brown and Pringle (1971), rather than that of Curtis, Duggan, Felix and Johnston (1970).
- (iv) Conformational studies made so far cannot reliably characterise the conformational modes of GABA likely to be present in the biophase. It would seem, however, that a spectrum of modes is likely to be present, and that this is important pharmacologically.

CHAPTER 8

Concluding Remarks

In conclusion, some brief comments of a general nature would seem to be in order.

As has been said, at the moment it does not seem possible to reliably determine which conformational modes of molecules such as GABA may be present in the biophase. However, a great deal of work continues to be done in this area, and developments in two particular methods, theoretical calculations and NMR, seem promising.

Theoretical calculations are becoming more sophisticated and it is probable that some combination of the "super molecule" and "continuum" approaches may give useful results for molecules in aqueous solution. It should be said that the computational demands involved in such a synthesis are large. Also there is a danger that the resulting method may contain elements of problems inherited from both the parent schemes: it will still presumably be necessary to determine reasonable positions for the water molecules of the first hydration shell, and some decision will have to be made concerning the form of the classical Onsager-type cavity. Assuming these difficulties can be successfully overcome, it may prove possible to achieve some measure of agreement between theoretical calculations and improved experimental results in the form of NMR studies.

However, even after having reached such a desirable state of affairs, the pharmacological interpretation of the results may not be straightforward. If the presence in solution of a spectrum of conformational modes for a molecule such as GABA is confirmed, it will still be necessary to decide which of the conformers is, or are, biologically important. Also it must be said that the most

important part of the biophase is likely to be that around the receptor, and the characteristics of this region may differ greatly from those of aqueous solution, so far as drug molecules are concerned.

The ultimate goal must, of course, be to study events actually at the receptor. Some form of "observation" of the interaction between drug and receptor is now urgently required: Electron microscopic techniques may or may not eventually make this possible, although first, of course, a receptor must be satisfactorily isolated in a form suitable for examination. Another useful step would be an X-ray crystallographic study of some "model" receptor eg a synthesised simple protein-agonist complex.

It may also eventually prove feasible to theoretically study molecules in bound states. The calculations would, of necessity, be extremely lengthy and gross approximations would inevitably again be required, at least initially: however, with the techniques of large-scale computing becoming more widely appreciated, and experience being gained in work such as the solvent-effect calculations, the idea need not be too lightly dismissed.

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APPENDIX A

Specification of the Conformation
of Molecules

APPENDIX A

SPECIFICATION OF THE CONFORMATION OF MOLECULES

The convention adopted in this work for conformational angles is: the conformational angle ABCD defined by four atoms, A, B, C, D is that angle through which CD must be moved to be eclipsed by AB, when viewed along BC. The anticlockwise direction is taken as positive. The conformational angles in the molecules of interest here may be conveniently designated by:

for GABA (Fig.A1)

$$\text{H14 N7 C1 C2} = T_1$$

$$\text{N7 C1 C2 C3} = T_2$$

$$\text{C1 C2 C3 C4} = T_3$$

$$\text{C2 C3 C4 O5} = T_4$$

for β GP (Fig.A2)

$$\text{O8 C1 C2 C3} = T_1$$

$$\text{C1 C2 C3 N4} = T_2$$

$$\text{C2 C3 N4 C5} = T_3$$

$$\text{C3 N4 C5 N6} = T_4$$

for muscimol (Fig.A.1)

$$\text{H9 N7 C6 C5} = T_1$$

$$\text{N7 C6 C5 O1} = T_2$$

for BIC (Fig.A.2)

$$\text{N3 C1 C23 O24} = T_1$$

Thus, for example, using the convention given, the "all-*trans*" conformation of GABA is that for which $T_1 = T_2 = T_3 = T_4 = 180^\circ$.

In discussing the minimum energy conformations of GABA and β GP, it is found convenient to use the terms "fully-folded", "fully-extended", "more extended" and "folded". These terms are used with the following significance.

fully-folded : with $T_2 < 100^\circ$ and $T_3 > 260^\circ$
or $T_3 < 100^\circ$ and $T_2 > 260^\circ$

fully-extended : with both T_2 and T_3 in the region of *trans* .

more-extended : conformers other than "fully-folded".

folded : conformers other than "fully-extended".

In all cases, the exact specification of the angles is given in Tables of values.

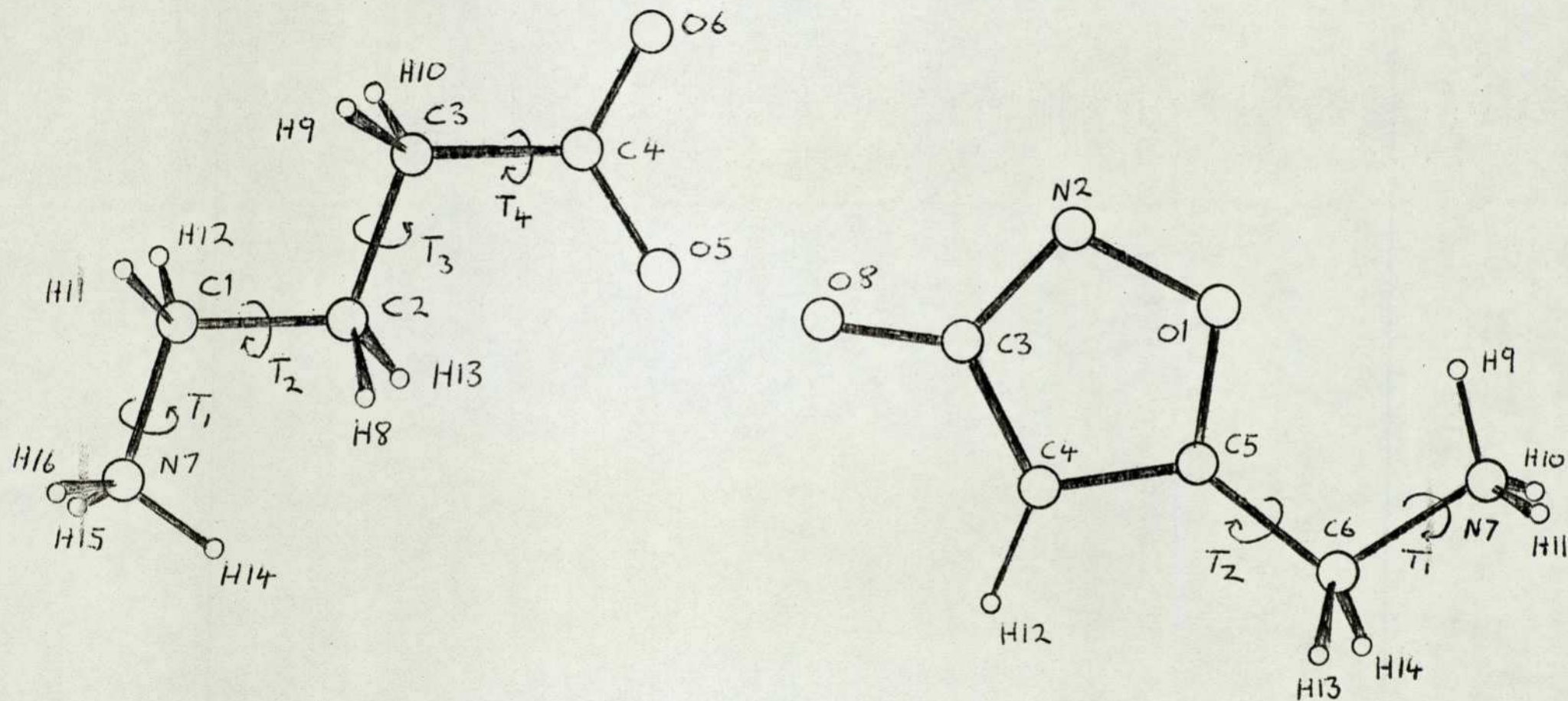
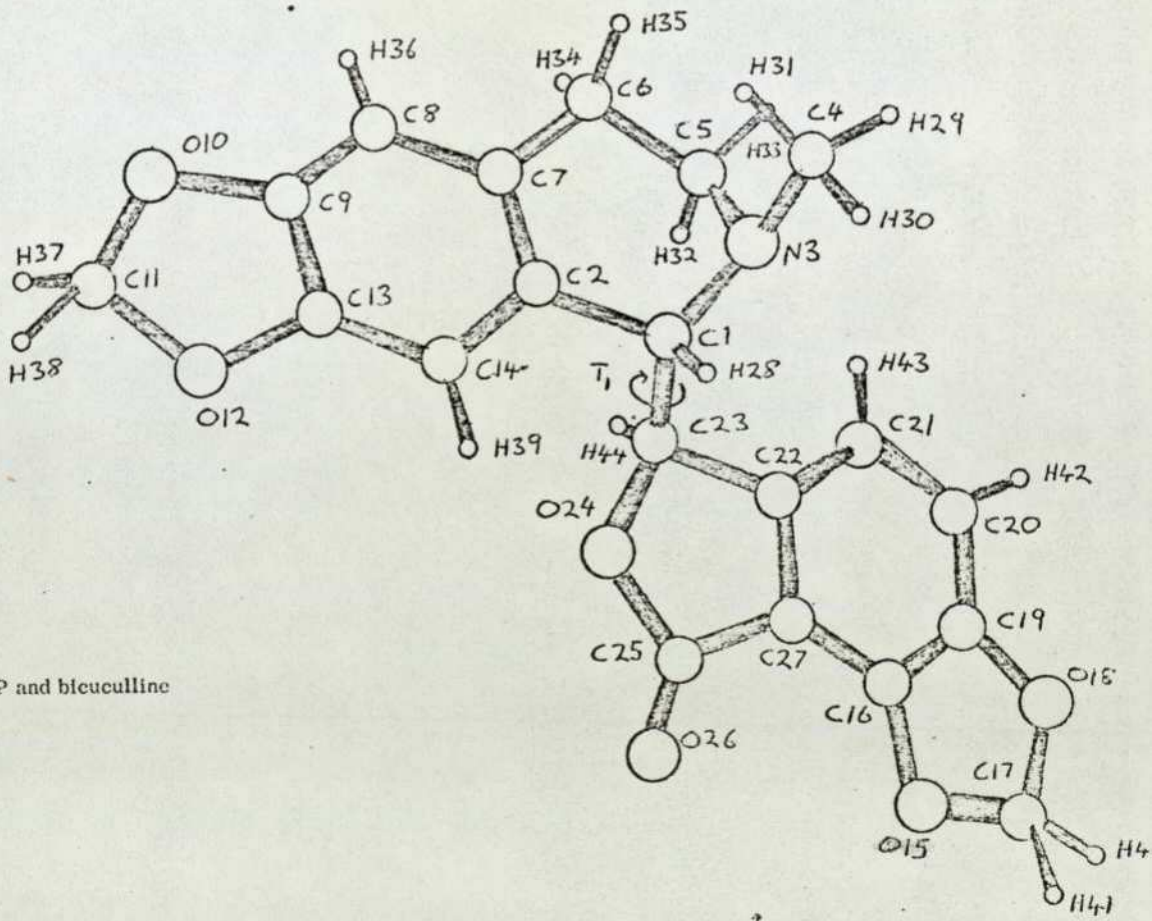


Fig. A.1 GABA and muscimol



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APPENDIX B

The Use of the X-RAY 70 System

APPENDIX B

THE USE OF THE X-RAY 70 SYSTEM

The X-ray 70 system (Stewart, Kundell and Baldwin, 1970) consists of a large number of inter-related programs written in FORTRAN. The system is designed to provide a complete set of software facilities for X-ray structural analysis from data collection to the final stages of refinement. It has recently been implemented on the ULCC CDC 7600 and was used in the structural studies described in Chapter 3. In this appendix a brief description of the use of the system is given.

An X-ray structure analysis proceeds step by step through the raw data stage, the procedure for scaling the data and obtaining temperature parameters, the production of an initial model for the structure, to the final refinement of the atomic parameters. Consequently X-RAY 70 is designed as a large number of subprograms, each of which carries out a specific task in the process of structure solution. The link between the various programs is the Binary Data File (BDF). As each routine is used it updates the BDF and hence, as the analysis proceeds, the data base holds more and more information on the molecule being studied.

During each single computer-run the BDF is saved on disc. If, as is normally the case, at the end of a run the analysis has not been completed, the contents of the BDF may be transferred to magnetic tape for later use. The retention of the BDF in this manner ensures that subsequent runs are able to reference the most recent version of the BDF, without the need to repeat previous sections of the analysis.

A complete list of all the elements of X-RAY 70 will not be given

here. Instead, Fig.B.1 gives a flow-chart of the main points in a typical X-ray structure determination and also shows the subprograms of the X-RAY system used. These subprograms are listed below, with their principal functions.

1. DATRDN - Treats the raw intensity data. Carries out Lorentz and polarisation corrections and produces values for the relative structure factors.
2. DATFIX - Produces initial estimates for scale factors and the overall temperature factor. A group scaling factor is obtained for each separate subset of data. The program then calculates the normalised structure factors and provides a summary of their statistical properties.
3. SIGMA2 - Searches the list of reflections to find those groups of three whose indices satisfy the conditions necessary for the application of the symbolic addition procedure of Karle and Karle. An ordered list of relationships is produced.
4. PHASE - Takes the output of SIGMA2 and produces, for centrosymmetric crystals, an initial set of phases and estimates of the probability that they are correct.
5. FOURR - Carries out Fourier syntheses. A variety of coefficients may be used: e.g. normalised structure factors, observed structure factors, or $|F_{\text{obs}}|^2$ (for a Patterson synthesis).

6. LOADAT - Loads into the BDF data such as; atomic coordinates; temperature factors; scale factors.
7. BONDLA - Calculates bond-lengths and bond-angles for the stored molecular model.
8. FC - Computes and lists observed and calculated structure factors. Calculates the R-factor.
9. CRYLSQ - Carrys out least-squares refinement. Options include: full-matrix; block diagonal; all conventional temperature factor modes.
10. LSQPL - Calculates geometrical properties of the stored molecular structure.

Of the subprograms listed above, those with the longest run-times were SIGMA.2, PHASE and CRYLSQ. Even these routines were not excessively expensive in computer time: most execution times were < 60 seconds. However, many of the subprograms in X-RAY 70 were found to produce large amounts of printed output much of which was not required. This resulted in a loss of priority for the job in the work-queue for the computer. A convenient way to ameliorate the situation was found. Within X-RAY 70 a mechanism exists for changing the disposition of output to peripheral devices. Using this it was found possible to divert data directed to the line printer to disc storage. This unwanted output was then lost at the end of the job. In this way a rapid turn-round of jobs was obtained.

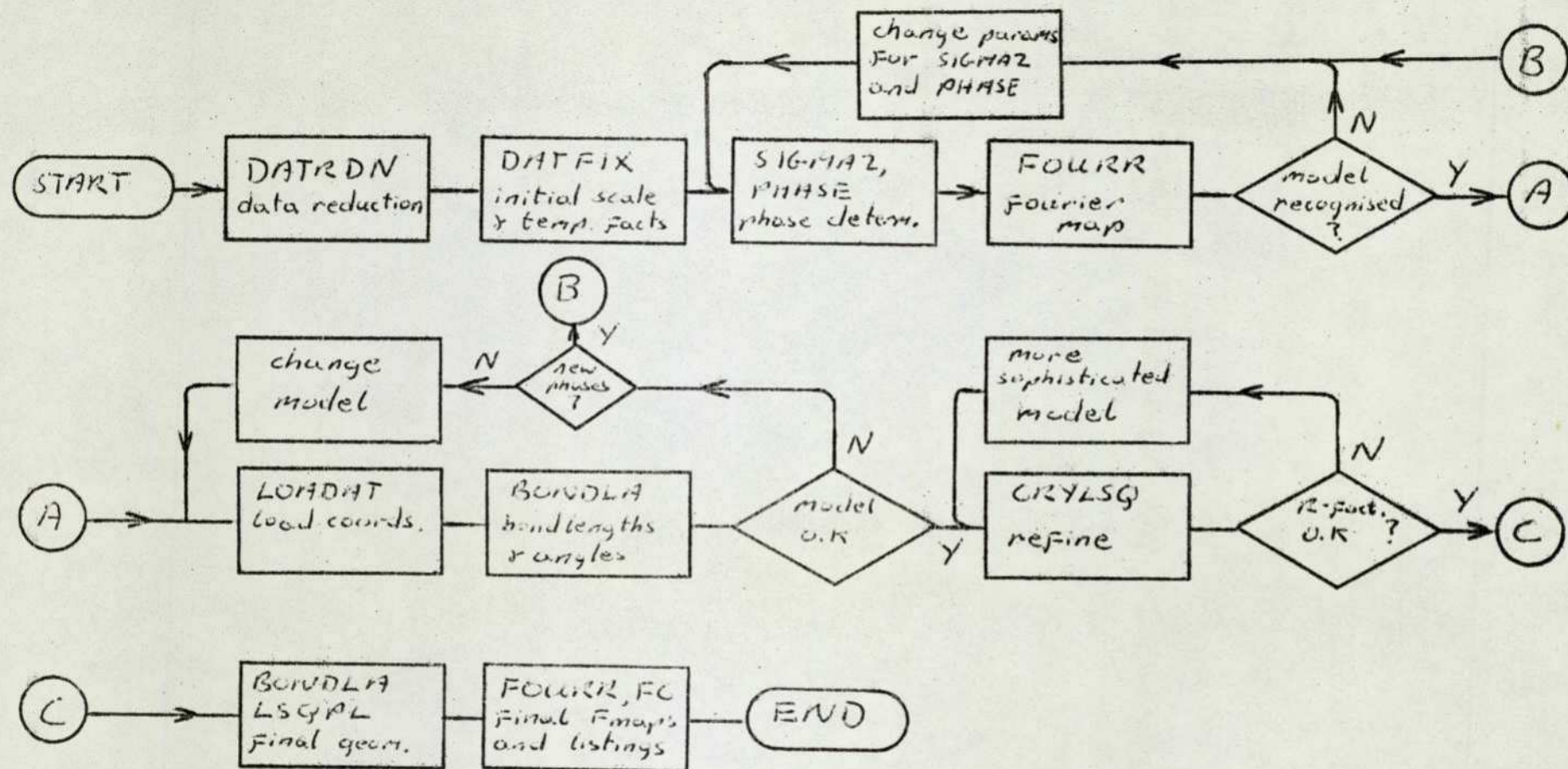


Fig. B.1 Flow chart showing the main points of a crystal structure determination

APPENDIX C

Structure Factor Tables for GABA.HCl

H	K	L	F OBS	F CALC
2	0	0	22.71	26.53
2	0	-2	18.27	13.55
3	0	-1	29.53	32.73
2	0	-1	28.49	36.80
3	0	0	4.10	2.96
4	0	0	21.72	18.43
5	0	0	23.97	23.33
0	0	5	4.47	3.92
1	0	5	17.01	16.97
2	0	5	2.33	2.54
3	0	5	6.80	5.60
4	0	5	4.15	3.37
5	0	5	3.56	3.04
0	0	4	2.30	2.36
1	0	4	4.99	3.96
2	0	4	3.66	4.35
3	0	4	1.45	1.56
4	0	4	11.58	10.09
5	0	4	6.19	5.52
6	0	4	6.90	5.57
7	0	4	10.32	8.39
0	0	3	17.68	16.34
1	0	3	10.39	8.87
2	0	3	1.38	.63
3	0	3	26.51	24.35
4	0	3	20.83	19.57
5	0	3	1.73	1.37
6	0	3	3.32	3.11
7	0	3	4.14	2.90
8	0	3	1.86	1.32
0	0	2	32.90	33.19
1	0	2	32.65	33.74
3	0	2	2.60	2.15
5	0	2	11.03	10.59
6	0	2	5.55	6.58
7	0	2	24.96	22.88
8	0	2	3.59	3.74
0	0	1	17.92	20.24
4	0	1	26.66	27.55
5	0	1	9.70	10.06
6	0	1	11.33	10.76
7	0	1	10.24	10.42
8	0	1	11.65	11.11
1	0	-1	32.36	33.18
3	0	-1	26.56	28.10
5	0	-1	13.05	14.51
6	0	-1	7.46	8.16
7	0	-1	4.33	5.32
8	0	-1	3.89	3.74
1	0	-2	4.47	3.01
2	0	-2	17.28	17.90
3	0	-2	17.70	18.09
4	0	-2	.32	2.60
5	0	-2	14.25	14.54

H	K	L	F OBS	F CALC
6	0	-2	1.34	1.46
1	0	-3	6.14	6.03
2	0	-3	4.97	5.08
3	0	-3	5.87	5.80
7	0	-2	.88	1.67
4	0	-3	5.07	6.14
5	0	-3	6.23	7.35
6	0	-3	4.95	6.33
1	0	-4	14.17	14.30
2	0	-4	5.98	5.45
3	0	-4	10.17	9.93
4	0	-4	.50	1.15
5	0	-4	4.24	4.25
1	0	-5	7.67	7.07
2	0	-5	2.68	3.14
3	0	-5	9.90	10.33
4	1	0	.94	4.93
3	1	0	18.24	20.34
0	1	2	18.72	17.19
0	1	1	15.65	11.70
8	1	-1	7.82	8.20
7	1	-1	5.71	6.56
6	1	-1	3.93	5.11
5	1	-1	13.80	13.44
4	1	-1	6.03	3.26
3	1	-1	6.40	7.34
1	1	-1	2.28	3.08
1	1	-1	45.51	48.54
2	1	-1	25.71	26.42
3	1	-1	2.11	4.96
4	1	-1	1.04	.66
5	1	-1	17.21	16.59
7	1	-1	8.84	9.99
8	1	-1	5.30	6.73
8	1	-2	3.35	2.71
7	1	-2	16.37	13.82
6	1	-2	6.79	7.49
5	1	-2	5.47	4.82
4	1	-2	19.22	18.89
3	1	-2	10.76	9.28
2	1	-2	41.20	41.48
1	1	-2	16.35	15.51
1	1	-2	4.40	5.19
2	1	-2	9.25	7.18
3	1	-2	14.35	14.80
4	1	-2	4.82	4.95
5	1	-2	16.83	19.12
6	1	-2	5.39	7.53
7	1	-2	9.67	11.63
8	1	-3	7.57	6.77
7	1	-3	.85	.64
6	1	-3	19.67	18.02
5	1	-3	10.73	9.90
4	1	-3	14.33	13.45

H	K	L	F OBS	F CALC	
3	1	-3	1.68	1.12	
2	1	-3	15.08	13.75	
1	1	-3	8.61	7.22	
0	1	-3	12.46	11.62	
-1	1	-3	27.98	27.11	
-2	1	-3	7.16	8.23	
-3	1	-3	15.89	16.55	
-4	1	-3	7.08	7.66	
-5	1	-3	12.75	14.89	
-6	1	-3	11.05	11.64	
7	1	-4	10.38	7.47	
6	1	-4	.83	1.52	
4	1	-4	4.11	3.68	
3	1	-4	7.44	6.17	
2	1	-4	30.17	29.41	
1	1	-4	9.52	8.02	
0	1	-4	10.16	9.09	
-1	1	-4	9.82	10.41	
-2	1	-4	3.26	5.24	
-3	1	-4	5.42	6.81	
-4	1	-4	8.00	8.88	
-5	1	-4	3.84	4.94	
5	1	-5	10.25	8.31	
4	1	-5	12.19	10.27	
3	1	-5	5.08	3.94	
2	1	-5	11.40	10.65	
1	1	-5	9.67	9.13	
5	1	-4	1.23	1.31	
6	1	-1	1.22	1.15	
0	1	-5	3.80	3.90	
-1	1	-5	7.27	6.50	
-2	1	-5	8.44	9.58	
-3	1	-5	8.34	9.66	
-1	2	1	.84	3.02	
2	2	-2	11.35	10.78	
-2	2	0	18.69	20.71	
-5	2	0	17.72	19.26	
6	2	-1	10.86	9.82	
7	2	-1	9.04	9.07	
6	2	-1	9.28	9.21	
5	2	-1	9.12	9.50	
4	2	-1	22.91	22.65	
3	2	-1	24.18	25.91	
2	2	-5	1.74	2.04	
8	2	-3	1.71	1.43	
-6	2	-2	1.68	1.25	
-4	2	-2	1.41	2.86	
0	2	-1	15.47	15.65	
-2	2	-1	10.88	9.64	
-3	2	-1	23.20	22.66	
-4	2	-1	15.45	16.65	
-5	2	-1	11.37	12.56	
-6	2	-1	6.56	7.37	
-7	2	-1	3.65	4.83	

H	K	L	F OBS	F CALC
-8	2	-1	3.24	3.42
8	2	-2	3.78	3.75
7	2	-2	23.24	20.25
6	2	-2	6.44	5.87
5	2	-2	10.11	9.40
4	2	-2	2.59	2.69
3	2	-2	1.77	1.63
1	2	-2	27.19	26.09
0	2	-2	26.50	26.07
-1	2	-2	4.02	3.96
-2	2	-2	14.61	15.06
-3	2	-2	14.13	14.88
-5	2	-2	11.42	12.36
-7	2	-2	1.26	1.87
7	2	-3	2.95	2.47
6	2	-3	3.14	2.75
5	2	-3	1.83	1.92
4	2	-3	17.79	16.51
3	2	-3	20.98	20.69
2	2	-3	2.65	1.96
1	2	-3	9.02	7.96
0	2	-3	13.51	13.51
-1	2	-3	4.88	5.20
-2	2	-3	2.10	4.27
-3	2	-3	5.31	5.71
-4	2	-3	4.64	5.74
-5	2	-3	5.43	6.62
-6	2	-3	4.50	5.80
7	2	-4	9.02	7.64
6	2	-4	6.16	5.06
5	2	-4	7.03	5.90
4	2	-4	10.12	8.83
3	2	-4	2.49	1.61
2	2	-4	3.04	3.67
1	2	-4	3.73	3.44
0	2	-4	1.12	1.70
-1	2	-4	12.03	12.67
-2	2	-4	5.58	5.01
-3	2	-4	8.62	8.93
-4	2	-4	0.00	1.54
-5	2	-4	3.44	3.82
5	2	-5	2.82	2.61
4	2	-5	4.21	3.08
3	2	-5	6.44	5.25
1	2	-5	15.87	15.02
0	2	-5	4.05	3.74
-1	2	-5	7.16	6.57
-2	2	-5	2.49	2.76
-1	1	0	4.79	6.98
0	2	0	79.61	90.34
-10	0	0	2.79	2.72
-9	0	0	10.25	8.88
-9	1	0	1.01	1.80
-9	2	0	8.50	6.02

H	K	L	F OBS	F CALC
18	0	0	6.45	6.11
18	1	0	6.36	6.20
18	2	0	6.03	5.72
18	3	0	5.14	4.88
18	4	0	4.68	4.63
17	0	0	13.00	12.79
17	1	0	5.60	4.92
17	2	0	12.14	11.54
17	3	0	3.85	4.09
17	4	0	8.22	8.68
17	5	0	2.32	2.94
16	0	0	14.98	12.19
16	1	0	1.20	2.20
16	2	0	11.55	10.66
16	3	0	1.47	2.99
16	4	0	6.83	7.67
11	2	0	2.70	2.75
12	4	0	14.55	12.58
12	5	0	3.72	2.85
12	6	0	7.65	7.64
12	7	0	2.53	2.10
12	3	0	3.99	2.82
15	3	0	13.16	15.15
15	4	0	9.15	12.10
15	5	0	7.53	9.51
15	6	0	4.89	6.77
15	1	0	20.92	20.48
14	2	0	13.82	14.30
13	5	0	10.36	9.94
12	1	0	3.50	2.83
14	3	0	4.02	4.56
14	4	0	6.13	7.69
14	5	0	3.56	3.62
13	2	0	3.10	2.52
13	3	0	15.17	14.56
13	4	0	2.06	1.95
11	3	0	7.00	6.02
11	4	0	4.61	3.67
11	5	0	3.06	3.52
11	6	0	3.58	3.25
11	7	0	1.17	1.76
13	6	0	1.62	1.40
14	6	0	2.08	3.49
16	5	0	1.88	2.96
0	4	0	46.54	43.83
0	6	0	21.25	21.29
1	2	1	27.67	23.95
12	2	1	31.35	28.24
12	1	1	27.10	27.96
11	0	1	.37	.04
4	0	1	20.03	19.22
2	0	1	10.53	11.69
1	5	1	17.08	15.56
12	3	1	19.46	17.02

H	K	L	F OBS	F CALC
5	4	1	7.37	8.71
5	3	1	9.75	11.48
9	2	1	.80	.57
9	1	1	3.20	4.96
9	0	1	0.00	.72
8	3	1	4.64	5.70
7	3	1	6.10	7.86
6	4	1	3.95	5.61
6	3	1	3.20	4.40
5	6	1	3.70	5.27
5	5	1	4.62	6.47
4	6	1	7.27	8.49
4	5	1	0.00	.52
4	4	1	10.98	12.46
4	3	1	.41	.49
3	6	1	6.01	7.41
3	5	1	4.77	4.95
3	4	1	13.79	13.69
3	3	1	5.18	5.42
2	7	1	3.61	4.59
2	6	1	3.54	3.63
2	5	1	8.72	9.18
2	4	1	5.60	6.13
2	3	1	17.47	16.99
1	7	1	7.70	8.54
1	6	1	6.40	6.28
1	4	1	14.55	12.29
1	3	1	30.96	28.61
0	6	1	3.33	4.23
0	5	1	2.24	2.50
0	4	1	8.80	8.54
0	3	1	2.23	4.66
1	7	1	3.56	3.28
1	6	1	2.68	2.85
1	5	1	4.71	3.96
1	4	1	2.30	3.37
1	3	1	3.93	3.32
12	7	1	3.19	3.90
12	6	1	10.15	9.41
12	5	1	8.60	8.45
12	4	1	10.74	16.38
13	6	1	7.73	8.69
13	5	1	2.37	2.75
13	4	1	16.24	15.52
13	3	1	5.57	4.91
14	6	1	7.50	8.60
14	5	1	.88	1.30
14	4	1	15.59	14.56
14	3	1	2.29	2.28
15	6	1	4.81	5.83
15	5	1	6.15	6.17
15	4	1	7.27	8.01
15	3	1	10.76	9.85
16	5	1	2.62	2.84

H	K	L	F OBS	F CALC
16	4	1	5.58	6.19
16	3	1	2.28	2.45
8	4	1	1.71	2.76
10	1	1	1.86	2.41
0	7	1	1.98	2.01
6	5	1	2.85	3.28
7	4	1	2.20	3.69
7	5	1	4.02	3.59
7	4	1	6.39	6.32
7	3	1	6.31	5.28
8	4	1	6.95	7.02
8	3	1	7.68	6.85
9	3	1	5.43	4.70
9	2	1	5.58	4.03
9	1	1	6.52	5.68
9	0	1	6.29	4.47
10	0	1	4.12	3.86
7	3	2	8.05	9.36
6	5	2	3.74	4.99
6	3	2	5.18	6.67
5	5	2	8.10	10.09
5	3	2	11.96	15.06
2	5	2	1.23	4.14
2	3	2	1.22	5.97
4	0	2	3.29	1.41
9	1	2	2.95	2.94
9	0	2	3.31	4.32
8	3	2	.65	1.76
8	2	2	6.97	8.68
8	0	2	8.45	9.66
7	4	2	1.04	1.99
7	0	2	0.00	1.67
6	4	2	0.00	.86
5	4	2	7.02	8.19
4	6	2	1.44	2.56
4	3	2	4.45	4.24
3	6	2	4.02	4.89
3	5	2	5.63	6.69
3	4	2	8.37	9.23
3	3	2	9.92	10.81
2	6	2	5.96	6.99
2	4	2	9.93	10.60
1	6	2	4.27	3.87
1	5	2	3.42	2.39
1	4	2	4.56	4.62
1	3	2	5.24	3.44
0	6	2	8.10	9.09
0	5	2	7.58	6.84
0	4	2	16.18	15.69
4	5	2	2.92	3.11
4	4	2	2.38	3.08
0	7	2	3.02	3.85
8	3	2	2.14	2.02
5	5	2	2.47	2.59

H	K	L	F OBS	F CALC
0	3	2	13.43	11.56
1	7	2	4.34	4.20
1	6	2	7.66	7.65
1	5	2	7.62	6.94
1	4	2	16.08	14.65
1	3	2	12.68	10.74
2	6	2	4.08	4.01
2	5	2	15.41	15.36
2	4	2	6.40	6.71
2	3	2	30.14	27.38
3	6	2	0.00	.18
3	5	2	2.75	2.48
3	4	2	0.00	.66
3	3	2	0.00	4.92
4	6	2	2.56	2.64
4	5	2	9.64	9.54
4	4	2	2.84	3.22
4	3	2	15.28	14.23
5	6	2	3.90	4.69
5	4	2	6.65	7.05
5	3	2	3.54	3.87
6	5	2	3.58	3.75
6	4	2	4.72	4.31
6	3	2	5.80	5.76
7	5	2	7.01	6.45
7	4	2	16.74	14.58
7	3	2	12.59	10.40
8	4	2	3.42	3.36
9	3	2	3.74	2.82
9	2	2	9.47	7.43
9	1	2	3.92	3.13
9	0	2	10.65	8.17
10	1	2	7.71	5.58
10	0	2	.64	.99

APPENDIX D

Structure Factor Tables for GABA

H	K	L	F OBS	A	H	K	L	F OBS	A
-2	0	1	28.64	-32.62	-3	9	1	2.42	2.61
-9	2	1	.85	.15	-3	10	1	1.47	-1.48
-9	3	1	2.17	2.42	-3	11	1	1.73	-1.57
-9	4	1	2.52	1.75	-1	3	1	7.67	7.67
-8	2	1	.77	-.42	-1	4	1	15.42	15.04
-8	3	1	6.68	6.65	-1	5	1	12.19	-12.08
-8	4	1	7.47	-6.96	-1	6	1	.91	-1.27
-8	5	1	.56	.12	-1	7	1	1.60	-1.61
-8	6	1	7.82	7.07	-1	8	1	11.35	11.64
-8	7	1	.91	-.45	-1	9	1	5.84	5.52
-7	2	1	9.18	8.84	-1	10	1	1.11	1.62
-7	3	1	.18	-.13	-1	11	1	2.81	-2.43
-7	4	1	1.23	-.89	-1	12	1	5.64	-4.83
2	2	1	31.38	-29.41	0	2	1	4.24	3.83
-2	2	1	29.57	30.63	0	3	1	41.55	42.93
-2	12	1	.89	-1.12	0	4	1	22.16	21.54
-7	5	1	4.70	4.74	0	6	1	15.54	-15.34
-7	6	1	4.79	-4.37	0	7	1	12.38	-11.43
-7	7	1	1.59	1.08	0	8	1	7.06	-7.66
-7	8	1	11.82	11.34	0	9	1	2.50	2.43
-6	2	1	7.30	-7.56	0	10	1	11.72	11.50
-6	3	1	1.61	-1.77	0	11	1	5.46	5.63
-6	4	1	0.00	.04	0	12	1	.52	-1.23
-6	5	1	1.16	-1.23	1	2	1	36.35	-36.65
-6	6	1	.25	-1.31	1	3	1	19.62	-18.07
-6	7	1	4.80	4.93	1	5	1	25.34	23.57
-6	8	1	1.85	1.99	1	7	1	3.89	3.63
-6	9	1	1.49	1.77	1	8	1	13.28	-12.67
-5	2	1	0.00	.44	1	9	1	5.07	-4.19
-5	3	1	13.26	-13.14	1	11	1	.78	.62
-5	4	1	12.93	-13.72	1	12	1	3.34	3.04
-5	5	1	3.24	3.37	2	6	1	11.61	13.62
-5	6	1	5.16	-5.81	3	2	1	2.31	-1.82
-5	7	1	2.32	.75	3	3	1	5.47	5.84
-5	8	1	.89	-.08	3	4	1	12.90	-11.50
-5	9	1	3.97	4.16	3	5	1	7.45	-7.47
-5	10	1	1.89	2.18	3	6	1	8.16	-7.58
-4	2	1	6.28	5.96	3	7	1	.42	-.71
-4	3	1	13.76	-14.08	3	8	1	8.70	-8.48
-4	4	1	2.84	3.29	3	9	1	2.93	2.42
-4	5	1	24.12	-23.58	3	10	1	5.17	5.00
-4	6	1	5.95	-7.05	3	11	1	7.18	6.84
-4	7	1	4.77	4.59	4	2	1	1.98	1.07
-4	8	1	2.58	-2.63	4	3	1	5.99	-6.43
-4	9	1	0.00	-.46	4	4	1	4.95	-5.85
-4	10	1	1.98	2.34	4	5	1	5.30	5.69
-4	11	1	.42	.23	4	6	1	10.63	10.39
-3	2	1	2.53	2.62	4	7	1	8.57	-9.33
-3	3	1	49.62	39.99	4	8	1	2.87	2.68
-3	4	1	9.73	-9.61	4	9	1	7.78	-8.65
-3	5	1	14.00	-13.83	4	10	1	9.77	-10.00
-3	6	1	6.46	-5.89	5	2	1	1.71	.75
-3	7	1	10.95	-12.73	5	3	1	6.43	-6.42
-3	8	1	4.99	-5.61	5	4	1	7.59	-7.25

H	K	L	F	OBS	I	A	H	K	L	F	OBS	F	A
5	5	1	5.27			5.80	-6	6	0	4.72			5.11
5	6	1	11.35			-11.54	-6	7	0	.39			.24
5	7	1	10.81			10.77	-6	8	0	1.83			-1.90
5	8	1	8.81			8.51	-6	9	0	6.08			-5.22
5	9	1	8.30			-8.35	-5	2	0	5.69			5.89
5	10	1	2.55			2.13	-5	3	0	5.70			4.59
6	2	1	3.89			3.59	-5	4	0	2.54			-2.64
6	3	1	4.89			-5.27	-5	5	0	8.01			7.58
6	4	1	4.96			4.80	-5	6	0	6.32			6.83
6	5	1	4.27			-3.53	-5	7	0	2.03			1.20
6	6	1	4.13			-3.71	-5	8	0	3.27			3.71
6	7	1	6.54			6.42	-5	9	0	7.01			6.03
6	8	1	8.33			-7.84	-5	10	0	3.87			3.69
6	9	1	7.10			7.00	-4	2	0	.72			-1.07
7	2	1	3.90			4.38	-4	3	0	23.76			-21.31
7	3	1	9.72			-9.92	-4	4	0	2.06			2.29
7	4	1	3.45			3.45	-4	5	0	15.17			14.93
7	5	1	4.49			-4.35	-4	6	0	8.18			-8.16
7	6	1	3.80			3.89	-4	7	0	4.03			3.44
7	7	1	2.48			-2.04	-4	8	0	3.05			2.70
8	2	1	3.48			-3.49	-4	9	0	2.92			-3.26
8	3	1	9.59			9.09	-4	10	0	6.55			6.95
8	4	1	2.38			1.92	-4	11	0	8.09			7.90
8	5	1	5.40			-4.91	-3	3	0	27.67			-25.13
-2	0	0	36.36			-33.84	-3	4	0	8.59			7.98
0	12	0	.68			-1.30	-3	5	0	22.64			-21.25
0	10	0	1.73			1.10	-3	6	0	5.90			-5.77
0	8	0	1.25			-.68	-3	7	0	12.88			13.97
0	6	0	2.35			2.18	-3	8	0	11.81			-12.21
-8	0	0	1.34			-1.29	-3	9	0	3.71			3.11
-6	0	0	1.68			1.41	-3	10	0	.17			.12
-9	2	0	2.75			2.36	-3	11	0	0.00			.19
-9	3	0	7.02			6.09	-2	3	0	16.78			15.67
1	2	0	74.95			103.48	-2	4	0	13.31			13.31
0	2	0	2.37			-1.53	-2	5	0	18.14			-19.11
-2	2	0	4.20			-6.53	-2	6	0	6.47			6.88
-1	1	0	9.56			8.95	-2	7	0	15.97			-21.02
-9	4	0	3.17			-3.50	-2	8	0	3.42			-3.17
-8	2	0	9.19			8.30	-2	9	0	4.64			5.13
-8	3	0	2.72			-2.76	-2	10	0	7.69			-8.87
-8	4	0	2.08			2.10	-2	11	0	2.27			2.39
-8	5	0	3.53			-3.38	-2	12	0	1.02			.89
-8	6	0	7.93			-7.09	-1	3	0	1.82			1.14
-7	2	0	.88			.57	-1	4	0	3.09			-3.81
-7	3	0	6.76			6.78	-1	5	0	18.12			18.32
-7	4	0	11.80			11.77	-1	6	0	8.44			8.40
-7	5	0	2.80			-2.62	-1	7	0	6.06			-7.07
-7	6	0	2.88			-2.28	-1	8	0	1.56			1.80
-7	7	0	9.28			-9.57	-1	9	0	8.79			-9.45
-7	8	0	2.27			-2.63	-1	10	0	1.53			-1.15
-6	2	0	4.72			-4.84	-1	11	0	0.00			.45
-6	3	0	10.99			10.12	-1	12	0	2.86			-3.01
-6	4	0	4.45			4.73	-9	1	2	1.44			-.51
-6	5	0	10.91			10.62	-2	1	2	40.70			44.85

H	K	L	F OBS	A
-9	3	2	6.41	-5.78
-8	1	2	1.04	-1.65
-8	3	2	4.22	-3.98
-8	4	2	9.80	8.30
-8	5	2	2.08	-1.84
-8	6	2	3.27	2.64
-7	1	2	3.06	3.29
-7	3	2	5.06	-5.11
-7	4	2	1.52	1.71
-7	5	2	9.18	-9.01
-7	6	2	7.14	6.53
-7	7	2	2.09	2.12
-7	8	2	1.31	-1.40
-6	1	2	2.08	1.85
-6	3	2	8.71	9.13
-6	4	2	21.01	-20.97
-6	5	2	8.86	-8.61
-6	6	2	4.20	3.95
-6	7	2	15.37	-14.12
-6	8	2	3.73	3.43
-6	9	2	1.88	1.12
-5	1	2	3.09	-3.54
-5	3	2	6.04	6.83
-5	4	2	4.24	-4.49
-5	5	2	20.56	19.87
-5	6	2	10.75	-10.51
-5	7	2	8.87	-7.90
-5	8	2	2.23	-1.72
-5	9	2	12.93	-10.98
-5	10	2	.65	.43
-4	1	2	14.82	-14.22
-4	3	2	16.44	-19.13
-4	4	2	.94	-.54
-4	5	2	10.29	10.47
-4	6	2	5.76	5.94
-4	7	2	12.44	12.22
-4	8	2	6.88	-6.34
-4	9	2	4.90	-4.69
-4	10	2	4.33	-3.90
-4	11	2	2.38	-2.35
-3	1	2	39.24	39.32
-3	3	2	17.45	-17.94
-3	4	2	4.14	-4.54
-3	5	2	11.18	-10.56
-3	6	2	5.67	5.73
-3	7	2	6.78	6.85
-3	8	2	5.17	4.82
-3	9	2	3.18	-2.83
-3	10	2	2.15	-1.70
-3	11	2	0.00	-.21
-2	3	2	27.47	27.89
-2	4	2	7.56	7.90
-2	5	2	1.94	-2.25
-2	6	2	2.57	-1.91

H	K	L	F OBS	F	A
-2	7	2	8.20		8.49
-2	8	2	4.24		3.98
-2	9	2	.66		-.62
-2	10	2	2.00		-1.67
-2	11	2	5.46		-4.44
-1	4	2	10.93		10.02
-1	5	2	.63		.32
-1	6	2	2.85		2.19
-1	7	2	8.11		9.11
-1	8	2	6.04		5.93
-1	9	2	3.83		3.47
-1	10	2	.92		.92
-1	11	2	3.75		-3.49
-1	12	2	1.14		-.96
0	4	2	4.10		-3.50
1	3	2	4.81		4.19
1	4	2	11.97		-11.85
1	5	2	1.10		-.84
1	6	2	10.11		-9.74
1	7	2	10.60		-10.72
1	8	2	2.55		2.25
1	9	2	14.92		14.38
1	10	2	5.98		-5.81
1	11	2	5.20		4.38
2	3	2	14.17		-13.39
2	4	2	15.30		16.17
2	5	2	4.13		-5.26
2	6	2	3.90		-3.63
2	7	2	11.28		-11.42
2	8	2	.53		-.62
2	9	2	5.06		-4.96
2	10	2	6.24		5.69
2	11	2	11.32		10.28
3	1	2	6.72		7.03
3	3	2	9.40		10.80
3	4	2	12.92		-13.46
3	5	2	2.95		3.49
3	6	2	5.98		5.42
3	7	2	5.73		-4.66
3	8	2	3.18		-2.26
3	9	2	7.10		-6.51
3	10	2	2.45		2.21
3	11	2	1.83		-2.32
4	1	2	2.62		2.77
4	3	2	1.24		-1.13
4	4	2	2.46		1.06
4	5	2	3.92		3.18
4	6	2	11.48		-12.17
4	7	2	2.78		3.28
4	8	2	1.75		1.91
4	9	2	1.27		-.40
4	10	2	.83		-.24
5	1	2	1.86		-2.16
5	3	2	11.35		11.36

H	K	L	F OBS	F	A	H	K	L	F OBS	A
5	4	2	16.26		17.05	8	0	-2	21.96	-22.72
5	5	2	4.09		-4.39	10	0	-2	1.45	2.43
5	6	2	2.93		2.74	4	0	-1	5.67	-5.32
5	7	2	1.85		-2.36	6	0	-1	12.12	-11.50
5	8	2	5.43		-5.10	8	0	-1	4.87	5.22
5	9	2	1.21		-.37	2	0	1	10.68	10.46
6	1	2	8.92		-9.51	4	0	1	7.45	7.19
6	3	2	3.42		3.15	6	0	1	8.31	8.29
6	4	2	6.00		-5.89	8	0	1	2.58	-2.75
6	5	2	10.47		10.34	2	0	2	20.93	-20.35
6	6	2	3.85		3.69	4	0	2	1.89	1.67
6	7	2	1.16		-1.35	6	0	2	5.98	6.90
6	8	2	.47		.04	8	0	2	3.88	-3.32
7	1	2	.47		-.53	2	0	3	9.10	8.46
7	3	2	6.99		-6.76	4	0	3	8.73	8.27
7	4	2	5.97		4.57	6	0	3	17.11	-16.87
7	5	2	2.75		2.41	2	0	4	1.84	1.76
7	6	2	2.69		-2.51	4	0	4	11.42	-11.79
8	1	2	2.85		-3.00	6	0	4	11.38	11.35
8	3	2	2.71		-2.99	2	0	5	13.62	-14.16
8	4	2	2.29		2.40	4	0	5	8.75	8.74
4	0	0	23.53		-23.10	2	0	6	1.11	-1.33
0	0	8	9.13		9.05	4	0	6	8.19	7.55
0	0	7	3.91		-3.85	2	0	7	.30	1.65
0	0	6	9.95		11.13	0	1	-3	33.72	30.55
0	0	4	28.57		26.30	0	1	-4	9.99	8.80
0	0	3	20.91		20.58	2	1	-8	3.12	-2.28
0	0	2	14.52		-14.99	3	1	-8	4.94	4.24
0	0	1	6.14		-6.60	4	1	-8	2.06	2.05
2	0	-8	.40		-.48	5	1	-8	12.38	-10.96
4	0	-8	1.42		-1.72	6	1	-8	14.69	-13.35
6	0	-8	4.87		5.68	7	1	-8	13.40	12.14
2	0	-7	1.45		1.85	2	1	-7	14.31	13.83
4	0	-7	12.63		-12.29	3	1	-7	15.93	-15.26
6	0	-7	1.30		-.32	4	1	-7	5.25	-4.99
8	0	-7	0.00		6.24	5	1	-7	3.75	-3.13
2	0	-6	19.96		-18.69	6	1	-7	8.69	-8.25
4	0	-6	15.60		18.50	7	1	-7	4.83	4.78
6	0	-6	4.54		4.12	8	1	-7	3.54	-3.10
8	0	-6	8.17		-8.76	2	1	-6	2.40	-2.80
2	0	-5	20.74		19.74	3	1	-6	12.41	15.95
4	0	-5	.62		1.20	4	1	-6	24.12	23.83
6	0	-5	5.21		-4.86	5	1	-6	8.65	-8.81
8	0	-5	6.91		6.73	6	1	-6	5.66	-6.08
2	0	-4	13.20		-12.34	7	1	-6	1.49	1.43
4	0	-4	17.93		-17.17	8	1	-6	1.47	.65
6	0	-4	11.48		-10.60	9	1	-6	4.74	-4.63
8	0	-4	.30		.82	3	1	-5	18.32	22.07
4	0	-3	31.40		30.13	4	1	-5	1.94	2.27
6	0	-3	17.15		-17.43	5	1	-5	.57	.61
8	0	-3	3.41		-3.35	6	1	-5	2.89	3.94
10	0	-3	0.00		.31	7	1	-5	1.02	-1.65
4	0	-2	34.04		35.59	8	1	-5	3.30	2.90
6	0	-2	29.34		31.14	9	1	-5	4.38	-3.97

H	K	L	F OBS	A
2	1	-4	9.37	-8.60
3	1	-4	4.72	-4.35
4	1	-4	8.60	8.74
5	1	-4	13.28	-14.16
6	1	-4	8.30	-8.70
7	1	-4	2.84	2.61
8	1	-4	9.01	-8.77
9	1	-4	8.27	7.60
10	1	-4	5.76	5.74
2	1	-3	14.74	13.92
3	1	-3	20.40	-20.55
1	1	2	12.62	-13.12
2	1	2	3.66	2.81
1	1	1	30.49	-32.04
1	1	-1	25.51	25.25
1	1	-2	34.38	-33.40
10	1	-2	3.78	3.83
4	1	-3	3.68	-3.94
5	1	-3	2.46	2.50
6	1	-3	5.37	3.63
7	1	-3	10.98	11.05
8	1	-3	5.08	5.08
9	1	-3	9.37	-9.04
10	1	-3	6.03	-5.90
2	1	-1	24.44	24.99
3	1	-1	1.98	-2.57
4	1	-1	17.43	-17.86
5	1	-1	2.28	-2.30
6	1	-1	4.56	4.38
7	1	-1	7.15	-7.35
8	1	-1	3.20	-3.23
9	1	-1	2.00	1.85
2	1	0	.89	1.34
3	1	0	14.39	14.52
4	1	0	21.43	20.60
5	1	0	18.87	-18.57
6	1	0	3.24	-2.78
7	1	0	5.12	5.09
8	1	0	3.11	2.99
9	1	0	4.43	-4.23
2	1	1	6.63	5.27
3	1	1	10.92	-10.68
4	1	1	18.01	-17.96
5	1	1	9.38	9.63
6	1	1	3.30	-4.21
7	1	1	13.23	14.51
8	1	1	15.96	15.83
9	1	1	6.30	-8.45
2	1	3	19.69	19.70
3	1	3	8.76	9.38
4	1	3	18.23	17.25
5	1	3	19.05	-18.60
6	1	3	8.87	-8.37
7	1	3	1.70	1.80

H	K	L	F. OBS	A
2	1	4	24.64	-24.16
3	1	4	13.66	13.47
4	1	4	15.30	14.70
5	1	4	5.14	-5.35
6	1	4	1.53	1.41
2	1	5	1.51	-1.81
3	1	5	7.90	-7.96
4	1	5	8.30	-8.49
5	1	5	6.61	6.84
2	1	6	8.07	-8.14
3	1	6	9.21	9.54
4	1	6	6.60	6.91
2	1	7	1.50	1.80
3	1	7	1.01	2.44
0	2	-3	18.07	16.23
0	2	-4	18.26	16.93
0	2	-2	17.58	-16.51
3	2	0	17.76	16.58
1	2	-8	1.53	1.96
2	2	-8	3.32	3.15
3	2	-8	2.37	-2.37
4	2	-8	2.02	-.86
5	2	-8	2.57	-2.25
6	2	-8	6.48	-6.51
7	2	-8	6.07	5.82
1	2	-7	2.33	-2.32
2	2	-7	8.51	-8.72
3	2	-7	7.20	6.51
4	2	-7	4.79	4.85
5	2	-7	5.13	-4.50
6	2	-7	5.25	5.49
7	2	-7	4.49	-5.14
8	2	-7	3.16	-3.19
1	2	-6	16.59	16.45
2	2	-6	12.66	-12.34
3	2	-6	18.79	-18.39
4	2	-6	9.58	8.66
5	2	-6	6.78	6.71
6	2	-6	3.36	3.78
7	2	-6	9.64	10.04
8	2	-6	2.42	-2.45
9	2	-6	0.00	-.09
2	2	-5	1.84	-1.86
3	2	-5	4.15	4.17
4	2	-5	1.28	.94
5	2	-5	1.23	-1.23
6	2	-5	1.70	-1.24
7	2	-5	4.57	-3.63
8	2	-5	9.07	-8.51
9	2	-5	2.12	2.43
1	2	-4	27.22	25.74
2	2	-4	8.25	8.93
3	2	-4	7.36	7.58
4	2	-4	5.46	-6.83

H	K	L	F OBS	A
5	2	-4	2.68	-3.52
6	2	-4	2.95	1.08
7	2	-4	6.93	-7.29
8	2	-4	3.32	3.67
9	2	-4	11.04	-10.18
10	2	-4	2.95	-2.36
1	2	-3	4.77	4.88
2	2	-3	25.10	-24.79
3	2	-3	7.97	-7.53
4	2	-3	5.35	-5.77
5	2	-3	24.55	26.26
6	2	-3	11.22	11.55
7	2	-3	5.14	-5.36
8	2	-3	3.77	3.57
9	2	-3	3.24	-3.31
10	2	-3	2.25	-2.72
2	2	-2	6.66	6.63
3	2	-2	42.13	-44.61
4	2	-2	2.46	2.31
5	2	-2	9.37	-10.51
6	2	-2	3.90	-4.30
7	2	-2	27.52	29.16
8	2	-2	2.12	-2.92
9	2	-2	7.29	-6.81
1	2	2	10.90	8.84
2	2	2	3.13	-2.47
3	2	2	10.50	-12.16
4	2	2	18.98	18.33
5	2	2	5.79	-6.25
6	2	2	5.23	-5.61
7	2	2	4.76	4.88
8	2	2	5.49	-5.89
2	2	3	7.49	-6.54
3	2	3	2.35	-2.06
4	2	3	4.41	3.88
5	2	3	16.33	15.25
6	2	3	.92	.99
7	2	3	5.61	-5.90
1	2	4	7.90	8.24
2	2	4	12.32	-11.49
3	2	4	6.06	5.87
4	2	4	6.04	-6.26
5	2	4	12.03	-11.97
6	2	4	3.01	2.91
1	2	5	1.64	2.29
2	2	5	7.80	-7.51
3	2	5	10.41	-10.04
4	2	5	0.90	-.24
5	2	5	3.45	3.65
1	2	6	7.54	7.19
2	2	6	.84	1.27
3	2	6	.96	-.85
4	2	6	3.67	3.78
1	2	7	6.51	-6.71

H	K	L	F OBS	A
2	2	7	3.67	3.84
0	0	5	18.35	-17.63
0	5	1	21.92	20.48
0	4	0	51.96	-49.63
0	1	2	2.55	-3.27
0	1	5	2.87	3.01
0	1	6	15.64	14.59
0	1	7	5.05	-4.41
0	1	8	3.99	3.10
0	2	5	8.97	9.08
0	2	6	2.06	-2.40
0	2	7	6.08	5.18
0	2	8	2.63	2.28
0	3	2	1.91	1.11
0	3	3	16.42	-13.18
0	3	4	6.88	7.19
0	3	5	1.56	.80
0	3	6	.94	1.36
0	3	7	8.83	-7.36
0	3	8	4.97	-3.79
0	4	3	4.82	-4.43
0	4	4	10.19	-11.05
0	4	5	.67	-.06
0	4	6	15.87	-15.55
0	4	7	8.19	8.67
0	1	1	5.67	-4.65
0	5	2	16.59	-15.74
0	5	3	4.57	-6.10
0	5	4	8.09	8.32
0	5	5	3.87	5.51
0	5	6	10.27	-13.00
0	5	7	3.94	4.59
0	6	2	3.39	3.40
0	6	3	11.15	-11.69
0	6	4	2.70	-2.80
0	6	5	13.35	-13.32
0	6	6	1.60	1.57
0	6	7	2.66	-3.96
0	7	2	1.93	2.60
0	7	3	4.04	-3.99
0	7	4	3.99	-4.02
0	7	5	8.18	8.28
0	7	6	1.83	-1.52
0	8	2	2.13	1.61
0	8	3	1.93	-2.45
0	8	4	2.20	2.21
0	8	5	6.38	6.76
0	8	6	4.50	5.43
0	9	2	9.80	10.20
0	9	3	0.00	.15
0	9	4	0.00	-.01
0	9	5	0.00	1.01
0	10	2	3.44	3.55
0	10	3	4.13	4.24

H	K	L	F OBS	A
0	10	4	6.52	-7.52
0	11	2	8.07	-8.58
0	11	3	3.74	-3.80
0	12	2	3.01	3.94
1	3	-2	1.39	1.80
1	2	-1	17.17	17.48
1	10	1	4.58	-3.83
1	6	1	22.65	22.38
1	4	1	33.62	32.30
1	2	3	11.04	9.71
1	1	-8	1.66	-.46
1	1	-7	1.21	1.87
1	1	-6	1.26	1.23
1	1	-5	8.48	-9.26
1	1	-4	1.07	1.90
1	1	-3	9.41	-6.98
1	1	4	7.21	-6.90
1	1	5	8.36	-9.16
1	1	6	7.51	-8.85
1	1	7	9.73	9.27
1	2	-2	4.20	.49
1	2	-5	15.00	-17.69
1	3	-8	4.34	4.24
1	3	-7	2.58	-3.19
1	3	-6	17.56	-17.17
1	3	-5	11.62	11.84
1	3	-4	23.79	22.67
1	3	-3	12.79	-11.43
1	3	3	19.82	-21.61
1	3	4	12.06	11.79
1	3	5	1.50	.81
1	3	6	8.35	8.52
1	3	7	3.30	-3.34
1	4	-8	.86	.65
1	4	-7	8.37	8.41
1	4	-6	5.32	5.40
1	4	-5	9.73	10.23
1	4	-4	.99	-.91
1	4	-3	26.02	25.46
1	4	3	9.78	11.29
1	4	4	18.95	18.98
1	4	5	11.13	11.47
1	4	6	3.69	-4.36
1	4	7	2.48	2.10
1	5	-7	0.00	.56
1	5	-6	9.00	.64
1	5	-5	6.06	6.25
1	5	-4	5.83	-6.11
1	5	-3	1.87	-.89
1	5	3	2.87	2.89
1	5	4	5.60	5.21
1	5	5	5.99	-6.23
1	5	6	1.70	1.09
1	5	7	2.75	-2.63

H	K	L	F OBS	A
1	6	-7	7.35	8.02
1	6	-6	14.82	-14.96
1	6	-5	7.93	-8.18
1	6	-4	4.66	-5.23
1	6	-3	12.51	12.61
1	6	3	14.07	-13.13
1	6	4	3.32	-3.36
1	6	5	2.46	-2.69
1	6	6	6.85	-6.46
1	7	-7	2.90	-3.27
1	7	-6	9.01	8.77
1	7	-5	1.72	-1.88
1	7	-4	5.23	-5.25
1	7	-3	12.76	12.93
1	7	3	7.57	7.28
1	7	4	4.22	-4.33
1	7	5	3.65	3.47
1	7	6	1.43	1.31
1	8	-6	2.94	-1.76
1	8	-5	11.06	-10.92
1	8	-4	9.21	9.63
1	8	-3	11.12	-10.82
1	8	3	7.92	-7.34
1	8	4	.94	.90
1	8	5	3.14	-2.91
1	8	6	2.79	-2.29
1	9	-5	4.52	-4.20
1	9	-4	1.29	-1.86
1	9	-3	4.89	-4.89
1	9	3	5.93	-5.45
1	9	4	5.18	-4.39
1	9	5	5.21	4.76
1	10	-5	2.77	2.76
1	10	-4	4.02	-3.90
1	10	-3	5.64	-4.82
1	10	3	.94	-.76
1	10	4	2.81	2.38
1	11	-4	.86	-.25
1	11	-3	1.53	-1.34
1	11	3	6.07	-5.92
2	1	-5	21.93	-22.41
2	0	-2	51.63	-51.54
2	0	-3	5.88	-6.37
2	3	-8	6.61	-5.65
2	3	-7	7.12	6.81
2	3	-6	7.96	-6.92
2	3	-5	12.60	-14.76
2	3	-4	11.87	11.15
2	3	-3	11.98	11.48
2	3	-1	1.58	-.83
2	3	1	21.01	-20.63
2	3	3	3.59	3.55
2	3	4	5.52	-5.19
2	3	5	3.60	3.28

H	K	L	F OBS	A
2	3	6	4.88	-5.53
2	3	7	3.40	3.63
2	4	-7	3.89	-3.75
2	4	-6	15.57	15.42
2	4	-5	0.00	-.38
2	4	-4	9.02	8.77
2	4	-3	15.96	-15.05
2	4	-1	12.34	12.03
2	4	1	25.37	-25.25
2	4	3	14.94	15.42
2	4	4	0.00	.07
2	4	5	3.80	3.60
2	4	6	0.00	1.10
2	4	7	1.29	-1.50
2	5	-7	4.61	-4.05
2	5	-6	6.05	-5.77
2	5	-5	3.17	3.11
2	5	-4	10.60	10.89
2	5	-3	12.59	-12.57
2	5	-1	17.93	-15.85
2	5	1	2.05	-1.93
2	5	3	17.56	-16.74
2	5	4	22.39	21.71
2	5	5	6.35	-5.94
2	5	6	5.31	-4.30
2	6	-7	4.58	4.54
2	6	-6	10.63	10.13
2	6	-5	5.64	5.80
2	6	-4	11.94	-11.18
2	6	-3	21.36	20.82
2	6	-1	5.80	-4.96
2	6	3	10.07	11.83
2	6	4	4.34	5.35
2	6	5	0.00	1.35
2	6	6	5.45	4.87
2	7	-6	1.31	-2.19
2	7	-5	2.70	3.18
2	7	-4	1.06	1.82
2	7	-3	1.65	1.85
2	7	-1	1.76	1.86
2	7	1	8.19	7.43
2	7	3	8.06	8.73
2	7	4	4.61	4.91
2	7	5	4.59	-4.89
2	7	6	0.00	-.37
2	8	-6	8.96	-9.24
2	8	-5	2.73	-3.18
2	8	-4	0.00	1.06
2	8	-3	7.90	8.38
2	8	-1	.94	-1.14
2	8	1	10.90	11.83
2	8	3	13.12	-13.32
2	8	4	1.87	-2.58
2	8	5	4.53	4.36

H	K	L	F OBS	A
2	9	-5	3.53	3.30
2	9	-4	3.60	2.71
2	9	-3	10.67	11.03
2	9	-1	2.47	2.86
2	9	1	0.00	-.61
2	9	3	9.69	9.66
2	9	4	1.44	-1.71
2	10	-4	4.02	5.14
2	10	-3	11.31	-11.33
2	10	-1	8.59	-8.23
2	10	1	.63	.95
2	10	3	6.60	-7.54
2	10	4	2.07	2.38
2	11	-3	5.68	-5.70
2	11	-1	1.64	1.27
2	11	1	1.92	1.68
2	12	-1	1.99	-1.12

APPENDIX E

Structure Factor Tables for β GP

H	K	L	FO	FC	H	K	L	FO	FC
2	0	=9	1,02	,57	0	0	1	18,28	16,59
2	0	=8	0,00	,02	6	0	1	9,91	7,99
4	0	=8	2,32	1,93	8	0	1	21,50	21,28
6	0	=8	4,36	4,27	10	0	1	9,57	9,51
8	0	=8	2,39	2,03	12	0	1	12,53	12,88
2	0	=7	11,00	10,46	14	0	1	6,73	6,68
4	0	=7	6,57	5,97	2	0	2	14,04	13,82
6	0	=7	5,18	4,37	4	0	2	25,37	25,24
8	0	=7	9,77	8,63	6	0	2	1,02	,77
10	0	=7	4,27	4,07	8	0	2	4,62	5,75
2	0	=6	1,64	1,19	10	0	2	8,69	9,72
4	0	=6	2,92	1,81	12	0	2	3,60	4,14
6	0	=6	19,54	18,94	0	0	3	38,10	36,58
8	0	=6	8,64	7,76	2	0	3	28,92	28,46
10	0	=6	5,62	4,96	4	0	3	14,01	14,04
12	0	=6	7,50	6,66	6	0	3	2,31	2,58
2	0	=5	20,51	20,17	8	0	3	9,66	11,14
4	0	=5	7,92	7,82	10	0	3	12,58	12,81
6	0	=5	6,00	6,37	12	0	3	4,83	5,23
8	0	=5	13,86	13,25	0	0	4	19,90	17,73
10	0	=5	13,70	12,70	2	0	4	7,00	6,58
12	0	=5	2,13	2,09	4	0	4	4,16	4,32
2	0	=4	28,68	27,98	6	0	4	10,78	10,40
4	0	=4	23,30	22,29	8	0	4	13,73	14,57
6	0	=4	16,24	15,30	10	0	4	9,57	10,29
8	0	=4	9,06	10,13	12	0	4	6,04	6,31
10	0	=4	6,64	6,47	0	0	5	14,01	13,22
12	0	=4	7,13	6,17	2	0	5	12,67	14,21
2	0	=3	8,34	7,75	4	0	5	2,58	2,31
4	0	=3	2,81	1,95	6	0	5	1,09	1,31
6	0	=3	3,17	2,67	8	0	5	2,11	1,50
8	0	=3	3,15	3,56	10	0	5	11,37	12,14
10	0	=3	1,85	1,88	0	0	6	10,82	10,63
12	0	=3	1,30	,96	2	0	6	4,80	4,09
14	0	=3	,76	,26	4	0	6	4,48	4,69
4	0	=2	38,00	38,36	6	0	6	,75	,57
6	0	=2	5,43	5,82	8	0	6	6,41	6,79
8	0	=2	11,28	11,41	0	0	7	3,19	3,16
10	0	=2	2,14	2,02	2	0	7	5,73	5,47
12	0	=2	4,91	5,31	4	0	7	6,74	6,73
14	0	=2	2,15	1,92	6	0	7	5,59	5,60
4	0	=1	47,21	48,71	0	0	8	2,27	2,25
6	0	=1	12,51	12,76	2	0	8	1,86	1,46
8	0	=1	1,26	1,76	4	0	8	7,20	7,45
10	0	=1	10,39	12,02	1	2	=8	4,90	5,68
12	0	=1	8,83	7,77	2	2	=8	4,44	5,58
14	0	=1	4,50	4,97	3	2	=8	2,04	2,70
2	0	0	33,38	36,02	4	2	=8	3,74	4,89
4	0	0	15,00	14,90	5	2	=8	1,34	1,58
6	0	0	4,82	4,15	6	2	=8	3,16	3,91
8	0	0	19,02	19,73	7	2	=8	1,73	1,91
10	0	0	2,56	3,31	1	2	=7	3,24	3,58
12	0	0	1,10	1,48	2	2	=7	3,16	3,80
14	0	0	1,31	1,47	3	2	=7	4,51	5,22

H	K	L	F0	FC	H	K	L	F0	FC
4	2	=7	2,31	2,70	12	2	=3	9,71	9,78
5	2	=7	4,28	4,96	13	2	=3	9,18	9,01
6	2	=7	8,66	9,84	14	2	=3	3,83	3,28
7	2	=7	1,95	2,30	1	2	=2	21,82	22,71
8	2	=7	2,03	1,95	2	2	=2	9,96	8,55
9	2	=7	1,55	1,37	3	2	=2	1,25	2,11
10	2	=7	3,12	3,61	4	2	=2	13,98	13,44
1	2	=6	,87	,98	5	2	=2	27,35	26,59
2	2	=6	1,67	1,81	6	2	=2	24,04	22,50
3	2	=6	2,91	3,78	7	2	=2	4,75	4,72
4	2	=6	6,79	8,36	8	2	=2	4,60	4,79
5	2	=6	3,09	3,42	9	2	=2	12,42	11,89
6	2	=6	3,90	4,15	10	2	=2	6,59	6,73
7	2	=6	,40	,34	11	2	=2	4,02	3,19
8	2	=6	1,74	2,02	12	2	=2	1,54	1,33
9	2	=6	2,00	2,20	13	2	=2	7,46	7,16
10	2	=6	1,08	,96	14	2	=2	3,40	3,36
11	2	=6	,67	,44	1	2	=1	2,23	2,94
1	2	=5	8,56	10,15	2	2	=1	27,75	26,29
2	2	=5	4,90	6,00	3	2	=1	11,88	11,87
3	2	=5	11,01	13,78	4	2	=1	20,15	18,09
4	2	=5	9,69	12,11	5	2	=1	9,75	9,06
5	2	=5	2,72	3,98	6	2	=1	1,79	,82
6	2	=5	6,78	7,95	7	2	=1	2,60	2,79
7	2	=5	3,69	3,86	8	2	=1	5,97	5,93
8	2	=5	1,94	2,07	9	2	=1	15,87	15,08
9	2	=5	5,06	5,46	10	2	=1	6,12	5,81
10	2	=5	3,37	3,68	11	2	=1	,64	,51
11	2	=5	1,40	1,66	12	2	=1	1,58	1,35
12	2	=5	6,79	7,48	13	2	=1	3,23	3,09
1	2	=4	26,86	23,44	14	2	=1	3,87	3,69
2	2	=4	5,56	4,91	1	2	=0	14,79	14,92
3	2	=4	3,49	3,94	2	2	=0	34,08	35,37
4	2	=4	5,39	5,47	3	2	=0	14,49	13,50
5	2	=4	5,35	5,97	4	2	=0	12,79	12,32
6	2	=4	16,73	15,50	5	2	=0	8,84	7,78
7	2	=4	2,75	2,87	6	2	=0	7,99	7,51
8	2	=4	7,96	7,14	7	2	=0	4,25	3,50
9	2	=4	12,59	11,13	8	2	=0	11,48	12,80
10	2	=4	6,29	5,66	9	2	=0	3,12	2,86
11	2	=4	,56	,27	10	2	=0	0,00	,03
12	2	=4	2,06	1,89	11	2	=0	,64	,66
13	2	=4	0,00	,86	12	2	=0	9,15	8,83
1	2	=3	0,00	,87	13	2	=0	5,60	5,20
2	2	=3	29,07	30,57	14	2	=0	6,11	5,99
3	2	=3	30,52	30,84	0	2	=1	18,87	17,93
4	2	=3	10,09	10,32	1	2	=1	37,12	37,90
5	2	=3	20,71	22,50	3	2	=1	8,46	7,21
6	2	=3	4,80	4,84	5	2	=1	2,69	2,78
7	2	=3	9,42	9,53	6	2	=1	17,03	16,57
8	2	=3	4,59	4,90	7	2	=1	14,40	16,17
9	2	=3	6,31	6,36	8	2	=1	7,73	8,35
10	2	=3	9,32	8,88	9	2	=1	2,14	2,29
11	2	=3	2,54	2,46	10	2	=1	7,89	7,82

H	K	L	FO	FC	H	K	L	FO	FC
11	2	1	2,06	1,89	1	2	6	12,99	10,53
12	2	1	2,91	2,66	2	2	6	8,90	7,42
13	2	1	2,24	2,25	3	2	6	9,33	8,36
0	2	2	31,77	35,04	4	2	6	11,00	9,66
1	2	2	11,64	11,46	5	2	6	4,81	4,57
2	2	2	24,40	23,85	6	2	6	2,76	2,47
3	2	2	25,94	23,98	7	2	6	2,27	1,96
4	2	2	9,24	8,12	8	2	6	1,67	1,63
5	2	2	15,73	15,65	9	2	6	4,67	4,90
6	2	2	9,12	9,87	0	2	7	7,64	7,17
7	2	2	5,56	5,74	1	2	7	4,79	4,71
8	2	2	20,91	21,52	2	2	7	5,47	5,08
9	2	2	5,24	4,78	3	2	7	2,31	1,53
10	2	2	3,99	3,78	4	2	7	4,16	4,07
11	2	2	6,06	6,34	5	2	7	2,62	2,70
12	2	2	11,69	11,98	6	2	7	3,20	2,83
13	2	2	2,67	2,28	7	2	7	2,77	2,79
0	2	3	8,16	8,74	0	2	8	3,40	3,26
1	2	3	28,24	24,76	1	2	8	6,48	5,95
2	2	3	25,46	23,11	2	2	8	10,50	8,64
3	2	3	24,76	23,65	3	2	8	2,48	2,35
4	2	3	3,56	3,19	4	2	8	3,53	3,17
5	2	3	6,07	7,72	1	3	8	2,10	2,74
6	2	3	14,30	16,87	2	3	8	2,56	3,30
7	2	3	12,27	14,04	3	3	8	3,01	2,61
8	2	3	1,30	1,13	4	3	8	2,40	1,93
9	2	3	2,88	2,54	5	3	8	2,59	2,42
10	2	3	7,84	6,90	6	3	8	2,91	1,10
11	2	3	5,65	5,16	1	3	7	2,06	1,69
12	2	3	3,04	2,90	2	3	7	3,45	3,48
0	2	4	7,06	7,28	3	3	7	2,58	2,77
1	2	4	21,03	20,66	4	3	7	6,08	5,62
2	2	4	4,16	4,15	5	3	7	1,44	1,83
3	2	4	2,97	1,42	6	3	7	4,36	3,84
4	2	4	2,17	2,12	7	3	7	2,44	2,21
5	2	4	9,94	9,98	8	3	7	1,65	1,97
6	2	4	15,59	13,90	9	3	7	3,12	2,86
7	2	4	12,33	11,34	1	3	6	7,63	7,79
8	2	4	11,12	9,91	2	3	6	8,62	8,50
9	2	4	10,86	9,48	3	3	6	1,60	1,70
10	2	4	4,45	4,38	4	3	6	0,00	0,01
11	2	4	2,26	1,96	5	3	6	2,92	1,42
0	2	5	2,25	1,70	6	3	6	2,99	1,04
1	2	5	8,68	7,37	7	3	6	4,98	4,96
2	2	5	1,68	1,17	8	3	6	4,09	3,90
3	2	5	14,35	12,64	9	3	6	2,32	1,95
4	2	5	1,26	1,04	10	3	6	2,03	1,41
5	2	5	7,12	6,17	11	3	6	5,34	5,02
6	2	5	4,91	2,98	1	3	5	11,30	10,91
7	2	5	2,46	1,83	2	3	5	5,74	5,39
8	2	5	2,32	2,66	3	3	5	10,32	9,67
9	2	5	0,00	2,41	4	3	5	4,11	3,47
10	2	5	1,32	2,35	5	3	5	2,79	1,08
0	2	6	7,60	6,36	6	3	5	2,82	2,45

H	K	L	F0	FC	H	K	L	F0	FC
7	3	=5	0,00	,47	10	3	=1	13,04	12,37
8	3	=5	2,67	2,72	11	3	=1	3,03	3,03
9	3	=5	4,72	4,78	12	3	=1	7,56	7,38
10	3	=5	4,58	4,34	13	3	=1	1,04	,68
11	3	=5	4,27	3,86	1	3	0	15,08	15,28
12	3	=5	1,04	1,09	2	3	0	9,31	8,94
1	3	=4	4,26	3,98	3	3	0	16,62	15,99
2	3	=4	2,11	1,64	4	3	0	22,66	22,99
3	3	=4	1,13	,26	5	3	0	23,38	23,67
4	3	=4	5,35	5,63	6	3	0	2,79	2,59
5	3	=4	2,63	2,34	7	3	0	3,65	4,23
6	3	=4	1,11	,48	8	3	0	20,69	20,68
7	3	=4	7,65	6,85	9	3	0	5,65	5,49
8	3	=4	2,03	1,93	10	3	0	15,42	15,20
9	3	=4	4,56	4,30	11	3	0	11,17	11,06
10	3	=4	5,37	4,82	12	3	0	4,89	4,74
11	3	=4	6,12	5,66	13	3	0	10,15	9,93
12	3	=4	3,11	3,00	0	3	1	25,18	27,36
13	3	=4	,09	,36	1	3	1	29,31	27,82
1	3	=3	7,68	9,06	2	3	1	3,34	2,98
2	3	=3	9,66	10,34	3	3	1	6,63	6,69
3	3	=3	5,27	5,54	4	3	1	11,08	10,43
4	3	=3	11,42	11,49	5	3	1	4,25	4,16
5	3	=3	7,98	8,06	6	3	1	5,52	5,51
6	3	=3	9,22	9,16	7	3	1	4,25	4,24
7	3	=3	2,86	3,41	8	3	1	16,11	16,17
8	3	=3	,46	,06	9	3	1	26,17	26,51
9	3	=3	12,12	12,02	10	3	1	7,25	7,18
10	3	=3	8,55	8,25	11	3	1	3,71	3,54
11	3	=3	6,10	5,71	12	3	1	8,67	8,20
12	3	=3	8,39	7,75	13	3	1	2,43	2,46
13	3	=3	,71	,44	0	3	2	23,67	25,17
1	3	=2	18,68	19,01	1	3	2	8,55	8,98
2	3	=2	11,79	11,12	2	3	2	24,42	23,14
3	3	=2	2,99	3,52	3	3	2	11,35	11,59
4	3	=2	3,18	3,30	4	3	2	2,64	1,93
5	3	=2	13,58	13,06	5	3	2	17,85	16,66
6	3	=2	7,74	7,37	6	3	2	15,41	15,57
7	3	=2	2,95	3,00	7	3	2	4,60	5,01
8	3	=2	2,64	2,16	8	3	2	23,35	23,71
9	3	=2	9,16	8,62	9	3	2	3,33	3,05
10	3	=2	18,73	17,90	10	3	2	6,21	6,30
11	3	=2	9,40	8,70	11	3	2	1,81	2,35
12	3	=2	3,08	3,06	12	3	2	5,80	5,90
13	3	=2	3,69	3,20	0	3	3	12,96	12,92
1	3	=1	17,95	17,83	1	3	3	17,19	17,83
2	3	=1	20,66	19,17	2	3	3	12,09	11,94
3	3	=1	5,98	5,50	3	3	3	2,60	1,92
4	3	=1	4,22	4,50	4	3	3	8,87	8,88
5	3	=1	15,50	15,92	5	3	3	9,18	9,50
6	3	=1	11,62	11,65	6	3	3	13,60	13,36
7	3	=1	1,90	1,97	7	3	3	6,22	6,19
8	3	=1	7,85	7,48	8	3	3	1,58	1,33
9	3	=1	28,29	28,06	9	3	3	11,19	11,65

H	K	L	FO	FC	H	K	L	FO	FC
10	3	3	3,98	4,22	8	1	=8	0,00	,74
11	3	3	6,48	6,53	1	1	=7	5,42	4,86
12	3	3	8,93	9,77	2	1	=7	7,81	7,20
0	3	4	20,28	21,08	3	1	=7	0,00	,58
1	3	4	15,17	15,87	4	1	=7	,71	,81
2	3	4	10,90	9,34	5	1	=7	2,26	2,48
3	3	4	1,63	1,55	6	1	=7	2,86	2,75
4	3	4	21,95	22,44	7	1	=7	4,31	4,51
5	3	4	11,25	12,06	8	1	=7	,41	,28
6	3	4	9,15	9,03	9	1	=7	2,42	2,19
7	3	4	1,00	,87	10	1	=7	4,50	3,87
8	3	4	7,11	7,32	1	1	=6	5,48	5,44
9	3	4	6,33	6,26	2	1	=6	,39	,24
10	3	4	8,22	7,95	3	1	=6	11,61	11,53
11	3	4	4,40	4,59	4	1	=6	11,84	11,75
0	3	5	3,41	3,12	5	1	=6	2,56	2,49
1	3	5	13,65	13,28	6	1	=6	5,77	5,27
2	3	5	5,15	5,20	7	1	=6	3,66	3,52
3	3	5	13,25	13,74	8	1	=6	2,50	2,50
4	3	5	6,24	6,07	9	1	=6	9,52	9,70
5	3	5	3,69	3,26	10	1	=6	,33	,05
6	3	5	6,92	6,43	11	1	=6	,85	,58
7	3	5	2,96	2,33	12	1	=6	,49	,77
8	3	5	8,64	9,23	1	1	=5	3,84	4,18
9	3	5	5,56	6,30	2	1	=5	,22	,20
10	3	5	4,99	5,46	3	1	=5	3,28	3,64
0	3	6	2,78	2,82	4	1	=5	6,74	6,69
1	3	6	11,74	11,17	5	1	=5	7,41	8,04
2	3	6	7,91	7,96	6	1	=5	,76	,66
3	3	6	1,52	1,56	7	1	=5	9,77	9,94
4	3	6	7,35	7,56	8	1	=5	12,57	12,35
5	3	6	9,08	10,06	9	1	=5	7,75	7,38
6	3	6	2,69	2,49	10	1	=5	,79	1,19
7	3	6	5,23	5,17	11	1	=5	2,33	2,01
8	3	6	3,37	3,17	12	1	=5	5,23	4,88
0	3	7	4,35	4,02	13	1	=5	5,90	5,65
1	3	7	7,19	6,73	1	1	=4	,59	,72
2	3	7	2,11	2,18	2	1	=4	10,01	10,28
3	3	7	8,75	8,99	3	1	=4	25,10	24,46
4	3	7	2,00	2,09	4	1	=4	27,29	26,93
5	3	7	1,36	1,58	5	1	=4	11,26	11,24
6	3	7	4,42	4,53	6	1	=4	5,63	6,11
0	3	8	2,31	2,36	7	1	=4	2,94	2,76
1	3	8	1,33	1,67	8	1	=4	,54	,73
2	3	8	4,22	4,34	9	1	=4	9,04	9,17
3	3	8	2,61	2,75	10	1	=4	2,36	2,41
2	1	=9	5,00	4,78	11	1	=4	4,72	4,69
1	1	=8	0,00	,44	12	1	=4	8,75	8,41
2	1	=8	3,19	3,09	13	1	=4	1,73	1,73
3	1	=8	2,64	2,73	1	1	=3	10,93	11,76
4	1	=8	,41	,39	2	1	=3	5,13	5,45
5	1	=8	0,00	,41	3	1	=3	2,43	2,34
6	1	=8	6,39	6,06	4	1	=3	4,41	4,73
7	1	=8	4,94	4,62	5	1	=3	3,12	3,25

H	K	L	FO	FC	H	K	L	FO	FC
6	1	3	7,60	7,37	6	1	1	3,48	3,00
7	1	3	21,30	20,56	7	1	1	25,10	24,63
8	1	3	13,92	15,85	8	1	1	4,00	4,05
9	1	3	18,76	19,08	9	1	1	1,70	1,54
10	1	3	10,04	10,38	10	1	1	4,66	4,92
11	1	3	4,43	4,43	11	1	1	3,17	2,63
12	1	3	7,03	6,82	12	1	1	4,53	5,02
13	1	3	0,00	,34	13	1	1	1,48	,31
14	1	3	,73	,01	14	1	1	,69	,62
1	1	2	37,29	39,07	1	1	2	30,20	31,12
2	1	2	1,89	2,68	2	1	2	20,68	20,20
3	1	2	39,22	41,04	3	1	2	15,01	13,76
4	1	2	19,10	17,89	4	1	2	28,34	28,73
5	1	2	8,24	7,34	5	1	2	22,95	22,47
6	1	2	6,94	6,63	6	1	2	11,07	10,68
7	1	2	7,74	7,32	7	1	2	12,94	12,80
8	1	2	7,50	7,01	8	1	2	5,64	7,10
9	1	2	,58	,52	9	1	2	3,37	3,56
10	1	2	,83	,15	10	1	2	2,21	2,36
11	1	2	6,93	7,29	11	1	2	3,48	3,88
12	1	2	5,54	5,58	12	1	2	2,16	1,88
13	1	2	2,86	2,98	13	1	2	4,12	4,84
14	1	2	7,18	7,08	0	1	3	25,75	25,45
2	1	1	23,19	25,12	1	1	3	10,08	9,87
3	1	1	2,59	1,33	2	1	3	13,11	13,54
4	1	1	36,48	36,64	3	1	3	28,18	28,47
5	1	1	27,50	26,36	4	1	3	14,35	13,31
6	1	1	5,66	5,25	5	1	3	9,80	9,58
7	1	1	37,01	35,80	6	1	3	7,43	7,68
8	1	1	,43	,53	7	1	3	1,65	,15
9	1	1	11,47	11,90	8	1	3	11,18	11,19
10	1	1	3,82	4,54	9	1	3	2,49	2,51
11	1	1	5,11	5,66	10	1	3	10,50	10,96
12	1	1	8,18	7,84	11	1	3	3,85	4,10
13	1	1	4,94	4,45	12	1	3	3,24	3,23
14	1	1	1,95	1,98	0	1	4	1,32	,54
1	1	0	18,00	17,62	1	1	4	17,57	17,25
2	1	0	9,61	9,88	2	1	4	7,28	7,16
3	1	0	28,36	29,00	3	1	4	22,44	21,85
4	1	0	19,65	18,95	4	1	4	15,22	15,54
5	1	0	39,46	38,23	5	1	4	2,72	2,86
6	1	0	8,12	8,03	6	1	4	3,55	3,18
7	1	0	14,51	13,63	7	1	4	,54	1,16
8	1	0	6,80	7,14	8	1	4	6,05	6,06
9	1	0	1,12	,83	9	1	4	3,32	2,77
10	1	0	0,00	,26	10	1	4	2,45	2,08
11	1	0	2,74	3,88	11	1	4	2,37	2,62
12	1	0	4,20	4,15	12	1	4	3,46	4,01
13	1	0	7,26	7,61	0	1	5	17,63	17,25
14	1	0	4,27	3,23	1	1	5	3,08	3,43
2	1	1	29,42	31,21	2	1	5	,66	,94
3	1	1	36,72	37,91	3	1	5	4,10	4,55
4	1	1	3,68	3,36	4	1	5	6,53	6,79
5	1	1	19,55	18,89	5	1	5	9,54	9,65

H	K	L	F0	FC
6	1	5	4,64	3,95
7	1	5	5,78	5,28
8	1	5	2,71	3,16
9	1	5	,68	,46
10	1	5	1,88	1,98
0	1	6	4,95	4,86
1	1	6	6,16	5,90
2	1	6	2,34	2,13
3	1	6	8,19	7,62
4	1	6	8,38	8,50
5	1	6	,81	,64
6	1	6	2,55	2,40
7	1	6	6,82	6,99
8	1	6	4,97	4,99
9	1	6	6,94	7,70
0	1	7	4,90	5,35
1	1	7	1,83	1,72
2	1	7	5,01	4,51
3	1	7	1,34	1,40
4	1	7	3,75	3,49
5	1	7	6,12	6,08
6	1	7	,57	,54
7	1	7	1,47	1,53
0	1	8	2,02	2,01
1	1	8	2,09	1,90
2	1	8	6,77	6,24
3	1	8	,93	1,04
4	1	8	2,33	2,79
0	4	0	,60	,60
0	6	0	0,00	,39
0	8	0	,56	,76
1	4	0	17,22	18,19
1	5	0	3,05	2,56
1	6	0	1,58	1,45
1	7	0	15,87	16,83
1	8	0	2,45	2,48
2	4	0	15,76	15,44
2	5	0	3,69	3,13
2	6	0	9,05	8,90
2	7	0	8,91	8,47
2	8	0	11,83	12,30
3	4	0	23,92	27,24
3	5	0	8,15	7,99
3	6	0	2,77	2,52
3	7	0	11,01	11,35
3	8	0	2,67	2,35
4	4	0	13,71	13,99
4	5	0	4,80	4,75
4	6	0	3,31	3,25
4	7	0	11,62	11,53
4	8	0	3,41	3,46
5	4	0	17,55	15,75
5	5	0	3,59	2,43
5	6	0	10,25	10,44

H	K	L	F0	FC
5	7	0	,98	,96
5	8	0	1,10	,82
6	4	0	3,95	3,06
6	5	0	14,45	14,50
6	6	0	3,55	3,50
6	7	0	6,57	6,69
6	8	0	6,18	6,76
7	4	0	4,02	3,58
7	5	0	7,18	6,19
7	6	0	1,03	1,72
7	7	0	2,38	2,10
8	4	0	21,13	20,60
8	5	0	12,23	11,67
8	6	0	1,59	1,11
8	7	0	8,67	8,65
9	7	0	,87	1,05
9	6	0	7,29	6,91
9	5	0	1,54	1,71
9	4	0	12,66	12,06
10	4	0	10,48	9,41
10	5	0	7,34	6,61
10	6	0	3,79	3,84
11	4	0	2,66	2,59
11	5	0	8,72	7,92
12	4	0	1,80	1,52
12	5	0	5,41	5,01
13	4	1	5,16	7,05
12	4	1	8,97	9,06
12	5	1	1,04	,83
11	4	1	3,19	2,75
11	5	1	4,06	4,04
10	4	1	5,86	6,07
10	5	1	5,43	4,91
10	6	1	,55	,04
9	4	1	6,00	6,05
9	5	1	1,31	,52
9	6	1	3,61	4,62
9	7	1	1,12	1,56
8	4	1	13,57	13,11
8	5	1	9,63	8,82
8	6	1	7,04	7,10
8	7	1	5,21	5,89
7	4	1	11,39	10,58
7	5	1	5,12	4,31
7	6	1	,33	,17
7	7	1	2,91	2,75
6	4	1	13,54	12,48
6	5	1	7,46	7,75
6	6	1	7,83	7,78
6	7	1	7,06	7,01
6	8	1	,88	1,07
5	4	1	14,20	13,42
5	5	1	6,51	5,81
5	6	1	3,45	3,51

H	K	L	FO	FC	H	K	L	FO	FC
#5	7	1	11,96	11,58	6	6	1	1,03	,84
#5	8	1	2,93	2,91	6	7	1	8,85	8,52
#4	4	1	19,86	19,01	6	8	1	2,21	2,44
#4	5	1	1,35	,76	7	4	1	12,66	14,79
#4	6	1	6,05	6,14	7	5	1	9,24	9,02
#4	7	1	5,77	5,52	7	6	1	5,04	4,24
#4	8	1	4,50	4,60	7	7	1	2,55	2,79
#3	4	1	2,18	2,09	8	4	1	2,69	2,40
#3	5	1	8,31	7,21	8	5	1	2,54	2,30
#3	6	1	6,51	7,64	8	6	1	0,00	,18
#3	7	1	6,13	5,72	8	7	1	3,91	3,55
#3	8	1	8,60	8,86	10	4	1	5,47	5,55
#2	4	1	13,78	12,51	10	5	1	1,20	,02
#2	5	1	7,72	7,11	10	6	1	1,23	1,07
#2	6	1	4,14	4,32	11	4	1	4,35	4,49
#2	7	1	5,95	5,63	11	5	1	3,35	3,20
#2	8	1	5,29	5,58	12	4	1	8,87	8,79
#1	4	1	1,46	1,75	#12	4	2	3,23	2,31
#1	5	1	6,11	5,73	#11	4	2	8,13	7,12
#1	2	1	20,74	21,72	#11	5	2	6,93	6,02
#1	7	1	9,83	9,55	#10	4	2	2,73	2,30
#1	8	1	5,76	5,72	#10	5	2	1,40	1,13
0	4	1	17,05	16,61	#10	6	2	0,00	,54
0	5	1	1,26	1,24	#9	4	2	8,11	7,12
0	6	1	3,87	1,84	#9	5	2	6,49	6,04
0	7	1	9,91	9,58	#9	6	2	1,63	1,81
0	8	1	2,53	2,21	#8	4	2	8,58	7,51
1	4	1	8,99	8,23	#8	5	2	17,64	17,39
1	5	1	5,68	5,69	#8	6	2	2,49	2,59
1	6	1	14,33	17,83	#8	7	2	9,52	9,36
1	7	1	8,07	8,02	#7	4	2	10,48	9,29
1	8	1	8,68	8,35	#7	5	2	11,73	11,34
2	4	1	5,86	5,10	#7	6	2	2,38	1,92
2	5	1	1,22	,08	#7	7	2	3,19	2,88
2	6	1	9,39	10,03	#6	4	2	1,55	1,89
2	7	1	2,69	2,89	#6	5	2	9,74	9,53
2	8	1	7,86	8,42	#6	6	2	4,94	4,41
3	4	1	8,71	8,68	#6	7	2	8,02	7,05
3	5	1	8,26	7,05	#6	8	2	3,21	3,04
3	6	1	3,92	3,54	#5	4	2	13,14	14,48
3	7	1	7,87	7,57	#5	5	2	13,45	13,02
3	8	1	5,08	5,31	#5	6	2	8,21	7,97
4	4	1	15,00	14,54	#5	7	2	,40	1,51
4	5	1	7,22	7,80	#5	8	2	2,25	1,94
4	6	1	2,57	2,31	#4	4	2	13,58	14,94
4	7	1	8,78	8,60	#4	5	2	12,27	10,80
4	8	1	3,54	3,35	#4	6	2	9,32	8,47
5	4	1	16,71	17,25	#4	7	2	6,81	6,43
5	5	1	1,09	,40	#4	8	2	2,48	2,69
5	6	1	3,67	2,51	#3	4	2	18,21	17,65
5	7	1	13,41	14,54	#3	5	2	2,17	2,07
5	8	1	4,98	4,77	#3	6	2	7,08	6,69
6	4	1	10,50	11,03	#3	7	2	1,34	1,41
6	5	1	4,48	3,42	#3	8	2	3,66	3,42

H	K	L	F0	FC
2	4	2	12,88	12,42
2	5	2	11,97	13,99
2	6	2	15,53	16,18
2	7	2	4,79	5,12
2	8	2	12,36	11,73
1	4	2	10,11	9,38
1	5	2	1,63	1,42
1	6	2	5,07	4,23
1	7	2	11,40	10,99
1	8	2	6,64	5,96
0	4	2	2,53	3,16
0	5	2	2,70	2,86
0	6	2	7,16	7,20
0	7	2	7,41	6,58
0	8	2	5,90	5,31
1	4	2	22,54	24,74
1	5	2	,65	,40
1	6	2	1,81	1,87
1	7	2	8,89	8,34
1	8	2	4,93	4,97
2	4	2	9,35	8,37
2	5	2	1,89	2,13
2	6	2	2,08	2,77
2	7	2	5,27	5,11
2	8	2	3,94	3,57
3	4	2	13,45	16,16
3	5	2	1,43	1,86
3	6	2	15,27	16,98
3	7	2	9,25	9,50
3	8	2	5,55	5,14
4	4	2	9,44	9,83
4	5	2	6,04	5,99
4	6	2	3,91	4,03
4	7	2	8,68	8,80
4	8	2	3,09	3,25
5	4	2	2,70	2,77
5	5	2	4,88	5,20
5	6	2	0,00	1,90
5	7	2	2,50	2,16
5	8	2	2,41	2,06
6	4	2	,87	,07
6	5	2	15,00	16,21
6	6	2	2,06	2,34
6	7	2	8,00	7,99
7	4	2	11,44	11,84
7	5	2	4,74	4,72
7	6	2	2,10	1,96
7	7	2	1,54	1,06
8	4	2	9,37	9,60
8	5	2	5,62	5,41
8	6	2	3,24	2,94
8	7	2	1,40	1,10
9	4	2	6,87	7,00
9	5	2	5,57	5,36

H	K	L	F0	FC
9	6	2	3,24	3,06
10	4	2	1,56	1,63
10	5	2	8,28	8,05
11	4	2	1,19	1,33
11	5	2	6,41	6,60
12	4	2	4,87	4,64

APPENDIX F

The modification of Program

QCPE 91

APPENDIX F

THE MODIFICATION OF PROGRAM QCPE 91

In order to have a suitable tool for making CNDO/2 calculations on the conformations of molecules, large changes were necessary in Segal's original (1966) QCPE 91 program. The changes were made over a period of time as need arose, but for convenience are described here in one section.

It was first necessary to adapt the program to run on the CDC 7600 machine rather than the CDC 6000 series computers for which the original version was designed. As described in Chapter 2, the CDC 7600 has a rather limited amount of 'small core memory' (SCM) ie. directly accessible central storage. QCPE 91, however, contains four large arrays: for storing the Fock-matrix, two bond-order matrices, and intermediate results. If N is the maximum number of basis functions used, each of these arrays requires $N \times N$ words of storage. By moving the two bond-order matrices into large core memory (LCM), the requirement of using less than 24K SCM could be met, though with a consequent loss of run-time efficiency. Since LCM data cannot be used as parameters in FORTRAN FUNCTION statements, some further changes involving the setting up of certain dummy variables in SCM was also required.

The original program did not have any facilities for manipulating molecular geometries and was thus unsuitable for the computation of conformational energy maps. To overcome this difficulty subroutines designed to make possible the systematic variation of molecular geometry as a function of dihedral angles were added. It was then possible to map large areas of energy surfaces of small molecules in each computer run.

With the program so-modified it was possible to carry out calculations on some molecules fairly rapidly. However, it was found that in certain cases the normal SCF process did not converge, but instead

oscillated. Some simple method was required to force convergence: one such which has previously been used (for example Howland and Flurry (1972)) has been to average values of the density matrix elements obtained in successive iterations. If P_i is a particular element of the density matrix at the i th iteration of the SCF process, then:

$$P_{i+1} = \frac{P_{i+1} + P_i}{2}$$

In QCPE 91 it was found to be more convenient to use, instead, successive values of the Fock matrix, and doing so normally produced satisfactory convergence; it was sometimes found useful to use the more general weighted mean:

$$F_{i+1} = (\chi) \cdot (F_i) + (1-\chi) \cdot (F_{i+1}) \dots (F_1)$$

$$\text{where } \chi \leq 1$$

$$\text{and } i > 1$$

In all cases, after convergence had been achieved a further iteration, not using the averaging procedure, was made to confirm that the solution obtained was indeed self-consistent. The method described above requires the storage of another large matrix, making five in all. It was possible to accommodate this additional array in LCM.

It became apparent that the program would have to be enlarged to carry out the calculations envisaged for some larger molecules: the original limits of up to 32 atoms and 72 basis functions did not, for example, allow studies of bicuculline, the GABA antagonist, to be made. It was decided to produce a program capable of treating a system of 50 atoms with 150 basis functions. This modification presented some difficulties: it was no longer possible to keep the Fock-matrix SCM resident. Further, only two large arrays could be held simultaneously in LCM: this necessitated the use of backing store. Data may be transferred to and from the disc stores available on the CDC 7600 at high rates (up to 580K words per second) but even so, access times are at least 2 orders of magnitude slower than from

LCM. By a careful analysis of the program it was found that certain of the large arrays were only used infrequently, eg the array holding the previous values of the Fock-matrix was only used once per iteration, for averaging purposes. Also most of the program statements only needed two arrays to be core resident at any given time. The exception occurred in the sections of coding concerned with open shell molecules: FORTRAN statements here needed to access elements of three arrays simultaneously. As an interim solution, a program suitable for use with large closed-shell molecules only was developed. Each of the large arrays was assigned to a distinct file on disc backing store. The bond-order matrices etc, could then be called into core when required; it was found that large numbers of swops to and from disc were not required. It was necessary to transfer the whole of a given file at one time, maintaining the order of the data elements making up the file. This situation was ideally suited to the use of sequential file format - the simplest form of organisation of information on disc, and one which corresponds to that on magnetic tape. The use of this type of file also minimised the extra core store required by the SCOPE operating system for input/output buffers. The program was therefore modified to use disc files in this way and worked satisfactorily.

To produce an enlarged program which could also treat open-shell molecules required a different strategy. The major problem was that of FORTRAN coding of the form:-

```

      DO 60 K = 1,N
      DO 60 I = 1,N
      DO 60 J = 1,N

      60  TY(I,J) = TY(I,J) + P(K,I)* F(K,I)

```

where TY, P and F were all large matrices and could not be core-resident simultaneously. It would, of course, have been possible to recode the program, changing the difficult sections or, making

use of sequential files, to read in small sections of each array, carry out the computations involving those elements only and then write the results to disc before reading in the next segment. The latter method, which would also have required extensive recoding of the program, is an untidy solution and, more important, is an inefficient way of using sequential files. A better method and the one adopted, was to place one array (that used only for intermediate results) on disc as a random access file. Each element of a random access file is immediately accessible without the need for positioning the file by REWIND or other commands which would be required when using a sequential file. Similarly, each element of the file may be rewritten without regard to previous WRITE commands which may have accessed other sections of the file. Using the random access form of file organisation has, however, one disadvantage: the operating system requires more space in LCM for input/output buffer areas. In this case it proved possible to keep within the LCM limit of 70K words and the program could be run with no further modification.

The CNDO/2 program at this stage was capable of dealing with calculations on small molecules, molecules for which SCF convergence was difficult, and large molecules. In the latter two cases, run times could be extremely lengthy. Also, even for the case of small molecules, the computing time for a complete energy surface was considerable. It was therefore desirable to find some way of making the calculations more rapidly. As described in section 4.2, to begin the SCF procedure an initial estimate of the values of the LCAO coefficients is required. In QCPE 91 this estimate is obtained by diagonalising a Hückel-type matrix. Winter and Dunning (1971) have demonstrated that the choice of a good initial guess at the coefficients can be very important in speeding the subsequent calculations. In the studies described here a good guess is normally available: it is the final coefficients obtained from a calculation on the molecule in a similar conformation. Considering the case of a

conformational energy surface being plotted using a 20° grid the use of the final eigenvectors produced at an adjacent grid point as the starting point for the new calculation can save significant amounts of computing time. Some examples are listed in Table F.1. Using a 5° grid the savings may be even more significant. For example, in the case of muscimol the time per conformation was reduced from 19 seconds to 8 seconds.

The above procedure was also found to be helpful in cases where convergence was difficult. A normal calculation on ibotenic acid anion did not converge and averaging of the Fock-matrix was necessary. However, using the saved vectors from a previous calculation led immediately to convergence with no averaging necessary; the time per conformation was drastically reduced as detailed in Table F.1.

Computationally, no great difficulties were encountered in implementing the procedure. Since the saved eigenvectors had only to be accessed and up-dated once per conformation, the data could be kept on disc as a sequential file with little loss in efficiency.

As the development of the above procedure was completed, what is apparently a similar method was briefly mentioned by Singh and Ferro (1974) in a paper concerned with hydrogen-bonded systems.

One further development was carried out. The values of the coefficients obtained from successive SCF iterations form a series. Aitkens generalised δ^2 method can be used to find, by extrapolation, the limit of such a series. The use of such extrapolations in *ab initio* molecular orbital calculations has been described by Winter and Dunning (1971). They extrapolated on the molecular orbital coefficients, but a more satisfactory approach is that of Howland and Flurry (1972) who used the density matrix. The method described was a five-point extrapolation procedure and thus required the storage of five intermediate versions of the density matrix.

molecule	time/conformation: normal calculation (seconds)	time/conformation: coefficients saved from a previous calculation (seconds)
muscimol	19	9
Ibotenic acid anion ($C_5H_5N_2O_4^-$)	35	21
GABA	9	7
α -fluoro γ -aminobutyric acid ($C_4H_8FNO_2$)	11	11
ATA	9	7

TABLE F.1 Examples of calculations using saved vectors

If $P_O^0, P_O^1, P_O^2, P_O^3$ and P_O^4 are the values of a particular element of the density matrix obtained from successive SCF iterations, the extrapolation algorithm is:

$$P_{i+1}^m = P_{i-1}^{m+1} + \frac{1}{P_i^{m+1} - P_i^m}$$

When $i = 0$, P_{i-1}^{m+1} is taken as zero and the extrapolation pyramid below may be built up:

$$\begin{array}{ccccccc} & & & & & & P_O^0 \\ & & & & & & P_O^1 & P_1^0 \\ & & & & & & P_O^2 & P_1^1 & P_2^0 \\ & & & & & & P_O^3 & P_1^2 & P_2^1 & P_3^0 \\ & & & & & & P_O^4 & P_1^3 & P_2^2 & P_3^1 & P_4^0 \end{array}$$

P_4^0 is then the predicted value of the matrix element.

A subroutine to implement the method was coded for future inclusion in the CNDO program. The precautions for the case of a nearly linear series, described by Howland and Flurry, were included; an additional trap to deal with problems which occurred when successive iterations produced values close to an exact geometric series was also coded. The subroutine was developed and tested on-line on the ICL 1905. A flow chart of the routine is given in Fig F .1

The extrapolation procedure was the final major development carried out but other, more minor, changes aimed at making the CNDO program more convenient to use have also been made. These include: the ability to vary the amount of printed output from a minimal summary to a complete listing; the ability to punch cards for the atomic coordinates of the conformation being studied; the facility to change the criterion for SCF convergence; and the facility to alter the diagonalisation criterion for the eigenvector-eigenvalue routine used in

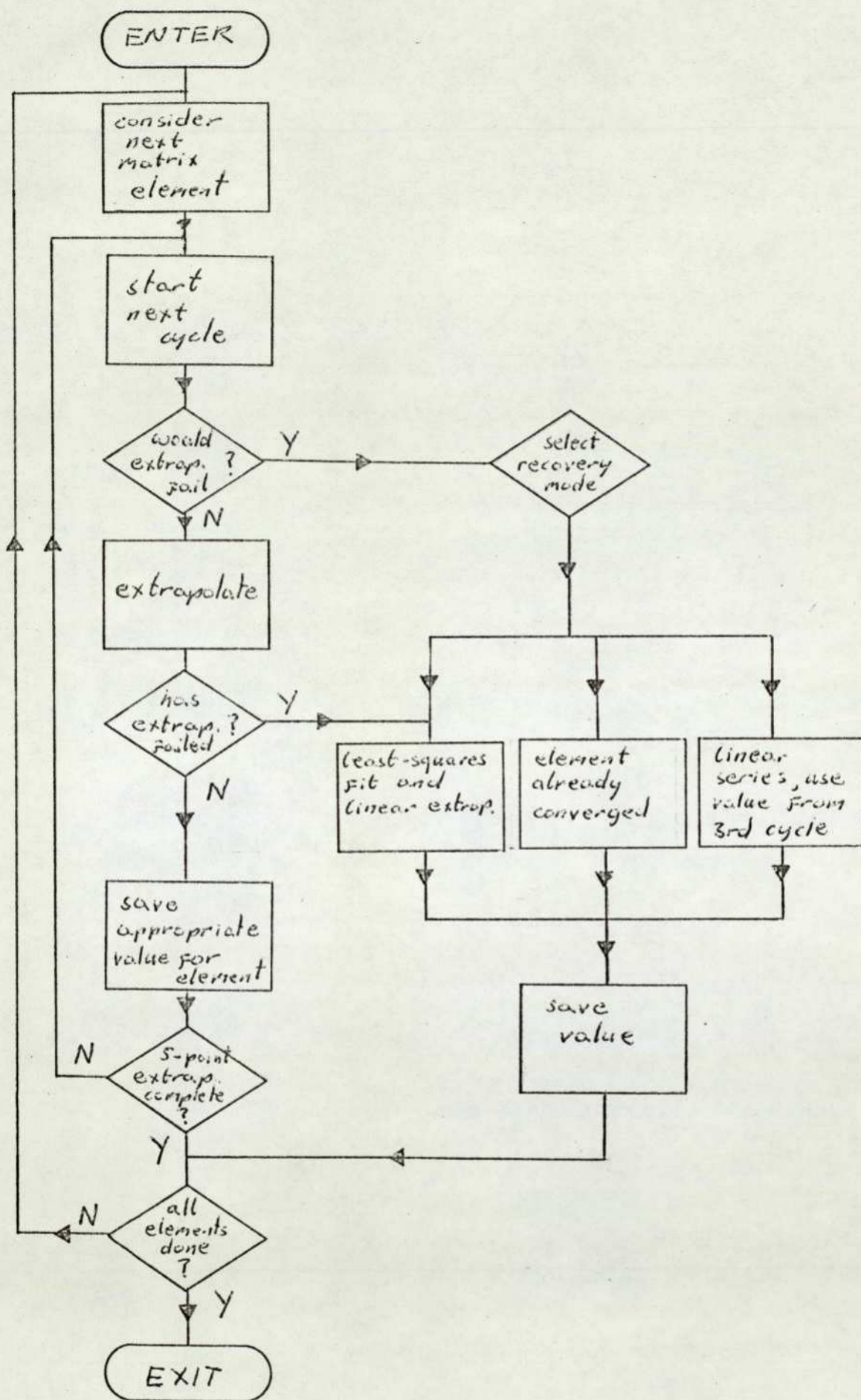


Fig. F.1 Logic flow-chart for the extrapolation procedure

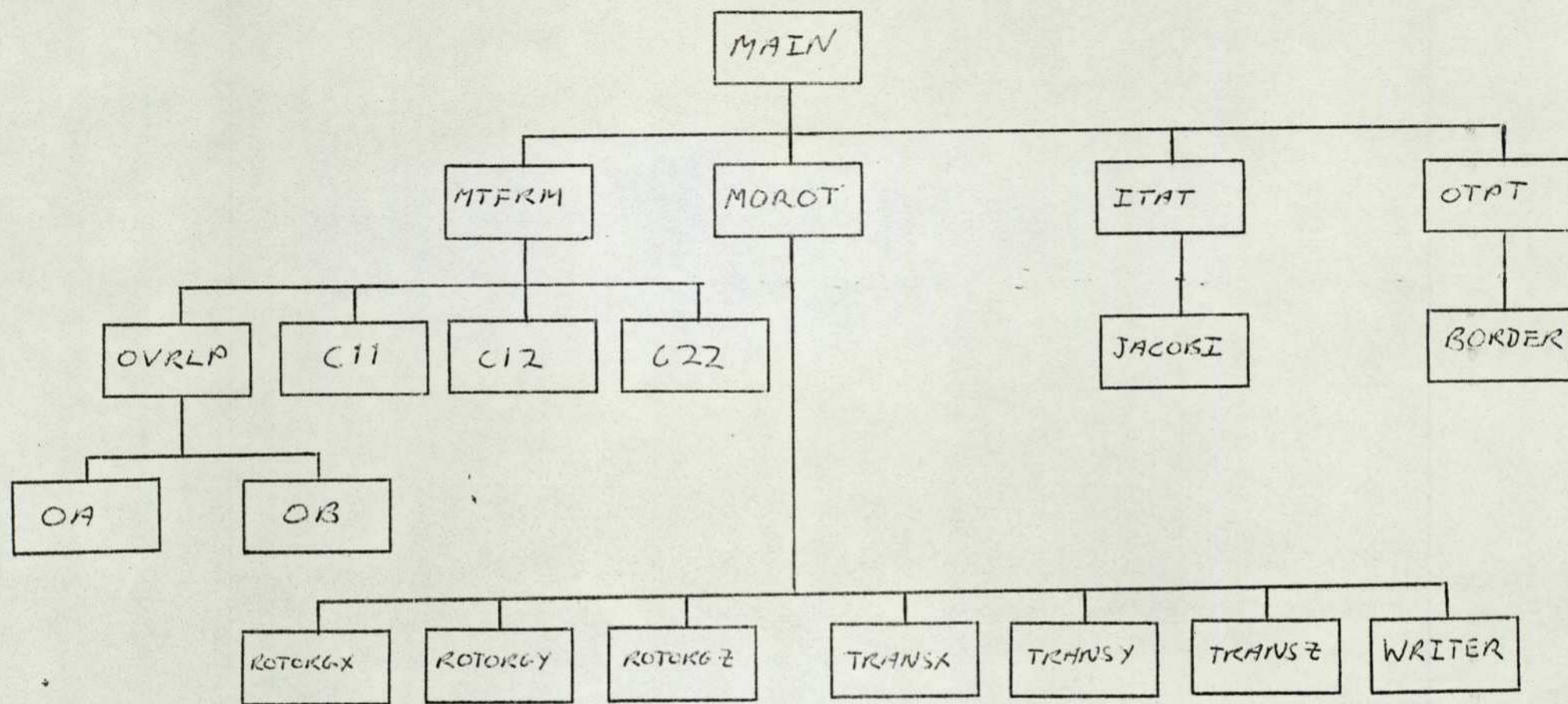


Fig. F.2 Hierarchy diagram of the modified CNDO/2 program

the program.

The program resulting from all these changes is described by the block diagram in Fig F.2. This modified QCPE 91 has proved to be extremely flexible and convenient to use. Its generality may be demonstrated by considering the options which may be specified on the control card which is the first input record to the program.

This card has 10 fields which are designated: IPART, IPROUT, IPUUT, IAV, KMARK, ISAVE, IEXTRA, SCONST, SDIAG, AVWEIGHT. These parameters may take the values listed below. The form of the list is

NAME = a/b/.....: effect of a/effect of b/.....

ie, 'NAME' may be assigned the values 'a' or 'b' and the result will be 'effect of a' or 'effect of b'.

IPART = 0/1/2/3: no further calculations/expect to read a complete set of data, change the conformation and carry out a CNDO calculation /expect to read only the data for a change of conformation, produce the new conformation and make a CNDO calculation/expect to read data specifying a series of conformations (eg 20° rotations about a given bond); produce a new conformation and carry out a CNDO calculation - do this as many times as specified.

IPROUT = 0/1/2/3: print only final energies, electronic populations and dipole moments/additionally print the electronic energy for each iteration/additionally print a limited summary of geometrical information on the molecule together with coulomb repulsion integrals/print whole of geometrical data, coulomb integrals, electronic energy for each iteration, final values of the density and overlap matrices, electronic populations, and dipole moments.

IPUUT = 0/1/2: no punched cards/punch cards for the new conformation/punch cards only for the atoms whose coordinates have changed.

IAV = 0/1: do not average the Fock matrix/average successive values of the Fock-matrix.

KMARK = 0/1: carry out the coordinate transformations exactly as specified by IPART but do not make a CNDO calculation/carry out calculations normally.

ISAVE = 0/1: normal Hückel-type starting point for SCF calculation/
saved eigenvectors of a previous conformation to be used as the
starting point..

IEXTRA = 0/1: no extrapolation/extrapolate after every sixth iteration
(this option included for the future development of the extrapolation
procedure).

SCONST = a real positive number: the energy criterion for SCF
convergence . If SCONST = 0, this is set to .0001 by default.

SDIAG = a real positive number: the criterion for matrix diagonalisation.
If SDIAG = 0, it is set to $(SCONST)^2$ by default.

AVWEIGHT = a real positive number: the factor (x in equation F.1) for
weighting successive values of the Fock-matrix in the averaging process.
If AVWEIGHT = 0, it is set to 0.5 by default.

APPENDIX G

The Busing Computer Program for
Classical Potential-Energy Calculations

APPENDIX G

THE BUSING COMPUTER PROGRAM FOR CLASSICAL POTENTIAL-ENERGY CALCULATIONS

A copy of the program described by Busing (Busing, 1972) was obtained and converted to run on the CDC 7600. The program proved to be very complex - consisting of some 3000 FORTRAN statements - and sophisticated. In fact, it had many more facilities than were required for the project in hand. Most attention was therefore paid to converting and testing those sections of the program which were needed immediately.

A block diagram showing the hierarchy of the subroutines within the program is given in Fig.G.1. Below a list of the routines and their functions is given.

- | | |
|--------|---|
| WMIN | - The executive routine of the program. Carries out some input and output, organises data storage and retrieval and controls the flow of the energy calculations. |
| WDYN | - Calculates frequencies and normal modes of oscillation. |
| WPRE | - Reads input data and makes preliminary calculations on coordinates. |
| WCALC | - Carries out energy calculations as required. Controls the generation of atomic coordinates from the initial specification and the necessary rotations and translations. |
| GCWX | - Calculates the energy of bond distances, bond-angles, and torsion angles. |
| IBYPAS | - Sets an indicator within the program to avoid the repetition of identical calculations in successive cycles of computations. |

TIMOUT - Analyses and prints times for the various stages of the energy calculation.

ANG - Calculates an angle defined by three atoms.

AVV - Calculates the angle between two vectors.

AXELBX - Reduces the problem

$$A * X = \lambda * B * X$$

to the form

$$P * Z = \lambda * Z$$

where A and B are symmetric matrices.

AXES - Stores three orthogonal unit vectors.

CNSTRN - A user-supplied subprogram to determine which parameters of the calculation are to be variables.

CONF - Calculates the dihedral angle defined by four atoms.

CWX - A user-supplied subroutine to calculate any non-standard contributions to the energy.

DIFV - Calculates the difference of two vectors.

DST - Computes the distance between two atoms.

GMADD - Adds two general matrices.

GMPRD - Multiplies two general matrices

GMTRA - Finds the transpose of a general matrix.

GTPRD - Premultiplies a general matrix by the transpose of another general matrix.

HOUSEH }
VALVEC } - Subprograms to solve the eigenvector-eigenvalue problem.

INVRs - Forms the inverse and evaluates the determinant of a 3x3 matrix.

LOK	- Computes a vector subscript for an element in a matrix of specified storage mode.
MCPY	- Copies a matrix.
MINV	- Inverts a general matrix.
MM	- Multiplies two 3x3 matrices.
MPRD	- Multiplies two general matrices.
MV	- Computes : matrix * vector.
NORM	- Forms a vector normal to 2 given vectors.
REJECT	- A user-supplied routine to avoid the calculation of space-group absences.
REPL	- A user-supplied routine to calculate repulsion energy
SETA	- A user-supplied routine to set constraints due to lattice symmetry.
SUMV	- Add two vectors.
TRANS	- Stores the transpose of a 3x3 matrix.
TRANSF	- Transforms eigenvectors produced by VALVEC.
UNIT	- Produces a unit vector in a given direction.
VEC	- Computes the vector defined by 2 atoms.
VMV	- Computes : transposed vector * matrix * vector.
WRAG	- Evaluates gaussian function.
WRAD	- Evaluates second derivative of gaussian function.

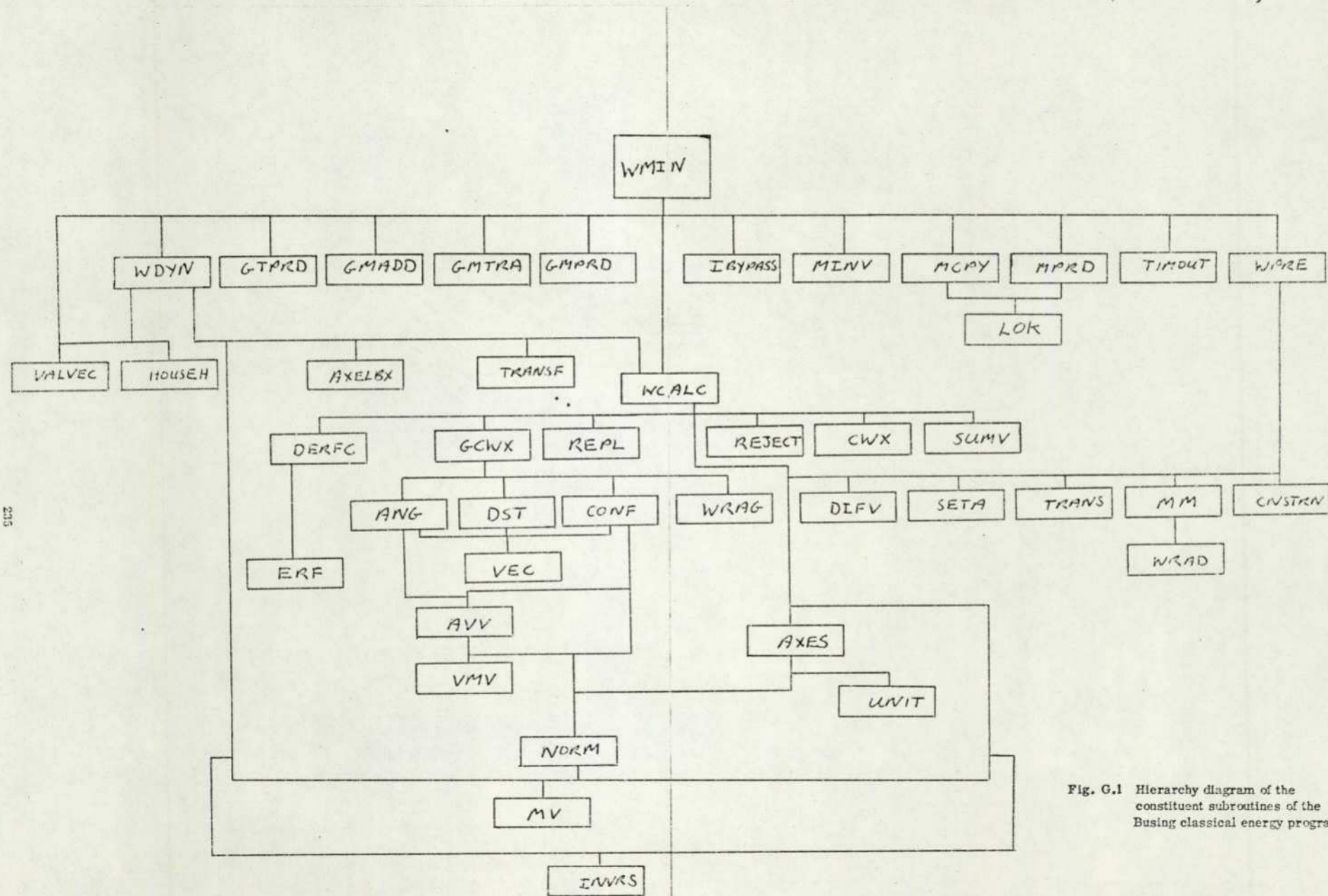


Fig. G.1 Hierarchy diagram of the constituent subroutines of the Busing classical energy program.