SYNTHESIS AND SOME PHARMACOLOGICAL PROPERTIES OF SOME N-SUBSTITUTED CYCLIC IMIDES

BY

IVAN OGHENEOCHUKO EDAFIOGHO

A

THESIS

Submitted to

Ahmadu Bello University

In partial fulfillment of the requirements for the degree c

MASTER OF SCIENCE IN PHARMACY

1 9 8 0

THESIS APPROVAL

This thesis by Ivan Ogheneochuko Edafiogho meets the regulations governing the degree of Master of Science in Pharmacy of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

Internal examiners

(a) Pharmaceutical Chemistry:

.....
J. Ayoola Owoyale, B.Sc., MSP, Ph.D.,
Department of Pharmacology and
Pharmaceutical Chemistry.

(b) Pharmacology:

.....
Gabriel E. Osuide, B.Pharm., Ph.D.,
F.S.A.N., F.A.S.,
Prof. of Pharmacology,
Department of Pharmacology and
Pharmaceutical Chemistry.

.....
G.D. Lahan, B.Sc.(Pharm.), Ph.D.,
Department of Pharmacology and
Pharmaceutical Chemistry.

External Examiner

.....
A.A. Olaniyi, B.Pharm.,
Ph.D.,
Ag. Head,
Department of Pharmaceutical Chemistry,
Faculty of Pharmacy,
University of Ife,
Ile-Ife.

Date: MARCH, 1980

DECLARATION

This is to certify that the work reported in this thesis was carried out by me under the supervision of Professor Gabriel E. Osuide, Dr. J. Ayoola Owoyale and Dr. G.D. Lahan of Department of Pharmacology and Pharmaceutical Chemistry. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.

Ivan Ogheneochuko Edafiogho,
Department of Pharmacology and Pharmaceutical
Chemistry, Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
ZARIA.

DEDICATION

To Freddy Edafiogho

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to Professor Gabriel Osuide, Dr. Ayoola Owoyale, and Dr. Gbola Lahan for their excellent supervision and guidance.

My gratitude also goes to Professor Frank Stansfield, and Dr. Steve Okecha of Chemistry Department for useful advice and helping to send samples of my compounds to Denmark for elemental analysis.

I am grateful to Mr. N.A. Ogunwole (Principal Technologist), the Pharmacy Technologists and Technicians for their technical assistance; Mallam Sheidu Ibrahim for typing this manuscript, and to my friends for their moral support.

GLOSSARY OF ABBREVIATIONS

ACH	-	Acetylcholine
AOAA	-	Aminooxyacetic acid
Cm	-	Centimeter
$Cdcl_3$	-	Deuterochloroform
CNS	-	Central Nervous System
CSF	-	Cerebrospinal fluid
DA	-	Dopamine
def	-	Deformation
DPA	-	Sodium di-n-propylacetate
EEG	-	Electroencephalogram, -graph,-graphic
e.g.	-	For example
ENPA	-	Ethyl N-phthalimidoxyacetate
ENP-2-P	-	Ethyl N-phthalimidoxy-2-propionate
EOS	-	Ethanolamine-O-sulphate
EPGABA	-	γ-ethyl-γ-phenyl-γ-aminobutyric acid
EPP	-	5-ethyl-5-phenyl-2-pyrrolidinone
ETOH	-	Ethanol
ENSA	-	Ethyl N-succinimidoxyacetate
GABA	-	Gamma-aminobutyric acid
GABA-T	-	Gamma-aminobutyric acid - α-oxoglutarate transaminase

GAD-1	-	Glutamic acid decarboxylase
h.	-	hour
HPA	-	Hydrazinopropionic acid
i.e.	-	That is
INH	-	Isonicotinic acid hydrazide
i.p.	-	Intraperitoneal,-ly
i.r.	-	Infra red
min.	-	Minute
mp	-	Melting point
MNPA	-	Methyl N-phthalimidoxyacetate
MNSA	-	Methyl N-succinimidoxyacetate
MPP	-	5-methyl-5-phenyl-2-pyrrolidinone
n.m.r.	-	Nuclear magnetic resonance
No.	-	Number
s.c.	-	Subcutaneous
sec.	-	Second
TLC.	-	Thin layer chromatography
uv	-	Ultra violet

TABLE OF CONTENTS

	Page
THESIS APPROVAL	i
DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
GLOSSARY OF ABBREVIATIONS	v
TABLE OF CONTENTS	vii
LIST OF FIGURES	x
ABSTRACT	xiii
CHAPTER	
I INTRODUCTION	1
Early Concepts of Causes and Treatment of Epilepsy	2
Modern Approach to Seizure Mechanisms..	6
Biochemical Approach to Seizure Mechanism	8
Acetylcholine	8
5-Hydroxytryptamine (5-HT)	9
Systemic electrolytes	10
Potassium and Sodium	11
Calcium	11
Amino Acids	11
Gamma-aminobutyric acid (GABA) ...	13
Hydroxylamine	15
Hydrazides	16
GABA analogues	16

TABLE OF CONTENTS(CONTINUED.....)

CHAPTER		Page
	Hydrazinopropionic Acid (HPA)	17
	Ethanolamine-O-Sulphate (EOS)	18
	Sodium di-n-propylacetate (DPA)... ...	18
	Aminooxyacetic acid (AOAA)	19
	General Survey of Anti-convulsant Agents Tried in the Therapy of Epilepsy...	20
	Testing of Anti-convulsant Agents ...	22
	Purpose of this Research	22
	Lipophilic substituents	24
2.	CHEMICAL SYNTHESES	26
	Choice of Compounds	26
	Hybrid products	27
	Survey of Various Synthetic Methods	28
	Mechanism of Reaction	29
	General Method	33
	ENPA	36
	ENP-2-P...	36
	MNPA	37
	ENSA	37
	MNSA	38
	Experimental	39
	General	39
	Ethyl N-phthalimidoxyacetate (ENPA) ...	40
	Ethyl N-phthalimidoxy-2-propionate (ENP-2-P)	41
	Methyl N-phthalimidoxyacetate (MNPA)...	42
	Ethyl N-succinimidoxyacetate (ENSA) ...	42
	Methyl N-succinimidoxyacetate (MNSA)...	43
3.	PHARMACOLOGICAL SCREENING	45
	Behavioural Studies	45
	Materials and Methods	46

TABLE OF CONTENTS (CONTINUED.....)

CHAPTER		Page
	Results of Behavioural Studies...	48
	AOAA	48
	ENP-2-P...	50
	MNPA	51
	ENSA	51
	MNSA	51
	Experimentally-induced Seizures ...	52
	Materials and Methods	52
	Electrically-induced Seizures ...	52
	Chemically-induced Seizures ...	54
	Results	56
	Influence of AOAA on experimentally-induced seizures..	56
	Influence of ENPA on experimentally-induced seizures..	57
	Influence of Phenytoin (DPH) on electroshock seizures ...	57
	Influence of Trimethadione (TMO) on chemical seizures	58
	ENP-2-P	58
	MNPA	59
	ENSA	60
	MNSA	61
4.	DISCUSSION	62
	Use of Chicks	62
	Use of Cages	63
	N-substituted Phthalimides	65
	N-substituted Succinimides	72
	Possibility of Using N-Substituted Cyclic Imides as leads	75
	Conclusion	80
	REFERENCES	83
	BIOGRAPHICAL SKETCH	90

LIST OF FIGURES

Figure

2(i) N.M.R. spectrum of ethyl N-phthalimidoxy2-propionate, ENP-2-P in deutorochloroform.

2(ii) Infra-red spectrum of ethyl N-phthalimidoxy-2-propionate, ENP-2-P in nujol.

2(iii) N.M.R. spectrum of methyl N-phthalimidoxyacetate, MNPA in deutorochloroform.

2(iv) Infra-red spectrum of methyl N-phthalimidoxyacetate, MNPA in nujol.

2(v) N.M.R. spectrum of ethyl N-succinimidoxyacetate, ENSA in deutorochloroform.

2(vi) Infra-red spectrum of ethyl N-succinimidoxyacetate, ENSA in nujol.

2(vii) N.M.R. spectrum of methyl N-succinimidoxyacetate, MNSA in deutorochloroform.

2(viii) Infra-red spectrum of methyl N-succinimidoxyacetate, MNSA in nujol.

2(ix) Thin layer chromatography (TLC) of the N-substituted cyclic imides and their starting materials (N-hydroxyphthalimide, NHP and N-hydroxysuccinimide, NHS) in methanol: chloroform (1:9).

3(i) Histograms showing the effects of 200 mg/kg of some N-substituted phthalimides on ataxia, tonic seizure and lethality in 4-day old chicks. n = 15 per histogram.

3(ii) Histograms showing the effects of 200 mg/kg of some N-substituted succinimides on ataxia, tonic seizure and lethality in 4-day old chicks. n = 15 per histogram.

3(iii) Histograms showing the effects of 50,100 and 150 mg/kg of ethyl N-phthalimidoxy-2-propionate, ENPA-2-P on susceptibility of 4-day old chicks to electroshock. n = 15 per histogram.

3(iv) Histograms showing the time course of anti-convulsant effect of ethyl N-phthalimidoxy-2-propionate, ENP-2-P (150 mg/kg) in 4-day old chicks. n = 15 per histogram.

3(v) Histograms showing the effects of 10,20 and 50 mg/kg of methyl N-phthalimidoxyacetate (MNPA) on susceptibility of 4-day old chicks to electroshock. n=15 per histogram.

3(vi) Histograms showing the time course of anti-convulsant effect of methyl N-phthalimidoxyacetate, MNPA (10 mg/kg) in 4-day old chicks. n = 15 per histogram.

3(vii) Histograms showing the effects of 5,10 and 50 mg/kg of ethyl N-succinimidoxyacetate, ENSA on susceptibility of 4-day old chicks to electroshock. n = 15 per histogram.

3(viii) Histograms showing the time course of anti-convulsant effect of ethyl N-succinidoxyacetate, ENSA (10 mg/kg) in 4-day old chicks. n = 15 per histogram.

3(ix) Histograms showing the effects of 5,10 and 20 mg/kg of methyl N-succinimidoxyacetate, MNSA on susceptibility of 4-day old chicks to electroshock. n = 15 per histogram.

3(x) Histograms showing the time course of anti-convulsant effect of methyl N-succinimidoxyacetate, MNSA (5 mg/kg) in 4-day old chicks. n = 15 per histogram.

3(xi) Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on AOAA-induced seizure and lethality in 4-day old chicks. n = 15 per histogram.

3(xii) Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on bicuculline-induced seizure and lethality in 4-day old chicks. n = 15 per histogram.

3(xiii) Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on leptazol-induced seizure and lethality in 4-day old chicks. n = 15 per histogram.

3(xiv) Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on picrotoxin-induced seizure and lethality in 4-day old chicks. n = 15 per histogram.

3(xv) Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on strychnine-induced seizure and lethality in 4-day old chicks. n = 15 per histogram.

Abstract

SYNTHESIS AND SOME PHARMACOLOGICAL
PROPERTIES OF SOME N-SUBSTITUTED CYCLIC IMIDES

by

Ivan Ogheneochuko Edafiogho

1980

Major Area: Pharmaceutical Chemistry

Aminooxyacetic acid (AOAA) is a potent anti-convulsant agent but it has convulsive properties at high doses. However, an analogue of AOAA, ethyl N-phthalimidoxyacetate (ENPA) has been reported to lack the convulsant property of AOAA at high doses but to be less potent than AOAA in its anti-convulsant activities. It was decided to prepare other cyclic imides, in particular hybrid compounds consisting of a cyclic imide associated with anti-convulsant properties such as the succinimides and AOAA with the hope of obtaining some potent anti-convulsant agents which are devoid of the convulsant tendency at

high doses. Other properties which would enhance the lipophilicity of AOAA were also considered. Therefore, ethyl N-phthalimidoxy-2-propionate (ENP-2-P) and three new compounds, ethyl N-succinimidoxyacetate (ENSA), methyl N-phthalimidoxyacetate (MNPA), and methyl N-succinimidoxyacetate (MNSA) were synthesized and screened for behavioural and anti-convulsant effects in 4-day old chicks. The results showed that whereas ENP-2-P and MNPA were excitatory and convulsant respectively at high doses, ENSA and MNSA did not produce any noticeable effects on the behavioural activity. All the compounds possessed some degree of anti-convulsant activity but none of them was as potent as AOAA. However, ENSA and MNSA lacked the convulsant property of AOAA at high doses. When compared with standard anti-convulsant drugs, the N-substituted cyclic imides were inferior to phenytoin and trimethadione in protecting against electroshock and chemical seizures respectively. Since ENSA particularly shows activities against electrically- and chemically-induced seizures which resemble clinical grand-mal and petit-mal epilepsies, it is being recommended for further investigation towards the management of both disorders.

CHAPTER I

INTRODUCTION

Epilepsy is a relatively common disease in the world today, and despite increased medical knowledge which had reduced the mortality of many otherwise fatal disease, the problem of epilepsy still remains with us. Other diseases, like meningitis, encephalitis and brain tumours have also contributed to the problem of epilepsy. This is because, these diseases often leave behind irreversible changes in some patients which predispose them to developing epilepsy.[1]

The term 'seizure' is the modern expression for epilepsy and the definition is a tribute to many workers. It is often stated as follows: "Seizures are occasional, paroxysmal, excessive rapid and local neuronal discharges of the grey matter[2] usually accompanied by paroxysmal dysrhythmia of EEG and hypersynchrony. They are characterised by recurrent paroxysmal aberrations of brain functions and are usually brief and self-limiting. These discharges which result in seizures usually originate from normal nerve cells surrounding a scar or other pathological lesions in the brain. Discharges from abnormal units contribute very little to the

seizure process".[3,4] Although it is claimed that seizure originates from the grey matter, any functional part of the brain can initiate seizure, and the characterization of seizure depending on the basis of its focus in the brain e.g. temporal lobe seizure, and cortical seizure, implies this fact. However, some parts of the brain are more susceptible to seizure than others. This is because the abundance of excitatory and inhibitory neurons respectively increases or decreases susceptibility of various areas of the brain to excessive discharges.[5]

Early Concepts of Causes and Treatment of Epilepsy

In those days, epilepsy was looked upon as an evil curse, and believed to be inflicted on the victim by unseen spirits. It was not surprising that its treatment was always sought with witch doctors and was full of magical procedures and incantations. The witch doctor must recommend the placation of the offended spirit or he must expel, mostly with purgatives, the evil spirits from the patient.

Due to the influence of ancient belief on epilepsy, the term 'aura' which is now known as a feeling of the approach of an attack of cerebral origin was thought by

those who lived in the days of Pelops, the master of Galen (130-210 AD) to be the cause of epileptic attacks and the point at which any pain was felt then was regarded as the location of the pathological process. Thus at that time, it was common to read from physicians records that epilepsy was of liver, spleen or kidney origin.[5]

Before the acceptance of the fact that epilepsy is a disease of the brain, one reads of attempts to treat epilepsy by application of local ligatures or local surgical treatment (cautery) of the parts where an aura originated - obviously still assuming a peripheral origin of epilepsy. It is not surprising, therefore, that no success was recorded except in a few cases where pathological causes, removable by surgery, were encountered.

The best that could possibly be said in favour of early therapeutic attempts at treating epilepsy is that it was recognised, perhaps accidentally of course that some factors like diet and alcohol could affect the disorder, and there were recommendations regarding diet, purgatives, and advice against alcoholic excesses.

Now, it is known that the failure of the early

physician could be traced to their inability to explain or interpret accurately the basic mechanisms of epilepsy.

The first report that epilepsies are of brain origin was probably by Charles le Pois (1563-1636),[6] but the modern concept of epilepsy is often credited to Hughlings Jackson (1834-1911)[7] who gave the idea of focal seizures being a starting point for the study of all seizures.

Experimentally-induced Seizures

In the laboratory, seizures can be induced in a number of ways.

The common ones are:

(i) Use of chemical convulsants such as Picrotoxin Pentylenetetrazol and Strychnine[8], which simulate clinical petit-mal epilepsy.

(ii) Electroshock method[9], which simulates clinical grand-mal epilepsy.

(iii) Producing acoustic stimulus (in the case of susceptible audiogenic mice).[10]

iv) Flickering light at a certain wavelength (in the case of susceptible photogenic species).[11,12]

Anticonvulsants

Anticonvulsants are chemical substances which are used to prevent, control or diminish the severity of

epileptic attacks. Anticonvulsants employed clinically are known as anti-epileptics. The first effective anti-epileptics were bromides.[13] This gave the idea that seizure was an abnormality of the neuronal activity which could be controlled without interferring with other functions of the central nervous system (C N S). The main drawback of the bromides was their undesirable side effect known as bromism manifest by skin rash, irritability, and hallucination. Phenobarbitone was introduced into therapy in 1912 as the first synthetic compound which was effective in the treatment of grand-mal epilepsy[14]. It therefore opened way for a deliberate synthetic search for anti-epileptic agents. This yielded dividends in 1938 with Merritt and Putnam's discovery of Phenytoin, another drug of great importance which was effective not only in grand-mal but also in psychomotor epilepsy which was refractory to the previously existing drugs. Phenytoin, in addition, lacked the sedative action of Phenobarbitone.[15] Trimethadione was discovered in 1946.[16,17] The importance of the discovery of anti-convulsant action of Trimethadione lay not only in providing a new pharmacological tool for research into the physiological mechanisms of the petit mal epilepsies, but more in emphasizing the value

of a systematic search for new selective anti-convulsants. It established the principle that clinical specificity may reside in various haptophoric moieties (groups involved in binding the drug molecule to the receptor).[17] This principle stimulated the search for new chemical classes of anti-convulsants.

Synthetic approach over the years has led to the production of new anti-epileptic agents.

Modern Approach to Seizure Mechanisms

All neurons are potentially epileptic because of their properties which include their excitability, the explosive nature of their response in generating action potentials, and their tendency to repetitive discharge.[15] However, there are natural inhibitory mechanisms in the central nervous system (C.N.S.) which prevent epilepsy from happening in normal states. All normal neurons are constantly under the influence of excitatory and inhibitory mechanisms, and an appropriate balance is maintained for the normal state to persist. Therefore, seizures occur as a result of gross inbalance in central excitatory and inhibitory states within an

interconnected assembly of neurons; this may mean the possible reduction in inhibitory input or an increase in excitatory control.

The cure-rate of epilepsy is about 2% of the afflicted population.[5] Complete control (not cure) with conventional therapeutic approach is in 33%, and perhaps some improvement in another 33% of epileptic patients.

The modern aproach to seizure mechanism is directed at finding the physiological, pathological and biochemical factors which make tissues at epileptogenic foci different from those at non-epileptogenic sites. The physiological factor is involved in the way in which the brain depends on glucose for its supply of energy;[19,20] but the involvement of a defective energy metabolism in seizures has not been demonstrated.[21]

Pathological factors are involved in seizure mechanism since depressed skull fracture, hypoxia at birth, tumour of the brain, and vascular occlusion can cause seizures. Once identified, a surgical operation can be used to effect a cure. Infact, this accounts for most of the earlier successes in epileptic cure, and therefore this area does not form a new study in seizure mechanism.

Biochemical Approach to Seizure Mechanism

Advancement in the understanding of seizure mechanisms has been slow. It is only recently that attention has been focused on the biochemical factors which cause epilepsy.[22] The concept of biochemical basis of seizures emerged because the visible histological changes, such as almost always occur with pathological and physiological factors of seizure were not always present in epileptogenic foci.[23]

On biochemical basis, the causes of seizures have been attributed to changes in the level of important transmitters such as acetylcholine, gamma-aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT) in the brain.

Acetylcholine

Acetylcholine (ACH) level in the brain is highly increased during seizures because of the inability of epileptogenic tissues to bind ACH. Direct measurements of ACH levels in epileptic and non epileptic foci substantiate the fact that cholinesterase activity is increased in epileptogenic cortical foci.[24,25] This is explained as a compensatory phenomenon for the increased levels of ACH at such foci. Since epileptogenic tissues cannot store ACH,[26] the level soon reaches excitatory

threshold values which cause seizures. Today, it is known that ACH is an excitatory transmitter substance in a number of brain areas and it is involved in seizure activities.

Anticholinergic effect has been implicated in the mechanism of action of drugs that counteract seizures initiated by the excitation of cholinergic neurons. This could be supported by the fact that Trimethadione and Phenytoin have atropine-like action by reducing high cortical acetylcholine output and suppressing the convulsive symptoms in epileptic guinea pig.[27]

5-Hydroxytryptamine (5-HT)

5-Hydroxytryptamine (5-HT) is involved in seizure mechanism; but there are conflicting reports on the effects of electroshock on steady-state concentrations of 5-HT.[28] However, two reports have shown an increase in 5-HT synthesis following electroconvulsive shock.[28,29] These reports differed in their explanation of the mechanisms involved. Tagliamonte et al,(1972) suggested that the mechanism was via the increase in brain tryptophan that they observed. Shields (1972), while finding the increased 5-HT synthesis, as suggested by a rise in 5-HIAA concentrations, found no change in brain tryptophan concentration. It has not been determined whether

electroconvulsive shock causes a true increase in synthesis rate, and whether this is produced by changes in tryptophan concentrations or by a shift in 5-HT available for release at the nerve ending.[30]

Systemic Electrolytes

Seizures are a frequent and well-known complications of systemic disorders but the possible significance of such disorders is undetermined. Therefore, Gordon Millichap tried to elucidate this significance.[4] However, long before Millichap's review, the importance of potassium in neuronal abnormalities in convulsed brain had been reported.[34] The ratio of intraneuronal sodium to extraneuronal potassium is controlled within fixed limits by sodium-potassium Atpase for normal activity. During seizure, the neuronal concentration of sodium ion is increased greatly while that of potassium is decreased. To stabilise the membrane, sodium-potassium Atpase is stimulated to enhance outflow of sodium and inflow of potassium into the cell.

Potassium and Sodium. The stabilization of the cell, membrane by maintaining the ionic distribution of sodium and potassium within certain limits leads to inhibition. An interference with the mechanism responsible for maintaining adequate levels of intracellular potassium would render the neurons concerned more liable to depolarization which is characteristic of neurons in epileptogenic brain areas. A fall in the level of potassium is accompanied by a build up of sodium, and such a state of hypernatraemia is known to lead to increased neuronal excitability and hence seizure.[94]

Calcium. Calcium is needed as a pre-requisite for selective permeability of cell membranes to sodium and potassium, hence hypocalcaemia has the same effect as hypokalaemia or hypernatraemia.[94,32,33] Hypocalcaemia (low level of calcium) both in the serum and cerebrospinal fluid following electroconvulsive therapy of severe depressive illness has been reported recently.[34]

Amino Acids

The inability of epileptogenic tissues to bind ACH can be reversed by glutamine,[24] and this suggested some interference with amino-acid systems. Glutamic acid is the most abundant amino acid in the brain. This high brain

concentration has been used to argue that glutamic acid must have a special function, more than catalytic to perform, and in 1954 Meister reviewed its involvement in transamination reactions.[35] Epileptogenic tissues show a defect in glutamic acid metabolism as evidenced by a marked drop in the level of this amino acid during incubation of cortical slices from an epileptic cortex.[36] These findings make the exact role of glutamic acid in seizures difficult to rationalize, especially when it even became known that this amino acid could be an excitatory transmitter in the CNS.[37]

According to Tower (1956) there are three most likely routes for metabolism of glutamic acid namely to glutamine, to α- oxoglutarate, or via gamma aminobutyric acid (GABA) to succinate.

There is some evidence to suggest that glutamine has anti-convulsant action. For example, it sometimes suppresses seizures in epileptic patients and may also be used to correct the inability of slices of epileptogenic cortex to accumulate glutamic acid.[38] Further, glutamine synthetase inhibitors e.g. methionine sulphoxide and methionine sulphoximine have convulsant actions on the

cerebral cortex.[39] Therefore a decrease in brain level of glutamine and hence a fall in glutamate and GABA level would cause seizures.

Gamma-aminobutyric acid (GABA)

GABA occurs naturally as a constituent of the mammalian brain,[40,41,42] and its distribution suggests inhibitory functions since high levels are found in areas of the brain associated with inhibitory functions; i.e. the grey matter.[43,44] Since GABA is produced from L-Glutamic acid by the action of GAD-1 in the presence of vitamin B_6, convulsant hydrazides caused a decrease of pyridoxal phosphate,[45] GABA, and a reduction in glutamic acid decarboxylase (GAD-1) activity.[46] Topically applied or intraventricularly administered GABA effectively counteracted the convulsive activity of thiosemicarbazide, one of the most potent hydrazides.[47] Parenteral administration of various forms of vitamin B_6 also prevented the seizures. It was suggested that the hydrazides might be producing seizures as a result of induced fall in brain GABA levels. This was most probably as a result of inhibition of GAD-1 activity arising from vitamin B_6 deficiency concurrently induced by these compounds.[47]

GABA can be obtained in a very pure form, but when it is administered to epileptic subjects, it does not penetrate the normal blood-brain barrier due to the presence of tight endothelial junctions, and the absence of pinocytosis.[48,49] Following the discovery of the inhibitory role for GABA in the CNS and the report that high brain levels of it protect animal species against seizures,[50,51] many researchers directed attention to ways of obtaining high levels of GABA in an attempt to control seizures. Since GABA itself does not penetrate the normal blood brain barrier to any significant extent, attention was focused on drug induced elevation of brain GABA levels.

Experiments have shown that the steady state concentrations of GABA in various areas of the brain depend on GAD-1 activity and not on GABA-α-oxoglutaric acid Transaminase (GABA-T) activity.[52]

Altering the intracellular pH of neurons in the CNS over a narrow range towards acidic pH, e.g. by increasing the extracellular carbon dioxide (CO_2) concentration is a physiological means of increasing intracellular acidity,[53] which increases GAD-1 activity and hence GABA production. However, the activity of carbonic anhydrase normally prevents such increase in intracellular CO_2. Antagonism of this enzyme forms the

basis of anti-convulsant activity of acetazolamide against some forms of seizures including myoclonic, akinetic, and menstrual seizures.

The transamination reaction responsible for the breakdown of GABA is the only truly reversible reaction in the whole system and probably represents the step which might appear feasible to be displaced in one direction or the other. Attention has therefore been concentrated on inhibition of the GABA-T activity so as to reduce the rate of breakdown of GABA, and hence achieve high brain GABA levels.

A few compounds tested on this basis inhibited GABA-T by complexing with its co-enzyme, pyridoxal phosphate.[54] These include hydroxylamine, the hydrazides, GABA analogues, hydrazinopropionic acid, ethanolamine-O-sulphate, sodium di-n-propylacetate, and aminooxyacetic acid.

Hydroxylamine

Although hydroxylamine is a potent GABA-T inhibitor and causes a profound rise in brain GABA levels in experimental animals,[50,55] it is non-specific, and it

produces some methaemoglobinaemia. This therefore calls for a better GABA-T inhibitor for use as an anti-convulsant.

The Hydrazides

Isonicotinic acid hydrazide (INH) in low doses elevated brain GABA levels but at high doses caused convulsions associated with a slow fall in GABA levels and then a rise.[56,57] Further, it has been reported that the dose of INH which produced a 25 to 50% increase in brain GABA was two to three times higher than the maximal therapeutic dose in Man.[45,56] Hence its use is unjustified.

GABA analogues.

Carvajal, Russek, Tapia, and Massieu (1964) postulated some requirements for GABA analogues which might be expected to inhibit the transaminase enzymes. The hydrogen on the gamma carbon atom of GABA must be substituted to avoid formation of the Schiff base necessary for the transamination. The substituent, in addition, must confer enough hydrophobicity on the substance to enable it to bind to the enzyme but it must still retain a small degree of polarity to aid easy penetration of the blood-brain barrier.[58]

Based on these postulates, Carvajal and his co-workers prepared three compounds namely, 5-methyl-5-phenyl-2-pyrrolidinone (MPP); 5-ethyl-5-phenyl-2-pyrrolidinone (EPP) and γ-ethyl-γ-phenyl-γ - amino butyric acid (EPGABA). All three compounds protected rats against chemically-induced seizures. However, they have certain disadvantages such as production of narcosis, or anaesthesia, long onset of action, and poor penetration through blood-brain barrier.

Hydrazinopropionic Acid (HPA).

HPA is a close structural analogue of GABA the only difference being that the gamma carbon atom is replaced by a nitrogen atom. HPA and GABA are 'bioisosteres' and because both compounds are similar in terms of molecular size, spatial configuration and molecular charge distribution, they can assume identical conformations.[59] HPA was found to be very potent as an anticonvulsant, and its actions on GABA-T activity could not be reversed by pyridoxal phosphate.[60] However, HPA causes seizures at high doses, and it is non specific on its inhibitory action on GAD-1.[61]

Ethanolamine-O-Sulphate (EOS).

Ethanolamine-O-sulphate (EOS) is claimed to be the most specific GABA-T inhibitor available.[62] It has been used as an anti-convulsant against audiogenic seizures in genetically susceptible mice.[10] Its disadvantage is that it does not cross the blood-brain barrier since the route of administration employed has always been intracerebroventricular.

Sodium di-n-propylacetate (DPA).

The parent compound of DPA is dipropylacetic acid, and it has been known since 1818 when it was synthesised by Burton although there was no reported investigation into its anti-convulsant properties until 1963 when these were accidentally discovered.[63] DPA was merely being used as a solvent for some substances being screened for possible pharmacological activities when in an attempt to establish the inertness of their solvent - a routine step in drug screening - Meunier et al. discovered to their astonishment that the anti-convulsant action obtained with the solution of their compounds in DPA was infact due to the solvent rather than the solutes.[63]

The first clinical trial of DPA was in 1964,[64] and since then publications have attested to its anti-convulsant efficacy in both experimental and clinical epilepsy. The compound is now available on the market in many parts of the world, including Nigeria, under various trade names like Epilim, Depakine, Depakene, Eurekene, Ergeyl, Labazine and Atemperator.

The mode of action of DPA was thought to be by inhibition of GABA-T,[65,66] but now it is known to be a potent inhibitor of succinic semialdehyde dehydrogenase (SSADH)[67].

The adverse effects of DPA include tremor, thrombocytophenia, alopecia, isomnia, anxiety, headache, weakness of the limb, and sedation,[68,69] Inspite of these adverse effects, DPA still enjoys wide usage in the treatment of photoconvulsive responses, temporal lobe attacks, and focal epilepsy.[11]

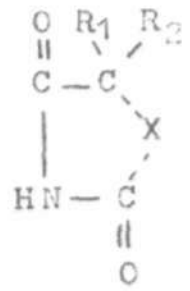
Aminooxyacetic acid (AOAA).

Aminooxyacetic acid (AOAA) is a very potent GABA-T inhibitor,[62,71] and hence a potent anti-convulsant agent. It is a much more potent inhibitor of GABA-T than hydroxylamine, DPA and EOS,[72,73] AOAA protects chicks,

and many mammalian species including rats, mice, and guinea pigs against various forms of experimentally-induced seizures including thiosemicarbazide-,picrotoxin-, strychnine-,pentylenetetrazol-,hyperbaric oxygen- and electrically-induced ones.[51,74,75] It also prevents barbiturate abstinence convulsions.[76] It has been tested clinically in Man and in dogs with good results.[75,76] However, AOAA convulses many animal species in high doses,[74,77] and therefore, there is a need for better anti-convulsant agents.

General Survey of Anti-convulsant Agents Tried in the Therapy of Epilepsy

The anti-convulsant agents which have been applied in the treatment of epilepsy include substances with the general formula:[78]



The substituents at X are:

- COHN- as in Barbiturates e.g. Phenobarbitone
- CH_2-NH- as in Deoxybarbiturates e.g. Primidone
- NH- as in Hydantoins e.g. Phenytoin
-O- as in Oxazolidinediones e.g. Trimethadione
-NH_2- as in Acetyl ureas e.g. Phenacemide
-CH_2- as in the Succinimides e.g. Ethosuximide.

However, there are miscellaneous chemical substances which do not fall under the general formula given above. These compounds include carbamates and other alcoholic esters e.g. Meprobamate; the derivatives of benzodiazepine e.g. diazepam; various chemical compounds including sulphonamides e.g. acetazolamide, local anaesthetics e.g. lidocaine, steroids e.g. progesterone, adrenocorticotropic hormone (ACTH), and anti-malarials e.g. quinacrine. Other anti-convulsant agents include gamma-amino butyric acid analogues such as hydrazinopropionic acid (HPA), dipropyl-acetic acid (DPA), aminoxyacetic acid (AOAA), and ethyl N-phthalimidoxyacetate (ENPA).[79]

A search conducted through an extensive screening for anti-convulsant activity among aliphatic and heterocyclic mides revealed the high activity within a

series of N-substituted succinimides.[80] Thus, the succinimides evolved from systematic search for effective anti-convulsant agents with less toxicity. The compounds include phensuximide (milantoin), methsuximide, ethosuximide, and diphenyl succinimide.

Testing of Anti-convulsant Agents.

Compounds are evaluated for anti-convulsant activity in two seizure models: the maximal electroshock seizure (ES) test; and the subcutaneous pentylenetetrazole metrazol(R) seizure threshold (ScMet) test. These two tests have been shown to identify all compounds known to demonstrate anti-convulsant activity.[9]

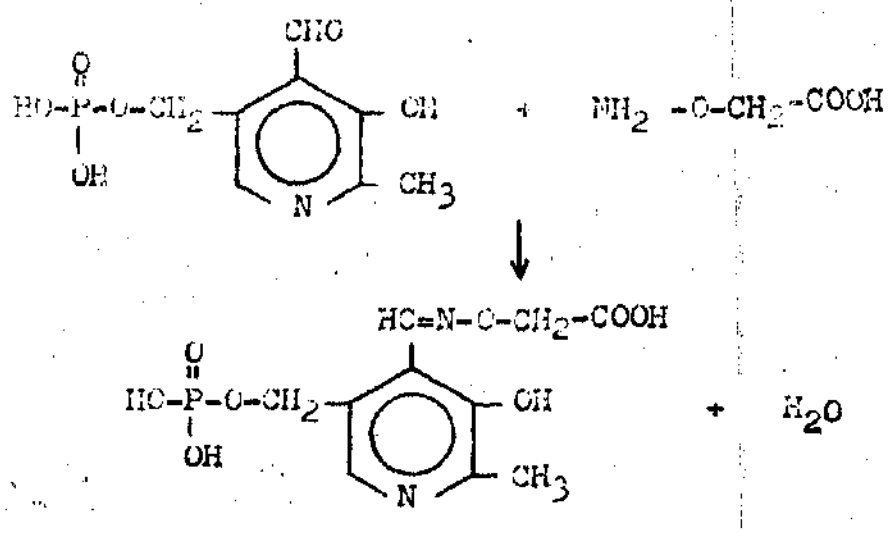
Purpose of this Research.

Epilepsy is a common health hazard in the world today, and the cure-rate has been quite low.[5] The epileptic patient has many problems which include the fear of an attack, social rejection, and limited job opportunities. To alleviate these problems, it is important to apply the principles of drug design to produce better anti-convulsant agents which would have less side-effects, long duration of action, and provide an effective control if not cure.

The modern approach to finding anti-convulsant drugs is based on biochemical factors; increased brain GABA levels

have been accepted as useful for protection against seizures.[62,71] On biochemical basis, AOAA has been found to be very potent as an anti-convulsant agent, but at high doses it has a convulsant property.[71,74]

It is also known that the free amino group of AOAA is a very reactive group and so might react with a number of other groupings of very vital substances like the aldehyde group of pyridoxal phosphate. Such reactions might create a low level of the vitamin which, as known may lead to seizures. The fact that concurrent administration of this vitamin in equimolar amounts with AOAA prevents AOAA convulsion[74] and enhances AOAA's anti-convulsant action in human patients[75] would appear to be in support of this possibility.



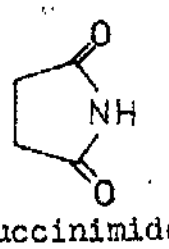
Substitution at that end was therefore thought to be a worthwhile exercise which might produce better anti-convulsant agents.

Lipophilic Substituents

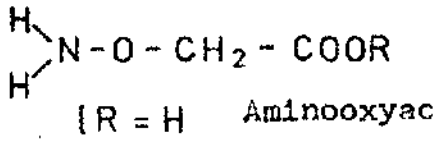
For the N-substituted cyclic imides, the starting materials were N-hydroxyphthalimide (NHP) and N-hydroxy-succinimide (NHS). Further, AOAA penetrated the blood-brain barrier in sufficient amounts to cause inhibition of GABA-T[57,73] and in postulating criteria for inhibition of GABA-T, Carvajal et al. (1964)[58] indicated that the GABA analogue being designed for this function should carry lipophilic substituent and retain some degree of polarity. Based on those findings N-hydroxyphthalimide and N-hydroxysuccinimide were considered suitable substituents at the amino end of AOAA. Morever, an analogue of AOAA known as ENPA was recently reported to be protective in most types of seizures induced in chicks but devoid of the convulsant property of AOAA.[79] Since it appears advisable to derivatize the free amino group of AOAA and since ENPA has shown good results, it was thought that the formation of hybrid compounds based on AOAA and such moieties like succinimides and glutarimides which are associated with anti-convulsant

properties might produce better anti-convulsant ag

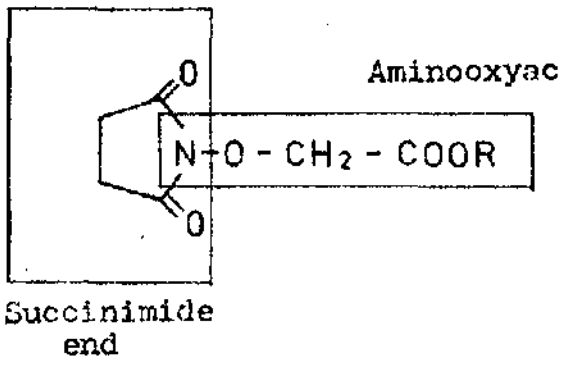
E.g.



Succinimide



Aminooxyacetic acid



Succinimide end

Aminooxyacetic acid end.

Accordingly, it was decided to synthesize some derivatives of phthalimide and succinimide. Though it is known that the unsubstituted succinimide does not possess any anti-convulsant property,[81] the substituted succinimides such as diphenyl succinimide, ethosuximide and phensuccinimide are effective anti-convulsant agents but not much is known about cyclic imides whose nitrogen atom carries an oxygen function.

CHAPTER 2

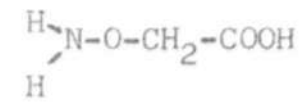
CHEMICAL SYNTHESES

Choice of Compounds

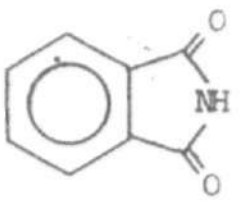
As stated at the close of Chapter 1, it was decided to synthesize hybrid compounds made up of AOAA and moieties known to have anti-convulsant properties like succinimide and glutarimide e.g. ethyl N-succinimidoxyacetate (ENSA) and methyl N-succinimidoxyacetate (MNSA). It will be recalled that the free amino group of AOAA is a very reactive group which could possibly play a significant role in the convulsant property of AOAA. It was felt that suitable substituents at this end might yield better anti-convulsant agents. Therefore, both the phthalimide and succinimide derivatives described here can also be considered as N-derivatives of AOAA. In addition, other properties of AOAA which might enhance penetration of the blood-brain barrier were used as criteria for synthesizing the new compounds. These included esterification of the carboxylic group (e.g. ethyl N-phthalimidoxyacetate (ENPA), ethyl N-phthalimidoxy-2-propionate (ENP-2-P), ENSA, methyl N-phthalimidoxyacetate (MNPA), and MNSA) and the introduction of other

lipophilic groups (e.g. ENP-2-P). With these hybrid compounds in the form of N-substituted cyclic imides it was hoped that better anti-convulsant agents would be obtained.

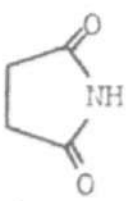
The chemical structures of AOAA, succinimide, phthalimide and the hybrid products are shown below:



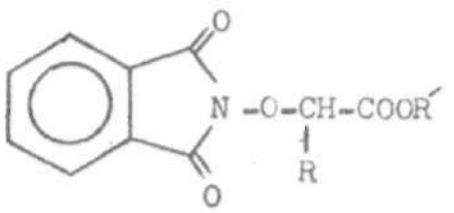
Aminooxyacetic acid (AOAA)



Phthalimide

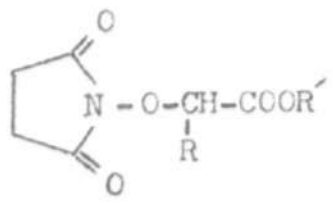


Succinimide



Hybrid products

	R	R´
Ethyl N-phthalimidoxyacetate (ENPA)	H	C_2H_5
Ethyl N-phthalimidoxy-2-propionate (ENP-2-P)	CH_3	C_2H_5
Methyl N-phthalimidoxyacetate (MNPA)	H	CH_3



	R	R´
Ethyl N-succinimidoxyacetate (ENSA)	H	C_2H_5
Methyl N-succinimidoxyacetate (MNSA)	H	CH_3

Survey of Various Synthetic Methods

The introduction of aminooxy group into a molecule via the phthalimidoxy derivative was first described in 1960[82] and since then aminooxyalkyl compounds have been prepared by other methods via suitable derivatives of hydroxylamine (NH_2OH)[83]. However, ethyl N-phthalimidoxyacetate (ENPA) was first synthesized by Neighbor,[84] and later by

Suresh and Malkani[85], and the method described by the latter has been used in this research.

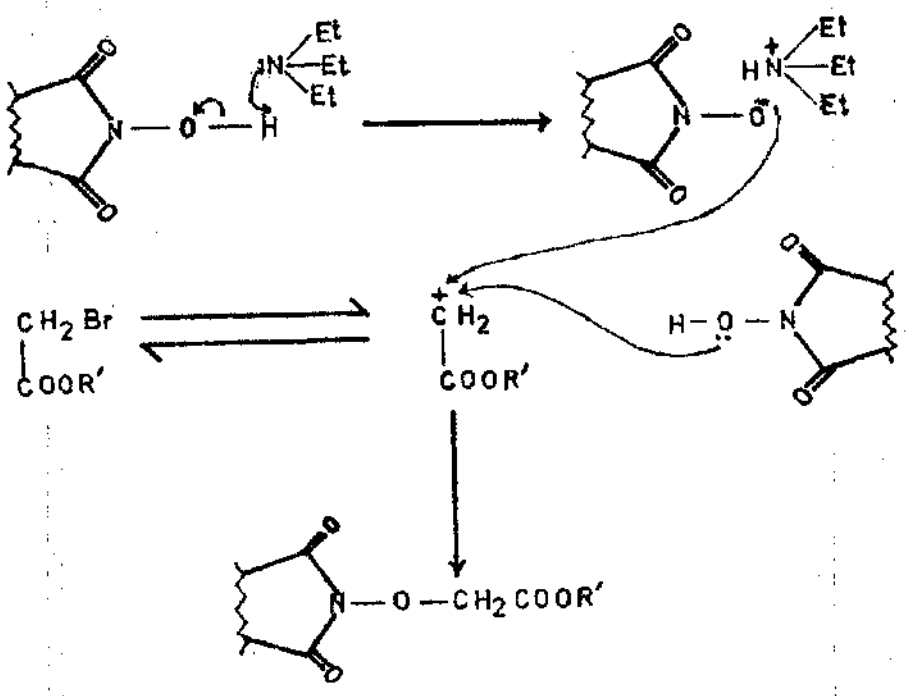
Apart from the herbicidal effect reported by Neighbor, ENPA has been reported to protect chicks against experimentally-induced seizures and to be devoid of the convulsant property of AOAA at high doses[79]. Hence, it was chosen as a reference compound in the screening exercise for the anti-convulsant properties of the other N-substituted cyclic imides.

Mechanism of Reaction

In the reaction of N-hydroxyimides and halogenated esters in suitable solvents, there is the substitution of the imide group for the halogen atom and the mechanism of reaction is likely to be nucleophilic substitution (S_N). The reagent required for the removal of what amounts to a molecule of acid (HBr) is a base such as triethylamine. It is feasible to suggest two types of S_N mechanism for the reaction; the S_N1 mechanism and S_N2 mechanism.[86]

The S_N1 mechanism takes place in two steps; the slow step which is the rate-determining step and the fast step. Either the free hydroxyl group of the N-hydroxyimide or the ionized form following the removal of a proton by triethylamine could attack the carbonium ion formed from the α-halogenated ester.

S_N1 - Nucleophilic substitution unimolecular.

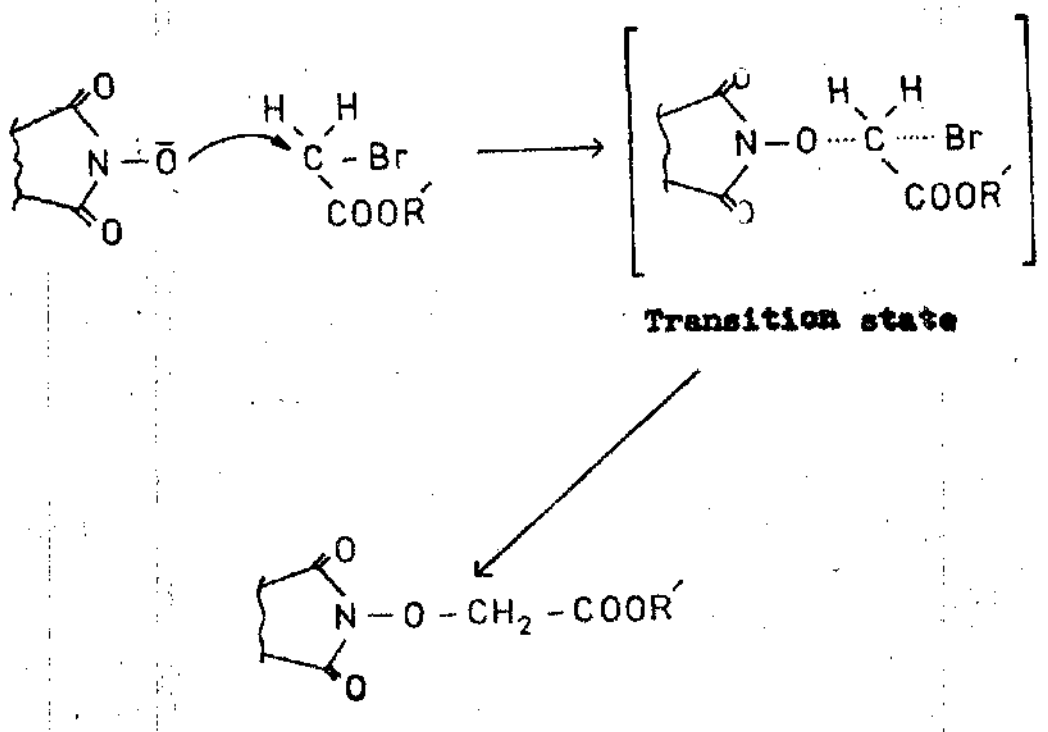


Scheme I

However, a primary carbonium ion is usually expected not to be very stable. Secondly, the presence of a carbonyl function - a good electron withdrawing group - further destabilizes the carbonium ion. If the reaction proceeds by S_N1, one would expect that the rate of reaction should be the same whether N-hydroxyphthalimide or N-hydroxy-succinimide is the nucleophile. On the contrary, the rate of reactions using the same medium were found to be different as described on page 33 . So the S_N1 mechanism is not likely to be the principal reaction path.

The S_N2 mechanism takes place in one step and it follows second order kinetics. Like in the S_N1 mechanism, free or ionized hydroxyl group of the N-hydroxyimide could attack the halogenated ester. However, the reaction would be expected to be faster when the attacking nucleophile is a negatively-charged specie rather than its conjugate acid. The S_N2 mechanism is generally better with primary halogens since steric factors are minimal. Accordingly, the S_N2 reaction shown below is being proposed as the major mechanism of the reaction of these N-hydroxyimides with primary α -haloesters.

S_N2-Nucleophilic substitution bimolecular



Scheme II

In the case of ENP-2-P where a secondary α-haloester is involved, a mixture of S_N1 and S_N2 may be operating.

General Method

A method similar to that for the preparation of ENPA[85] was thought to be feasible. This involves dissolving N-hydroxyphthalimide in N,N-dimethyl formamide (DMF) and mixing it with the solution of the halogenated ester in triethylamine (TEA) and after about 30 mins. of reaction, pouring the filtrate from the reaction mixture onto crushed ice and allowing the product to separate out.

N-hydroxyphthalimide (NHP) and the products obtained from it e.g. ENPA, and MNPA are insoluble in water whereas N-hydroxysuccinimide (NHS) is hygroscopic and moisture-sensitive. The products obtained using NHS and DMF after 12 h. of reaction could not be separated by pouring the corresponding filtrate onto crushed ice thus indicating their high solubility in water. Preparative thin layer chromatography (TLC) did not give good results because of interference by DMF. Partitioning the reaction mixture with ether gave crystals of the desired product but this still contained triethylamine hydrobromide crystals (TLC).

Accordingly, an alternative procedure was developed to obtain the desired compounds. Instead of DMF, tetrahydrofuran (THF) was used since THF like DMF is a polar and aprotic solvent but with reasonably low boiling point.

Thus the phthalimide series of compounds were prepared using DMF while the succinimide series involved THF and consequent concentration in vacuo to obtain the crude product followed by recrystallization from suitable solvents.

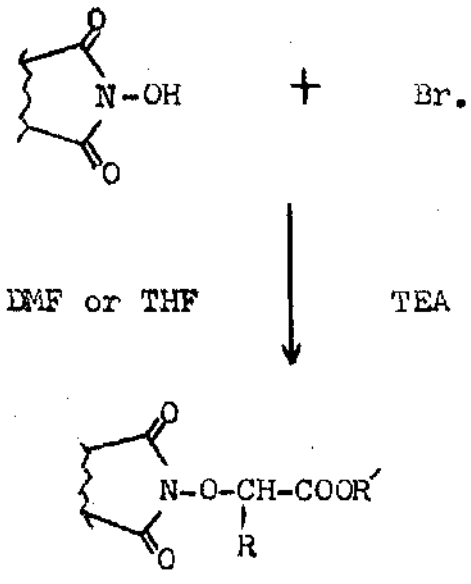
The by-product from the reaction was triethylamine hydrobromide which was insoluble in the organic solvents used, hence it was easily filtered off. Some of the desired product was also removed along with the by-product and in some cases such a loss was substantial e.g. MNSA. Thus using preferential solubility of the by-product in cold ethanol, most of it was removed and the desired product was retained.

The advantages of the reaction include the following:

(a) it provides a one-step synthesis which ensures a greater percentage yield of product than when several steps are involved.

(b) the reaction is carried out at room temperature and this is very convenient and economical. Besides, it would have been impossible to prepare ENSA if heating were involved; the starting material, NHS is normally decomposed by warm water[87].

(c) the reaction acts as a self-indicator since a colour normally develops at the beginning of the reaction and disappears when the reaction is completed.



Scheme III

ENPA. ENPA was prepared by reacting N-hydroxyphthalimide (NHP) and ethyl bromoacetate in the presence of N,N-dimethyl formamide (DMF) and triethylamine (TEA) and pouring the filtrate from the reaction mixture onto crushed ice whereupon the product separated out and was obtained as colourless fluffy shiny flakes mp 94° (Lit. 95-97° [85]) hRf = 65.9 - fig. 2(ix).

ENP-2-P. Ethyl N-phthalimidoxy-2-propionate (ENP-2-P) was prepared by treating NHP in DMF with ethyl 2-bromo-propionate in TEA. The product was separated out from water and recrystallized to give white shiny crystals mp 76-77° (Lit. 79-80° [85]). The n.m.r. spectrum - fig.2(i)- gave δ values for a singlet at 7.62 for four aromatic protons, the ethyl group being represented at 4.16 (CH_2, quadruplet) and 1.26 (CH_3, triplet), the side chain CH_3 at 1.59 (doublet) and the C-H at 4.77 (quadruplet) - thus confirming the structure of ENP-2-P. The i.r. spectrum showed presence of ester group (C=O str. at 1790, and C-O-C_2H_5 str. at 1050) and the lack of absorption at 3100 indicating

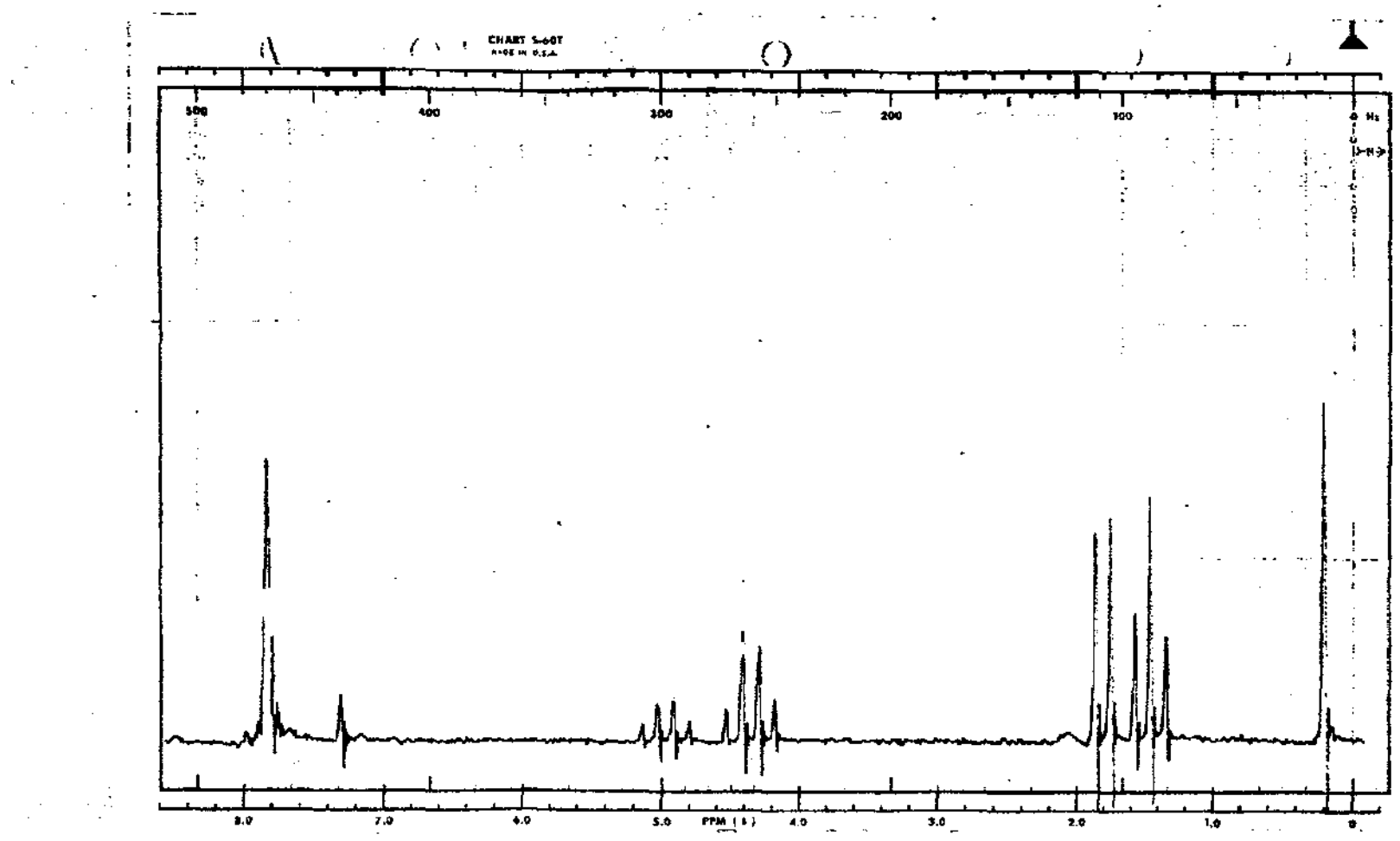


Fig. 2(i) N.M.R. spectrum of ethyl N-phthalimidoxy-2-propionate, ENP-2-P in deutorochloroform.

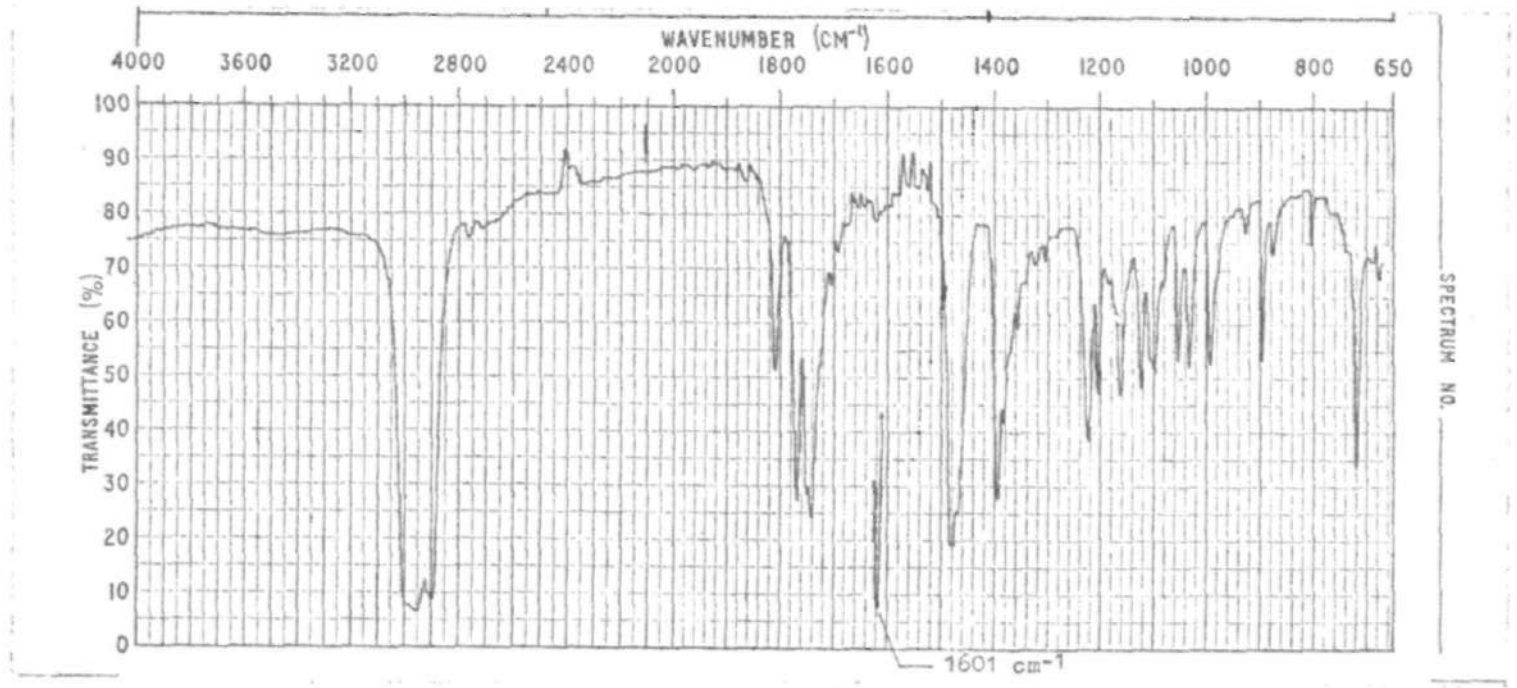


Fig. 2(ii) Infra-red spectrum of ethyl N-phthalimidoxy-2-propionate, ENP-2-P in nujol.

absence of hydroxyl group of the starting material, and C-H str. at 2850, C = C def. of benzene ring at 1460, C-H def. at 1230, and other peaks at 880, and 700 cm^{-1} - fig. 2(ii). hRf = 68.0 - fig. 2(ix).

MNPA. Methyl N-phthalimidoxyacetate (MNPA) was prepared by reacting a solution of NHP in DMF with a mixture of methyl bromoacetate in TEA and the product separated out from water to obtain white fluffy flakes mp 134-136°. It's n.m.r. spectrum - fig. 2(iii) - gave δ values for a singlet at 7.57 for aromatic protons, singlet at 4.73 for CH_2, and a singlet at 3.73 for the methyl group. The i.r. spectrum showed lack of absorption at 3100 cm^{-1} which indicated the absence of a hydroxyl group; presence of ester group, ν max C=O str. at 1790, and C-O-CH_3 str. at 1050 . Other peaks included C-H str. at 2900, C=C def. of benzene ring at 1460, C-H def. at 1230, and the peaks at 880 and 700 cm^{-1} - fig. 2(iv). hRf = 65.9 - fig.2(ix).

ENSA. Ethyl N-succinimidoxyacetate (ENSA) was prepared by reacting a solution of N-hydroxysuccinimide (NHS) in THF with ethyl bromoacetate in TEA and the filtrate concentrated

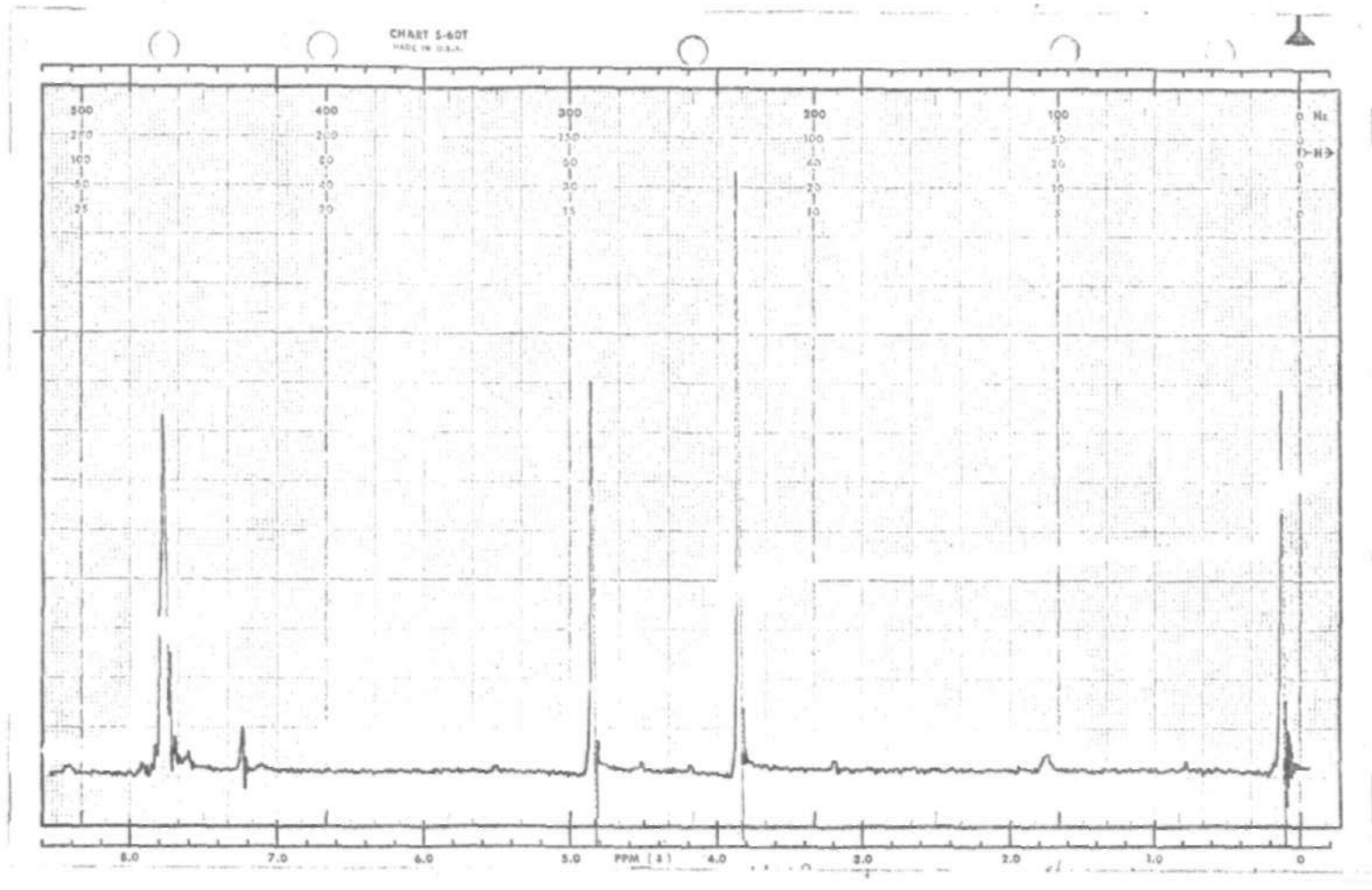


Fig. 2(iii) N.M.R. spectrum of methyl N-phthalimidoxyacetate, MNPA in deutorochloroform.

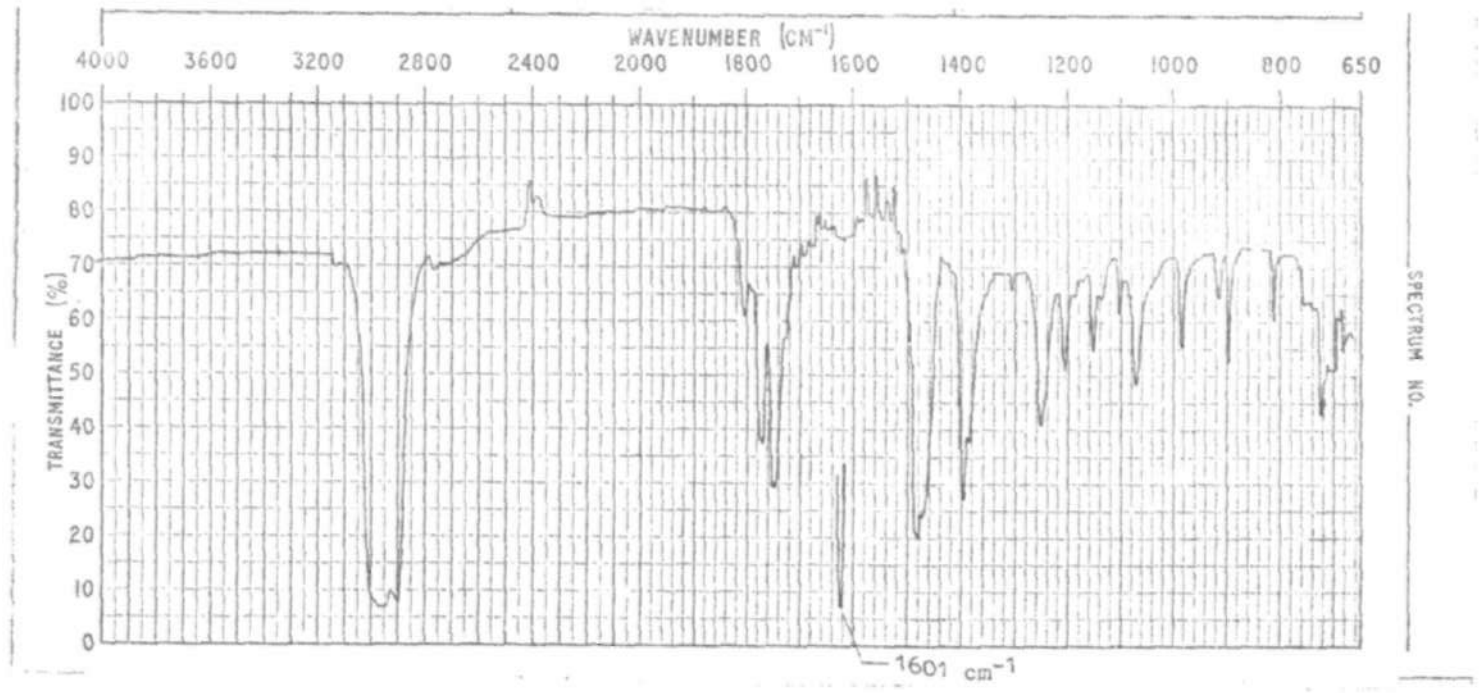


Fig. 2(iv) Infra-red spectrum of methyl N-phthalimidoxyacetate, MNPA in nujol.

to yield white fluffy flakes mp 80-81°. The n.m.r. spectrum - fig. 2(v) - gave δ value for a singlet at 2.70 for the cyclic protons, singlet at 4.60 for the methylene protons, and the ethyl group being represented at 4.17 (CH_2, quadruplet) and 1.27 (CH_3, triplet) - thus confirming the structure of ENSA. The i.r. spectrum showed ν max for an ester group (C=O str. at 1780, C-O-C_2H_5 at 1110), C-H str. at 2850, C-H def. at 1380, and others at 1225 and 820 cm^{-1}. The absence of absorption at 3200 cm^{-1} indicated the absence of a hydroxyl group of the starting material - fig.2(vi). hRf = 56.8 - fig. 2(ix).

MNSA. Methyl N-succinimidoxyacetate (MNSA) was prepared by reacting a solution of NHS in THF with methyl bromoacetate in TEA and the filtrate concentrated to yield the first crop. The precipitate was washed several times with aliquots of cold ethanol until the white crystals did not seem to dissolve any further. The remaining crystals yielded the second crop of the product as white crystals mp 128 - 130°. The n.m.r.

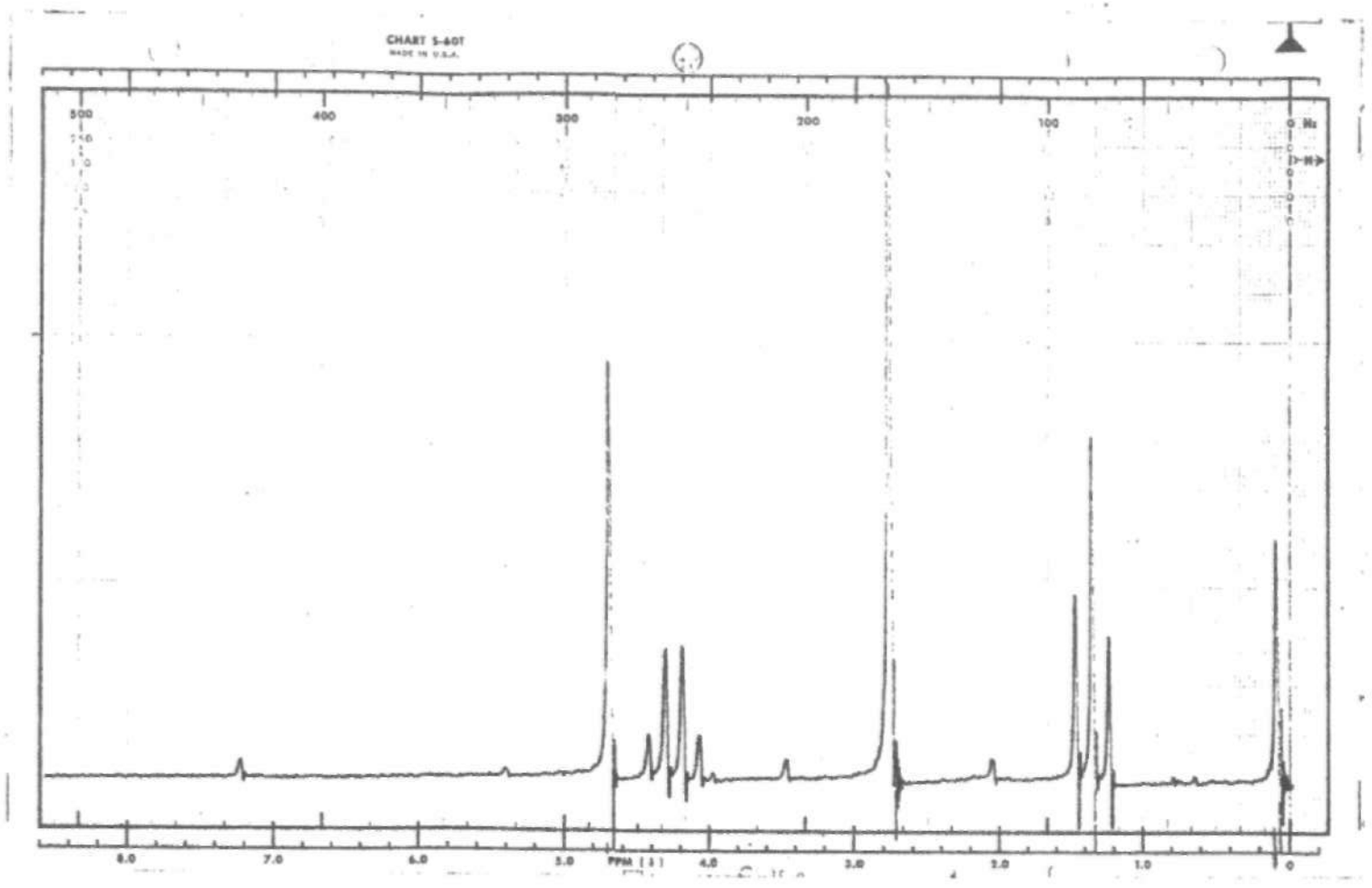


Fig. 2(v) N.M.R. spectrum of ethyl N-succinimidoxyacetate, ENSA in deutorochloroform.

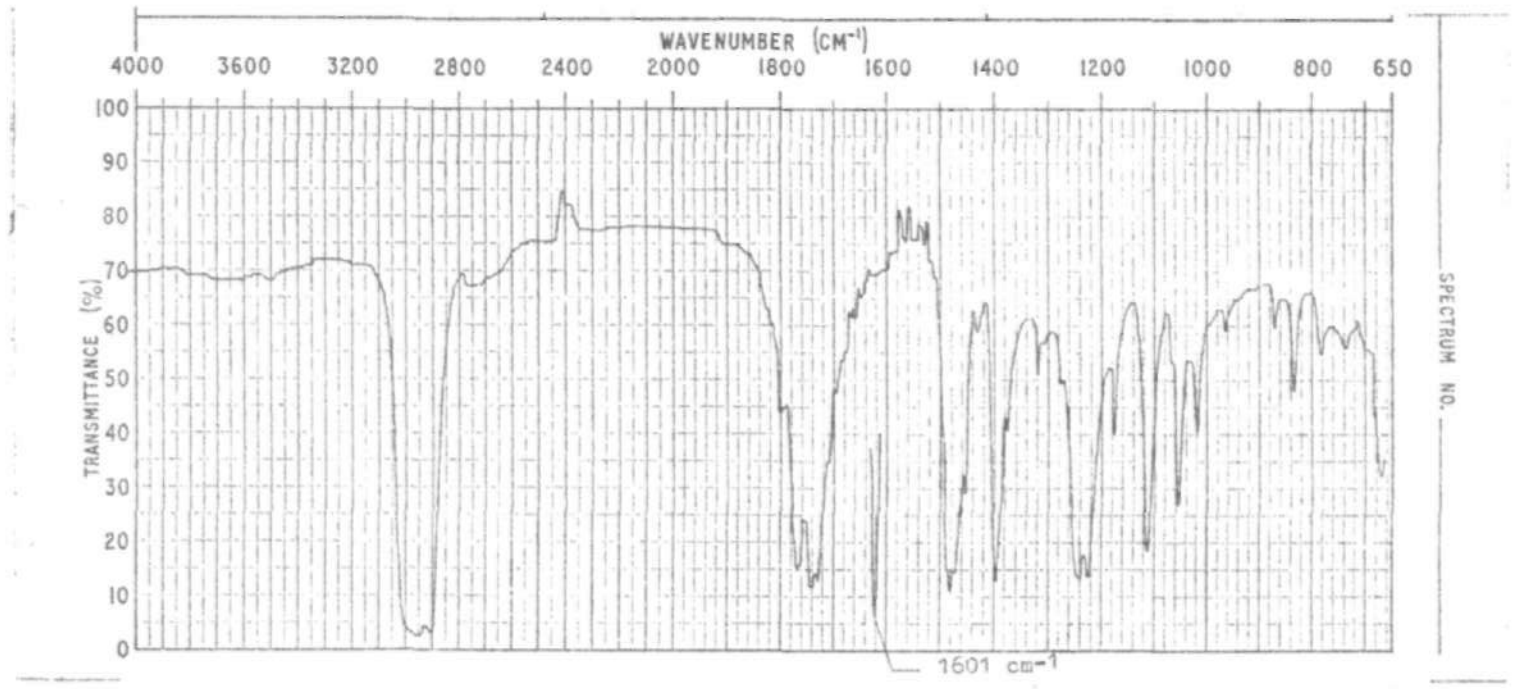


Fig. 2(vi) Infra-red spectrum of ethyl N-succinimidoxyacetate, ENSA in nujol.

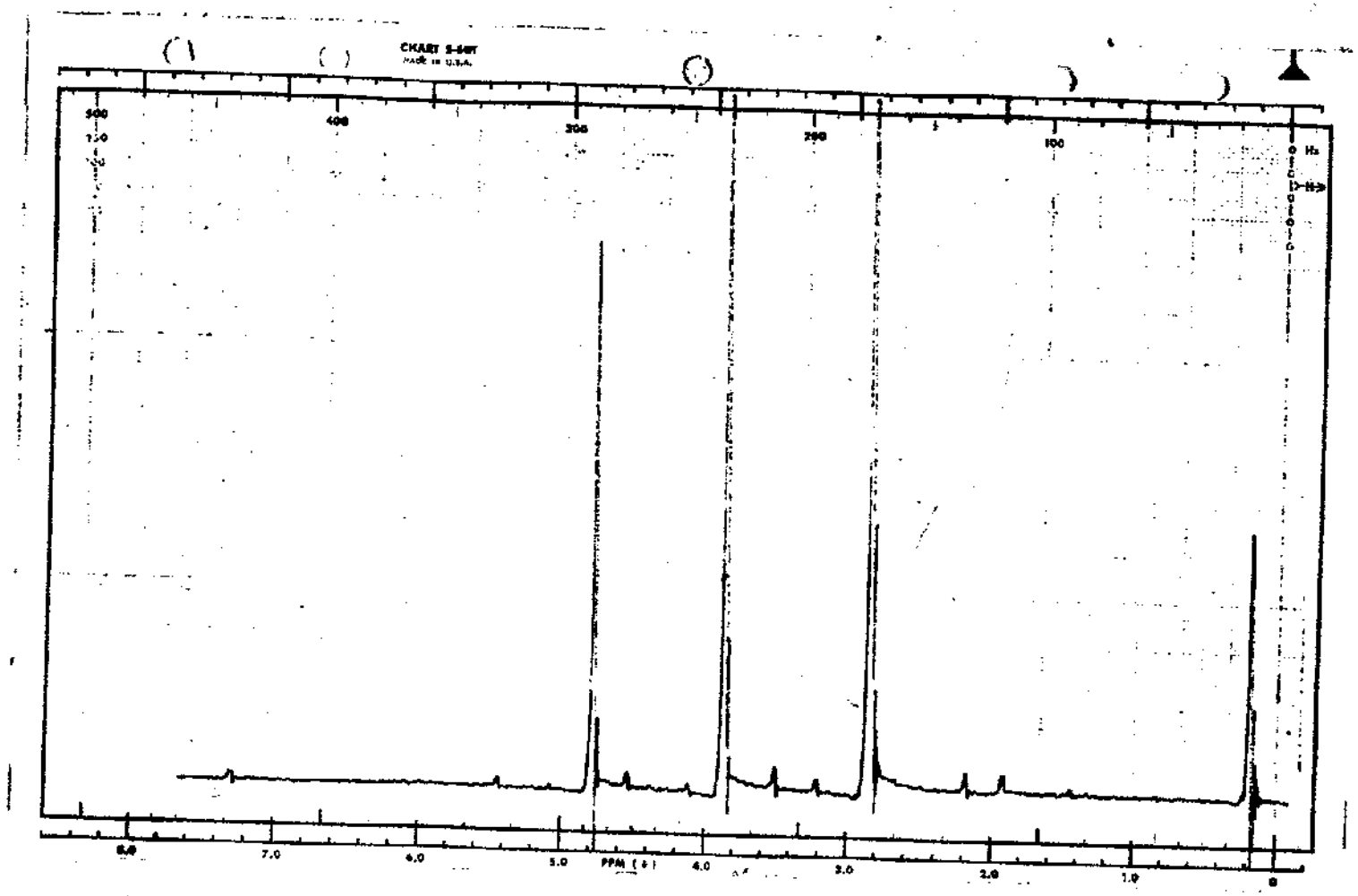


Fig. 2(vii) N.M.R. spectrum of methyl N-succinimidoxyacetate, MNSA in deutorochloroform.

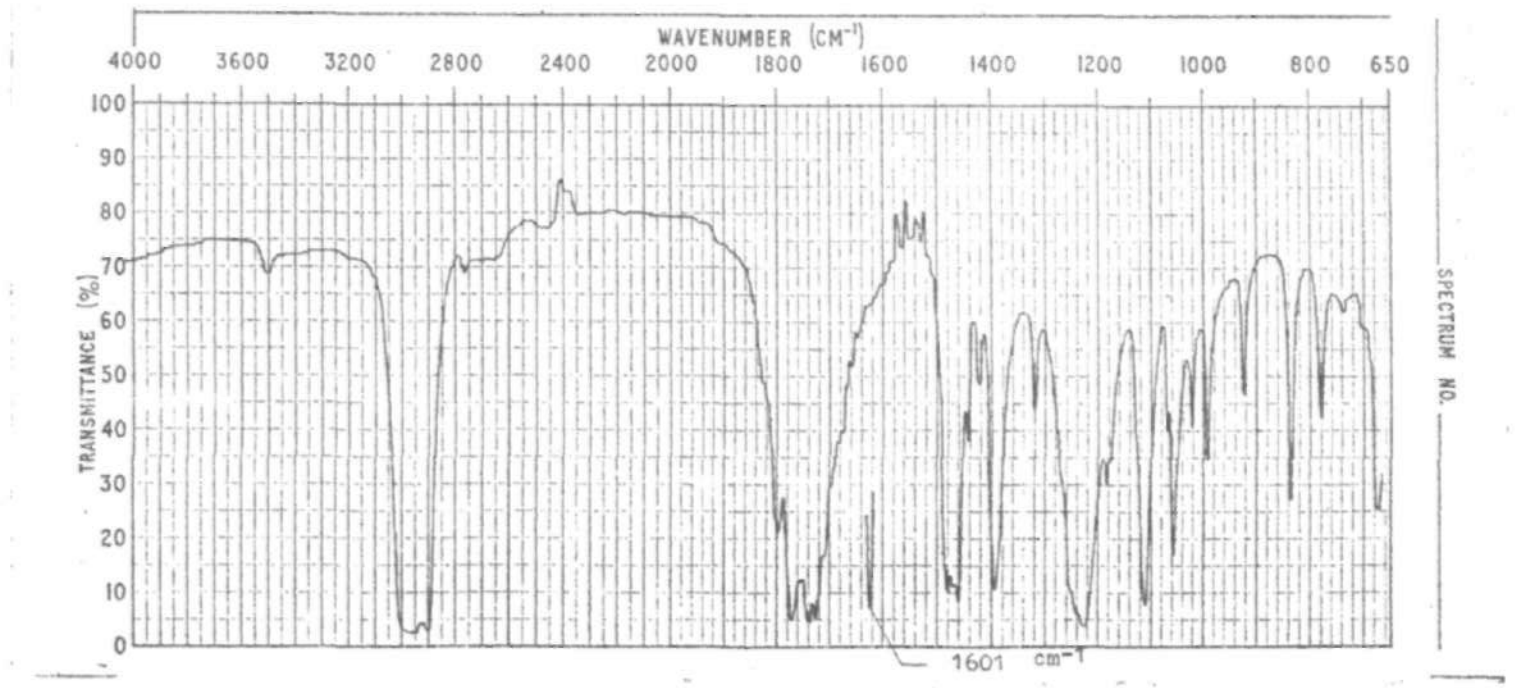


Fig. 2(viii) Infra-red spectrum of methyl N-succinimidoxyacetate, MNSA in nujol.

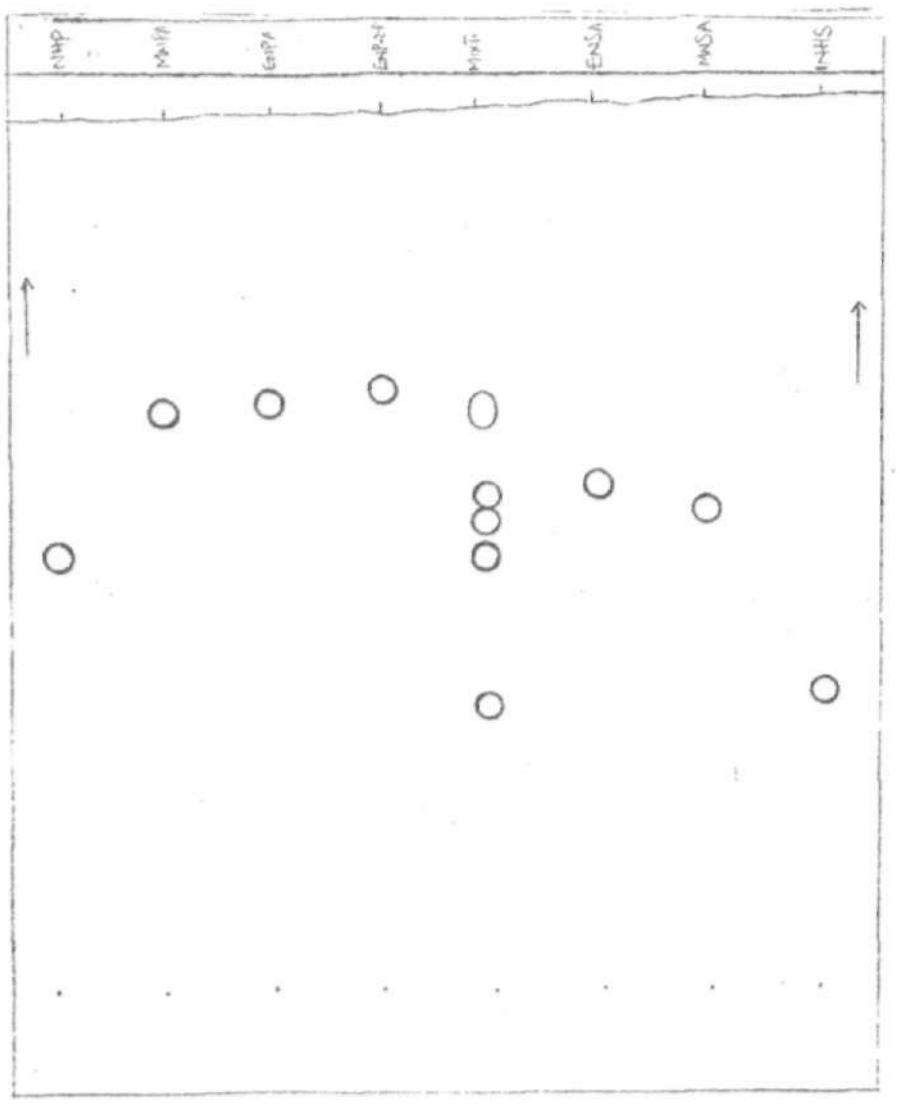


Fig. 2(ix) Thin layer chromatography (TLC) of the N-substituted cyclic imides and their starting materials (N-hydroxyphthalimide, NHP and N-hydroxysuccinimide, NHS) in methanol: chloroform (1:9).

spectrum - fig. 2(vii) - gave δ values for three singlets; at 2.63 for protons on heterocycle, 4.60 for methylene protons and 3.63 for methyl protons respectively. The i.r. spectrum - fig. 2(viii) - showed ν max for an ester function (C=O str. at 1720, C-O-CH_3 str. at 1110), C-H str. at 2950, C-H def. at 1380 and others at 1460, 1205, and 820 cm^{-1}. The lack of absorption at 3200 cm^{-1} indicated the absence of hydroxyl group in the starting material. hRf = 53.7 - fig. 2(ix).

Experimental

General. The N-substituted cyclic imide was prepared by adding a mixture of TEA and the halogenated ester to a solution of the N-hydroxyimide in a suitable solvent such as DMF or THF. The reaction mixture was allowed to stand at room temperature during which a colour developed and gradually changed to almost colourless. White crystalline precipitate was filtered off, and the product obtained from the filtrate.

Infra red (i.r.) and Nuclear Magnetic Resonance (n.m.r.) spectra were determined with Perkin Elmer SP 700, and

varian A-60A instruments respectively. The i.r. spectra of the solids were obtained from nujol mulls, and n.m.r. spectra were run in deuterochloroform, The internal reference for the n.m.r. spectra was tetramethylsilane (TMS)

Melting points were determined by an electrothermal melting point apparatus - a Gallenkamp apparatus or kofler hot stage microscope - and are uncorrected.

Elemental analyses were carried out by Scandinavian Microanalytical Laboratories, Denmark.

All Chemicals were obtained from Aldrich Chemical Co. Ltd; Gillingham.

The thin layer chromatography (TLC) of the compounds were run on precoated sheets - Polygram SIL G/UV_{254} (Camlab Cambridge) - in a solvent system of 10% methanol in chloroform at room temperature.

Ethyl N-phthalimidoxyacetate (ENPA)

ENPA was prepared by adding a mixture of triethylamine (1.50 g, 0.015 mole) and ethyl bromoacetate (2.10 g, 0.0125 mole) to a solution of N-hydroxyphthalimide (1.63 g, 0.01 mole) in N,N-dimethyl formamide (10 ml, 0.13 mole)

and allowing to stand for 15 min. during which its colour changed from red to very light yellow and a white crystalline precipitate was filtered off. The filtrate was poured on crushed ice (80 g) whereupon 1.74 g of the product was obtained as clean colourless fluffy shiny flakes in 70% yield, mp 94° (lit. 95-97°[85]).

Ethyl N-phthalimidoxy-2-propionate (ENP-2-P)

A mixture of triethylamine (2.1 ml, 0.015 mole) and ethyl 2-bromopropionate (1.62 ml, 0.0125 mole) was added to a solution of N-hydroxyphthalimide (1.63 g, 0.0 1 mole), in N,N-dimethylformamide (10 ml, 0.13 mole). The reaction mixture was allowed to stand at room temperature for 30 min. during which its colour changed from blood-red to very light yellow. A white crystalline precipitate was filtered off and the filtrate was poured on crushed ice (80 g). The crude product separated out as a white solid, and it was collected on buchner funnel and dried in a vacuum desiccator. The product was recrystallized from petroleum ether (100-120°) to obtain 2.12 g of white shiny crystals in 80% yield, mp 76-77° (lit. 79-80°[85]).

Methyl N-phthalimidoxyacetate (MNPA)

A mixture of triethylamine (2.1 ml, 0.015 mole) and methyl bromoacetate (1.1 ml, 0.0125 mole) was added to a solution of N-hydroxyphthalimide (1.63 g, 0.01 mole) in N,N-dimethyl formamide (10 ml, 0.13 mole). The reaction mixture was allowed to stand at room temperature for 30 min. during which its colour changed from blood-red to very light yellow. A white crystalline precipitate was filtered off, and the filtrate was poured on crushed ice (80 g.). The crude product separated out as a white solid, and it was collected on a buchner funnel and dried in a vacuum desiccator. The product was recrystallized from ethanol to obtain 1.82 g of white fluffy flakes in 77% yield, mp 134-136°.

Anal. Calcd. for $C_{11}H_9NO_5$: C,56.17, H,3.82; N,5.95

Found : C,56.26, H,3.94; N,6.11.

Ethyl N-succinimidoxyacetate (ENSA)

A mixture of triethylamine (2.1 ml, 0.0 15 mole) and ethyl bromoacetate (1.4 ml, 0.0125 mole) was added to a

solution of freshly recrystallized N-hydroxysuccinimide (1.15 g, 0.01 mole) in tetrahydrofuran (12 ml, 0.15 mole). The reaction mixture was allowed to proceed at room temperature for 12 h. during which its colour changed from light blue to colourless. After filtering the reaction mixture, both the filtrate and precipitate were kept. The filtrate was concentrated and cooled to obtain the first crop of 1.51 g of ENSA in 75% yield. The white precipitate was extracted five times with 20 ml portions of ether to give the second crop which increased the total yield to 1.71 g (85%). ENSA was recrystallized from ethanol to obtain white fluffy flakes mp 80-81°.

Anal. Calcd. for $C_8H_{11}NO_5$: C,47.76; H,5.51; N,6.96

Found : C,47.82; H,5.50; N,7.01

Methyl N-succinimidoxyacetate (MNSA)

A mixture of triethylamine (2.1 ml, 0.015 mole) and methyl bromoacetate (1.1 ml, 0.0125 mole) was added to a solution of freshly recrystallized N-hydroxysuccinimide (1.15 g, 0.01 mole) in tetrahydrofuran (12 ml, 0.15 mole). The reaction mixture was allowed to proceed at room

temperature for 8 h. during which its colour changed from light blue to colourless. After filtering the reaction mixture, both the filtrate and precipitate were kept. The filtrate was concentrated and cooled to obtain the first crop of MNSA in 7% yield. The precipitate was washed several times with 10 ml. aliquots of cold ethanol until the white crystals did not seem to dissolve any further. The remaining crystals were recrystallized from ethanol to obtain the second crop which increased the total yield to 77%, (1.44g) mp 128-130°C.

Anal. Calcd. for $C_7H_9NO_5$: C,44.92; H,4.85; N,7.48

Found : C,45.03; H,4.88; N,7.47

CHAPTER 3

PHARMACOLOGICAL SCREENING

Behavioural Studies

Most screening programmes often begin with behavioural studies. In these studies, the investigator is expected to adopt an open mind towards the experiment even when the investigation has a particular aim from its onset. Conventionally referred to as a blind screening procedure, such approach tells the investigator whether his compound has some desired effects or not. It may also reveal some properties which might even be more useful than that for which the compound has been produced originally. For example, Pethidine was originally studied for atropine-like activity but was found during behavioural studies to produce the Straub reaction characteristic of morphine. Subsequent tests for analgesic actions showed it to be a potent morphine-like analgesic.

It is obvious immediately that behavioural pharmacology is not a new concept. What is relatively new is the systematic and open minded approach to the study. This is a consequence of increasing knowledge on general pharmacology.

In general, the aim of using centrally acting drugs is to alter behaviour, since by definition these influence the function of the nervous system and therefore inevitably affect behaviour. Since this research aims at developing compounds with central action, it is feasible to expect that the compounds may influence behaviour. This in addition to the possibility of discovering other possible activities of the compounds and gaining an insight into the possible toxicological aspects of the compounds formed the basis of making behavioural studies on the compounds.

Materials and Methods. Male Warren chicks aged 4 days (35-40 gm) were used for the experiments. The chicks were kept singly in the apartments of a specially constructed multi-purpose observation box which consists of fifteen apartments, each measuring 25 x 25 x 15 cm and has a hinged cover on top. The cover is made of wire mesh so that activities inside the apartments can be observed easily and also to prevent animals from jumping from one apartment to the other. In addition, this cover can be opened or closed quietly without causing any

disturbance to the animals inside the cage. Observation time was 2 h. unless effects persisted for longer time when observation was continued till chicks recovered. All experiments were performed in as quiet a condition as possible and during these experiments the chicks were allowed free access to food and water placed in separate small receptacles inside the apartments. With the exception of bicuculline, the phthalimide derivatives (ENPA, ENP-2-P and MNPA) and reference drug, phenytoin which were formulated in 5% acacia in normal saline, all other compounds were made in normal saline. In all cases, control experiments were run concurrently for ease of direct comparison. Animals used in these control experiments received 0.2 ml of the appropriate solvents; this volume representing the maximum volume injected into any of the chicks used in the whole study. The route of administration was intraperitoneal and doses were chosen arbitrarily in the first instance using the dose of aminooxyacetic acid (AOAA) which had been reported to produce seizures (8.5 mg/kg - Osuide, 1972)[74] as a working guide.

Observations from these initial works dictated direction of change of dose.

Three experiments were performed under identical conditions. Five chicks were used per experiment, making a total of 15.

Results of Behavioural Studies

No statistical evaluation of the results expressed in this section was performed - because they represented results from screening exercises aimed at developing new compounds and it was felt that such results should not require significance test calculations or probability values to show their effects.

AOAA. When as a preliminary measure and for reference purpose AOAA was used, observations with the compound confirmed earlier reports. Doses below 2 mg/kg produced no observable change in behaviour of the 4-day old chicks. Above this dose the observed effect was dose-dependent. Low doses produced behavioural signs of CNS depression,

while higher doses produced excitatory signs. For example, in the 4-day old chicks, intraperitoneal administration of 5 mg/kg of AOAA produced signs of behavioural depression with chicks being inactive, stationary and dozing. At times, they even appeared to sleep but they were easily aroused from such sleep. However, 10 mg/kg produced signs of excitation characterised by myoclonic convulsions and loss of righting reflex but certainly no tonic seizure. Such behaviour usually followed an initial depressive state and was generally preceded by hyperkinetic activities which consisted mainly of increased crying and attempts to get out of the cage. The chicks generally became calm and very depressed after the excitatory activities. Recovery was usually marked by a regain of righting reflex. Higher doses (12.5 mg/kg and above) showed greater degree of excitation as the myoclonic seizures were always followed by tonic attacks and in some cases death. The incidence of death following such 100%

tonic seizures was 60-70%. Doses of 15 mg/kg and above nearly always produced 100% lethality. During the seizures that followed high doses of AOAA, there was increased salivation characterised by frothing and excessive flow of saliva.

Ethyl N-phthalimidoxy-2-propionate (ENP-2-P). Doses below 100 mg/kg ENP-2-P produced a general calming effect, but there were intermittent signs of normal activity like walking around and crying for a few minutes before returning to the quiescent state. When high doses such as 200 mg/kg - fig.3(i)- and above were used one out of 15 chicks showed some form of 'starting' behaviour which consisted of much crying, running around for a short time, remaining in one position and showing frequent wing spreading and trembling. There were some signs of fear in the chicks before losing their righting reflexes. There was no tonic seizure, the occasional stretching of the limbs observed being an attempt by the chicks to regain their righting reflexes.

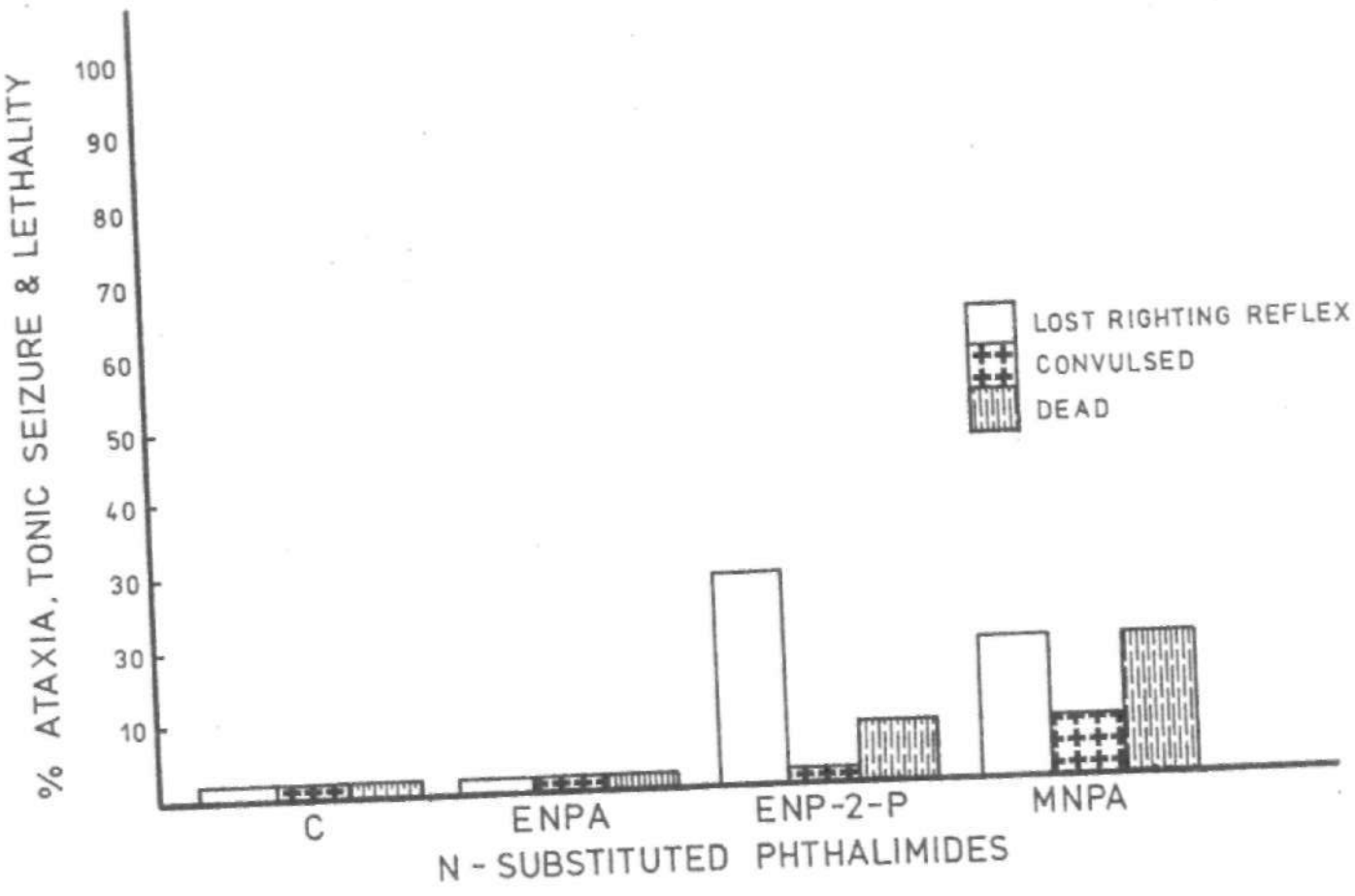


Fig. 3(i). Histograms showing the effects of 200 mg/kg of some N-substituted phthalimides on ataxia, tonic seizure and lethality in 4-day old chicks. n = 15 per histogram.

Methyl N-phthalimidoxyacetate (MNPA). Doses below 50 mg/kg of MNPA had no noticeable effect on the normal activities of the chicks. With 50 mg/kg MNPA, the chicks appeared normal although some were in crouching position. However doses above 100 mg/kg of MNPA were convulsant and lethal in 7% of chicks used. MNPA (200 mg/kg) gave about 20% lethality - fig. 3(i) - while 500 mg/kg gave 100% lethality. About 15 mins. after injecting the chicks with 200 mg/kg MNPA they started to make high-pitched noise as if in pain, running around furiously, jumping up, and appearing excited. The chicks then lost their righting reflexes and one out of fifteen chicks convulsed and died 2 h. later.

Ethyl N-succinimidoxyacetate (ENSA). ENSA was apparently without any noticeable effect on the normal activities of the chicks. Doses used ranged from 10 mg/kg to 500 mg/kg and neither depression even in form of drowsiness nor excitation in any form was observed - fig. 3(ii).

Methyl N-succinimidoxyacetate (MNSA). MNSA, like ENSA did not impart any noticeable effect on the normal activities of the chicks at the same dose range.

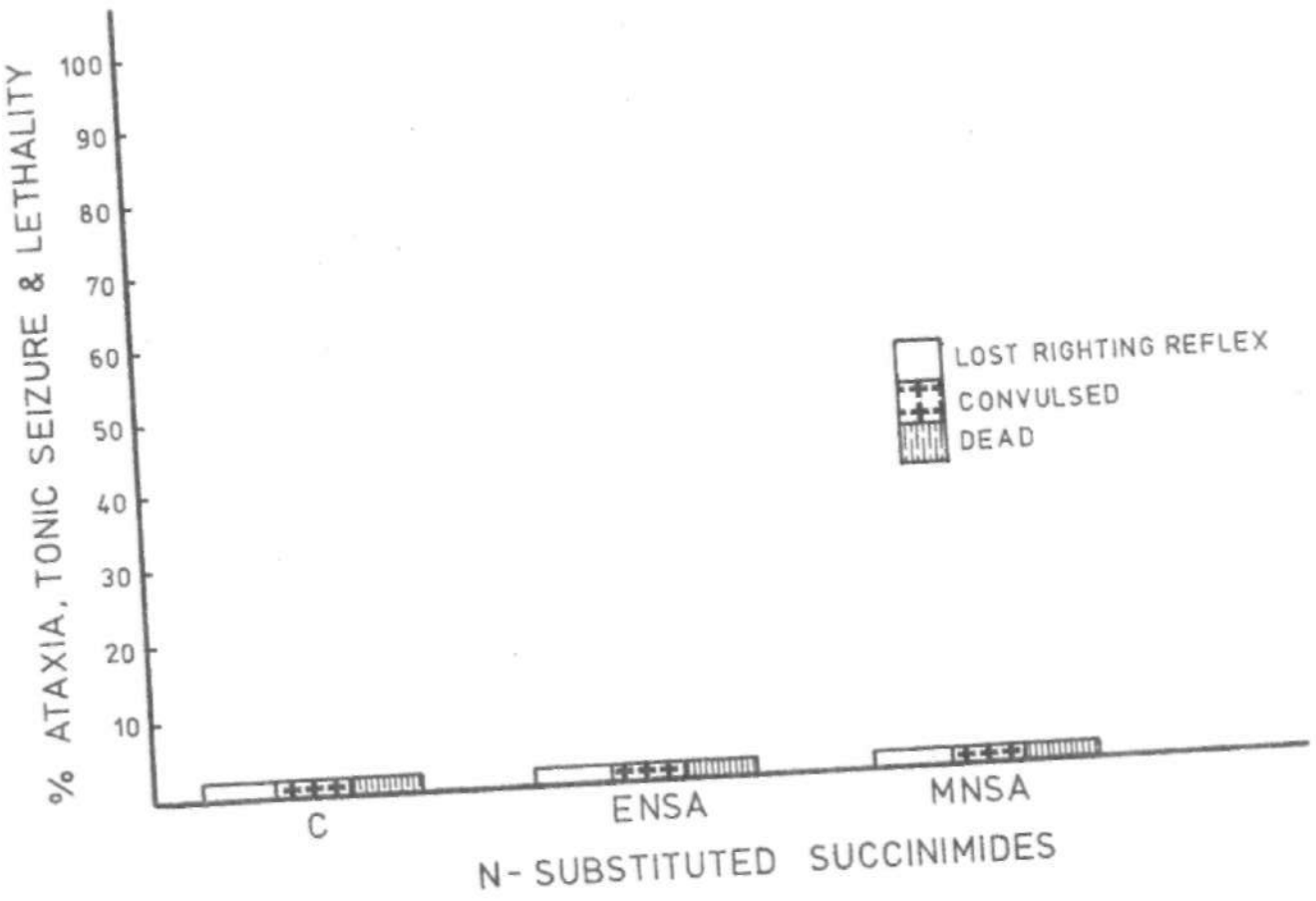


Fig. 3(ii). Histograms showing the effects of 200 mg/kg of some N-substituted succinimides on ataxia, tonic seizure and lethality in 4 - day old chicks. n = 15 per histogram.

Experimentally-induced Seizures

Some substituted imides have been known to be good anti-convulsant compounds[80]. Widely recognised too, is the high potency of aminooxyacetic acid (AOAA) in protecting many species against various forms of seizures. This effect is thought to be at least in part due to the ability of this compound to cause elevation of brain GABA levels[61]. However, at high doses, AOAA has convulsant action which is a major disadvantage[71,74]. Therefore, as described at the beginning of Chapter 2, various derivatives of AOAA were synthesized with the hope of producing better anti-convulsant agents.

Materials and Methods

The investigations were carried out using young male Warren chicks aged 4 days and weighing between 35 and 40 g.

Electrically-induced seizures. Maximal electroshock was delivered using the Ugo Basile ECT unit model 7800 set permanently at a pulse width of 0.6 ms, frequency of 100 pulses/sec. and shock duration of 0.8 sec. Steel electrodes were used, these being placed on the upper eyelids of the chicks.

The seizure parameter was clonic and tonic flexion of the legs. Abolition of the tonic extension phase only or the entire tonic phases by any of the compounds was regarded as protection. The electroshock experiments were done in three groups; the first one consisting of control animals which were treated with normal saline and used to establish the susceptibility of the chicks being used at any time to electroshock seizures. They were shocked with a current value already established in earlier preliminary experiments as being required to produce a desired level of seizure. The second group consisted of animals treated with various doses of the compounds under test and left for varying periods before testing with the same current value as used for control animals. The third group of experiments consisted of animals receiving an appropriate amount of the solvent used in formulating the compound and left for a length of time which had been identified in the second group of experiments as being most adequate for activity of the test compound. This was to establish whether

the observed effect in the second group of experiments was due to the compound or the solvent. Fifteen animals in three groups of five were used for each dose and each pretreatment time. The doses of the test compounds were arbitrarily chosen initially and results of the preliminary experiments dictated the direction of further changes. Phenytoin (Sigma Chemical Co.), aminooxyacetic acid (Sigma Chemical Co.) and ethyl N-phthalimidoxyacetate (ENPA) were used as reference drugs in evaluating the potencies of the test compounds in the electroshock experiments.

Chemically-induced seizures. Chemically-induced seizures were accomplished with aminooxyacetic acid (Sigma Chemical Co.), bicuculline (Sigma Chemical Co.), leptazol (Sigma Chemical Co.), picrotoxin (Sigma Chemical Co.), and strychnine (Sigma Chemical Co.) injected intraperitoneally and an "all or none" type of observation was made for both incidence of seizure and lethality in the chicks over a period of 1 h. following drug administration. Seizure parameter was the tonic extension of the neck and legs with or without initial hyperkinetic activity. Chicks which had convulsed once

were carefully taken out of their compartments in the observation cage to prevent them from inducing seizure in others as a result of post-seizure disturbances which usually accompanied their attempts to regain their righting reflexes. All surviving chicks at the end of the experiment were kept for a period of 24 h. to determine the percentage lethality over this period. Reduction in the percentage convulsed or dead in the chicks previously treated with any of the N-substituted cyclic imides when compared with the figures in the control population was regarded as protection by the compound. No chick was used more than once and all experiments were completed within the same periods of the day to avoid influence of diurnal variation in natural susceptibility to seizures.

The experiments were done in three parts as follows:
(a) Control group which received normal saline before the dose of the convulsant drug which had been established in preliminary experiments as being necessary to produce a desired percentage of seizure in the chicks. This group provided the information on the susceptibility of the batch

of chicks being used at any time to the action of the chemical convulsants. (b) Treated groups which consisted of chicks previously treated with the imide derivatives before being injected with the same dose of the convulsant drugs as used in (a) above. The dose of the imide derivatives and the pretreatment time used were those which had been established from the study on electroshock. (c) The solvent control group which received the solvent used in formulating the imide derivatives for the same length of time as used for the compounds before being injected with the convulsant drug. This was to establish whether the effects observed in (b) above were due to the solvent itself.

Results

Results obtained with both saline and all solvent-treated groups showed that these did not influence susceptibility to the seizures.

Influence of AOAA on experimentally-induced seizures.

5-6 mg/kg AOAA offered complete protection against both incidence of seizure and death when i.p. administered 6 h. before electroshock and doses of AOAA, bicuculline, leptazol

picrotoxin and strychnine. These chemicals and electroshock produced 60% seizure and up to 60% lethality in control chicks. Thus this compound proved just as potent against chemically-induced seizures as it was against electroshock.

Influence of ENPA on experimentally-induced seizures.

100 mg/kg ENPA offered complete protection against both incidence of seizure and death when i.p. administered 6 h. before electroshock or doses of either AOAA or strychnine which produced 60% seizures, and up to 60% lethality in control chicks. However, ENPA reduced the incidence of seizure to 13% in leptazol experiment, to 33% in picrotoxin experiment, but did not affect the incidence of seizure induced by bicuculline. ENPA reduced incidence of lethality from 60% to 53% in bicuculline experiment, from 33% to 0% in leptazol experiment and from 40% to 13% in picrotoxin experiment - figs. 3(xi) - 3(xv).

Influence of Phenytoin (DPH) on electroshock seizures.

10 mg/kg Phenytoin (DPH) offered complete protection against incidence of seizure when i.p. administered 3 h. before electroshock current producing 60% seizures in control chicks.

Influence of Trimethadione (TMO) on chemical seizures. 300 mg/kg Trimethadione (TMO) offered complete protection against both incidence of seizure and death when i.p. administered 1 h. before doses of AOAA, bicuculline, leptazol, picrotoxin and strychnine which produced 60% seizures and up to 60% lethality in control chicks - figs. 3(xi) - 3(xv).

Ethyl N-phthalimidoxy-2-propionate (ENP-2-P). 50-150 mg/kg of ENP-2-P offered some protections against electroshock seizures - fig . 3(iii). 150 mg/kg was chosen for routine study and the latency to peak effect was found to be 7 h. The degree of protection was about 80% - fig.(iv). At the pretreatment time of 7 h. 150 mg/kg of ENP-2-P offered 20% protection against AOAA seizure, and 45% protection against AOAA lethality - fig. 3(xi); 45% protection against both bicuculline seizure and lethality - fig. 3(xii); 22% protection against leptazol seizure, and 60% protection against leptazol lethality fig. 3(xiii); 90% protection against picrotoxin seizure and complete protection against picrotoxin lethality - fig. 3(xiv); but potentiated

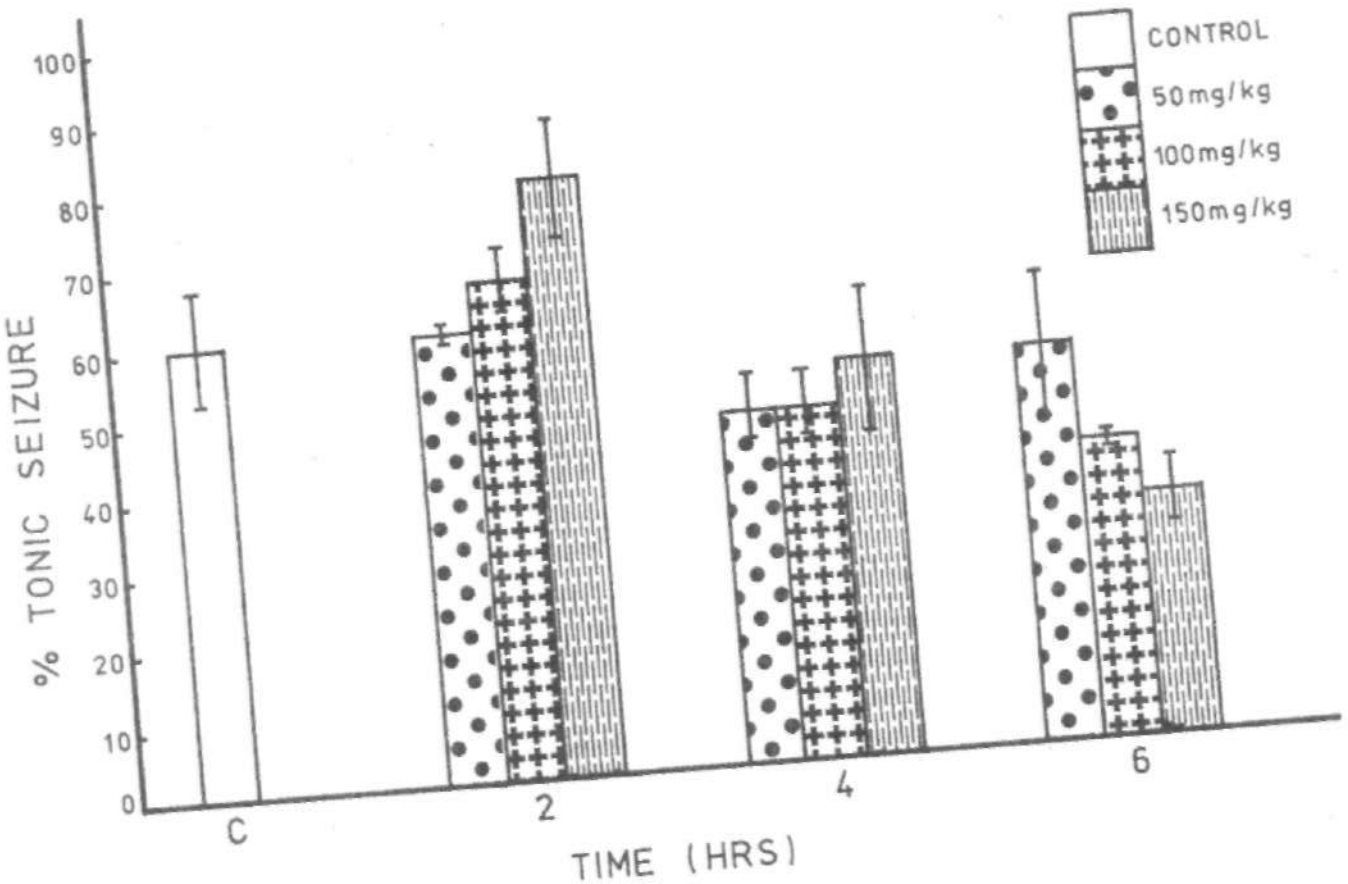


Fig. 3(iii). Histograms showing the effects of 50, 100 and 150 mg/kg of ethyl N-phthalimidoxy-2-propionate, EN-2-P on susceptibility of 4 - day old chicks to electroshock. n = 15 per histogram.

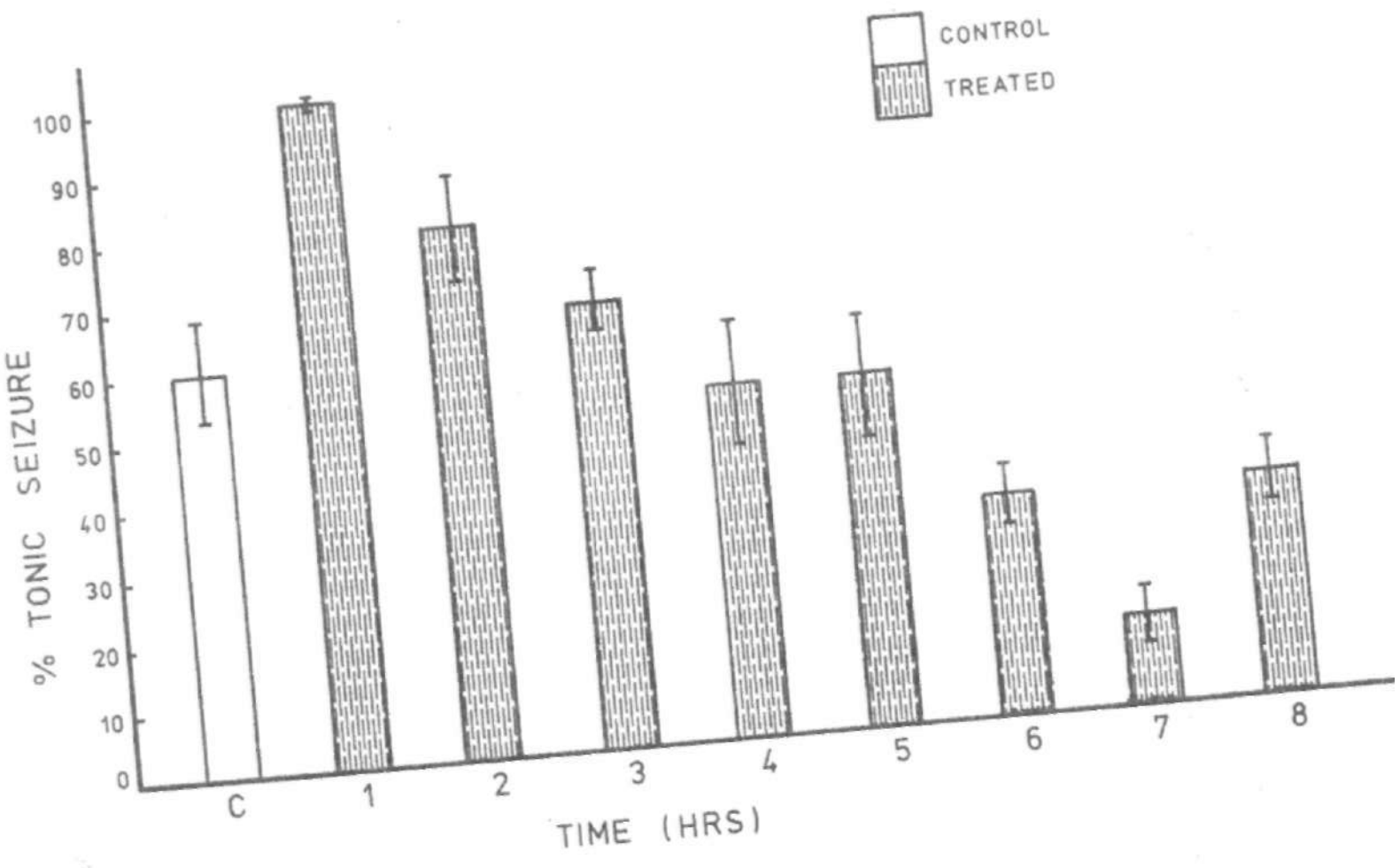


Fig. 3 (iv). Histograms showing the time course of anti-convulsant effect of ethyl N-phthalimidoxy-2-propionate, ENP-2-P (150 mg/kg) in 4 - day old chicks. n = 15 per histogram.

strychnine seizure to 87% without affecting strychnine lethality - fig. 3(xv).

Methyl N-phthalimidoxyacetate (MNPA). MNPA produced anti-convulsant activity in the chicks after 3 hours of administration. The test dose was 50 mg/kg and the effect was maximal at 6 h. following drug administration. It gave complete protection against electroshock seizures. Fig. 3(v) shows the effect of using 10,20 and 50 mg/kg of MNPA at 2,4, and 6 h. Fig. 3(vi) shows the effect of using the optimal dose of the compound over a wider period than covered in the initial studies. It produced a time-dependent increase in protection starting from 3 h. after administration and reaching a maximal level 6 h. later. The effect had started to decline by 8 h. after drug administration.

At the pretreatment time of 6 h. 10 mg/kg MNPA offered 80% protection against both the incidence of seizure and lethality induced by AOAA - fig. 3(xi); 33% protection against bicuculline seizures, and 66% protection against bicuculline lethality - fig. 3(xii); potentiated leptazol seizures to 100%, but offered complete protection

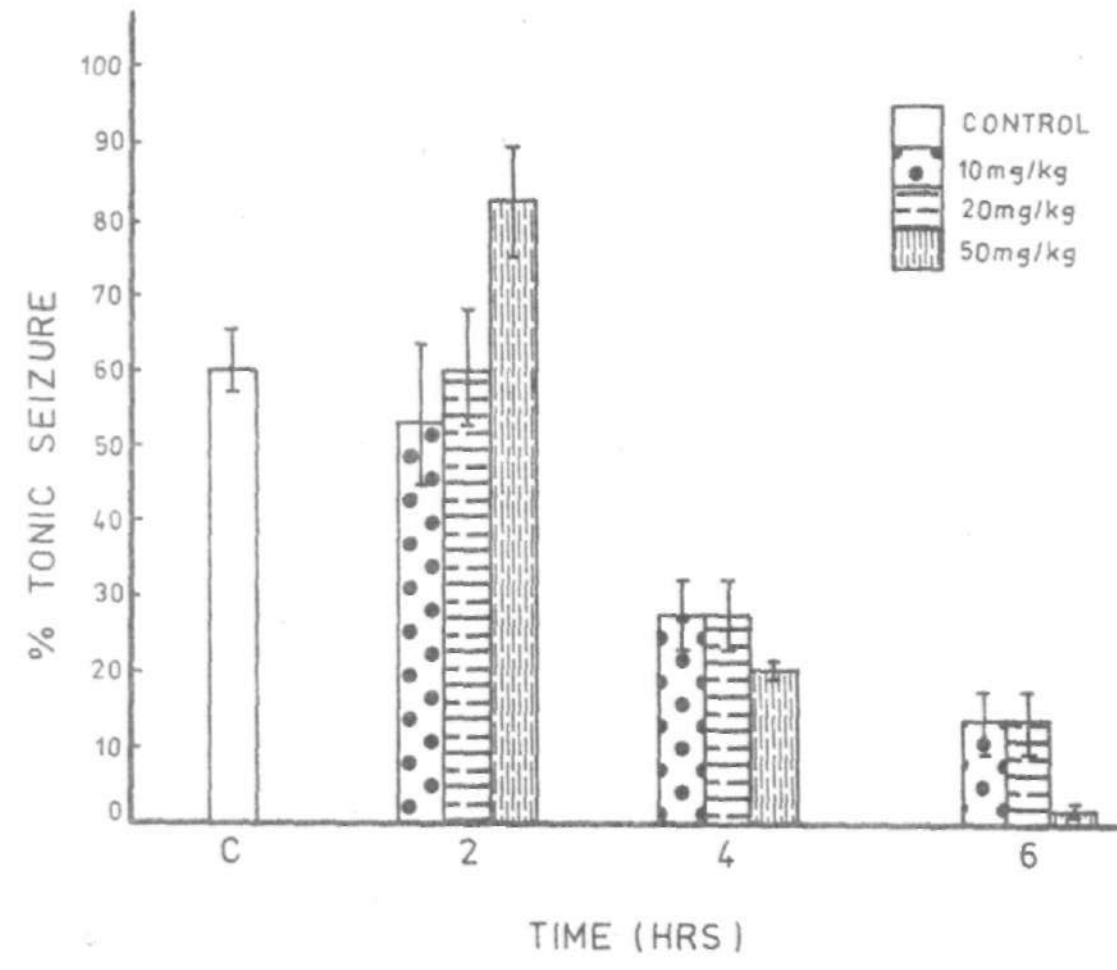


Fig. 3(v). Histograms showing the effects of 10,20 and 50 mg/kg of methyl N-phthalimidoxyacetate (MNPA) on susceptibility of 4- day old chicks to electroshock. n = 15 per histogram.

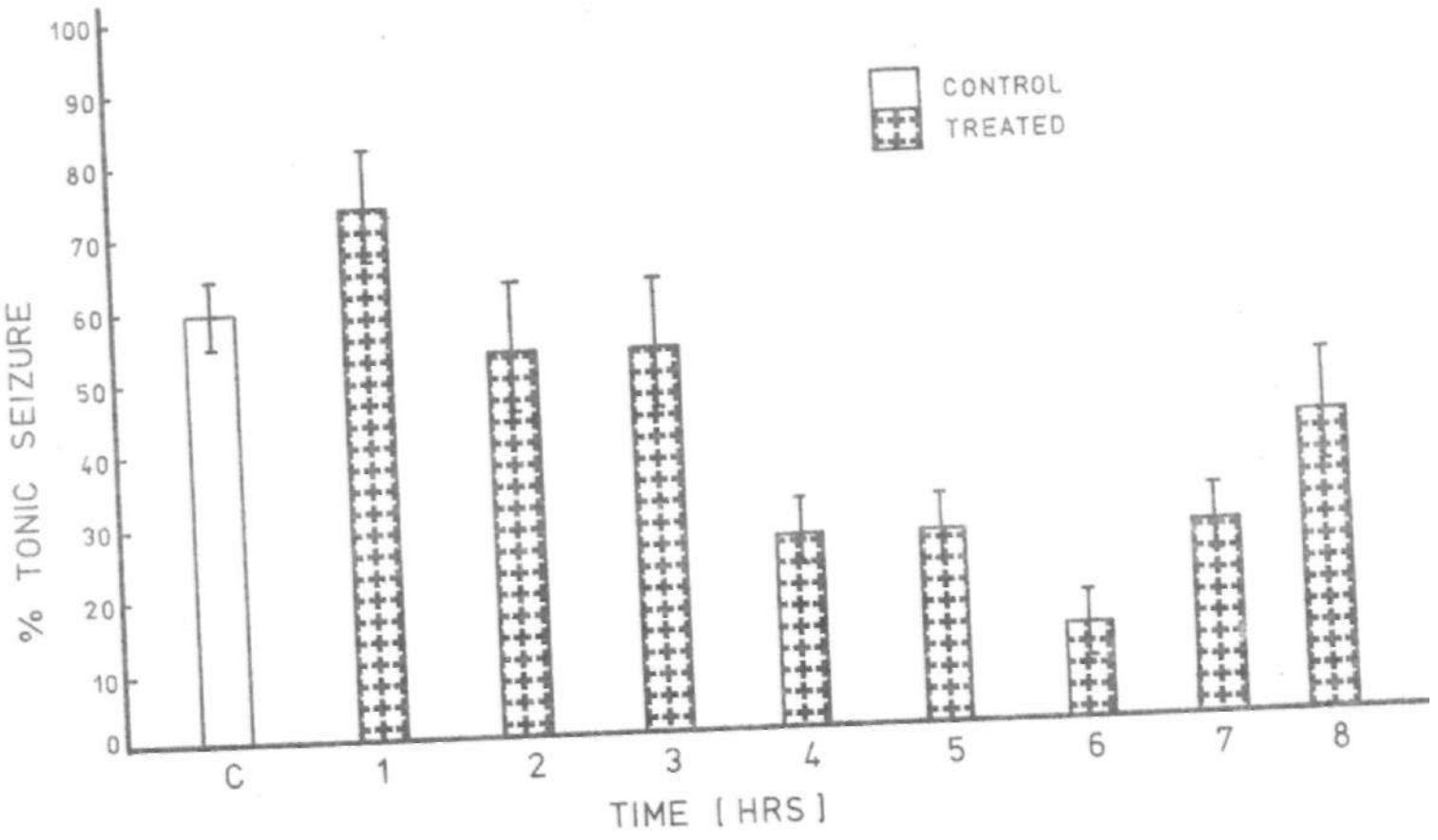


Fig. 3(vi). Histograms showing the time course of anti-convulsant effect of methyl N-phthalimidoxyacetate, MNPA (10 mg/kg) in 4 - day old chicks. n = 15 per histogram.

against leptazol lethality - fig. 3(xiii). MNPA did not affect the incidence of seizure and lethality caused by picrotoxin - fig. 3(xiv) but offered 66% protection against strychnine seizure, and complete protection against strychnine lethality - fig. 3(xv).

Ethyl N-succinimidoxyacetate (ENSA). ENSA produced a time-dependent anti-convulsant activity in chicks. Fig. 3(vii) shows the effect of using 5,10 and 50 mg/kg of ENSA at 2,4 and 6 h. Fig. 3(viii) shows the effect of using the best dose (10 mg/kg) of the compound over a wider period than that covered in the initial studies. The effect was maximal at 4 h. and the degree of protection was 80%. At the pretreatment time of 4 h. 10 mg/kg of ENSA offered good protections against the chemically-induced seizures and lethality; 33% protection against both AOAA seizure and lethality - fig. 3(xi); 55% protection against bicuculline seizure, and 66% protection against bicuculline lethality - fig. 3(xii); 33% protection against leptazol seizure, and 33% protection against leptazol lethality - fig. 3(xiii); 45% protection against picrotoxin seizure, and 68% protection against picrotoxin lethality -

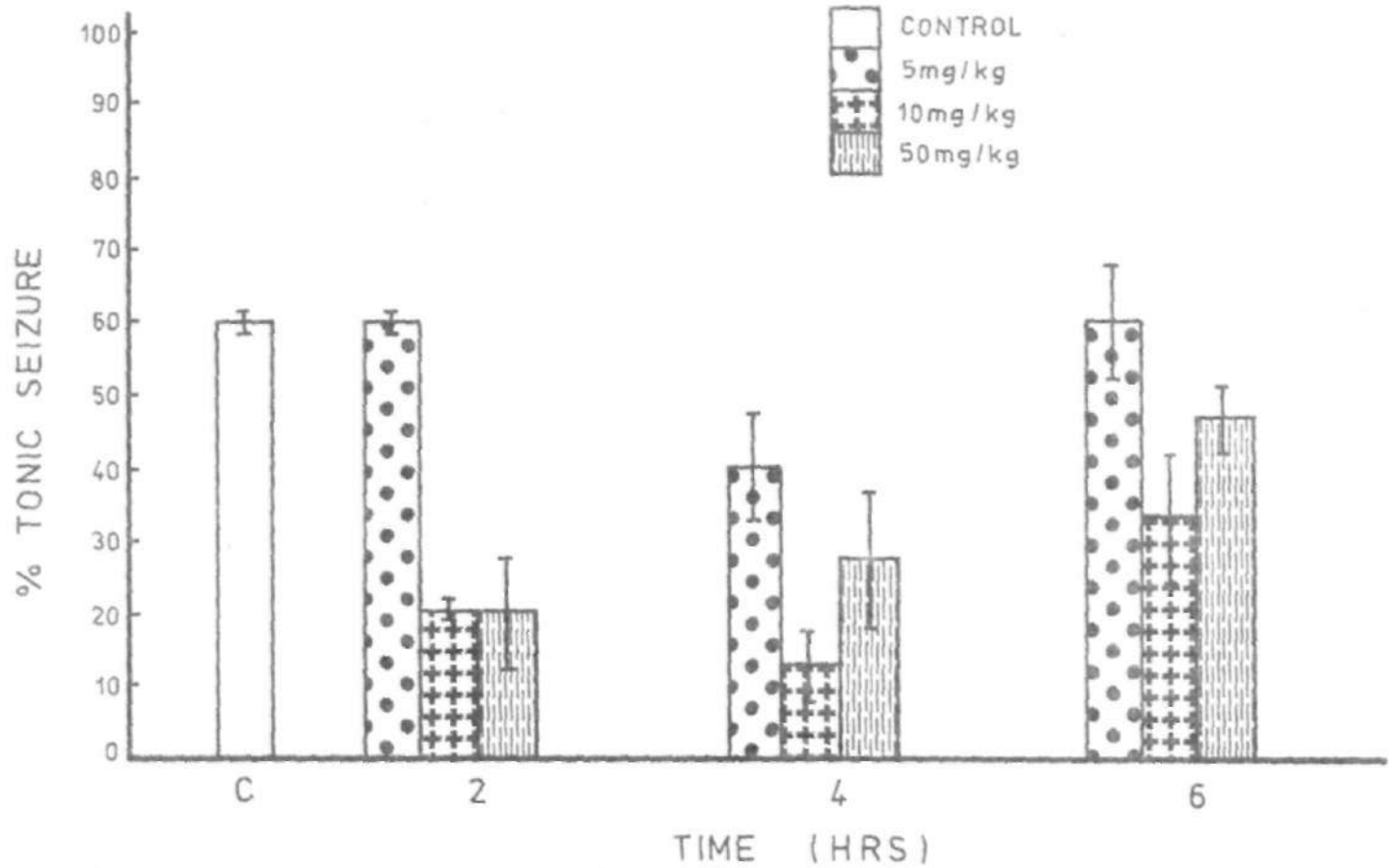


Fig. 3(vii). Histograms showing the effects of 5, 10 and 50 mg/kg of ethyl N-succinimidoxyacetate, [illegible] on susceptibility of 4-day old chicks to electroshock. n = 15 per histogram.

fig. 3(xiv); and 66% protection against strychnine seizure, and complete protection against strychnine lethality - fig. 3(xv).

Methyl N-succinimidoxyacetate (MNSA). MNSA offered some protection against electroshock seizures. The test dose was 5 mg/kg and the anti-convulsant effect was maximal at 2 h. following drug administration. Fig. 3(ix) shows the effect of using 5, 10 and 20 mg/kg of MNSA at 1,3, and 5 h. Fig. 3(x) shows the effect of using the optimal dose of the compound over a wider period than that covered in the initial studies. The latency to peak effect was 2 h and the degree of protection was 66%. At the pretreatment time of 2 h. 5 mg/kg of MNSA did not affect AOAA seizures, but offered 45% protection against AOAA lethality - fig. 3(xi). MNSA offered 33% protection against both bicuculline seizure and lethality - fig. 3(xii); 11% protection against leptazol lethality - fig.3(xiii); potentiated both picrotoxin seizure and lethality to 67% - fig. 3(xiv), and offered 45% protection against strychnine seizure, and complete protection against strychnine lethality - fig. 3(xv).

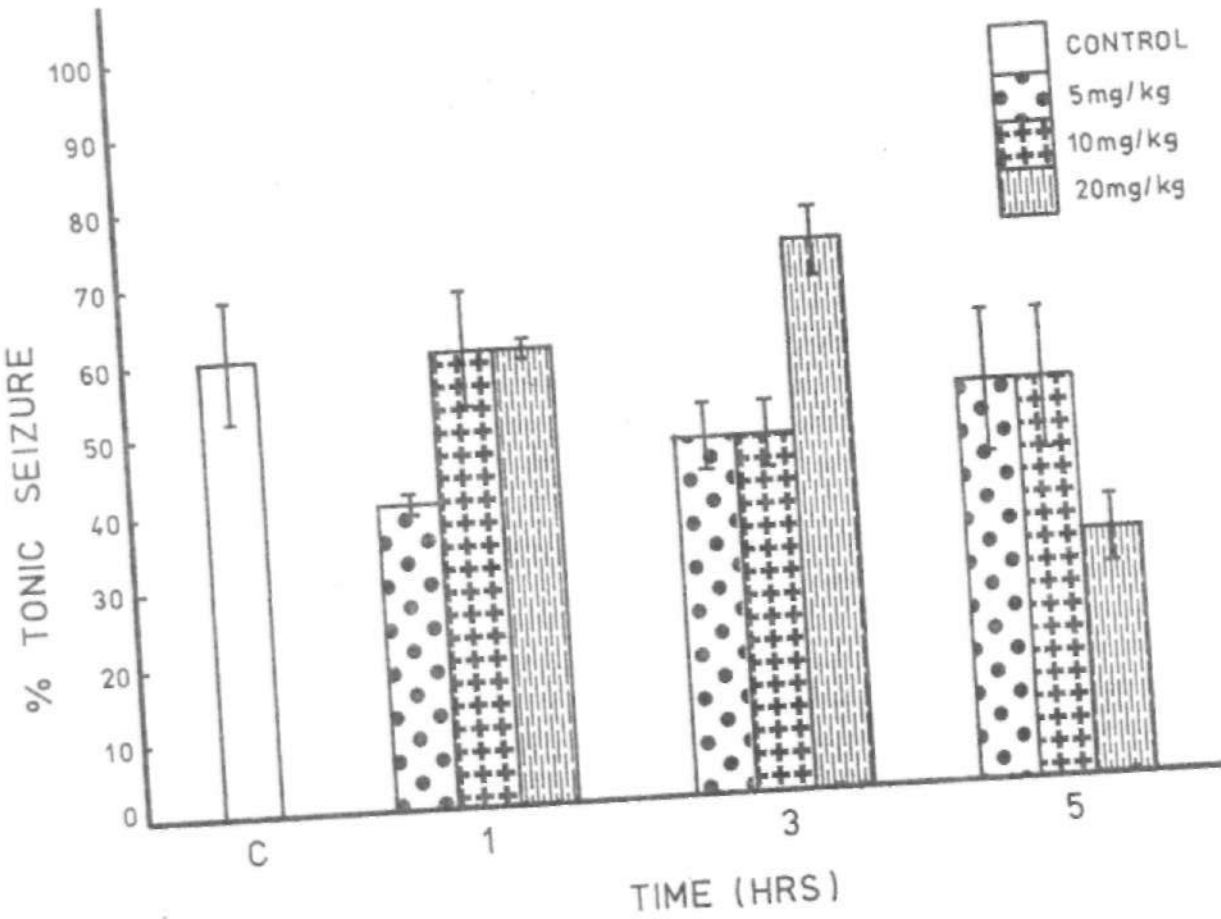


Fig. 3(ix). Histograms showing the effects of 5,10 and 20 mg/kg of methyl N-succinimidoxyacetate, MNSA on susceptibility of 4- day old chicks to electroshock. n = 15 per histogram.

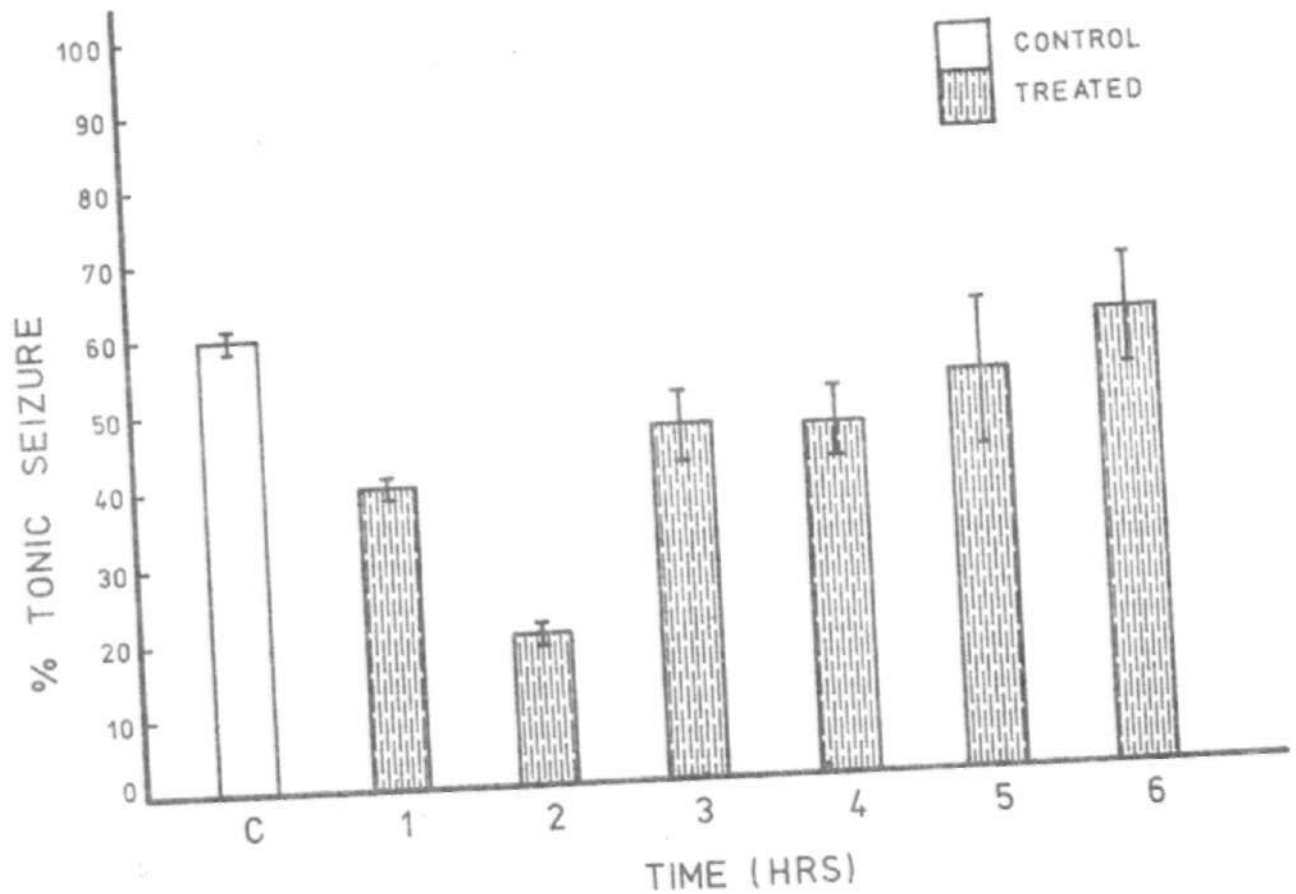


Fig. 3(x). Histograms showing the time course of anti-convulsant effect of methyl N-succinimidoxyacetate, [illegible] (5 mg/kg) in 4 - day old chicks. n = 15 per histogram.

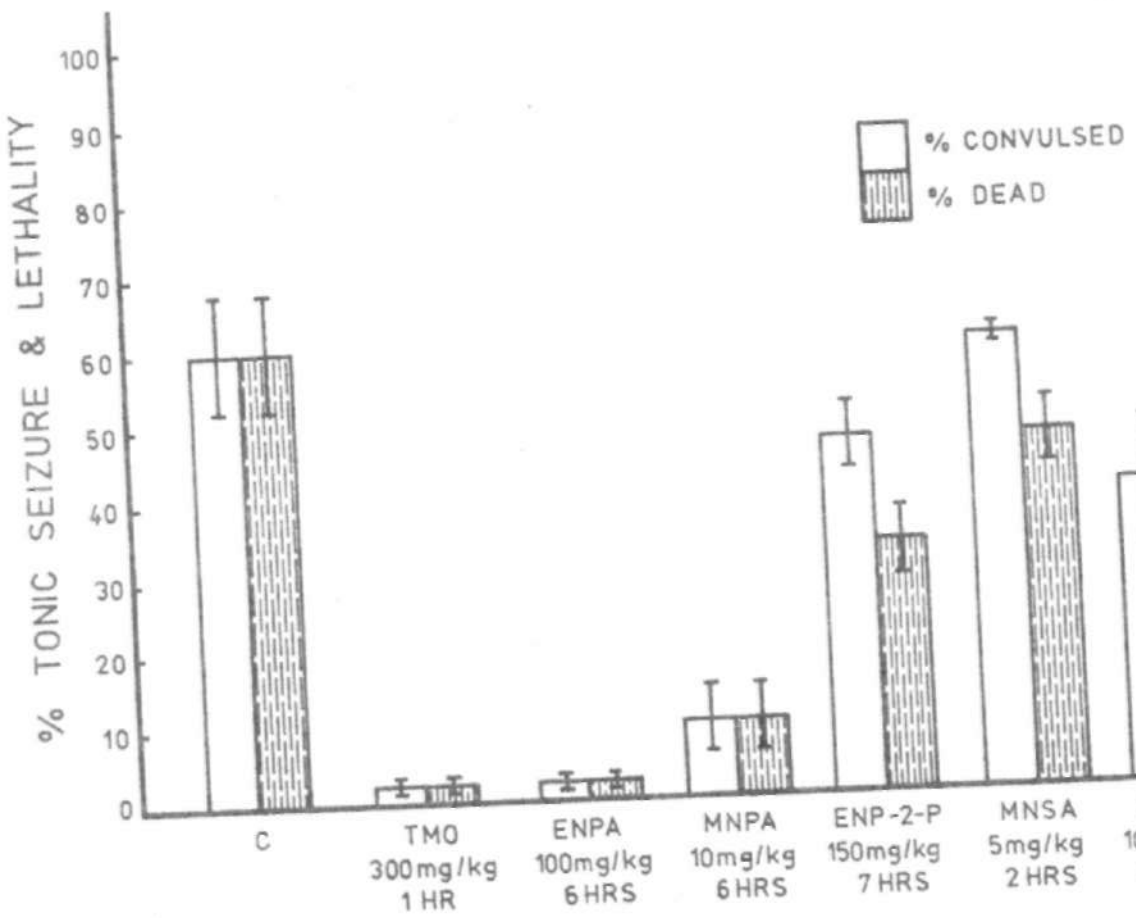


Fig. 3(xi). Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on AOAA-[illegible] and lethality in 4 - day old chicks

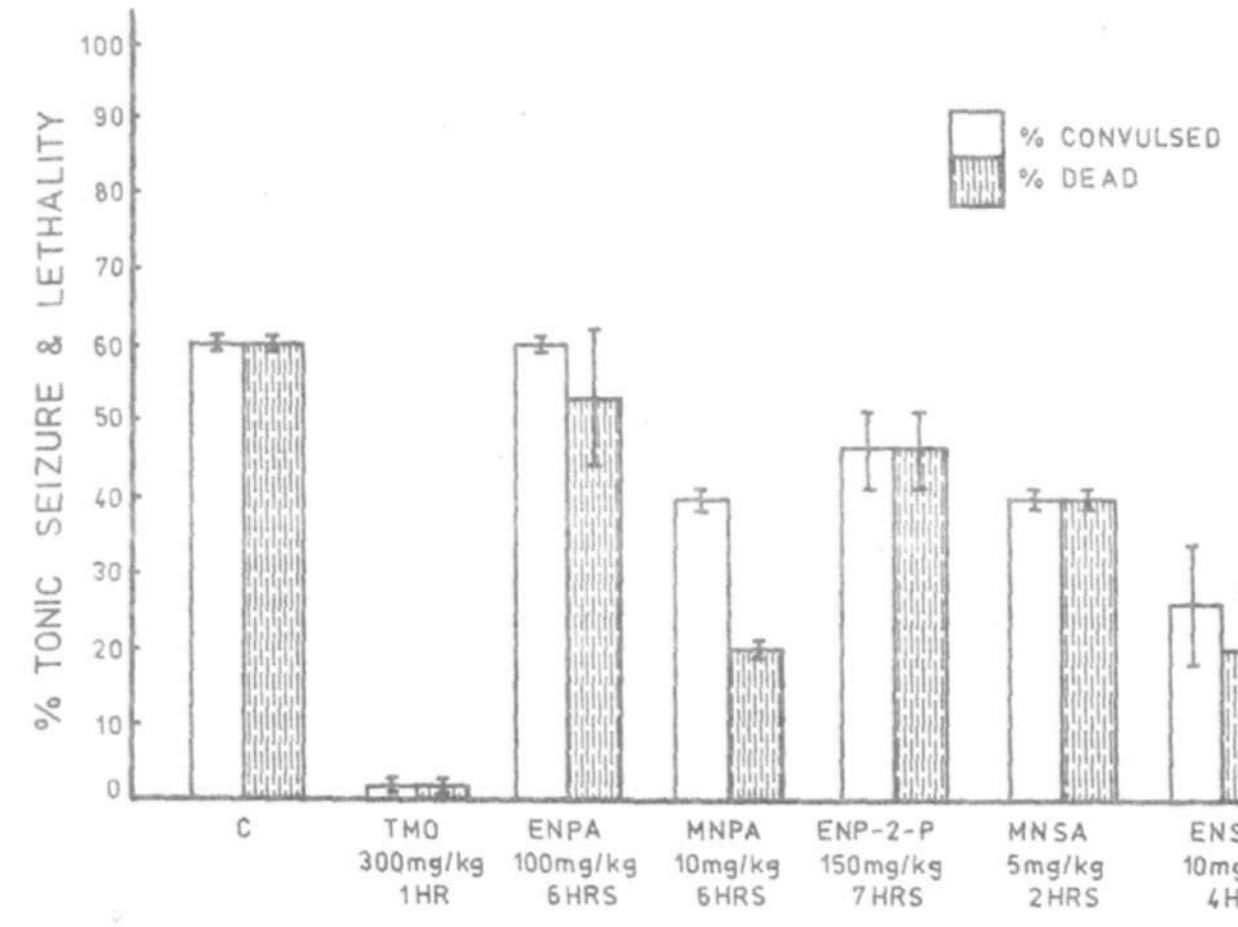


Fig. 3(xii). Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on bicuculline-induced seizure and lethality in 4- day old chicks. n = 15 per histogram.

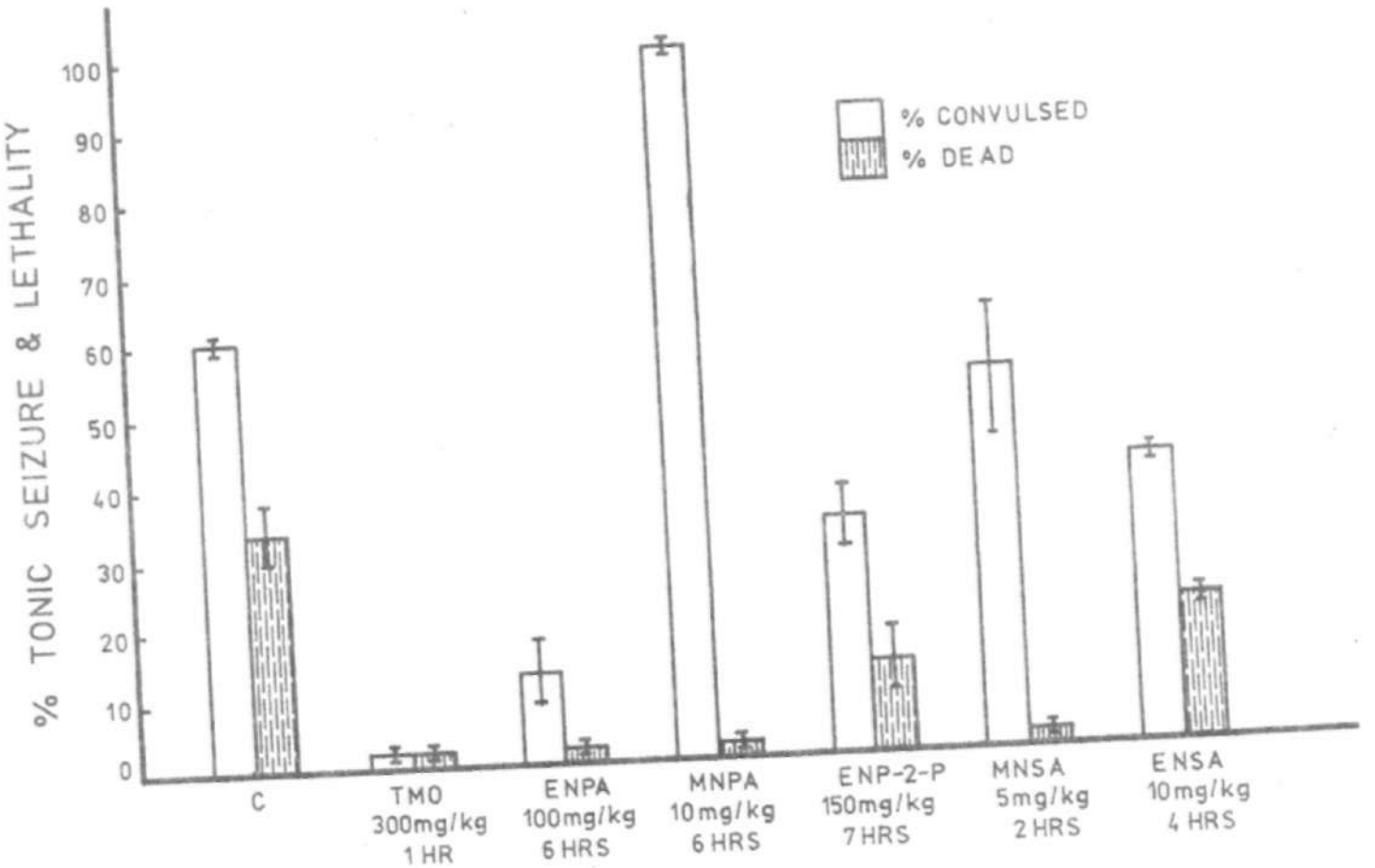


Fig. 3(xiii). Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on leptazol-induced seizure and lethality in 4-day old chicks. n = 15 per histogram.

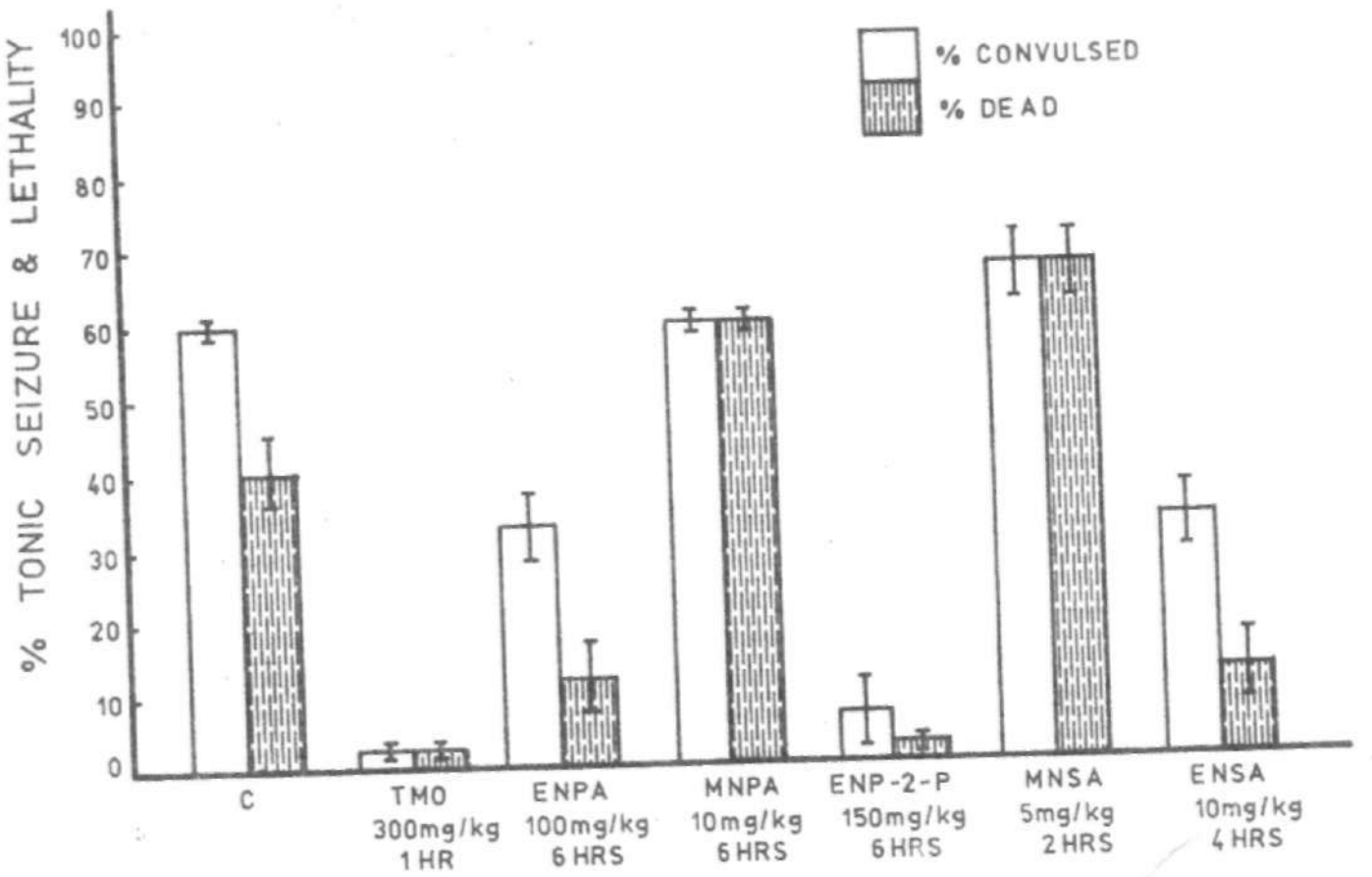


Fig. 3(xiv). Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on picrotoxin-induced seizure and lethality in 4 - day old chicks. n = 15 per histogram.

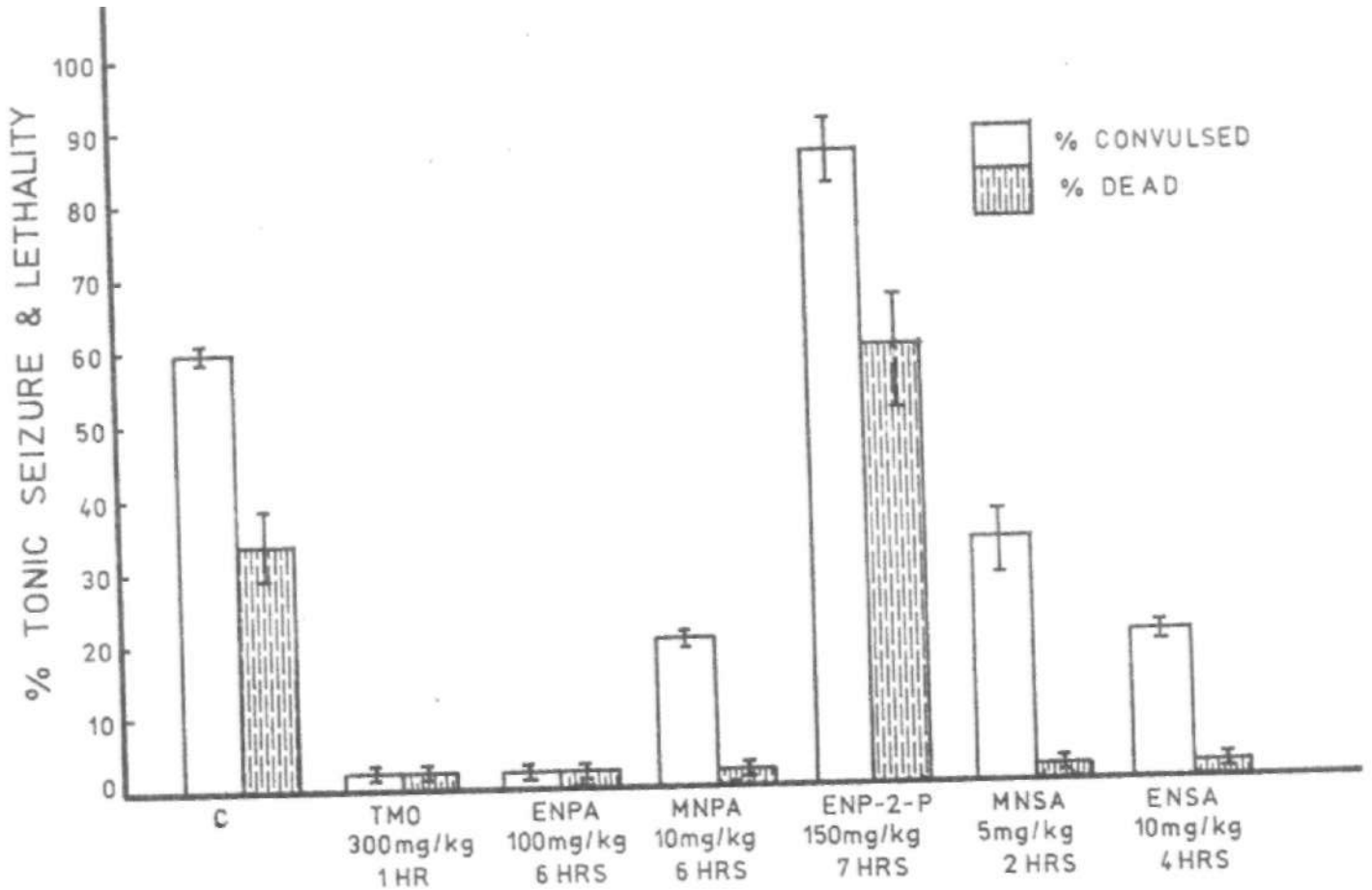


Fig. 3(xv). Histograms showing the effects of some N-substituted cyclic imides and the reference anti-convulsant compounds on strychnine-induced seizure and lethality in 4- day old chicks. n = 15 per histogram.

CHAPTER 4

DISCUSSION

A few points may be worth explaining from the onset. These include the use of chicks as experimental animals in a research which aims at developing drugs for eventual human consumption, and the use of cages.

Use of Chicks

The ease of handling, availability, cost of maintenance, and the nearness to man on evolutionary scale are some factors guiding the choice of experimental animals in a drug development exercise. Therefore most workers usually employ animals such as rats, mice, cats, dogs, guinea-pigs, rabbits etc., the choice being dictated largely by availability in any locality. In Nigeria, domestic fowl chicks are available in large numbers so that it is easy to obtain two hundred of them every week for the 'use once and discard' types of experiments. Perhaps the most relevant point in justifying the use of chicks for the work reported in this thesis was the finding by Osuide[88] that results obtained from his investigations on the effects of some

centrally acting drugs in chicks were similar to those obtained by other workers in mammalian species. Besides, more information may be obtained from chick experiment because postural and behavioural effects of some centrally acting drugs have been reported to be more apparent in chicks than in mammalian species.[89] However, similarities in results obtained with AOAA in chicks and mammalian species [51,71,74] as well as the finding that the chick is the most sensitive specie to AOAA-induced seizures,[88] would justify the use of chicks. Since the aim of this work is to develop compounds which lack the convulsive tendency of AOAA, it is reasonable to use the specie most sensitive to the unwanted property.

Use of Cages

Cages were found to be very useful not only for ease of observation but also for a more complete revelation of drug effects. Experience showed that when left unrestricted most animals would either hide away from human beings and open spaces thereby making

observation difficult, or be so involved in the process of space exploration that some drug effects might be masked.[61] Therefore the cage serves as a restricting environment which allows a wide range of observations to be made.

As reported earlier, results from saline-treated chicks and those treated with just the solvents used in formulating the compounds indicated that the solvents, did not contribute to the observed effects. Apparently, there are some limitations in trying to draw a structure-activity relationship between members of the N-substituted cyclic imides. These include the types and fewness of compounds obtained. More emphatic relationship would only be drawn with more compounds in which some have the amino end of AOAA substituted by the cyclic imides and the carboxylic end free, and the others have the carboxylic end esterified and the amino end unsubstituted. However the success with ENPA in protecting against experimentally-induced seizures, as well as the hypothesis suggested at the close of

Chapter 1 may give one some basis for drawing certain structure-activity relationships as discussed below.

N-Substituted Phthalimides

Our results appear to suggest that the replacement of the ethyl ester group in ENPA with a methyl ester group e.g. MNPA, or the substitution of a methyl group on number 2 carbon atom of ENPA, e.g. ENP-2-P may confer excitatory property on the compounds only at high doses. For example, MNPA was convulsant while ENP-2-P was excitatory at high doses. A dose of 100 mg/kg of MNPA produced some behavioural convulsant actions in 7% of 4-day old chicks. This was surprising in view of its close structural similarity to ENPA - one of the reference compounds - which was not convulsant even at doses as high as 500 mg/kg in a similar set of experimental animals and conditions. It is possible to hydrolyse MNPA under conditions similar to those reported useful for ENPA[85] since the conditions required are easily attainable within the body (enzymatic activity and body

temperature) and the end product is likely to be an ester of AOAA. Therefore it is feąsible to suggest that the rate of metabolism is fast enough to result in appreciable amounts of the 'active' compound - which like AOAA could cause a fall in brain GABA levels by inhibiting GAD-1 activity to result in seizures. Similarly, the excitatory action of ENP-2-P at high dose may be explained on the basis of its rate of metabolism but in this case the level of the 'active' compound is only high enough to cause stimulant effects, but not convulsion.

Other factors may be responsible for the excitatory effects of MNPA and ENP-2-P. For example, they may have a direct excitatory action of their own quite unrelated to brain GABA levels or they may be affecting some other inhibitory substances. It is to be realised that the GABA system is only one of the many inhibitory factors controlling CNS activity, and alterations in any of them can affect neuronal stability.

MNPA offered complete protection against the electric current that induced seizures in 60% of control

chicks - fig. 3(v). This was accomplished at half the corresponding dose of ENPA needed for complete protection. This result suggests that the anti-convulsant potency of ENPA is increased by esterification with a methyl instead of an ethyl group. On the contrary, the substitution of a methyl group at the number 2 carbon atom of ENPA appears to reduce the potency of ENPA because only 80% protection was offered - fig. 3(iv) - at one and a half times the dose of ENPA needed for complete protection.

One may suggest that the protective abilities of MNPA and ENP-2-P against electroshock seizures may be due to the elevation of brain GABA levels as a result of their inhibition of GABA-T. However, MNPA may be offering a higher degree of protection than ENP-2-P because the 'active' metabolites of the former are probably being formed at a faster rate than those of the latter compound. In addition the branching of the alkyl chain of ENP-2-P may prevent the molecules of the 'active' metabolites - compared to those of MNPA -

from fitting onto the receptors well enough to elicit anti-convulsant action.

Like AOAA, DPH and ENPA, MNPA provided complete protection against electrically-induced seizures but of the reference anti-convulsant drugs it resembled only AOAA in being convulsant at high doses and hence MNPA was considered inferior to DPH and ENPA. ENP-2-P was also considered inferior to the reference compounds because of its inability to provide complete protection against electroshock seizures.

In chemical seizures, MNPA had the greatest anti-convulsant effect, of all the compounds tested against AOAA-induced seizure and lethality by giving 80% protection against both percentage seizure and lethality. There seems to be a general opinion that the exact mechanism of action of AOAA is not known. The convulsant action of AOAA is thought to be due to either its direct action on brain excitability[74] or as a result of the inhibition of GAD-1 consequent to their ability to deprive the enzyme a supply of vitamin B_6 which is an essential

co-enzyme for its activity. The result of this is a fall in GABA level and occurrence of seizures.[47] Therefore the protective ability against AOAA-seizure and lethality by MNPA may probably be due to its occupation of AOAA receptors thereby making it difficult for AOAA to gain access to its receptors, and thus reducing AOAA seizures considerably. Perhaps, MNPA may be elevating brain GABA levels by inhibiting the activity of GABA-T to afford this high degree of protection against AOAA seizures.

Of all the compounds tested, ENP-2-P offered the best protection against leptazol and picrotoxin-induced seizures. It reduced the leptazol-induced seizure to 33% and picrotoxin-induced seizure to 10% (about 90% protection) while eliminating picrotoxin lethality. The leptazol seizure is claimed to be due to excitation of cerebral structures by decreasing the neuronal recovery time to make the neurones responsive to repetitive stimuli. Such seizures are relatively unopposed by inhibitions.[90] As regards the protection offered by ENP-2-P against picrotoxin seizure and lethality, one may suggest that

it is elevating brain GABA levels to effect this inhibitory process since picrotoxin is known to cause its convulsive action by blocking GABA - mediated pre-synaptic inhibition[91] and strychnine-resistant post-synaptic inhibition in CNS.[92]

MNPA reduced to a great extent the percentage seizure induced by bicuculline and strychnine, and offered complete protection against strychnine lethality but had no effect on picrotoxin-induced seizure and lethality. The results seem to suggest that MNPA is more potent than ENPA in protecting against bicuculline seizures but is inferior to AOAA and TMO in protecting against chemical seizures- figs.3(xi) - 3(xv). In addition, MNPA potentiated leptazol-induced seizure from 60% to 100%, although it offered complete protection against the leptazol lethality; and electroshock seizures. Since leptazol convulsions have been reported to resemble electroshock seizures[8] one would normally have expected the compound to behave identically towards leptazol-and electrically-induced seizures.

The difference would tend to suggest that although the convulsive patterns and manifestation in leptazol and electroshock seizures may be similar, there may be significant differences in their controls.

ENP-2-P afforded some protections against the incidence of seizure and lethality induced by AOAA and bicuculline, while it potentiated strychnine-induced seizure and lethality. ENP-2-P protected against leptazol-and bicuculline-induced seizures which are usually more difficult to protect than strychnine-induced seizures. One would not attempt to speculate any mode of action on the basis of this finding until more biochemical work is done. Since ENP-2-P could not offer complete protection against chemical seizures it was considered inferior to AOAA and TMO. However, it was superior to ENPA in providing a higher degree of protection against picrotoxin-induced seizure and lethality.

The N-substituted cyclic imides - ENP-2-P and MNPA- have afforded various modifications of AOAA which are

not as potent as AOAA itself but in which the convulsive tendency of AOAA at high doses has been drastically reduced.

N-Substituted Succinimides

The same limitations (types and fewness of compounds) as explained above would make it difficult to categorically state the structure-activity relationship between members of the N-substituted succinimides. However, the results show that none of the N-substituted succinimides had any noticeable effect on the behaviour of the 4-day old chicks at any of the doses administered. This may be explained on the basis of their rates of metabolism. Since ENSA and MNSA have more hydrophilic properties than ENP-2-P and MNPA, it would not be quite easy for them to cross the lipoidal membranes to have sufficient quantity hydrolysed into appreciable amounts of the 'active' metabolites to cause seizures. In addition ENSA and MNSA had remarkable protective abilities by affording 90% and 70% protection respectively against electroshock seizures; thus probably

suggesting that as for MNPA above, the compounds at optimum doses become metabolised into such levels of the corresponding esters of AOAA which may probably show anti-convulsant effects. ENSA had a wide-spectrum anti-convulsant activity against the chemically-induced seizures. Of all the compounds used, it had the highest protective ability against the incidence of seizure and lethality induced by bicuculline and strychnine. It reduced the percentage seizure to 27% and 20% respectively for bicuculline - and strychnine-induced seizures. It reduced bicuculline lethality from 60% to 20% and provided complete protection against strychnine lethality. The result of the bicuculline experiment probably strengthens the suggestion that ENSA may be eliciting its anti-convulsant action by elevating brain GABA levels since bicuculline is a GABA antagonist. It is well known that presynaptic and some post-synaptic inhibitory processes are GABA-mediated.[91,93] However the result of the strychnine experiment leads one to suggest that stablization of neurones by ENSA may probably be mainly responsible for

its anti-convulsant action although strychnine is believed to owe its convulsive action to blockade of post-synaptic inhibitory processes notably those utilizing glycine as transmitter, by competing with the inhibitory transmitter for the post-synaptic receptor sites.[93] It is therefore possible to classify inhibitory substances as "GABA-like" (antagonized by bicuculline) and "glycine-like" (antagonized by strychnine). Like ENSA, MNSA offered marked protection against strychnine-induced seizures; and its mode of action against strychnine seizures may be explained as above. However, both ENSA and MNSA were still considered inferior to DPH and ENPA for failing to provide complete protection against electroshock seizures. Also, ENSA and MNSA were inferior to TMO since they did not provide complete protection against the chemically-induced seizures. The advantage the N-substituted succinimides had over AOAA is that they were devoid of any convulsant tendency. ENSA and MNSA had an edge over ENPA in offering a higher degree of protection against bicuculline-induced seizure and lethality.

This suggests that the substitution of the succinimido group for the free amino end and the simultaneous esterification of the carboxylic end of AOAA tend to produce compounds which lack the convulsant tendency at high doses. Further more, this modification has converted the inactive succinimides to potent anti-convulsant agents and it is feasible to suggest that the succinimido group may be acting as a fixed carrier moiety which forms an intrinsic part of the 'active' molecule which could be acting like substituted succinimides.

Possibility of Using N-Substituted Cyclic Imides as Leads

Among the N-substituted phthalimides, ENP-2-P and MNPA have undesirable side effects in being excitatory and convulsant respectively at high doses. However, the reference compound ENPA is devoid of this convulsant property. Also, the N-substituted succinimides, ENSA and MNSA lack any convulsant tendency at high doses. Infact, there was no noticeable behavioural effects when high doses such as 500 mg/kg of ENSA and MNSA were

administered. These N-substituted succinimides elicited anti-convulsant activity at much lower doses than the N-substituted phthalimides. For example the optimum anti-convulsant doses of ENSA and MNSA were 10 and 5 mg/kg respectively, while those of ENP-2-P and MNPA were 150 and 50 mg/kg respectively. Compared to the phthalimide derivatives, the succinimide derivatives are easily soluble in water and therefore their formulation is easier than that of the former which are insoluble in water. In addition, ENSA and MNSA had quicker onset of action (4 and 2 h. respectively) than ENP-2-P and MNPA (7 and 6 h. respectively). This therefore suggests quicker onset of action with increased hydrophilicity. Nevertheless a delicate balance between lipophilic and hydrophilic moieties is probably necessary for a quicker onset of action. Due to their water solubility, ENSA and MNSA have quick onset of action and probably rapid elimination of the 'active' compound (metabolised or unmetabolised form) via the kidney. In this way, the brain level of the 'active' compound will

not be high enough to cause excitatory and convulsive actions. On the basis of the factors enumerated above, it would appear that the N-substituted succinimides are generally superior to the N-substituted phthalimides and therefore the former would make better leads for further investigation of compounds possessing desirable anti-convulsant properties.

There appears to be some structure-activity relationship between the phthalimide derivatives and the succinimide derivatives. MNPA is convulsant at high doses while MNSA is not. The only difference in their chemical structures lies between the phthalimido group of MNPA and the succinimido group of MNSA. However, ENPA is not convulsant and it is difficult to make any definite structure-activity relationship on the basis of MNPA and MNSA alone. In general, these modifications of AOAA appear to result in a reduction of its convulsant property although a substantial amount of its anti-convulsant property is also lost. This may be due to the overall physico-chemical property of the compounds.

Considering the methyl and ethyl esters, it would appear that the methyl analogues (MNPA and MNSA) have higher potencies than their corresponding ethyl analogues (ENPA and ENSA) in their protective ability against electroshock seizures. For example, 100 mg/kg of ENPA offered complete protection against electroshock seizures whereas 50 mg/kg MNPA also offered maximum protection under similar conditions. This is further substantiated by the results of the succinimide derivatives where percentage seizure in the electroshock experiment was reduced to 10% by 10 mg/kg of ENSA and 20% by 5 mg/kg of MNSA.

The advantage of the compounds - ENP-2-P ENSA, MNPA and MNSA - over AOAA is that they allow the safe administration of doses otherwise impossible for AOAA without lethal effects. For example, 50 mg/kg of any of the compounds was anti-convulsant to some extent. This is approximately 20 mg/kg of AOAA on molecular basis and such a dose was prohibited with AOAA realising

that even 15 mg/kg was 100% lethal to the 4-day old chicks under similar conditions.

The results appear to indicate that none of the compounds offered complete protection against percentage seizure induced by any of the chemical convulsants administered. So the compounds were considered inferior to TMO. However, all the compounds were superior to ENPA in reducing percentage seizure and lethality induced by bicuculline. Also, ENP-2-P was superior to ENPA in affording a better protection against the incidence of seizure and lethality caused by picrotoxin. Like the reference compounds - AOAA, ENPA and TMO - ENSA, MNPA and MNSA provided complete protection against lethality caused by strychnine. Furthermore, MNSA and MNPA, provided complete protection against leptazol lethality while only ENP-2-P provided maximum protection against lethality caused by picrotoxin. In these isolated cases, the compounds compared favourably with the reference drugs - AOAA and TMO.

The compounds which have shown high protective ability against electroshock seizures may be useful in clinical grand-mal epilepsy since electroshock seizures simulate clinical grand-mal epilepsy. In this regard one would suggest the use of ENSA, and MNPA. However, if MNPA finds its way into clinical usage especially with psychotic patients, caution should be exercised in view of its convulsant tendency at high doses. ENSA would also be useful in the management of petit-mal epilepsy since it possesses some protective ability against chemically-induced seizures which resemble clinical petit-mal epilepsy. Since ENSA has shown a wide-spectrum activity against electrically-and chemically-induced seizures, it is recommended for further investigations towards the management of grand-mal and petit-mal disorders.

Conclusion

The results from the pharmacological screening supported the hypothesis proposed in the introduction; that a hybrid compound consisting of a cyclic imide moiety such

as the succinimido group and aminooxyacetic acid (AOAA) would possess anti-convulsant properties. ENSA and MNSA possessed some degree of anti-convulsant activity but none of them was as potent as AOAA in protecting the young chicks against experimentally-induced seizures. However, they were superior to AOAA in lacking the convulsant tendency at high doses. Also, ENSA and MNSA are improvements over unsubstituted succinimides which are known to be inactive as anti-convulsant agents. Thus ENSA and MNSA have led to better anti-convulsant properties of AOAA, and enhanced activity of succinimides.

For the purpose of a more detailed structure-activity relationship it will be of interest to synthesize more compounds which would enable one elucidate the functions of the amino and carboxylic acid ends of AOAA as regards convulsant and anti-convulsant properties. These compounds should include those in which some anti-convulsant moieties such as Ethosuximide, Methsuximide and Phensuximide are used for replacing the amino end of AOAA. Also, biochemical assays can possibly be carried

out in order to investigate the effects of the N-substituted cyclic imides on the level of the naturally-occuring inhibitory transmitter, GABA and compare them with the effects of AOAA with a view to gaining an insight into their possible mode of action. On the other hand, more information on these compounds may also be obtained by monitoring their effects on EEG of young chicks in order to find out states of seizures and other changes not easily correlated with behaviour. From these suggested works, one would probably identify and preserve the moieties in AOAA having anti-convulsant properties and replace those groups having convulsant property in order to obtain highly potent anti-convulsant agents which will be devoid of the convulsant property.

REFERENCES

1. LIVINGSTON, S., In "Comprehensive Management of epilepsy in infancy, childhood and adolescence", Charles C. Thomas, Publishers. Springfield, Illinois. U.S.A. p. 5, (1972).

2. JACKSON,J.H., in "Selected writings of John Hughlings Jackson" Vol. 1., Taylor, J. Ed,, London, Hodder and Straughton, p. 94 (1931).

3. HOFFMAN, W.W., Neurology (Mineap.) 8, 210, (1958).

4. MILLICHAP, J.G., Postgrad. Med.,37, 22 (1965).

5. PENFIELD, N., and JASPER, H., Eds., in "Epilepsy and the functional anatomy of the human brain". Little, Brown and Co. Boston, p.22 (1973).

6. TEMKIN., in "The falling sickness" Baltimore, John Hopkins Press, (1954).

7. JACKSON, H.J., in "Selected writings of John Hughlings Jackson" Vol. 1., Taylor, J. Ed., London, Hodder and Straughton, p.193 (1931).

8. FRANZ,D.W., in "The Pharmacological Basis of Therapeutics" 5th edn. Goodman L.S. and Gilman, A. (eds.) Macmillan Publishing Co., New York, Toronto, London, pp.359-362,(1977).

9. MERIT, C., and PUTNAM,T.J., in "Anticonvulsant Drugs",Mercier,J. ed. Vol. 1,205 (1941).

10. COLLINS, R.L., in "Experimental Models of Epilepsy" Purpura,D.P., Penry, J.K., Tower, D.B., Woodbury, D.M., and Walter,R.D., eds., Raven Press, New York, Chap. 14 (1972).

11. GRANT,R.H.E., and BAROT, M., in "Clinical and Pharmacological Aspects of Sodium Valprocate (Epilim) in the Treatment of Epilepsy". Proc. of symposium at Nothingham University, LEGG, N.J. ed., pp. 130-135 (1975).

12. NAQUET, R., and MELDRUM, B.S., in "Experimental Models of Epilepsy", Purpura, D.P., Penry, J.K., Tower,D.B., Woodbury,D.M., and Walter,R.D., eds. Raven Press, New York, Chap.15 (1972).

13. WILSON,C.O., GRISVOLD, O., and DOERGE., in "Textbook of Organic, Medicinal and Pharmaceutical Chemistry", J.B. Lippincott Company,Philadelphia-Toronto, p.402 (1977).

14. SWINYARD,E.A., and GOODMAN, L.S., in "Antiepileptic drugs", Woodbury, D.M., Penry, J.K., and Schmidt, R.P., eds., Raven Press, New York, (1972).

15. MERRIT, H.H., and PUTNAM, T.J., J.Am.Med.Soc., III, 1068-1073, (1938).

16. GOODMAN,L.S., TOMAN,J.E.P., and SWINYARD,E.A., Am.J.Med. I, 213-228, (1946).

17. RICHARDS, R.K., and EVERETT, G.M., J. Lab.Clin.Med., 31, 1330-1336, (1946).

18. JASPER,H.H., in "Basic Mechanisms of the epilepsies", Jasper, H.H., Ward, A.A., and Pope, A., eds., Little, Brown and Co. Boston, pp.421-451, (1969).

19. LOWRY, O., and PASSONNEAV,J.V., J. Biol.Chem., 239, 31 (1964).

20. BACHELARD, H.S., and McILWAIN,H., in "Comprehensive Biochemistry, Florkin, M., and Stotz, E.H. eds., Amsterdam; Elsevier (1969).

21. ELLIOT, K.A.C., and PENFIELD, W., J. Neurophysiol. 11, 485 (1948).

22. LIVINGSTON, S., in "Comprehensive Management of epilepsy in infancy, childhood and adolescence", Charles C. Thomas, Publishers, Springfield, Illinois, U.S.A. p.7 (1972).

23. EARLE, K.M., BALWIN, M., and PENFIELD, W., Arch.Neurol. and psychiat. 69, 27 (1953).

24. TOWER, D.B., and ELLIOT, K.A.C., J. Appl. Physiol. 5, 375 (1953).

25. TOWER, D.B., and McEACHERN, D., Canad.J.Res. 27, 120 (1949).

26. TOWER, D.B., and ELLIOT, K.A.C., J. Appl. Physiol. 4, 669 (1952).

27. BIANCHI, C., BEANI, L., and BERTELLI, A., Neuropharmacol., 14, 237-332 (1975).

28. SHIELDS, P.J., J. Pharm. Pharmacol. 24, 919-920 (1972).

29. TAGLIAMONTE, A., TAGLIAMONTE, P., DICHARA, G., GESSA, R., and GESSA, G.L., J. Neurochem.19, 1509-1512 (1971).

30. GRAHAME-SMITH, D.G., and GREEN, A.R., Brit. J. Pharmacol. 52, 19-26 (1974).

31. HOCKADAY, T.D.R., KEYNES, W.M., and MCKENZIE, J., Brit.Med. J. 1, 85 (1966).

32. FOURMAN, P., RAWNSLEY, K., DAVIS, R.H., JONES, K.H., and MORGAN, D.B., Lancet. 11, 914 (1967).

33. ANDERSON, J., Proc. Roy.Soc.Med. 61, 1123 (1968).

34. CARMAN, J.S., POST,R.M., GOODWIN, F.K., and BUNNLY,Jr. W.E., Biol. Psychiat. 12, (1) 5-17 (1977).

35. TALLAN, H.H., in "Amino acid pools", Holden, J.T. ed. Amsterdam, Elsevier, P. 471 (1962).

36. TOWER, D.B., in "Progress in Neurobiology and Neurochemistry", Vol. VI Hoeber, P.B. ed., pp.169-197 (1956).

37. CURTIS, D.R., in "Basic mechanisms of the epilepties", Jasper, H.H., WARD, Jr.A.A., and POPE, A. eds., Little, Brown and Co. Boston, pp. 105-135 (1959).

38. PETERS, E.L., and TOWER, D.B., J. Neurochem. 5, 80-90 (1959).

39. SELLINGER, O.Z., and WEILER, P., Biochem.Pharmacol. 12,989-1000 (1963).

40. ANARAPA, J., LANDUA, A.J., GUEST, R., and SEALE, B., J. Biol. Chem. 187, 35-39 (1950).

41. ROBERTS, E., and FRANKEL, S., J. Biol.Chem. 187, 55-63(1950).

42. UDENFRIEND, S., J. Biol.Chem. 187,65-69(1950).

43. OKADA, Y. NITSCH-HASSLER, C., KIM, J.S., BAK, I.J., and HASSLER, R., Exp. Brain, Res. 13, 514-518 (1971).

44. PERRY, T.L., BERRY, K., HANSEN, S., DIAMOND, S., and MOK,C., J. Neurochem. 18, 513-519 (1971).

45. KILLAM, K.F., and BAIN, J.A., J. Pharmacol. Exp. Therap.,119, 255-262 (1957).

46. KILLAM, K.F., J. Pharmacol. Exp. Therap.,119, 263-271 (1957).

47. KILLAM, K.F., DASGUPTA, S.R., and KILLAM,E.K., in "Inhibition in the Nervous System and Gamma-aminobutyric acid", Roberts, E., ed., Pergamon Press, New York, pp. 302-316 (1960).

48. VAN-GELDER, N.M., and ELLIOT, K.A.C., J. Neurochem., 3, 139-143. (1958).

49. KURIYAMA, K., and SZE,P.Y., Neuropharmacology,10, 103-108 (1971).

50. EIDELBERGE, E., BAXTER, C.F., ROBERTS, E., SALDIAS, C.A., and FRENCH,J.D., Proc. Soc. expt. Biol. Med., 101, 815-817 (1960).

51. KURIYAMA, K., ROBERTS, E., and RUBENSTEIN, M.K., Biochem. Pharmac.,15, 221-236 (1966).

52. ROBERTS, E., and ELDELBERGE, E., Int. Rev. Neurobiol.,2, 279-332.(1960).

53. GRAY, W.D., MAREN, T.H., SISSON, G.M., and SMITH, F.H., J. Pharmacol. and Exp. Therap.,121, (2), 160-170 (1957).

54. ROBERTS, E., WEIN, J., and SIMONSEN, D.G., Vitamine Horm. 22, 503-556 (1964).

55. BAXTER, C.F., and ROBERTS, E., Proc. Soc. expt. Biol. Med. 101, 811-815 (1959).

56. PERRY, T.L., and HANSEN, S., J. Neurochem.,21, 1167-1175 (1973).

57. WOOD, J.D., and PEESKER, S.J., J. Neurochem.,20, 279-287 (1973).

58. CARVAJAL, G. RUSSEK, M., TAPIA, R., and MESIEU, G., Biochem. Pharmacol., 13, (7), 1059-1069 (1964).

59. VAN-GEIDER, N.W., J. Neurochem.,16, 1355-1360 (1969).

60. VAN-GEIDER, N.M., J. Neurochem., 15, 747-757 (1968).

61. WOOD, J.D., and PEESKER, S.J., J. Neurochem., 25, 277-282 (1975).

62. SAWAYA, M.C.B., HORTON, R.W., and MELDRUM, B.S., Epilepsia,16, 649-655 (1975).

63. MEUNIER, H., CARRAZ, G., MEUNIER, Y., EYMARD, P., and AIMARD, M., Therapie, 18, 435-438 (1963).

64. CARRAZ, G., FRAU, R., CHATEAU, R., and BONNIN, J., Am. Med. Psychol.,122, 577-585 (1964).

65. GOODIN, Y., HEINER, L., MARK,J., and MANDEL, P., J. Neurochem., 16, 869-873.(1969).

66. SIMLER, S., GESIELSKI, L., MAITRE, M., RANDRIANARISOA, H., and MANDEL, P., Biochem. Pharmacol.,22, 1701-1708 (1973).

67. HARVEY, P.K.P., BRADFORD, H.F., and DAVIDSON, A.N., Febs. Letts. 52, 251-254 (1975).

68. JEAVONS, P.M., and CLARK, J.E., Brit. Med. J.,2, 584-586. (1974).

69. BURIN, M., SAKS, G., GERBER, B.I., MEISCHENGUISER, R., and TALLIS, J., Sem. Med. (Buenos Aires), 140, 678-682 (1972).

70. GRANT, R.H.E., and BAROT, M., in "Clinical and Pharmacologic Aspects of Sodium Valproate (Epilim) in the Treatment of Epi... Proc. of symposium at Nothingham University, LEGG, N.J. ed., (1975).

71. WALLACH, D.P., Biochem. Pharmacol.,5, 323-331 (1961).

72. SAWAYA, M.C.B., HORTON, R.W., and MELDRUM. B.S., Epilepsia 16, 649-655 (1975).

73. BAXTER, C.F., and ROBERTS, E., Proc. Soc. expt. Biol. Med.,101, 811-815 (1959).

74. OSUIDE, G., Br. J. Pharmac., 44, 31-44 (1972).

75. CARTER, C.H., Cur.Ther.Res.,6, 608-618 (1964).

76. ESSIG, C.F., Arch. Int. Pharmacol., 176, 97-103 (1968).

77. SCHRUMANN, E.L., PAGUETTE, L.A., HEINZELMAN,R.U., WALLACH, D.P., DAVANZO, J.P. and CRIG, M.E., J. Med. Chem.,5, 464-477 (1962).

78. WOODBURY,D.M., and FINGL,,E., in "The Pharmacological Basis of Therapeutics", 5th edn., Goodman, L.S., and Gilman, A. eds, Macmillan Publishing Co., New York, Toronto, London, pp.201-226 (1977).

79. LAHAN,G.D., OSUIDE,G., and STANSFIELD,F., Proc.Brit.Pharmacological Soc., Dublin,67p (1979).

80. CHEN,G., WESTON,J.K., and BRATTON,A.C. Jr., Epilepsia, 4, 66-76 (1963).

81. ABBOT, E., and SCHWAB, D.M., New Eng. J. Med., 197, 256 (1954).

82. McKAY,A.F., GARMAISE,D.L., PARIS,G.Y., and GELBLUM,S., Can.J.Chem., 38, 343-358 (1960).

83. SCHUMANN,E.L., PAQUETTE,L.A., HEINZELMAN,R.V., WALLACH,D.P., DA VANZO, D.P., and GREIG M.E., J. Med.Chem., 5, 462-477 (1962).

84. NEIGHBOR,R.P., C.A., 73, 304 (35083 p) (1970).

85. SURESH,K.S., and MALKANI,R.K. Indian J.Chem., 10, 1068-1069 (1972).

86. MORRISON, R.T., and BOYD, R.N., in "Organic Chemistry", Allyn and Bacon, Inc. Boston, p.456-474, (1973).

87. AMES,D.E., and GREY,T.F., J.Chem.Soc., 1, 631-636 (1955).

88. OSUIDE, G., Eur. J.Pharmacol., 3, 283-293 (1968).

89. SCHNEDER,C., Nature, 191, 607-608 (1961).

90. LEWIN,J., and ESPLIN,D.W., J. Pharmac. Exp. Therap., 132, 245-250 (1961).

91. CURTIS,D.R., and JOHNSTON, G.A.R., Ergebn Physiol., 69, 97-188 (1974).

92. BANNAR,N.R., and JABBUR,S.J., Int. J. Neuropharmacol., 8, 299 (1969).

93. HULTON,H., JANKOWSKA,E., and LINDSTRON,S., J.Physiol.Lond., 218, 613-636 (1971).

94. COLFER,H.F., and ESSLY,H.E., Am. J. Physiol., 150 27 (1947).

BIOGRAPHICAL SKETCH

Ivan Ogheneochuko Edafiogho was born at Abraka in Bendel State of Nigeria on May 23, 1951. He attended Anglican Grammar School, Okpara Waterside, where he was Dining Hall Prefect, and School goal-keeper. He obtained Division One in the West African School Certificate (WASC) examination in December,1968. He obtained his higher School Certificate (HSC) from Edo College, Benin City, in November,1970. He was Edo College goal-keeper and school prefect.

The author taught at Anglican Grammar School, Okpara Waterside from January,1971 to August,1972, where he was also Games-Master. In September,1972 he entered Ahmadu Bello University, Zaria where he studied Pharmacy.

While at Zaria, he was the University goal-keeper, member of the University Swimming team and won half-colour in 1972-1973 session. He became University Swimming Captain, competed for the University and won medals at West African University Games (WAUG) at Kumasi-Ghana in December,1973 and Nigeria University Games (NUGA) at

University of Nigeria, Nsukka, in April,1974. At the end of that session he was awarded full colour in Swimming. In his final session he won medals at the First All-Africa University Sports (FASU) at Accra-Ghana in December,1974, and at the Kaduna State Sports Festival in April,1975. At the end of the session, he had bagged so many gold, silver and bronze medals that he was crowned the best sportsman of the year; thus becoming the first pharmacy student to win the highest honour any student could obtain in University Sports. He graduated with a Bachelor of Science (Second Class,Upper) degree in Pharmacy from Ahmadu Bello University, Zaria in June,1975.

Mr. Edafiogho did his internship at University of Benin Teaching Hospital (UBTH), Benin City, and participated in the National Youth Service Corps Scheme at Shendam, in Plateau State of Nigeria. Of course, he won many medals in swimming at the Plateau State Sports Festival in April,1977. He joined the academic staff of the Faculty of Pharmaceutical Sciences as a graduate Assistant in September,1977, and registered for the M.Sc. course in Pharmaceutical Chemistry. He was promoted

Assistant-Lecturer in October,1978, and since then he has been University Co-ordinator for Swimming, and Time-Table Officer for the Faculty of Pharmaceutical Sciences. He is a registered member of the Pharmaceutical Society of Nigeria (PSN) and he presented a scientific paper at the 52nd annual conference of PSN held at Kaduna, in November,1979.