PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF THE FRUIT, LEAVES AND BARK OF ERYTHRINA SENEGALENSIS D.C

BY

KPAMIOSEH ANTHONY OGBECHE

Being

A Thesis Submitted in fulfillment of the Requirements for the Award of Doctor of Philosophy (Pharmaceutical and Medicinal Chemistry) in the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

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CERTIFICATION

I hereby certify that this dissertation has been read and approved as having satisfied the conditions for the Award of Doctor of Philosopy (Ph.D) Degree.

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A B S T R A C T

The fruits, leaves and bark of Erythrina senegalensis D.C. (family leguminoseae), a savannah shrub widely distributed in West Africa and employed in traditional medicine in Northern Nigeria to treat jaundice and other ailments such as dropsy gonnorhoea and also as a muscle relaxanthave been chemically and pharmacologically investigated;

General phytochemical screening carried out on the crude extracts of the plant revealed the presence of alkaloids in high quantities in the bark as compared to the leaves and fruits while glycosides, saponins, steroidal substances were found in moderate quantities in all parts of the plant investigated.

Three components were isolated from the various extracts from the
of the plant coded ESMB as a new compound/methanolic extract of the stem bark,ESFP from the petroleum ether extract of the fruit and ESLP, from the petroleum ether extract of the leaves. On the basis of their chemical and spectral data (I.R, U.V., NMR and MS) .ESMB. was shown to be Methyl erythrina, while the petroleum spirit extract of the fruit contained among others 1 myristo-2-stearo-3-palmitin coded ESFP... The third component coded ESPL was isolated from the petroleum spirit extract of the leaves which on the basis of the spectral evidence was shown to be n-octade-canoic acid. Pharmacologically the methanolic extract of the fruits and leaves of the Erythrina senegalensis relaxed rhythmic contraction or pendular movement of the smooth muscle of rabbit jejunum while the methanolic extract of leaves and bark of the plant contracted isolated guinea pig ileum just like acetylcholine

which was blocked by a competitive antagonist atropine. The bioassay of the leaves, fruits and bark of methanolic extract showed a gradual increase in neuromuscular blockade as the doses of the extract was increased. This blockade occurs at the application of acetylcholine which produces contraction and methanolic extract of the leaves, fruits and bark to the skeletal muscle of the frog. The crude extract specifically blocked Ach induced spasms. The spasmolytic ED against Ach for methanolic leave extract was 1.26 x 10 g/ml, for fruits extract was 6.61 x 10 g/ml and for bark extract was 2.85 x 10 ' g/ml. Their potency ratio relative to methanolic fruit extract was found to be 3.46 x 10 g/ml while that of leaves extract was **1.82** x 10-^{3}g/ml and the bark 8.035 x 10"" g/ml. Since their potency ratio was less than 1, tubocurarine was found to be more potent than the crude extract of fruits and leaves of Erythrina senegalensis while the bark had similar potency as D-_tubocurarine.

DEDICATION

This piece of work is first and foremost dedicated to Almighty God for His gratefulness and mercy upon me and secondly to my Children.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to my supervisors Professor M. Ilyas, Dr. A. Mustapha and Dr. I. Abdu-Aguye for their immense encouragement and invaluable advice and suggestions throughout the entire programme;

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A word of thanks should also go to all members of academic, laboratory and administrative staff of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, A.B.U. Zaria especially Mr. A.M. Agonoh for his moral support and cooperation throughout the entire period.

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It would be ingratitude on my part if I fail to express how indebted I am to my late Father, Mother, Brothers and Sisters for their moral, unflinching and unsurpassable support they accorded me during the entire programme.

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ABBREVIATIONS

ε_{max}	-	Absorption maxima
Ach	-	Acetyl choline
Ad	-	Adrenaline
AP	-	Action Potential
cm	-	Centimetre
δ	-	Chemical shift
°C	-	Degree centrigrade
D-Tb	-	D-tubocurarine
EPP	-	End Plate Potential
g	-	Grammes
g/dm^3	-	Grammes per decimetre cube
g/ml	-	Grammes per millilitre
H	-	Hours
IR	-	Infrared
Kg	-	Kilogrammes
m/z	-	Mass to charge ratio
MS	-	Mass Spectrometer
mHz	-	Mega Hertz
MeOH	-	Methanol
m	-	Metres
µg/ml	-	Microgrammes per millilitre
mg	-	Milligrames
mm	-	Millimetres
min	-	Minutes

M	-	Molar concentration
$m^{+\cdot}$	-	Molecular ion
MAP	-	Muscle action potential
nm	-	Nanometres
NMR	-	Nuclear Magnetic Resonance
O_2	-	Oxygen
ppm	-	Parts per million
%	-	Percentage
^1H NMR	-	Proton NMR
Sec	-	Seconds
α	-	Specific rotation
TMS	-	Tetramethylsilane
TLC	-	Thin Layer Chromatography
TTS	-	Transverse Tubular System
UV	-	Ultraviolet
λ	-	Wavelength
cm^{-1}	-	Wave number (reciprocal centimetre)

θ	-	Optical Rotation
rf	-	Relative factor
ρ	-	density

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CHAPTER ONE

INTRODUCTION

1.1. INTRODUCTION:

One of the prerequisites for the success of primary health care is the availability and use of suitable drugs. Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principle. It is thus reasonable for researchers to identify locally available plants or plant extracts that could usefully be added to the national list of medicinal herbs that could even replace some pharmaceutical preparations that need to be purchased and imported.

Since most medicinal plants occur naturally in a large number of countries, a plant of potential importance in one country may well have been studied by scientists elsewhere. Considerable time and effort could be saved if their findings could be made available to all interested people. Pooled information is especially critical when it comes to drugs, as a value judgement on the safety or efficiency of a particular drug can rarely be based on the results of a single study.

In contrast, a combination of information indicating that a specific plant has been used in a local health care system for centuries, together with efficacy and toxicity data published by several groups of scientists, can help in deciding whether it should be considered acceptable for medicinal use (Akele, 1984).

No accurate data are available to assess the value and extent of the use of plants or of active principles derived from them in the health care systems of developing countries. WHO has estimated that perhaps 80% of more than 4,000 million inhabitants of the world in Africa, Asia, Latin America rely chiefly on traditional medicines for their primary health care needs, and it can safely be presumed that a major part of traditional therapy involves the use of plant extract or their active principles (Akele 1984).

In the developed countries, too, plant derived drugs may be of importance. In the U.S.A. for examples, 25% of all prescriptions dispensed from community pharmacies from 1959 to 1980 contained plant extracts or active principles prepared from higher plants. This figure did not vary by more than ± 1% in any of the 22 years surveyed (Farnsworth 1979) and in 1980 consumers in the U.S.A. paid more than $8,000 million for prescriptions containing active principles obtained from plants (Farnsworth 1985).

Industrial interests in exploiting plants as sources of new drugs is almost exclusively found in China and Japan. The plant kingdom has long served as prolific source of useful drugs, classical examples such as quinine, morphine, cocaine, reserpine, emetine, vinblastine, vincristine, atropine and digitalis, to mention a few, serve to remind us of the debt that medicine owes to plant derived compounds (Farnsworth 1984).

Each of these drugs has an interesting history of discovery and development culminating in their use in medicine. In recent years progress in analytical and preparatory methods stimulated a resurgence of interest in the field of natural drug products.

The introduction of a new drug is a laborious, expensive and highly sophisticated process. Many years and many millions of naira

separate the chemist's initial isolation or synthesis of a new substance and its becoming an acceptable new medicine.

In drug development from natural sources, synthetic modification of naturally derived molecules and the synthesis of similar compounds based on the natural product molecule as template have resulted in the formation of some new and effective drugs, for example, cocaine and its derivatives, a large number of synthetic anticholinergic drugs designed from tropane nucleus have been made and also steroidal hormones currently used in medicine are derived from diosgenin. It is obvious today that the major advances in the development of clinically useful antibacterial compounds came from the exploitation of natural products provided by micro-organism . For example, the successful isolation, structure elucidation, evaluation and practical development of penicillin was followed by an intensive search for others (Dejerassi 1969).

The practical advances in the therapeutic use of anti-inflammatory glucocorticoids and the introduction of oral contraceptives rest entirely upon the systematic purification of steroids from various natural sources and their use as starting materials from new organic materials (Kupchan 1969).

Although, much of the research into the development of new antibacterial compounds has centred around synthetic modification of existing one, the potential value of plants as sources of new antibacterial agents have been recognised.

The isolation and identification of active alkaloid vincristine and vinblastine in Catharantus roseus offered an example

of the application of modern analytical chemistry to the problem of separation and complete identification of very complete compounds. Until recently, few questioned the efficacy of traditional remedies, One who did was (Paracelsus 1920) a professor at the University of Busel, who regarded much of the contemporary practice of medicine as fraud.

Rejecting the authority of Galen, he spoke out in condemnation of the herbal mixtures, that useful drugs were diluted to impotent concentration by the abundance of inert ingredients present in the mixture. He also recognised the relationship between the desirable action of a drug and its toxic effects (Watt 1962).

The 18th century saw major advances in therapeutics. The cinchona alkaloids were imported from South America to Europe where it became obvious that they were highly effective in the treatment of malaria (Jarvis 1958)

(Withering 1937) identified the leaf of the purple foxglove Digitalis purpurea as the active principles in a folk remedy for "dropsy" (i.e. oedema caused by congestive heart failure) and thereby introduced into therapeutic one of the most important of all pharmacological agents.

Phytochemical investigation of plants plays a very significant role in the understanding of the chemotaxonomic relationship of plants. and in suggesting the necessary key substances in the actual biosynthetic sequence.

It was the scientific revolution in medicine during the 19th century led by men like Glaude Bernard that laid the basis for a rational therapy with the development of experimental methods for testing actions of drugs in animals (Bernard 1970).

The first isolation and crystallization of an active drug from a natural source was the accomplishment of a pharmacist's assistant,

Sertuner, who obtained pure morphine from opium in 1903; pure quinine was isolated from cinchona bark in 1820. In the later part of the century the German dye industry led the way towards the deliberate synthesis of newer drugs and the molecular modification of existing ones (Turner 1965).

Clearly, the pathway is open for scientists in developing countries to organise and implement interdisciplinary research programmes for the further utilization of this natural sources of drugs. These sources are usually available in abundance and can provide safe, stable, standardized and effective galenical products for use in primary health care or can lead to the discovery of new biological active plant principle that may be candidates for use as drugs (Hutchinson 1958).

Traditional medicine has been practised for last several thousand years but found a place in the WHO programme only 14 years ago (Akerele 1990). Traditional medicine in one form or another is widespread throughout the world. The practices are based on beliefs that were in existences often for hundreds of years before the development and spread of modern scientific medicine and that are still prevalent today As its name implies it is part of the tradition of each country where it is handed down from generation to generation. Its acceptance by a population is largely conditioned by cultural factors and much of traditional medicine, therefore may not be readily transferable from one culture to another (Sofowora 1982)

Medicinal plants are a small but important part of the biological heritage of the earth. Traditional Societies place a high value on this inheritance which they express through their intimate relationship

with nature. What is significant is the growing recognition from the industrialized world that these so called traditional values are valid for all people. Responding to the environment and ecological deterioration that threatens health and development everywhere a worldwide movement has arisen to awaken people to the dangers facing our planet and help preserve its integrity.

Primary health care requires the utilization of all appropriate and available local resources which in developing countries almost always include traditional medicine and practitioners. Where traditional medicine is well patronized by communities it makes good sense to adopt safe and useful traditional practices and incorporate them in the design and implementation of National Health Systems. However, this means putting traditional medicine on a scientific basis.

Countries should make a critical examination of the local material medica (if any) and practices, accurately identify the plants and other natural substances employed and decide which remedies and practices are useful and suppress those which are patently ineffective or unsafe. It is an undeniable fact that in today's world, herbal medicine plays a vital role in the health care for large sections of the population, especially in developing countries, in many cases they bridge the gap between the availability of and the demand for modern medicine (Akerele 1990). The use of medicinal plants in traditional medicine finds its natural expression and further development in primary health care P.H.C. (an integral part of the National Drug Policy). It is at this level that the transition from traditional practice to medical care can

most easily be made. In China for example, medicinal plants are an integral part of the formal health system and are used in about 40 percent of cases at the primary care level. Formerly medicinal plants were mostly collected in the wild bush, but as more land is brought under cultivation, the natural sources are becoming depleted. Special encouragement has therefore, been given for the cultivation of medicinal plants and agricultural departments at all levels to take part in formulating policy and establishing plantations which now cover some 300,000 hectares (Akerele 1990).

A system based on clinical usage may be more straightforward for the thoroughly studied allopathic drugs used in Western medicine but difficulties can arise for plants used in traditional medicine because of the often numerous condition for which any one drug may be employed (Evans 1989).

Erythrina senegalensis belongs to sub-family papilionaceae (Fabaceae). The sub-family plants are useful in agriculture for their manure value in the soil, apart from any crop they may yield. This is because their roots are habitually invaded by minute organism with the ability to convert atmospheric nitrogen into water soluble nitrogen salts which may be used by plants to build up its protein (Nielsen 1965).

The extensive industrial application of Erythrina senegalensis in formulation of pharmaceutical dosage forms and their local use as an anti-infective agents in Jos locality has been an undoubted stimulus to the natural product research geared

towards the phytochemical and pharmacological investigation of the fruit and bark of the plant.

1.2. DESCRIPTION OF THE PLANT:

(a) BOTANITAL SOURCE:-

Botanical Name	-	Erythrina senegalensis D.C.
Family	-	Leguminosae
Sub-Family	-	Papilionaceae

VERNACULAR NAMES:

Hausa	-	Majiriya
Tiv	-	Shor
Yoruba	-	Ologun Sheshe
Ibo	-	Echichi

(b) GEOGRAPHICAL LOCATION:

The plant is commonly found in Savannah Areas of West Africa like Nigeria, Ghana and Ivory Coast (Dalziel 1955). There are about 108 species of Erythrina distributed all over the world today. Such species exist in different forms as trees, shrubs and herbs in Africa, Asia, America and Australia (Games 1974).

(c) MACROSCOPIC DESCRIPTION OF THE PLANT:

The plant is a savannah tree usually 13m - 15m high and shrubby but sometimes reaching 16m in height and about 50cm in girth with crooked branches forming an irregular crown. The bark is very rough and fissured (Fig. 1) in old trees with slash fibrous and yellowish brown. While the leaves are

rather variable with a few thorns and prominent glands. The leaves are also elliptic to broadly ovate in shape (Fig. 2) and rounded to bluntly pointed at the apex. The paired leaflets are lanceolates smaller and usually rather narrower. They are 7 - 15cm long and 4.0cm broad. The petioles are slightly grooved. The fruits are shortly velvet, twisted irregularly constricted splitting down one side and then coiling up irregularly. The fruits are about 12cm long. The seeds are red glossy about 0.45cm long with a white hilum. The plant flowers between September to January, and in individual flowers groups of 2 or 3 with slender stalks about 3cm long. The flowers are slightly thickened at the apex and with a projecting more or less acute tip. The calyx of the flower are scarlet at the initial period turning to blackish of about 3.25 - 4.88cm long. Standard petals are 6.5 - 13cm long usually they are straight and folded very flat so that the stamens are usually wider (Keay 1974).



Fig. 1: A branch of the plant of Erythrina senegalensis showing thorns, the stem with paired leaflets.



Fig. 2: A tree of Erythrina senegalensis with crooked branches forming an irregular crown.

1.3. APPLICATION OF THE PLANT IN TRADITIONAL MEDICINE:

The bark of Erythrina senegalensis is used by traditional healers in Northern Nigeria to treat jaundice and other ailments. The bark infusion with spices and honey is widely used for the treatment of gonorrhoea and as muscle relaxant It is also used as diuretic for horses (Dalziel 1955).

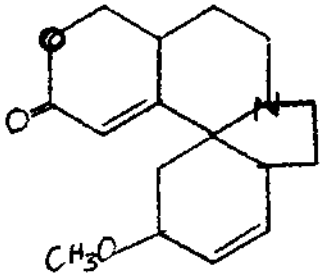
In Ghana, the bark is recommended as an emmenagogue and in French Guinea it is given to women after child birth. In Senegal the bark is considered a remedy for dysentery and colitis. The fruits of Erythrina senegalensis are used in Jos locally for treatment of malaria fever. The roots are pounded and used as sponge by the Dagombas. The bark and leaves decoction are sometimes given as drinks, baths or as a tonic to children with ricketts and also to weak old men. The pounded bark and leaves in soup are used in Ghana for sterility in women (Games 1974).

1.4. PREVIOUS CHEMICAL AND BIOLOGICAL WORK DONE ON THE PLANT:

Erythrina is a very distinct genus of leguminosae family consisting of 108 species of wide distribution in the world. As a result of studies carried out by (Krukoff 1972) and his collaborators this genus of handsome trees, shrubs and a few herbs is probably better known in many respect than any other of comparable size and distribution.

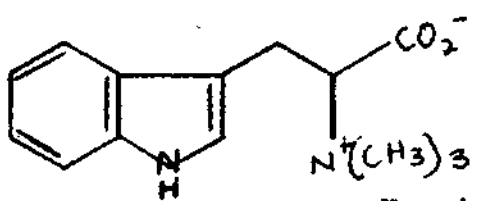
One of the most remarkable achievements of the work of Erythrina has been the complete or partial characterization of

the alkaloid and amino acid constituency of the seeds and in some cases other parts of the plant for over three quarters of the species of this large genus (Hargreaves 1974). Erythrina species are known to be the main source of tetracyclic Erythrina-type plant/ alkaloids Interestingly enough, however, cocculus species formerly menispermaceae also produce closely related alkaloids which differ from the true Erythrina alkaloids in their oxygenation pattern in ring A. An interesting insight into the possible catabolism of the Erhthrina type alkaloids is provided by such lactonic compounds Erythroidine[1] (Masouda 1991).



Erythroidine (1)

which are most likely products of in vivo oxidation of the aromatic ring D. Alkaloids are ubiquitous in the seeds of Erythrina with hypaphorine [2] probably present in all the species this compound has long been known to be of limited physiological potency (Raven 1974).



Hypaphorine (2)

The seed differ greatly in their alkaloid and amino acid constituents and when the alkaloid content is high the amino acid concentration is low (Raven 1974).

Alkaloids are present in the roots, stem, leaves, heart-wood and flowers of most species but in smaller quantities than in the seeds (Krukoff 1977).

Games et al 1974 indicated that in Erythrina senegalensis Erysodine [3] represented 75% of the total alkaloids while 11-oxoerysodine [4] represents 9% and 5% for 11-hydroxy-erysodine [5].

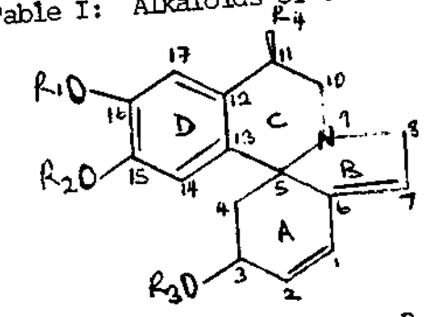
In erythrina excelsa 48% of the total alkaloid is represented by Erysodine [3] and 28% for Erysovine [6].

Many of the most common alkaloids are the same as those found in the American species. However, Erysodine [3] and Erysopine [7] are the most abundant of all the species and occur in every one of the 18 different species from Africa, Asia, Australia and America examined. Erythratidine [9] occurs in over half the species but only in small quantities. However, the most significant difference between the American species of Erythrina and many of those from other countinents is the presence of one or more 11-oxygenated alkaloids which are dienoid type. Thus the presence of an enzyme which can hydrolyse the 11-position in the tetracyclic Etrythina (Raven 1974).

alkaloid nucleus may be inferred in many of the African, Asian and Polynesian species whereas this enzyme is presumably absent in the American species (Games et al 1974).

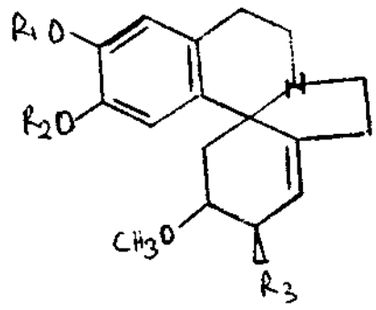
Today, over 30 erythrina alkaloids are known; these are conveniently classified into two main structural types, those containing a conjugated diene system in Rings A and B called dienoids (Table 1) and those containing one double bond in Ring A called Alkenoids (Table 2).

Table I: Alkaloids of the Dienoid Type:



		R_1	R_2	R_3	R_4
[3]	Erysodine	H	CH_3	CH_3	H
[4]	11-Oxoerysodine	H	CH_3	CH_3	O
[5]	11-Hydroxyerysodine	H	CH_3	CH_3	OH
[6]	Erysovine	CH_3	H	CH_3	H
[7]	Erysopine	H	H	CH_3	H
[8]	Erythraline		$-CH_2-$	CH_3	H O

Table 2: Alkaloids of Alkenoid Type (Folkers 1941)



	R_1	R_2	R_3
[9] Erythratidine	CH_3	CH_3	OH

The first curarizing Erythrina alkaloid was obtained in 1937 by Folkers et al from the seeds of E. americana . It was named Erythroidine [1] and was subsequently shown to be a mixture of two isomers of α- and β- erythroidine((Folkers 1939). In a systemic investigation of 50 species of Erythrina all were shown to contain alkaloids with curariform activity (Raven 1974.) Many of these species examined contained as their main alkaloid Erysodine [3] Erysovine [6] and Erysopine [7]. In addition 11-hydroxy erysodine [5] and Erythraline [8] have been discovered in the seeds of American, African, Asian and Australian species (Hargreaves et al 1974). The curarizing action based upon the gram of frog curarized per gram of seed has been examined by Folker and Unno . This relationship was 20,000X for E. senegalensis and 44,000X for E. sigmoidea.(Folkers 1939).

Erythroidine [1] and its derivatives have been used as muscle relaxants in a number of clinical applications such as the control of convulsions and in shock therapy for psychiatric patients and as an adjunct to general anaesthesia to produce the muscular relaxation desirable in some surgical operation (Bhattachary 19
The alkaloids have the advantage of being active if taken by mouth but are less active than curare. Erythrina alkaloids are quaternary bases and their actions are less lasting as they lose their activity through biotransformation to tertiary bases. They have, however, now been replaced by other drugs because of their high toxicity. Neuromuscular blocking, smooth muscle relaxant, CNS depressant, hydrocholoretic and anticonvulsant effects are also beeing observed (Ghosal 1972).

In general, it has been reported that the sulpho-acetic esters of Erysovine [6] and Erysopine [7] possess greater activity than the free alkaloids (El Olemy 1978).

CHAPTER TWO

MATERIALS AND METHODS

2.1. MATERIALS AND CHEMICALS:

(a) MATERIALS:-

Kymograph recording set up - Grass Polygraph Model 7D
Grass Instruments, Quincy M.A.

Dissection Kits

Organ baths with levers

Miniature air-pumps

Needles

Thread

Syringes (1ml)

Pipettes (10ml)

Soxhlet apparatus

Test tubes

Beakers

Spatula

Litmus paper

Filter paper

Measuring cylinder

Reagents bottles

Analytical weighing balance - Gallenkamp

Conical flask

Crucible

Watch glass

Capillary tubes

Stirring rod

Adrenaline Sigma

Atropine (Sigma)

Distilled water

Magnesium sulphate hexahydrate ($MgSO_2.7H_2O$) May and Baker

D-tubocurarine (Sigma)

(c) ANIMALS:-

Rabbit (1.7kg)

Frogs (75 - 100g)

Guinea Pig (500 - 750g)

(d) PHYSIOLOGICAL SALT SOLUTION:-

The pharmacological screening involved the use of rabbit jejunum, guinea pig ileum and the rectus abdominis muscle of the frog. Hence the tissue was bathed by the appropriate physiological solutions, in which the tissue survives best. Table 3 shows the type and composition used in this project.

Table 3: Composition of Physiological Solutions (Shetty 1982)

Composition	Frog - Ringer	Tyrode
NaCl	65g	80g
KCl (10%)	14ml	20ml
$MgSO_4.7H_2O$ (10%)	-	26ml
$NaH_2PO_4 2H_2O$ (5%)	1.3ml	13ml
D-glucose	20g	10g
$NaHCO_3$	4g	10g
$CaCl_2$ (Molar)	10.8ml	18ml
Distilled water to	10 Litres	10 Litres
Aerating Gas	air	0_2 or air
Typical Preparation	Amphibian tissue	Intestinal tissue

In order for the physiological salt solution to be free of heavy metal ions and toxic chemicals which may come from poor quality rubbers tubings pure materials of "Analar" grade and glass distilled water were used. The physiological salt solution was freshly made on the day of the experiment. To make 10 litres of the tyrode solution, 10g of Potassium chloride (KCl) was weighed on a balance and dissolved in 100ml of distilled water to make 10% solution KCL; 10g of Magnessium sulphate ($MgSO_2.7H_2O$) was carefully weighed on a balance and dissolved in 100ml of distilled water to make 10% solution of $MgSO_4.7H_2O$; 5g of Sodium hydrogen phosphate ($NaH_2PO_4.2H_2O$) was weighed and made into 5% solution of $NaH_2PO_4.2H_2O$.

To make 1 Molar Calcium chloride ($CaCl_2$) 40.08g of $CaCl_2$ was weighed in a balance and dissolved in 40.1ml of distilled water. 80g NaCl, 10g D-glucose, 10g $NaHCO_3$ was weighed in a balance and mixed together with 20ml of 10% KCl, 26ml of 10% $MgSO_4.7H_2O$, 13ml of 5% $NaH_2PO_4.2H_2O$ in a 10 litre container. After thorough mixing 18ml of 1M $CaCl_2$ was added and made up to 10 litre by distilled water.

To make 10 litres of frog-rinder solution 65g of Sodium chloride NaCl, 20g of D-glucose and 4g of $NaHCO_3$ was weighed in a balance and mixed thoroughly with 14ml of 10% KCl, 1.3ml of 5% NaH_2PO_4 with little amount of distilled water. After thorough mixing 10.8ml of 1M $CaCl_2$ was added with distilled water to make 10 litre frog-ringer solution.

2.2 SPRAY AND TEST REAGENTS - PHYTOCHEMICAL:

The composition of the test reagents and their methods of preparation are given below. The thin layer plates after spraying were heated to 110°C for 15 minutes unless otherwise stated.

Anisaldehyde reagent . Anisaldehyde (2g), acetic anhydride (50ml) were mixed with sulphuric acid (50%, 50ml) (Lisboa 1984).

Cerric Ammonium Sulphate reagent (CAS): Cerric ammonium sulphate (2g), water (90ml) were mixed with Conc. sulphuric acid (10ml) (Jacouljevic 1964)

Molybdophosphoric acid reagent : Molybdophosphoric acid (20g) was dissolved in ethanol (10%, 100ml) (Kritchevsky 1952).

Liebermann - Burchard reagent : Acetic anhydride (20ml) Conc. sulphuric acid (1ml) and chloroform (50ml) were mixed together (Tschesche 1961).

Methanolic Potassium Hydroxide : Potassium hydroxide (10g) was dissolved in methanol (100ml) (Shaffer 1962).

Ferric Chloride reagent : Ferric chloride (5g) was dissolved in water (100ml) (Fink 1949).

Sulphuric acid reagent : Sulphuric acid (50ml) was added to water (50ml) (Tschesche 1961).

Dragendoff's reagent : Bismuth nitrate (8g) was dissolved in nitric acid (20ml) potassium iodide (27.2g) was dissolved in water (50ml) (Bregoff 1953).

The two solutions were mixed and allowed to stand. When

potassium nitrate crystallized out, the supernatant was decanted and the solution was made up with water to 100ml.

Wagner's reagent : Iodine (1.27g) and potassium iodide (2g) were dissolved in water (5ml). The solution was diluted with water to 100ml (Bregoff 1953).

Mayer's reagent : Mercuric chloride (1.36g) was dissolved in water (60ml). To this was added a solution of potassium iodide (5g) in water (100ml). The final solution was diluted with water to 100ml (Bregoff 1953).

2.3. CHROMATOGRAPHY:

Thin layer chromatography (TLC) separation were carried out on glass plates of silica gel G preparation (M.F.C. Hopkins and Williams). Thickness 0.25mm, 0.50mm or 1.00mm.

Argentative TLC plates were prepared by incorporating silver nitrate (10%) into the adsorbent.

Columns were run with silica (Kieselgel 60, 60-80, 80-120 mesh ASTM, MERCK) as adsorbent.

The adsorbents were usually packed in glass columns using petroleum spirit 40-60°C or carbon tetrachloride as the case may be.

A proportion of the sample (1g) to silica gel (30g) was generally employed.

2.4. DETERMINATION OF SPECTRAL AND OTHER PHYSICAL CONSTANTS:

Melting points were determined using an Electrothermal melting points apparatus. All values recorded were uncorrected. The infra red (IR) spectra were run on a Perkin-Elmer 257 infra-red spectrophotometer using nujol mull. Ultra violet (UV) absorbance were determined on a PYE Unicam SP8-400 uv/vis spectrophotometer using benzene. The mass spectra were run on a Finnigan MAT 8200 mass spectrometer. While the Nuclear Magnetic Resonance (NMR) was run on a Bruker 250 MHz using trimethyl-silane TMS as internal standard. The materials were dissolved in deuteriated chloroform.

Optical rotation was determined using a Bellingham and Stanley R 820G8 Polarimeter while for pharmacological screening the contractions were recorded sequentially using Grass FT.03 strain gauge connected to Grass Polygraph Model 7D (Grass instruments Quincy, M.A.).

2.5. COLLECTION AND PREPARATION OF THE PLANT:

The fruits, leaves and bark of Erythrina senegalensis were collected in the premises of the Federal School of Forestry, Jos in the months of December 1990 through January 1991. The plant was authenticated by comparison with a herbarium sample of the Botany Department, Ahmadu Bello University, Zaria

Specimen No. FHI 24386.

Immediately after collection the fruits, leaves and bark were dried in a hot air oven at 40°C for seven days. The dried plant material were crushed and powdered with pestle and mortar. The powdered plant materials were weighed, kept in clean containers and labelled for easy identification.

2.6. EXTRACTION:

Powdered material of the fruit (1kg) was extracted with petroleum spirit 60 - 80°C in a soxhlet extractor for 36 hours until the draining solvent was clear. The marc was then exhaustively extracted with methanol for another 36 hours. About 2.5 litres of solvent was used for each extraction. 1kg of the powdered bark was also subjected to soxhlet extraction process using 2.5 litres of methanol and petroleum spirit 60 - 80°C for 36 hours until the draining solvent was clear. The same procedure was done for the leaves of the plant. After complete extraction had taken place the different marc obtained were

concentrated in vacuo and weighed.

Description of Extracts:

The methanolic extracts of fruits and bark were brown in colour while the methanolic extract of the leaves were dark green in colour.

The petroleium spirit extract of the fruits, leaves and bark gave a brown colour. On testing with litmus paper the methanolic extract gave blue colour with red litmus paper. This indicate that the extract is basic in nature while the petroleum spirit extract were slightly acidic in nature.

2.7. TREATMENT OF TISSUES (Shetty 1982):

One of the most common causes for the failure of pharmacological experiments is mishandling of living tissues. Hence greatest care was exercised in the handling of tissues (frog rectus abdominis muscle and rabbit jejunum) in order to avoid stretching, crushing or otherwise damaging the living cells. Drying of the tissue was avoided. The changing of bathing physiological salt solution was done slowly, by draining downwards and refilled.

Since some tissues are very sensitive to metals especially copper and mercury including iron pins used for impaling the tissue care was taken not to produce any sensitization on the tissues. The apparatus was

washed with detergent and distilled water before and immediately after use to discourage the growth of micro-organism.

(a) Oxygenation:

Oxygen is necessary for the survival of living tissue. Miniature air-pump was used to aerate the tissues and to maintain it throughout the experiment.

(b) Temperature:

Living tissues are very sensitive to changes in temperature . The organ bath with thermostats set at the correct temperature was used to produce the right tissue temperature and maintained throughout the experiment. Mammalian tissue require body temperature but since the rabbit jejunum used was to last for longer periods lower temperature of 32°C was used. Amphibian tissues will survive for long periods at room temperature hence a thermostat was not used for rectus abdominis muscle of a frog.

2.8. PREPARATION OF DRUG SOLUTIONS:

Acetylcholine solutions (Ach) was made up freshly by diluting a stock solution (10^{-1} g/dm^3 = 0.55M) kept at pH 4. which is stable. To make the stock solution 10g of Ach was weighed in analytical balance and dissolved in 100ml of distilled water.

10^{-2}g/ml, 10^{-3}g/ml, 10^{4}g/ml, 10^{-5}g/ml, and 10^{-6}g/ml Ach solutions was added up freshly from the stock solution by using the serial dilution method.

Adrenaline dilute solutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}g/ml) were prepared from a freshly stock solution kept at pH 4 by serial dilution method and the dilute solution contained ascorbic acid and (about 10^{-4} M) to delay oxidation. To make stock solution of 10^{-1}g/ml, 0.55M. 10g of adrenaline was weighed in analytical balance and dissolved in 100ml of distilled water. Atropine dilute solutions 10^{-2}, 10^{-3}, 10^{-4} and 10^{-6}g/ml was also made from freshly prepared stock solution of 0.55M kept at pH 4.

A stock solution of d-tubocurarine chloride (dtb) of 0.5g/ml, 64 x 10^{-2}M was made. From the stock solutions 50µg/ml was prepared by serial dilutions.

Most tissues respond to very small quantities of drugs, often as little as 10mg or less.0.16g of methanolic extract of the leaf weighed in an analytical balance and dissolved in 10ml of distilled water to make 1.6 x 10^{-2}g/ml. 1.5g of methanolic extract of the fruit was weighed in an analytical balance and dissolved in 10ml of distilled water to make 1.5 x 10^{-1}g/ml. From this concentration various serial dilutions were made. For the bark 1.5g of the methanolic extract was weighed and dissolved in 10ml of distilled water to make 1.5 x 10^{-1}g/ml stock solution.

CHAPTER THREE

RESULTS

3.1. CALCULATION OF PERCENTAGE YIELD:

Weight of plant material = 1000g

Weight of petroleum spirit extract concentrate of Fruit = 202 g

Percentage (%) yield = $\frac{202}{1000} \times 100$ = 20.2%

Weight of methanolic extract concentrate of Fruit = 120 g

Percentage (%) yield = $\frac{120}{1000} \times 100$ = 12%

Weight of methanolic extract of leaves concentrate = 160 g

Percentage (%) yield = $\frac{160}{1000} \times 100$ = 16%

Weight of petroleum spirit extract of leaves concentrate = 265g

Percentage (%) yield = $\frac{265}{1000} \times 100$ = 26.5%

Weight of methanolic extract of bark concentrate = 205 g

Percentage (%) yield = $\frac{205}{1000} \times 100$ = 20.5%

Weight of petroleum spirit extract of bark concentrate = 55 g

Percentage (%) yield = $\frac{55}{1000} \times 100$ = 5.5%

3.2. PRELIMINARY PHYTOCHEMICAL SCREENING OF THE PLANTS:

(i) Test For Alkaloids (Brain 1975):

About 3ml of methanolic extract of Erythrina senegalensis plant parts was stirred with 10ml of 1% Aqueous hydrochloric acid on a steam bath, the content was filtered and 1ml of the filtrate was treated with a few drops of Mayer's reagent, to give a buff precipitate. The second 1ml portion was similarly treated with Dragendorff's reagent to give an orange-red precipitate and the third portion was treated with Wagner's reagent to give a brown precipitate.

The same procedure was repeated using the petroleum spirit extract of the plant materials.

(ii) General Test For Glycosides (Trease and Evans 1983):

About 0.2ml of dilute sulphuric acid was added to 1ml of each of the extracts, heated on a water bath for 5 minutes and filtered. The filtrate was made neutral with sodium hydroxide solution. A drop of fehlings solutions A and B was added to it and heated on a water-bath for 2 minutes. The presence of reducing sugars is indicated by an orange-red presicipitate on boiling with fehlings solution.

(iii) Liebermann-Burchard Test for Steroidal Nucleus (Sofowora 1982)

Each of the extract was dissolved in chloroform and filtered. The filtrate was mixed with 2ml of acetic anhydride and 2 drops of conc. sulphuric acid. A colour change from violet to blue or bluish green indicates the presence of steroidal nucleus.

(iv) Test for Tannins (Robinson 1964)

A small quantity of each of the extract was mixed with water and heated on a water-bath. The mixture was filtered and sulphuric acid and 5% Ferric Chloride were added to the filtrate. A dark blue or dark green solution indicates the presence of tannins.

(v) Salkowskii Test for Steroidal Nucleus (Brain 1975)

0.5ml of the extract was dissolved in 2ml chloroform. This was followed by addition of dilute sulphuric acid. A reddish-brown colour at the interphase indicates presence of steroidal nucleus.

(vi) Test for Phlobatannins (Robinson 1964)

0.5ml of each of the extract was boiled with 5ml of 1% Hydrochloric acid. A red precipitate shows presence of Phlobatannins.

(vii) Frothing Test for Saponins (Brain 1975)

1ml of each of the extracts was shaken with water in a test-tube, followed by heating in a water-bath to the

boiling point. Frothing shows presence of saponins.

(viii) Test for Flavonoids (Malkin 1930)

1ml of each of the extract was dissolved in dilute sodium hydroxide acid. A yellow solution that turns colourless on addition of hydrochloric acid confirms the presence of flavonoids.

(ix) Radulescu Test for Morphine Alkaloids (Brain 1975)

1ml of each of the extract was evaporated to dryness and the residue dissolved in 0.6ml of 1% sulphuric acid. 2ml of distilled water was added and 2 drops of 10% sodium nitrite solution, The solution was made alkaline with dilute ammonia solution. A reddish-brown solution shows the presence of Morphine alkaloids.

(x) Thalleiquine Test for Quinoline Alkaloids (Brain 1975)

0.5ml of conc. hydrochloric acid and a few crystals of potassium chlorate was added to each of the extract. It was carefully evaporated and a drop of strong ammonia was added to it. A green colour is taken as positive.

(xi) Test for Indole Alkaloids (Brain 1975)

A few drops of conc. sulphuric acid and a crystal of potassium dichromate was added to each of the methanolic and petroleum ether extract. A violet colour is taken as positive.

(xii) Vitali-morin Test for Tropane Alkaloids (Trease & Evans 1983)

Each of the extract was evaporated to dryness. The residue was dissolved in a few drops of fuming nitric acid. This was then evaporated to dryness and allowed to cool. It was dissolved in 2ml dry acetone and few drops of fresh alcoholic potash solution (3%) was added. A violet colour is expected.

(xiii) Borntrager's Test for Anthraquinone Glycoside (Shinoda 1928)

Each of the extract was mixed with dilute hydrochloric acid, extracted with carbon tetrachloride and then shaken with dilute ammonia. A rose pink to cherry red colour shows the presence of anthraquinone glycosides.

(xiv) Keller-Killiani Test for Cardiac Glycoside (Brain 1975)

Each of the extract was mixed with 3ml of 3.5% ferric chloride in glacial acetic acid and transferred to the surface of 2ml conc. sulphuric acid. A reddish brown colour at the junction of the liquids with the upper layer pale green which gradually becomes blue is an indication of deoxy sugars.

(xv) Test for Cyanophoric glycosides (Brain 1975)

1ml of each of the extract were placed in test tubes. A piece of sodium picrate paper was suspended above the extracts by trapping the top edge between the cork and the test tube wall. This was allowed to stand for 30 minutes in a warm water of about 45°C. A change in colour of sodium picrate paper from yellow to brick red indicates the presence of cyanogenetic glycosides.

Table 4: METHANOLIC EXTRACT OF FRUITS OF ERYTHRINA SENEGALENSIS

GROUPS	TEST	OBSERVATION	INFERENCE
ALKALOID General/Test	Dragendorff's reagent Mayer's reagent Wagner's reagent	Orange-red ppt Buff precipitate Brown ppt	++ ++ ++
MORPHINE ALKALOID	Radulescu test	Reddish brown colouration	++
QUINOLINE ALKALOID	Thalleioquine test	No colour change	-
INDOLE ALKALOID	Extract + Conc. H_2SO_4 + Potassium dichromate	No colour change	-
TROPANE ALKALOID	Vitali-Morin test	No colour change	-
GLYCOSIDES (General Test)	Extract Hydrolysed + Fehlings solution A and B heat	Reddish Brown precipitate	++
ANTHRAQUINONE GLYCOSIDE	Born-trager's test	Brown colour	+++
CARDIAC GLYCOSIDE	Keller-Killiani test for deoxy sugars	Reddish brown colour at inter-phase with pale green colour at upper layer	++
SAPONIN GLYCOSIDE	Frothing test	Little frothing was observed	+
CYANOPHORIC GLYCOSIDE	Extract + Na picrate paper	No colour reaction	-
FLAVONOIDS	Extract + NaOH + HCl	No colour reaction	-
STEROIDAL NUCLEUS	Liebermann-Buchard test	Green colour from brown to blue	+
	Salkowskii test	Brown colour at the interphase	+
TANNINS (General Test)	Extract + Ferric chloride	No colour change observed	-
	Test for phlobatannins	No colour change	-

Key:

+	=	Faintly present
++	=	Moderately present
+++	=	Highly present
-	=	Not present

Table 5: METHANOLIC EXTRACT OF LEAVES OF ERYTHRINA SENEGALENSIS

GROUPS	TEST	OBSERVATION	INFERENCE
ALKALOID General/Test	Dragendorff's reagent Mayer's reagent Wagner's reagent	Orange-red ppt Buff precipitate Brown ppt	++ ++ ++
MORPHINE ALKALOID	Radulescu test	Reddish brown colouration	+
QUINOLINE ALKALOID	Thalleioquine test	No colour change	-
INDOLE ALKALOID	Extract + Conc. H_2SO_4 + Potassium dichromate	No colour change	-
TROPANE ALKALOID	Vitali-Morin test	No colour change	-
GLYCOSIDES (General Test)	Extract Hydrolysed + Fehlings solution A and B heat	Reddish Brown precipitate	+
ANTHRAQUINONE GLYCOSIDE	Born-trager's test	Brown colour	+
CARDIAC GLYCOSIDE	Keller-Killiani test for deoxy sugars	Reddish brown colour at inter-phase with pale green colour at upper layer	+
SAPONIN GLYCOSIDE	Frothing test	Little frothing was observed	+
CYANOPHORIC GLYCOSIDE	Extract + Na picrate paper	No colour reaction	-
FLAVONOIDS	Extract + NaOH + HCl	No colour reaction	-
STEROIDAL NUCLEUS	Liebermann-Buchard test	Green colour from Brown to Blue	++
	Salkowskii test	Brown colour at the interphase	+
TANNINS (General Test)	Extract + Ferric chloride	No colour change observed	-
	Test for phlobat-annins	No colour change	-

Key:

+ = Faintly present
++ = Moderately present
+++ = Highly present
- = Not present

Table 6: METHANOLIC EXTRACT OF BARK OF ERYTHRINA SENEGALENSIS

GROUPS	TEST	OBSERVATION	INFERENCE
ALKALOID General/Test	Dragendorff's reagent Mayer's reagent Wagner's reagent	Orange-red ppt Buff precipitate Brown ppt	+++ +++ +++
MORPHINE ALKALOID	Radulescu test	Reddish brown colouration	+++
QUINOLINE ALKALOID	Thalleioquine test	No colour change	-
INDOLE ALKALOID	Extract + Conc. H_2SO_4 + Potassium dichromate	No colour change	-
TROPANE ALKALOID	Vitali-Morin test	No colour change	-
GLYCOSIDES (General Test)	Extract Hydrolysed + Fehlings solution A and B heat	Reddish Brown precipitate	++
ANTHRAQUINONE GLYCOSIDE	Born-trager's test	Brown colour	+
CARDIAC GLYCOSIDE	Keller-Killiani test for deoxy sugars	Reddish brown co colour at inter-phase with pale green colour at upper layer	++
SAPONIN GLYCOSIDE	Frothing test	Little frothing was observed	+
CYANOPHORIC GLYCOSIDE	Extract + Na picrate paper	No colour reaction	-
FLAVONOIDS	Extract + NaOH + HCl	No colour reaction	-
STEROIDAL NUCLEUS	Liebermann-Buchard test	Green colour from Brown oto Blue	++
	Salkowskii test	Brown colour at the interphase	+
TANNINS (General Test)	Extract + Ferric chloride	No colour change observed	-
	Test for phlobatannins	No colour change	-

Key:

+ = Faintly present
++ = Moderately present
+++ = Highly present
- = Not present

Table 7: PETROLEUM SPIRIT EXTRACT OF FRUITS OF ERYTHRINA SENEGALENSIS

GROUPS	TEST	OBSERVATION	INFERENCE
ALKALOID General/Test	Dragendorff's reagent Mayer's reagent Wagner's reagent	Orange-red ppt No colour reaction Brown ppt	+++ +++ +++
MORPHINE ALKALOID	Radulescu test	Reddish brown colour	+
QUINOLINE ALKALOID	Thalleioquine test	Colour change from yellow to brown	-
INDOLE ALKALOID	Extract + Conc. H_2SO_4 + Potassium dichromate	No colour change	-
TROPANE ALKALOID	Vitali-Morin test	No colour change	-
GLYCOSIDES (General Test)	Extract Hydrolysed + Fehlings solution A and B heat	No colour change	+++
ANTHRAQUINONE GLYCOSIDE	Born-trager's test	No colour change	++
CARDIAC GLYCOSIDE	Keller-Killiani test for deoxy sugars	Brown at interphase and green at upper layer	+
SAPONIN GLYCOSIDE	Frothing test	No frothing noticed	-
CYANOPHORIC GLYCOSIDE	Extract + Na picrate paper	No colour reaction	-
FLAVONOIDS	Extract + NaOH + HCl	No colour change	-
STEROIDAL NUCLEUS	Liebermann-Buchard test	Colour change from Brown to dark green	+
-	Salkowskii test	No colour change	-
TANNINS (General Test)	Extract + Ferric chloride	No colour change	-
-	Test for phlobatannins	No colour change	-

Key:
+ = Faintly present
++ = Moderately present
+++ = Highly present
- = Not present

Table 8: PETROLEUM SPIRIT EXTRACT OF LEAVES OF ERYTHRINA SENEGALENSIS

GROUPS	TEST	OBSERVATION	INFERENCE
ALKALOID General /Test	Dragendorff's reagent Mayer's reagent Wagner's reagent	Orange-red ppt No colour reaction Brown ppt	++ ++ ++
MORPHINE ALKALOID	Radulescu test	Reddish brown colour	+
QUINOLINE ALKALOID	Thalleioquine test	Colour change from yellow to brown	-
INDOLE ALKALOID	Extract + Conc. H_2SO_4 + Potassium dichromate	No colour change	-
TROPANE ALKALOID	Vitali-Morin test	No colour change	-
GLYCOSIDES (General Test)	Extract Hydrolysed + Fehlings solution A and B heat	No colour change	++
ANTHRAQUINONE GLYCOSIDE	Born trager's test	No colour change	++
CARDIAC GLYCOSIDE	Keller-Killiani test for deoxy sugars	Brown at interphase and green at upper layer	+
SAPONIN GLYCOSIDE	Frothing test	No frothing noticed	-
CYANOPHORIC GLYCOSIDE	Extract + Na picrate paper	No colour reaction	-
FLAVONOIDS	Extract + NaOH + HCl	No colour change	+
STEROIDAL NUCLEUS	Liebermann-Buchard test	Colour change from Brown to dark green	-
-	Salkowskii test	No colour change	-
TANNINS (General Test)	Extract + Ferric chloride	No colour change	-
-	Test for phlobatannins	No colour change	-

Key:

+ = Faintly present
++ = Moderately present
+++ = Highly present
- = Not present

Table 9: PETROLEUM SPIRIT EXTRACT OF BARK OF ERYTHRINA SENEGALENSIS

GROUPS	TEST	OBSERVATION	INFERENCE
ALKALOID General/Test	Dragendorff's reagent Mayer's reagent Wagner's reagent	Orange-red ppt No cour reaction Brown ppt	+ - +
MORPHINE ALKALOID	Radulescu test	Reddish brown colour	+
QUINOLINE ALKALOID	Thalleioquine test	Cour change from yellow to brown	-
INDOLE ALKALOID	Extract + Conc. H_2SO_4 + Potassium dichromate	No colour change	-
TROPANE ALKALOID	Vitali-Morin test	No colour change	-
GLYCOSIDES (General Test)	Extract Hydrolysed + Fehlings solution A and B heat	No colour change	-
ANTHRAQUINONE GLYCOSIDE	Born-trager's test	No colour change	-
CARDIAC GLYCOSIDE	Keller-Killiani test for deoxy sugars	Brown at interphase and gree at upper layer	+
SAPONIN GLYCOSIDE	Frothing test	No frothing noticed	-
CYANOPHORIC GLYCOSIDE	Extract + Na picrate paper	No colour reaction	-
FLAVONOIDS	Extract + NaOH + HCl	No colour change	-
STEROIDAL	Liebermann-Buchard test	Colour change from Brown to dark green	+
-	Salkowskii test	No colour change	-
TANNINS (General Test)	Extract + Ferric chloride	No colour change	-
-	Test for phlobatannins	No colour change	-

Key:

+ = Faintly present
++ = Moderately present
+++ = Highly present
- = Not present

3.3. PARTITION:

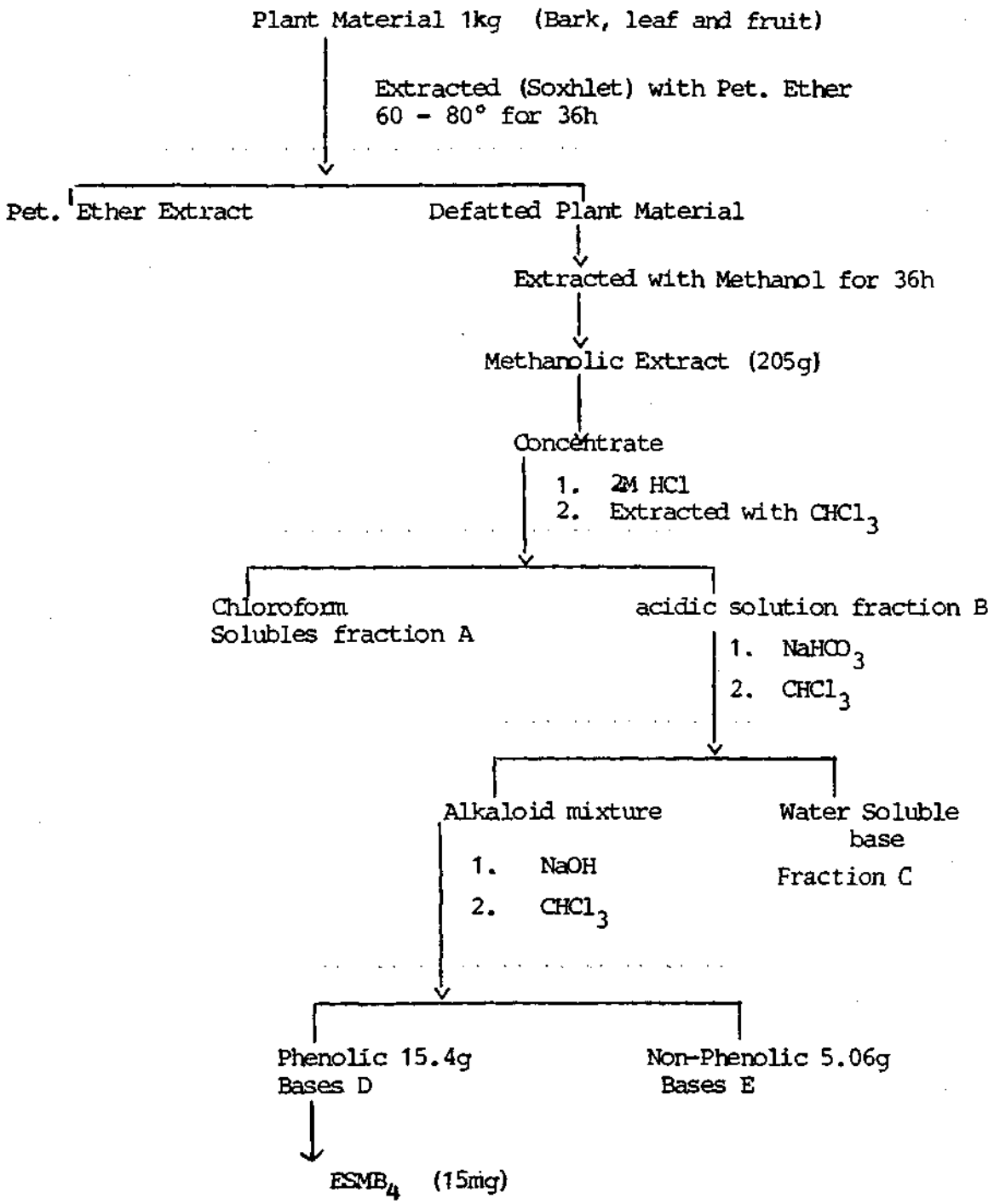
(a) Methanolic Extract -

The concentrated methanolic fraction of the bark (205g) was diluted with water (200ml) and acidified with 2M Hydrochloric acid (HCl). This was partitioned between water (200 ml) and chloroform (400 ml).

The acidic solution fraction B was basified with 5% $NaHCO_3$ and extracted with chloroform (400ml) to give the Alkaloidal mixture (coded C) and the water soluble bases.

Fraction C was again basified with NaOH 5% solution and extracted with chloroform 400ml to give the Phenolic bases and non-phenolic bases of fractions D and E of (15.4g) and(5.06g) respectively.

Summary of Extraction and Isolation of Alkaloids from Plant Material of Erythrina senegalensis (Ghosal 1972)



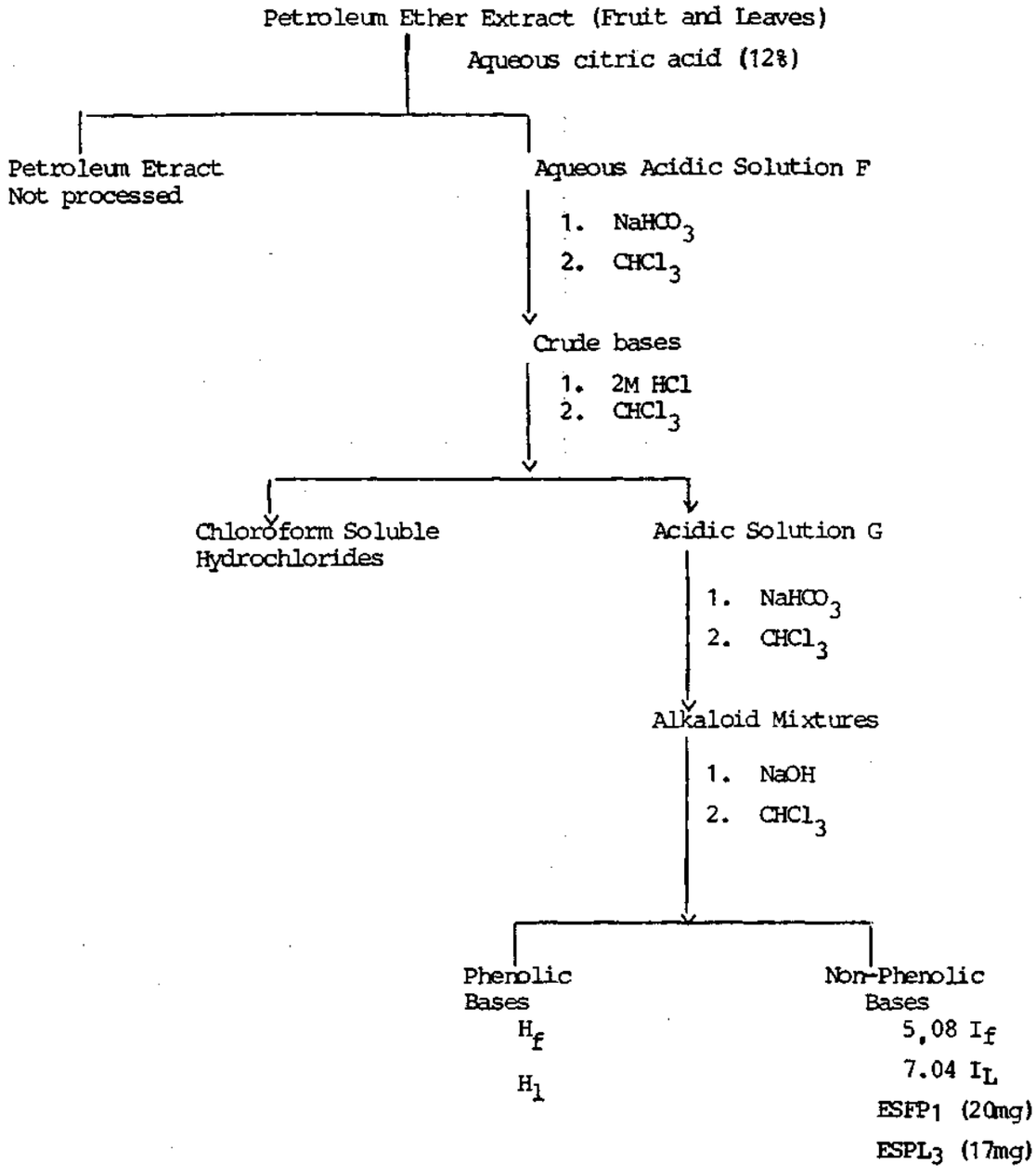
SCHEME 1

(b) Petroleum Spirit Extract -

The concentrated petroleum spirit of fruit (202g) and leaves (265g) were each treated with aqueous citric acid 12% and diluted to 200ml with water. This was extracted into petroleum ether extract and Aqueous acidic solution coded F.

Fraction F were treated with 5% $NaHCO_3$ and extracted with chloroform (400ml) to give chloroform soluble hydrochloride and the acidic solution coded G.

Fraction G were further treated with 5% $NaHCO_3$ and extracted with chloroform 400ml to give the alkaloidal mixtures which was basified with 5% NaOH and extracted with chloroform to give the phenolic bases of H_f (2.20g) and H_L (1.27g) for the fruit and leaves and the non-phenolic bases of I_f (6.08g) and I_L (7.04g) for the fruit and leaves respectively.


Petroleum Ether Extract (Fruit and Leaves)
Aqueous citric acid (12%)
Petroleum Etract
Not processed
Aqueous Acidic Solution F
1. NaHCO3
2. CHCl3
Crude bases
1. 2M HCl
2. CHCl3
Chloroform Soluble
Hydrochlorides
Acidic Solution G
1. NaHCO3
2. CHCl3
Alkaloid Mixtures
1. NaOH
2. CHCl3
Phenolic
Bases
Hf
H1
Non-Phenolic
Bases
5,08 If
7.04 IL
ESFP1 (20mg)
ESPL3 (17mg)

SCHEME 2

3.4. THIN LAYER CHROMATOGRAPHY OF BOTH METHANOLIC AND PETROLEUM SPIRIT EXTRACT OF THE PLANTS AND THE ISOLATED ALKALOID:

Vizualization Technique:-

After drying the plates in an oven at a temperature of 110°C for about 2 minutes, the plates were observed under U.V. light, the florescencing spots were marked. The plates were sprayed with the appropriate spray reagent, after which they were observed under U.V. light again. The plates sprayed with Anisaldehyde were put in the oven for 3 minutes to bring out the colour development.

The spot locations and colour reactions were noted. The R_f values were calculated using the formula:

$$RF = \frac{\text{Distance moved by compound}}{\text{Distance moved by solvent}}$$

The chromatograms are shown on Appendix

Table 10: TLC ANALYSIS OF FRACTION D (BARK)

Methanolic Extract of Erythrina Senegalensis

	R_f Value	View Under U.V.	Colour After Spraying
1.	0.04	Pink Flourescence	None
2.	0.14	Fluorescence	Green
3.	0.71	No Flourescence	Purple
4.	0.82	No Flourescence	Purple
5.	0.93	No Flourescence	Green

Solvent System = Benzene - Ethylacetate - Ammonia (20:10:2)

Spray Reagent: Dragendoff's

Distance Moved By Solvent = 10cm

Table 11: TLC ANALYSIS OF FRACTION E (BARK)

Methanolic Extract of Erythrina Senegalensis			
	R_f Value	View Under U.V.	Colour After Spraying
1.	0.01	No Flourescence	Purple
2.	0.04	No Flourescence	Purple
3.	0.14	Flourescence	Purple
4.	0.46	Flourescence	None
5.	0.71	No Flourescence	Purple
6.	0.82	Flourescence	Purple
7.	0.93	Flourescence	Green
8.	0.96	No Flourescence	Purple

Solvent System = Benzene - ethylacetate-ammonia (20:10:2)

Spray Reagent: Anisaldehyde in H_2SO_4

Distance moved by solvent = 10cm

Table 12: TLC ANALYSIS OF FRACTION H (FRUIT)

Petroleum Spirit Extract of Erythrina Senegalensis

	R_f Value	View Under U.V.	Colour After Spraying
1.	0.21	Flourescence	Orange
2.	0.32	Flourescence	Orange
3.	0.50	Flourescence	Orange
4.	0.61	Flourescence	None
5.	0.71	Flourescence	None
6.	0.93	Flourescence	Orange

Solvent System = n-hexane-ethylacetate (4:1)

Spray Reagent: Dragendorff's reagent

Distance moved by Solvent = 10cm —

Table 13: TLC ANALYSIS OF FRACTION I (FRUIT)

	Petroleum Spirit Extract of Erythrina Senegalensis		
	R_f Value	View Under U.V.	Colour After Spraying
1.	0.21	Flourescence	Orange
2.	0.32	Flourescence	Orange
3.	0.50	Flourescence	Orange
4.	0.93	Flourescence	Orange

Solvent System = n-hexane-ethylacetate (4:1)

Spray Reagent: Dragendorff's reagent

Distance moved by solvent = 10cm

Table 14: TLC PROPERTIES OF FRACTION H (LEAVE)

	Petroleum Spirit Extract of Erythrina Senegalensis		
	Rf Value	View under U.V.	Colour after spraying
1.	0.01	No Flourescence	Orange
2.	0.07	No Flourescence	Orange
3.	0.29	Flourescence	None
4.	0.71	No Flourescence	Orange

Solvent System = Benzene-ethylacetate (70:30)

Spray Reagent: Dragendorff's reagent

Distance moved by Solvent = 10cm

Table 15: TLC PROPERTIES OF FRACTION I (LEAVES)

	Petroleum Spirit Extract of Erythrina Senegalensis		
	R_f Value	View Under U.V.	Colour after spraying
1.	0.01	No Flourescence	Orange
2.	0.02	No Flourescence	Orange
3.	0.25	Pink Flourescence	None
4.	0.50	No Flourescence	Orange
5.	0.71	No Flourescence	Orange
6.	0.86	No Flourescence	Orange

Solvent System = Benzene - ethylacetate (70 : 30)

Spray Reagent: Dragendorff's reagent

Distance Moved by Solvent = 10cm

3.5. SEPARATION OF THE METHANOLIC EXTRACTS:

3.5.1. The Methanolic Extract of Bark:-

The methanolic extract of the bark having a very high percentage yield of 20.5% was subjected to the following separation technique as in Scheme 1 to give the phenolic bases fraction D (15.4g) and was examined by TLC analysis revealing five major components of R_f values 0.4, 0.14, 0.71, 0.82 and 0.93 as seen in Table 10 using Benzene : Ethyl Acetate : Ammonia 20 : 10 : 2 as solvent system.

The fraction D (15.4g) was mounted as a solution in petroleum spirit (40 - 60°C) 25ml on a Silica gel column. The principle of isolation and separation of the alkaloids involved utilization of differential solubility of the alkaloids in solvents of graded polarity starting with petroleum spirit, chloroform and methanol.

Individual alkaloids were obtained by repeated column chromatography monitoring eluates of similar components by TLC. Eluents having similar components were bulked together and this resulted into four compounds namely:

$ESMB_1$	as an oil
$ESMB_2$	as an oil
$ESMB_3$	as a solid (201mg)
$ESMB_4$	as a solid (415mg)

TLC of the Isolated Samples from Methanolic Extract of the Bark Fraction D:

The TLC examination of the isolated compounds using two

different solvent systems of n-butanol-acetic acid-water (4:1:5) with Dragendorff's reagent for staining purposes and Benzene-ethyl acetate - Ammonia (20 : 10 : 2) with Anisaldehyde in H_2SO_4 as spray reagent are shown in Tables 19 and 20 respectively.

Table 16: TLC Properties of Isolated Compounds from Fraction D (Egon Stahl 1969)

Fraction D - Methanolic Extract of Bark

Methanolic Extract Isolate of Bark			
Compounds	R_f Value	View Under U.V light	Colour After Spraying
$ESMB_1$	0.28	Flourescence	Orange
$ESMB_2$	0.41	No Fluorescence	Orange
$ESMB_3$	0.55	No Fluorescence	Orange
$ESMB_4$	0.71	Fluorescence	Orange

Solvent System: n-butanol : Acetic acid : Water (4:1:5)

Spray Reagent: Dragendorff's

Table 17: TLC Properties of Isolated Compounds of Fraction D

Methanolic Extract Isolate of Bark			
Compounds	R_f Values	View Under U.V light	Colour After Spraying
$ESMB_1$	0.14	Flourescence	Green
$ESMB_2$	0.71	Flourescence	Purple
$ESMB_3$	0.82	No flourescence	Purple
$ESMB_4$	0.93	No flourescence	Green

Solvent System: Benzene-Ethylacetate - Ammonia (20:10:2)

Spray Reagent: Anisaldehyde in H_2SO_4 (Egon Stahl 1969).

The solids $ESMB_3$ and $ESMB_4$ were recrystallized from boiling ethanol However, $ESMB_3$ was lost during recrystallization while the $ESMB_4$ gave a pure yield of 15mg.

$ESMB_4$ was subjected to TLC analysis as shown in Tables 18 and 19.

Table 18: TLC PROPERTIES OF RECRYSTALLIZED $ESMB_4$ (Egon Stahl 1969)

Solvent System : n-butanol:acetic acid:water (4:1:5)				
Compound	R_f Value	Reactions to Spray Reagents		
		View under UV light	Dragendorff	Anisaldehyde
$ESMB_4$	0.71	No Fluorescence	Orange	Purple

Table 19: TLC PROPERTIES OF RECRYSTALLIZED $ESMB_4$

Solvent System: Benzene-Ethylacetate-Ammonia (20:10:2)				
Compound	R_f Value	Reactions to Spray Reagents		
		View Under UV light	Dragendorff	Anisaldehyde
$ESMB_4$	0.82	No Fluorescence	Orange	Purple

3.6. SEPARATION OF THE PETROLEUM SPIRIT EXTRACT:

3.6.1. Petroleum Spirit Extract of Leaves:-

The Petroleum Spirit Extract of Leave 265g was subjected to separation and fractionalization by partitioning as shown in Scheme 2.

Fraction I leave of non-phenolic bases gave a yield of 7.04g which revealed the presence of six different components with TLC analysis as seen on Table 15 with R_f values of 0.01, 0.02, 0.25, 0.50, 0.71 and 0.86 respectively.

The fraction I of the leave petroleum spirit extract 7.04g was mounted as a solution in petroleum spirit (40 - 60°C), 10ml on a Silica gel column.

Gradient elution was made with petroleum spirit/benzene (9:1, 8:2, 7:3, 6:4, 5:5. 4:6, 3:7, 2:8, 1:9). The colum eluates were monitored by TLC.

Eluents having similar components were bulked and this resulted into three fractions $ESPL_1$, $ESPL_2$ and $ESPL_3$.

$ESPL_1$ from petroleum spirit : benzene 8:2 as oil

$ESPL_2$ from petroleum spirit : benzene 3:7 as oil

$ESPL_3$ from petroleum spirit : benzene 2:8 as solid of 17mg

TLC examination of the isolated compounds using two different solvent system of petroleum spirit: benzene (9:1) and Benzene ethylacetate (70:30) are shown in Tables 20 and 21

Table 20: TLC PROPERTIES OF ISOLATED COMPOUNDS FROM FRACTION I LEAVES

Petroleum Spirit Extract

Solvent System: Benzene 7 : Ethylacetate 3				
Compounds	R_f Value	Reactions to Spray Reagents		
		Cerric Ammonium Sulphate	Anisaldehyde	Dragendorff's reagent
$ESPL_1$	0.25	Brown	Purple	Orange
$ESPL_2$	0.45	Brown	Purple	Orange
$ESPL_3$	0.61	Brown	Purple	-

Table 21: TLC PROPERTIES OF ISOLATED COMPOUNDS FROM FRACTION I LEAVES

Petroleum Spirit Extract

Solvent System: Petroleum Spirit 60-80°C Benzene (9:1)				
Compounds	R_f Value	Reactions to Spray Reagents		
		Cerric Ammonium Sulphate	Anisaldehyde	Dragendorff's Reagents
$ESPL_1$	0.42	Brown	Purple	Orange
$ESPL_2$	0.55	Brown	Purple	Orange
$ESPL_3$	0.73	Brown	Purple	-

The solid $ESPL_3$ was recrystallized from boiling acetone/ benzene and was subjected to TLC analysis.

Table 22: TLC PROPERTY OF RECRYSTALLIZED $ESPL_3$

Solvent System: Benzene:Petroleum Spirit (60-80°C) 9:1				
Compounds	R_f Value	Reactions to Spray Reagents		
		Anisaldehyde	Cerric Ammonium Sulphate	Molybdophosphoric acid
$ESPL_3$	0.75	Light Purple	Brown	Blue

Table 23: TLC PROPERTIES OF RECRYSTALLIZED $ESPL_3$

Solvent System:		Benzene 7 Ethyl acetate 3		
Compounds	R_f Value	Reactions to Spray Reagents		
		Cerric Ammonium Sulphate	Anisaldehyde	Molybdophosphoric acid
$ESPL_3$	0.60	Brown	Light purple	Blue

3.6.2. PETROLEUM SPIRIT EXTRACT OF FRUIT:

The Petroleum spirit extract of fruit 202g was also subjected to partitioning as in Scheme 2 to give a non-phenolic fraction of fruit (5.08g) which revealed the presence of four compounds from the TLC analysis on table 13 of R_f values 0.21, o.32, 0.50 and 0.93.respectively.

The Fraction I fruit of 5.08g was mounted as a solution in petroleum spirit 40 - 60°C (10ml) on a Silica gel column.

Gradient elution was also carried out with petroleum spirit : benzene as follows (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9).

The column eluates were monitored by TLC. Eluents of similar components were bulked together and this resulted into two main compounds:

$ESFP_1$ from petroleum spirit:benzene 3:7 as solid

$ESFP_2$ from petroleum spirit:benzene 8:2 as oil

TLC examination of the isolated compound using two different solvent systems of Petroleum spirit : benzene 9:1 and Benzene:ethylacetate 7:3 are shown in Tables 24 and 25:

Table 24: TLC PROPERTIES OF ISOLATED COMPOUNDS FROM FRACTION I PETROLEUM SPIRIT OF FRUIT

Solvent System: Petroleum Spirit : Benzene 9:1				
Compounds	R_f value	Reactions to Spray Reagents		
		Anisaldehyde	Cerric Ammonium Sulphate	Dragen-dorff's
$ESFP_1$	0.82	Light Purple	Brown	-
$ESFP_2$	0.73	Puple	Brown	-

Table 25 : TLC PROPERTIES OF ISOLATED COMPOUNDS FROM FRACTION I PETROLEUM SPIRIT EXTRACT OF FRUIT

Solvent System : Benzene 7 Ethyl acetate 3				
Compounds	R_f Value	Reactions to Spray Reagents		
		Cerric Ammonium sulphate	Anisaldehyde	Dragendorff's
$ESFP_1$	0.88	Brown	Purple	-
$ESFP_2$	0.69	Brown	Purple	Orange

The solid $ESFP_1$ was also recrystallized from boiling acetone/ benzene and subjected to TLC analysis.

Table 26 : TLC PROPERTY OF RECRYSTALLIZED $ESFP_1$

Solvent System:		Benzene:Petroleum Spirit 60-80°C (9:1)		
Compounds	R_f Value	Reactions to Spray Reagents		
		Anisaldehyde	Cerric Ammonium sulphate	Molybdophosphoric acid
$ESFP_1$	0.73	Light Purple	Brown	Blue

Table 27: TLC PROPERTY OF RECRYSTALLIZED $ESFP_1$

Solvent System : Benzene 7 Ethyl acetate 3				
Compounds	R_f Value	Reactions to Spray Reagents		
		Cerric Ammonium Sulphate	Anisaldehyde	Molybdophosphoric acid
$ESFP_1$	0.85	Brown	Purple	Blue

3.6.3. CHARACTERISATION OF PRODUCTS ISOLATED FROM THE METHANOLIC EXTRACT OF BARK:

$\underline{ESMB_4}$: This was obtained as a white powder (15mg). It has a melting point of 148 - 150°C. It is soluble in methanol, dichloromethane and ethanol.

It has an R_f value of 0.71 in n-butanol:acetic acid:water (4:1:5) and 0.82 in Benzene-ethylacetate-ammonia (20:10:2)

UV λ max (MeOH) 279nm

ε max 0.256

$[\alpha]_D^{20}$ + 119° ($CHCl_3$)

IR (CCl_4)cm^{-1}

1000 (CH_2, rocking) 1200 (CH_3, bending)

1550 (C = C stretching)

2900 (C - H stretching aromatic).

^{1}H-NMR (250MHz, $CDCl_3$) δ (ppm)

0.88 (t, 3H) 1.26 (m, 11 H) 2.1 (m, 7H)

6.5 (m, 2H) 7.1 (m, 2H)

MS (70ev)m/z [m^+] 256 (25), 241 (11) 227 (100) 122 (26).

$ESMB_1$ and $ESMB_2$

This two compounds were obtained as oils with R_f values of 0.28 and 0.41 in n-butanol-acetic acid-water (4:1:5) respectively and also with R_f values of 0.14 and 0.71 in Benzene:ethyl acetate: ammonia (20:10:2) respectively. However, both $ESMB_1$ and $ESMB_2$ were not investigated any further as there is no equipment available for complete separation and purification of the oils. $ESMB_1$ and $ESMB_2$ were soluble in petroleum spirit 60 - 80°C, Dichloromethane

and benzene.

3.6.4. CHARACTERISATION OF PRODUCTS ISOLATED FROM THE PETROLEUM SPIRIT EXTRACT:

*$\underline{ESPL_3}$: This was obtained from the petroleum spirit extract of the leaves as a colourless solid of 17mg. It was found soluble in ethylacetate and benzene.

IR (Nujol) cm^{-1}, 730 (CH_2 - rocking)

1460 (CH - bending)

1710 (C=O - stretching of an acid)

UV λ max (benzene) λ max 296nm ε max 0.18

^{1}H NMR ($CDCl_3$) 250MHz δ (ppm)

0.88 (t, 3H), 1.26 (S, 28H), 1.61 (t, 2H) 2.34 (t, 2H)

MS (70ev) m/z [m^+] 284(11), 255(38), 185(23), 129(28), 73(100), 60(41), 57(38), 55(31), 43(52).

Melting Point 68 - 70°C.

Compound had an R_f values of 0.75 in benzene : petroleum spirit 60 - 80°C (9:1) and 0.60 in Benzene : ethyl acetate (7:3) respectively.

$\underline{ESFP_1}$: This compound was obtained from the petroleum spirit fraction of the fruit as a white crystalline powder with R_f values of 0.85 in Benzene : ethyl acetate (7:3) and 0.73 in Benzene - petroleum spirit 60 - 80°C.

* Spectral datas of $ESPL_3$ are shown in Appendix IX.

It is soluble in benzene, dichloromethane and petroleum spirit 60 - 80°C. It has a melting point of 57 - 59°C.

IR (CCl_4) cm^{-1}, 730-(CH_2) - rocking

1166, 1179 C-O stretching

1735 C=O stretching of an ester.

UV λ max (C_6H_6) 294nm ε max 0.175

^1H NMR ($CDCl_3$) 250MHz δ (ppm)

0.88 (t, 9H) 1.25 (S, 74H) 1.60 (m, 6H)

2.30 (t, 6H) 4.10 (m, 4H) 5.26 (m, 1H)

MS (70ev) m/z [m^+] 806(12) 608(35) 578(50) 550(79)

522(23) 314(18) 149(28) 71(100)

3.7. PHARMACOLOGICAL SCREENING OF THE PLANT EXTRACT:

3.7.1. Effect on Smooth Muscle of Rabbit Jejunum :

The effect of the methanolic extract of the leaves and fruits of Erythrina senegalensis was noted in isolated rabbit jejunum preparation. The rabbit jejunum was used in order to take advantage of the spontaneous activity of the tissue to study the action of drugs like adrenaline and the erythrina extract , which inhibit movement (Ghosal 1972 and Litchfield 1949).

An adult rabbit starved for 24h. was killed by a blow on the head and exsanguinated by cutting the throat. The abdomen was exposed and opened. The jejunum was identified by its mesenteric attachment. 8cm length was cut and transferred to a dish containing Tyrode's solution which was aerated with 95% O_2 and 5% CO_2 (the intestine was cut at a point 5 to 10cm, below the stomach and a length taken from here downwards towards the caecum). Since the rabbit jejunum does not usually require washing partly due to its spontaneous activity and because it is wider than guinea-pig intestine it was not washed.

2.3cm length was cut in portions, free from mesenteric attachment. The caudal end of the jejunum pieces was tied with thread to the bent-end of the aeration tube while the other end to a frontal-writing lever, taking care to see that the jejunum is left open and the threads do not close the lumen. The piece was then mounted in an organ-bath of 50ml capacity. Necessary kymograph arrangements for recording gut motility was made.

The tissue was allowed to stabilize for 15 minutes into the bath at 37°C through which air was bubbled . The Kymograph speed was 15 x 0.1mm/sec. for 152mm drum. (Shetty 1982).

Once the tissue had stabilized administration of drug and crude methanolic extract started according to the scheme in Table 4. To investigate the effects of the crude methanolic extract on the rhythmic contraction of the rabbit jejunum the following time cycle was used:

0min	start kymograph
1min	add drug/extract
1½min	stop the kymograph and wash off the drug/ extract from the bath. Waiting for the tissue to regain its normal rhythmic movement.
5min	start kymograph.

All preparations used in the present study were allowed to equilibrate for at least 60 - 90 min before challenge with drugs and during that time the fluid was replaced every 20 min and the passive tension was readjusted.

After all the drug/crude extract had been added and the response obtained , the response smoked paper was carefully removed from the drum, dipped in a special varnish and allowed to dry.

Table 28: Order of Exposure of Rabbit Jejunum to Drugs/Crude Extracts

Serial No.	Drugs /Crude extract	Conc. admin (g/ml)	Vol admin (ml)	Conc. of drug in bath (µg/ml)
1.	Acetylcholine	1×10^{-6}	0.1	0.002
2	Acetylcholine	1×10^{-6}	0.2	0.004
3	Adrenaline	1×10^{-6}	0.1	0.002
4	Adrenaline	1×10^{-6}	0.2	0.004
5	Methanolic Extract of fruit	1.5×10^{-1}	0.05	750
6	Methanolic Extract of fruit	1.5×10^{-6}	0.10	0.003
7	Methanolic Extract of fruit	1.5×10^{-6}	0.2	0.006
8	Methanolic Extract of fruit	1.5×10^{-4}	0.1	0.3
9	Methanolic Extract of fruit	1.5×10^{-4}	0.2	0.6
10	Methanolic Extract of fruit	1.5×10^{-2}	0.1	30
11	Methanolic Extract of fruit	1.5×10^{-2}	0.2	60
12	Methanolic Extract of leaves	1.6×10^{-2}	0.1	32
13	Methanolic Extract of leaves	1.6×10^{-2}	0.2	64
14	Methanolic Extract of leaves	1.6×10^{-2}	0.4	128
15	Methanolic Extract of leaves	1.6×10^{-2}	0.6	192

3.7.2. Effect on Guinea Pig Ileum:

The effect of the methanolic extract was also noted on the isolated guinea pig ileum preparation. The guinea pig ileum was used in order to study the effect of acetylcholine and atropine since they both act on the same receptor sites and also to determine whether the extract has a muscarinic effect.

A 24 hour starved white male guinea pig 520g was killed by stunning it on the head with a sharp blow. The abdomen was opened and the terminal portion of the ileum was removed. just above the patch of lymph tissue. The lumen was cleared with the tyrode solution using a 5.0ml bulb pipette,2.5cm length of the ileum was cut and suspended in a 50ml organ bath containing tyrode solution aerated with oxygen and maintained at 35°C by attaching the lower end of the muscle to a hook on the aerater glass tubing with a looped thread, while the upper end was attached to a transducer. The tension was adjusted on the muscle to 0.2g weight and the record of its movements on polygraph was observed as follows:

0min	-	start kymograph
1min	-	add drug extract
1½min	-	stop kymograph and wash off the drug/ extract; refill the bath.
5min	-	start kymograph

The dose was continuously increased until a maximal dose response was obtained for both the extracts and acetylcholine and the effect was followed by atropine to inhibit the effect of those doses.

Table 29: Order of Exposure of Guinea Pig Ileum to Drug/Crude Extract

(i) Methanolic Bark Extract

Serial No.	Drug/Crude Extract	Conc. admin (µg/ml)	Vol. admin (ml)	Conc. of Drug in bath µg/ml
1	Methanolic Bark Extract	1.6×10^3	0.5ml	16
2	Methanolic Bark Extract	1.6×10^3	1.0ml	32
3	Methanolic Bark Extract	1.6×10^3	3.0ml	96
4	Methanolic Bark Extract	1.6×10^3	10ml	320
5	Ach	1µg/ml	0.1ml	0.002
6	Ach	1µg/ml	0.3ml	0.006
7	Ach	10µg/ml	0.1ml	0.02
8	Ach	10µg/ml	0.3ml	0.06
9	Atropine	10µg/ml	0.3ml	0.06
10	Ach	1µg/ml	0.1ml	0.002
11	Ach	1µg/ml	0.3ml	0.006
12	Ach	10µg/ml	0.1ml	0.02
13	Ach	10µg/ml	0.3ml	0.06
14	Methanolic Bark Extract	1.6×10^3	0.5ml	16
15	Methanolic Bark Extract	1.6×10^3	1.0ml	32
16	Methanolic Bark Extract	1.6×10^3	3.0ml	96
17	Methanolic Bark Extract	1.6×10^3	10ml	320
18	Methanolic Bark Extract	1.6×10^3	20ml	640

Table 30: Order of Exposure of Guinea Pig Ileum to Drug/Crude Extract

(ii) Methanolic Leave Extract

Serial No.	Drug/Crude Extract	Conc. admin (µg/ml)	Vol. admin (ml)	Conc. of Drug in bath µg/ml
1	Methanolic Leave Extract	1.12×10^2	0.5ml	1.12
2	Methanolic Leave Extract	1.12×10^2	1.0ml	2.24
3	Methanolic Leave Extract	1.12×10^2	3.0ml	6.72
4	Methanolic Leave Extract	1.12×10^2	10ml	22.4
5	Ach	1µg/ml	0.1ml	0.002
6	Ach	1µg/ml	0.3ml	0.006
7	Ach	10µg/ml	0.1ml	0.02
8	Ach	10µg/ml	0.3ml	0.06
9	Atropine	10µg/ml	0.3ml	0.06
10	Ach	1µg/ml	0.1ml	0.002
11	Ach	1µg/ml	0.3ml	0.006
12	Ach	10µg/ml	0.1ml	0.02
13	Ach	10µg/ml	0.3ml	0.06
14	Methanolic Leave Extract	1.12×10^2	0.5ml	1.12
15	Methanolic Leave Extract	1.12×10^2	1.0ml	2.24
16	Methanolic Leave Extract	1.12×10^2	3.0ml	6.72
17	Methanolic Leave Extract	1.12×10^2	10ml	22.4
18	Methanolic Leave Extract	1.12×10^2	20ml	44.8

3.8. Effect on Skeletal Muscle: (Litchfield 1949)

To ascertain the effect of the methanolic extract of the leaves and fruits on skeletal muscle the rectus abdominis muscle of the frog was used . This muscle is straited, it does not behave like normal voluntary muscle and responds to acetylcholine by giving slow contraction. It is therefore, an extremely useful preparation for showing the actions of those compounds which block transmission at the neuromuscular junction by acting in the same way as an excess of acetylcholine.

The frog rectus preparation was made from a frog kept for two weeks at 4°C without feeding. The frog was stunned and decapitated and the spinal cord was destroyed. The frog was pinned out on a dissecting board and/skin of the abdomen, but the not the abdomen itself, was picked up with forceps and cut from the sternum down to the forth. It was then cut laterally to expose the whole of the abdomen. The two rectus muscles were plainly seen, running each side of the midline from the base of the sternum to the fork. The sternum was cut through just above the xiphisternum at its base and the pair of muscle attached to it were dissected out and transferred to a dish containing frog ringer solution at room temperature. The muscle was divided and thread were attracted to the top and bottom of each piece.

The preparation was kept at room temperature in frog-ringer solution. The capacity of the organ bath was 50ml and the kymograph speed was 40 x 0.01ml/sec for 152mm drum.

After the muscle has been made to contract, it will not relax rapidly when the drug has been washed out, hence it must be stretched gently by increasing the load. For this purpose a small piece of plasticine was added to the lever at the same distance from the other side. This was raised and lowered as was necessary.

The cycle of operations used was:

0min	Raise the weight and start the kymograph
2min	Add drug/extract
3½min	Stop the kymograph, wash the rectus and lower the weight.
6min	Raise the weight and start the kymograph.

When necessary kymograph arrangements for recording contracture was made and the tissue have stabilized drug treatment was started as shown below:

Order of procedure

1. Establish a sub maximal dose of Ach 0.2ml of 10^{-2}g/ml.
2. 0.05ml 60µg/ml D-TB followed by 0.2ml Ach
3. 0.2ml Ach
4. 0.1ml 50µg/ml D-TB followed by 0.2ml Ach
5. 0.2ml Ach
6. 0.2ml 50µg/ml D-TB followed by 0.2ml Ach
7. 0.2 Ach
8. 0.3ml 50µg/ml D-TB followed by 0.2ml Ach
9. 0.2ml Ach
10. 0.4ml 50µg/ml D-TB followed by 0.2ml Ach

11. 0.2ml Ach.

12. 0.05ml 1.5 x 10^{-1}g/ml methanol fruit extract followed by 0.2ml Ach

13. 0.2ml Ach.

14. 1ml 1.5 x 10^{-1}g/ml methanolic fruit extract followed by 0.2ml Ach.

15. 0.2ml Ach

16. 0.2ml 1.5 x 10^{-1}g/ml methanolic fruit extract followed by 0.2ml Ach.

17. 0.2ml Ach

18. 0.3ml 1.5 x 10^{-1}g/ml methanolic fruit extract followed by 0.2ml Ach

19. 0.2ml Ach

20. 0.4ml 1.5 x 10^{-1}g/ml methanolic fruit extract followed by 0.2ml Ach

21. 0.2ml Ach

22. 0.05ml 1.6 x 10^{-2}g/ml methanolic leaf extract followed by 0.2ml Ach

23. 0.2ml Ach

24. 0.1ml 1.6 x 10^{-2}g/ml methanolic leaf extract followed by 0.2ml Ach

25. 0.2ml Ach

26. 0.2ml 1.6 x 10^{-2}g/ml methanolic leaf extract followed by 0.2ml Ach

27. 0.2ml Ach

28. 0.3ml 1.6 x 10^{-2}g/ml methanolic leaf extract followed by 0.2ml Ach

29. 0.2ml Ach

30. 0.4 1.6 x 10^{-2}g/ml methanolic leaf extract followed by 0.2ml Ach.

31. 0.2ml Ach

32. 0.05ml 1.5 x 10^{-1}g/ml methanolic bark extract followed by 0.2ml Ach

33. 0.2ml Ach

34. 1ml 1.5 x 10^{-1}g/ml methanolic bark extract followed by 0.2ml Ach

35. 0.2ml Ach

36. 0.2ml 1.5 x 10^{-1}g/ml methanolic bark extract followed by 0.2ml Ach

37. 0.2ml Ach

38. 0.3ml 1.5 x 10^{-1}g/ml methanolic bark extract followed by 0.2ml Ach

39. 0.2ml Ach

40. 0.4ml 1.5 x 10^{-1}g/ml methanolic bark extract followed by 0.2ml Ach

3.9. EFFECT OF METHANOLIC EXTRACT ON FROG RECTUS ABDOMINIS:

Tabulation of Values for Plotting of Graphs:-

Table 31: DETERMINATION OF SUB-MAXIMAL DOSE ACETYLCHOLINE (Ach) 10^{-2}g/ml

HEIGHT (mm)	% RESPONSE	ml
3	50	0.1
4	67	0.2
6	100	0.3
6	100	0.4

Table 32(a): Ach = 0.2ml, dtb = 50µg/ml, organ bath = 50ml.

HEIGHT ACH ONLY	ACH (MM) WITH EXTRACT Dtb	INHIBITION	PERCENTAGE INHIBITION	VOL. ADMIN. (ML)	CONC. IN BATH (Dtb)	LOG CONC.
3	2	0.3333	33.33	0.05	0.05µg/ml	-7.301
2	1.5	0.25	25	0.1	0.1µg/ml	-7.000
2	1	0.50	50	0.2	0.2µg/ml	-6.699
25	1	0.60	60	0.3	0.3µg/ml	-6.523
2	1	0.50	50	0.4	0.4µg/ml	-6.400

Table 32(b):

HEIGHT ACH ONLY	ACH (MM) WITH EXTRACT	INHIBITION	PERCENTAGE INHIBITION	METHANOLIC LEAVE EXTRACT 1.6×10^{-3} g/ml Vol(ml extract	Conc extract	Log conc.
2	2	0	0	0.05	1.6×10^{-5}	-4.800
2.5	2	0.2	20	0.1	3.2×15^{-5}	-4.500
3.0	2	0.3333	33.33	0.2	6.4×10^{-5}	-4.200
2.5	1	0.6	60	0.3	9.6×10^{-5}	-4.020
2.0	1	0.50	50	0.4	12.8×10^{-5}	-3.90
1.5	1	0.3333	33.33	0.5	16×10^{-5}	-3.80

FIG. 9: THE BIOASSAY OF THE LEAF METHANOLIC EXTRACT OF *Erythrina senegalensis* on Frog rectus abdominis

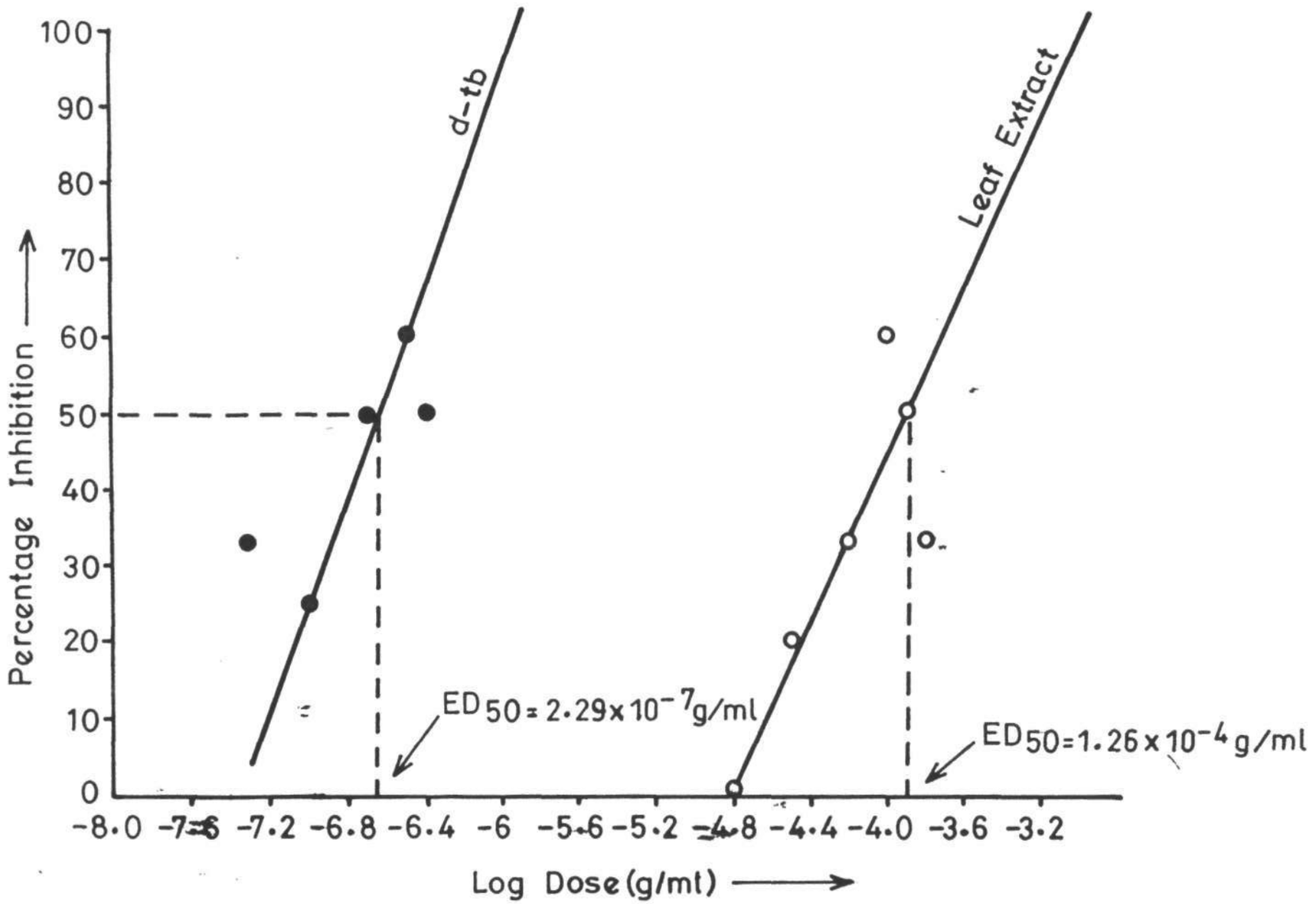


TABLE 32(c):

HEIGHT Ach Only	Ach (mm) with extract	Inhibition	% Age inhibition	Methanolic Vol (ml) extract	Fruit extract 15×10^{-2} 1G/ml Conc. extract	Log Conc.
2mm	1.5	0.25	25%	0.05	1.5×10^{-4}	-3.824
2mm	1	0.5	50%	0.1	3×10^{-4}	-3.523
2mm	1.5	0.25	25%	0.2	6×10^{-4}	-3.222
2mm	1	0.5	50%	0.3	9×10^{-4}	-3.046
2mm	1	0.5	50%	0.4	12×10^{-4}	-2.921

TABLE 32(d): Ach = 0.2ml, Methanolic Bark Extract, Organ Bath 50ml (65µg/ml)

HEIGHT Ach Only	Ach (mm) with extract	Inhibition	% Inhibition	Vol. Admin. (Ml)	Conc. In Bath µg/ml	Log Conc.
2mm	2mm	0	0%	0.05	0.064	-7.20
2mm	1.5mm	0.25	25%	0.1	0.145	-6.84
2.0mm	1mm	0.5	50%	0.2	0.285	-6.545
2.5mm	1mm	0.6	60%	0.3	0.398	-6.400
2.5mm	1mm	0.6	60%	0.5	0.631	-6.200

FIG. 10: THE BIOASSAY OF THE FRUIT METHANOLIC EXTRACT OF *Erythrina senegalensis* on Frog rectus abdominis

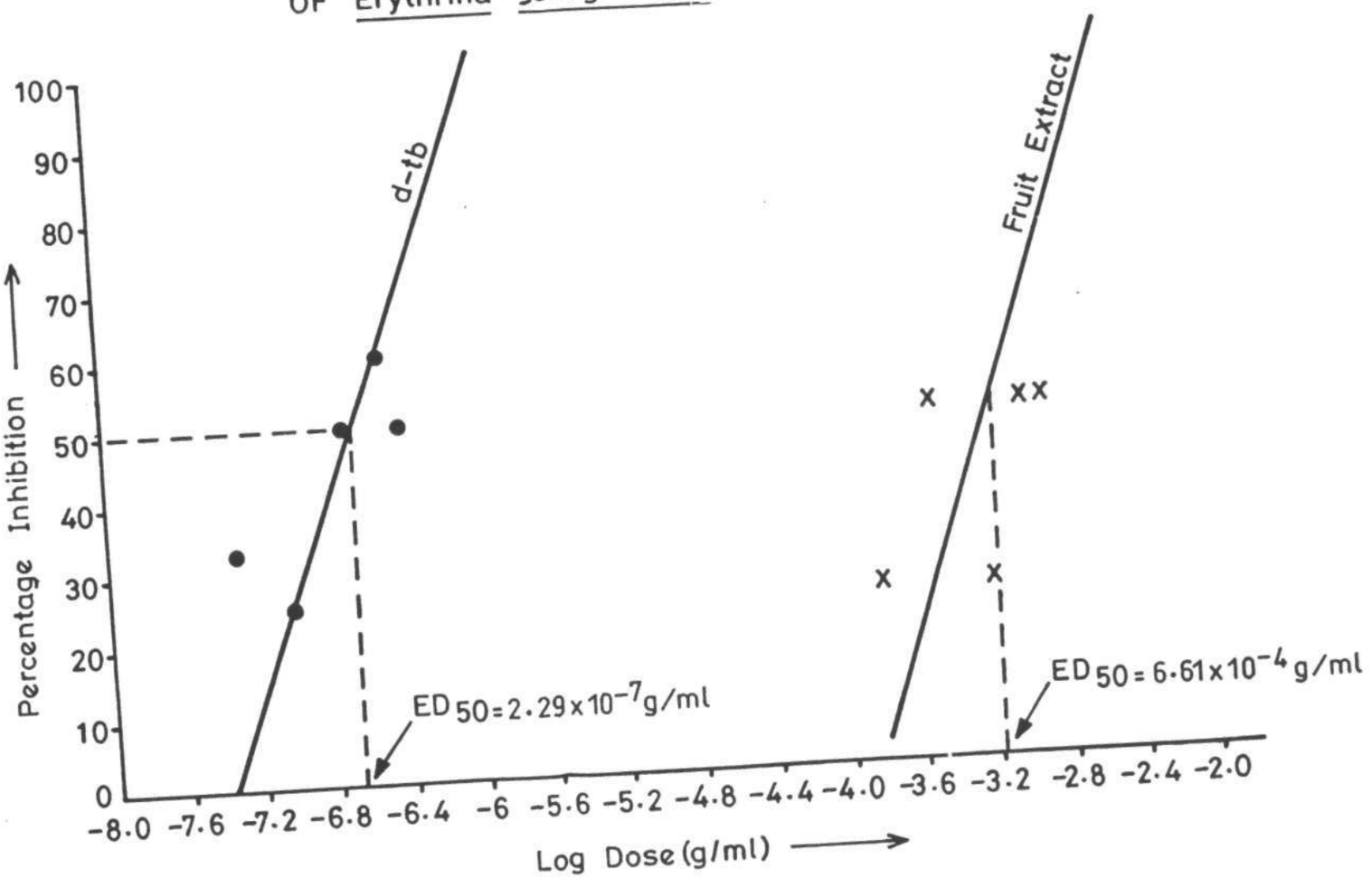
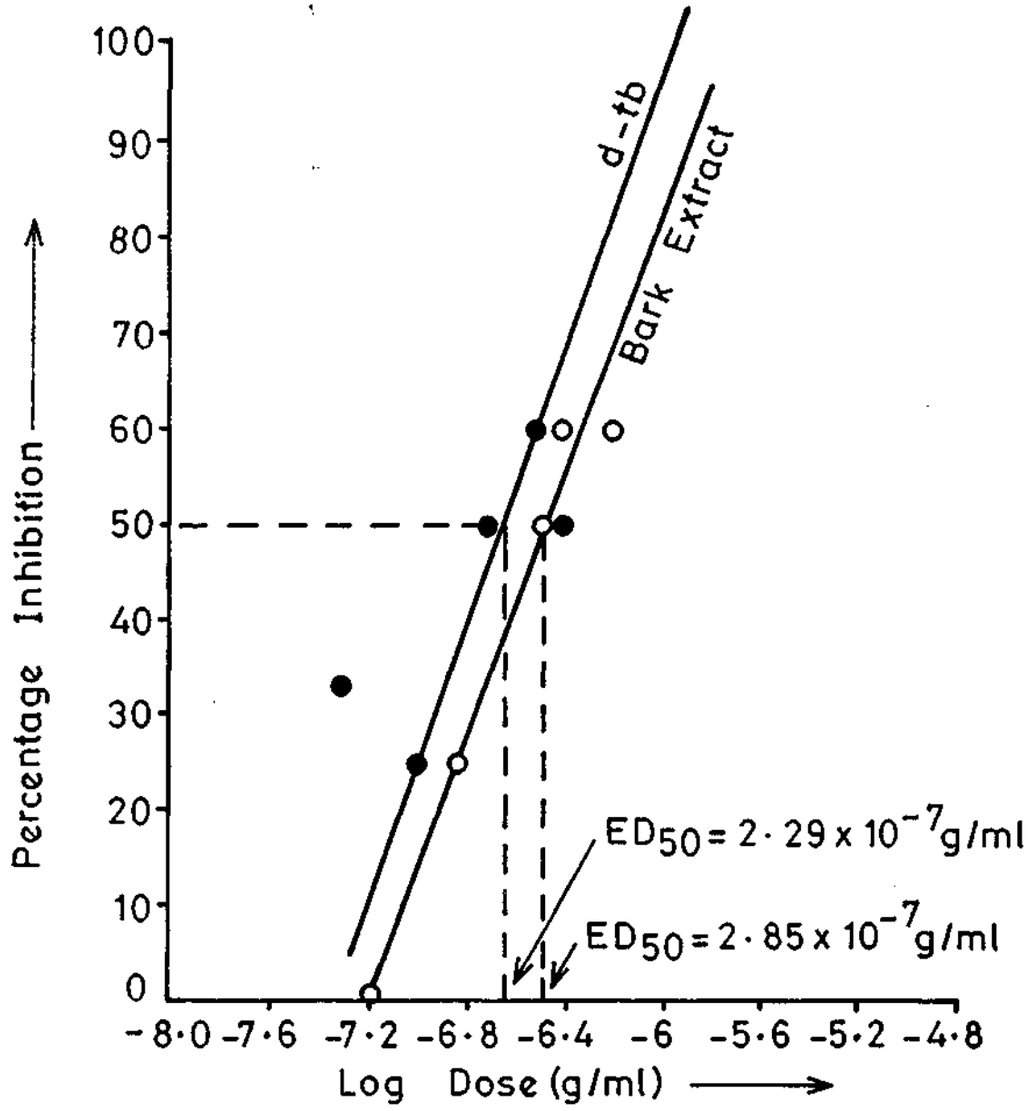


FIG. 11: The Bioassay of the Bark methanolic extract of Erythrina senegalensis on Frog rectus abdominis.



Calculation of Result Values:

For calculation of the percentage of inhibition, the formula used was:

$$\%\text{ age inhibition} = \frac{\text{Height of sub-maximal responde (Ach)} - \text{Height of response of Ach in presence of antagonis}}{\text{Height of sub-maximal response (Ach)}} \times 100$$

Example 1: When 0.05ml of 0.05µg/ml dtb is given

Height of Sub-maximal response (Ach) = 3mm

Height of response of Ach in presence of dtb = 2mm

Therefore, %age inhibition $= \frac{3mm - 2mm}{3mm} \times 100$

$= 33.33\%$

Example 2: When 0.05ml of 1.5×10^{-4}g/ml methanolic fruits extract is given.

Height of sub-maximal response (Ach) = 2mm

Height of response of Ach in presence of extract = 1.5mm

Therefore, %age inhibition $= \frac{2mm - 1.5mm}{2mm} \times 100$

$= 25\%$

Example 3: When 0.05ml of 1.6×10^{-5}g/ml methanolic leaves extract is given.

Height of sub-maximal response (Ach) = 2mm

Height of response of Ach in presence of extract = 2mm

Therefore, %age inhibition $= \frac{2mm - 2mm}{2mm} \times 100$

$= 0\%$

For calculation of ED_{50} of tubocurarine, methanolic leaf, fruits and bark extracts.

ED_{50} of tubocurarine which is the antiLog - 6.64(g/ml)

$$= \underline{2.29 \times 10^{-7} g/ml}$$

ED_{50} of methanolic fruit extract which is the antiLog - 3.18(g/ml)

$$= \underline{6.61 \times 10^{-4} g/ml}$$

ED_{50} of methanolic leaf extract which is the antiLog - 3.90(g/ml)

$$= \underline{1.26 \times 10^{-4} g/ml}$$

ED_{50} of methanolic bark extract which is the antiLog - 6.50(g/ml)

$$= \underline{2.85 \times 10^{-7} (g/ml)}$$

The potency ratio relative to methanolic fruit extract:

$$= \frac{ED_{50} \text{ Standard}}{ED_{50} \text{ Test}}$$

$$= \frac{2.29 \times 10^{-7} g/ml}{6.61 \times 10^{-4} g/ml} = \underline{3.46 \times 10^{-4} g/ml}$$

The potency ratio relative to methanolic leaf extract:

$$= \frac{2.29 \times 10^{-7} g/ml}{1.26 \times 10^{-4} g/ml} = \underline{1.82 \times 10^{-3} g/ml}$$

The potency ratio relative to methanolic bark extract:

$$= \frac{2.29 \times 10^{-7} g/ml}{2.85 \times 10^{-7} g/ml} = \underline{8.035 \times 10^{-1} g/ml}$$

3.9.1. EFFECT OF ACH AND METHANOLIC EXTRACT OF BARK AND LEAVES ON GUINEA PIG ILEUM:

Table 33(a): Tabulation of Values for Plotting Graphs

Leave Extract Only 1.12×10^2 µg/ml Organ Bath = 50ml

Volume Admin (ml)	Response (cm)	Conc Admin µg/ml	Log dose	Percentage Response %
0.5	1.4	1.12	0.05	28
1.0	3.0	2.24	0.35	60
3.0	4.2	6.72	0.82	84
10	5	22.4	1.35	100

(b): Leave Extract

In the presence of Atropine 10µg/ml Organ Bath = 50ml

Volume Admin (ml)	Response (cm)	Conc. Admin. µg/ml	Log dose	Percentage Response %
0.5	0.16	1.12	0.05	8
1.0	0.5	2.24	0.35	25
3.0	1.2	6.72	0.82	60
10	2	22.4	1.35	85

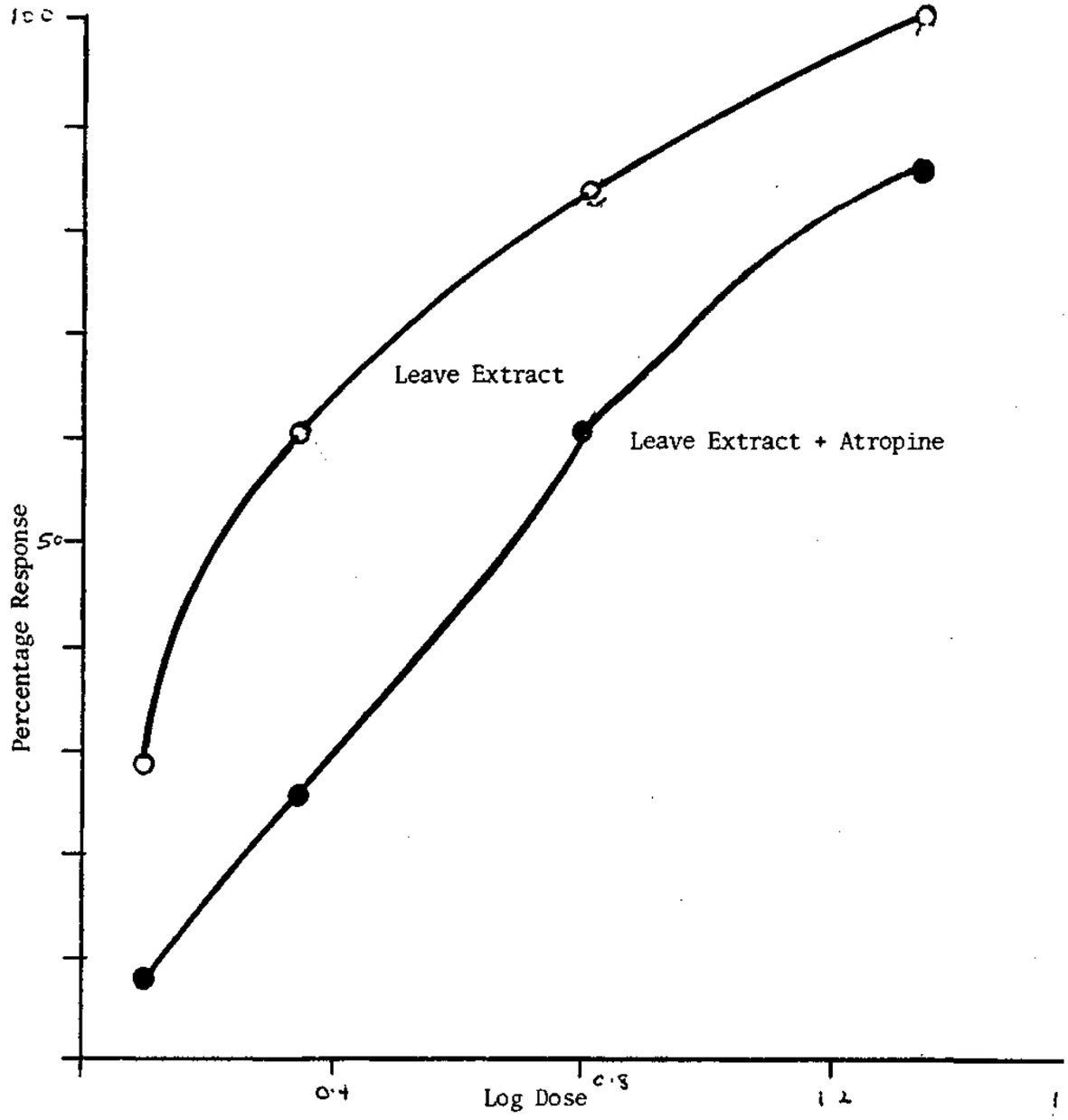


Figure 12: Dose Response Curve of Methanolic Extract of Leave on Guinea Pig Ileum.

Table 34(a): EFFECT OF ACH ONLY ON GUINEA PIG ILEUM

Volume Amin. (ml)	Response (cm)	Conc. Admin. (µg/ml)	Log dose	Percentage Response (%)
0.1 1µg/ml	0.6	0.002	-2.7	17
0.3 1µg/ml	1.2	0.006	-2.2	36
0.1 10µg/ml	2.6	0.02	-1.7	80
0.3 10µg/ml	3.3	0.06	-1.22	100

Table 34(b): Ach In the Presence of Atropine:

Volume Admin. (ml)	Response (cm)	Conc. Admin. (µg/ml)	Log dose	Percentage Response (%)
0.1 1µg/ml	0.6	0.002	-2.7	12
0.3 1µg/ml	1.4	0.006	-2.2	28
0.1 10µg/ml	4	0.02	-1.7	66
0.3 10µg/ml	5	0.06	-1.22	90

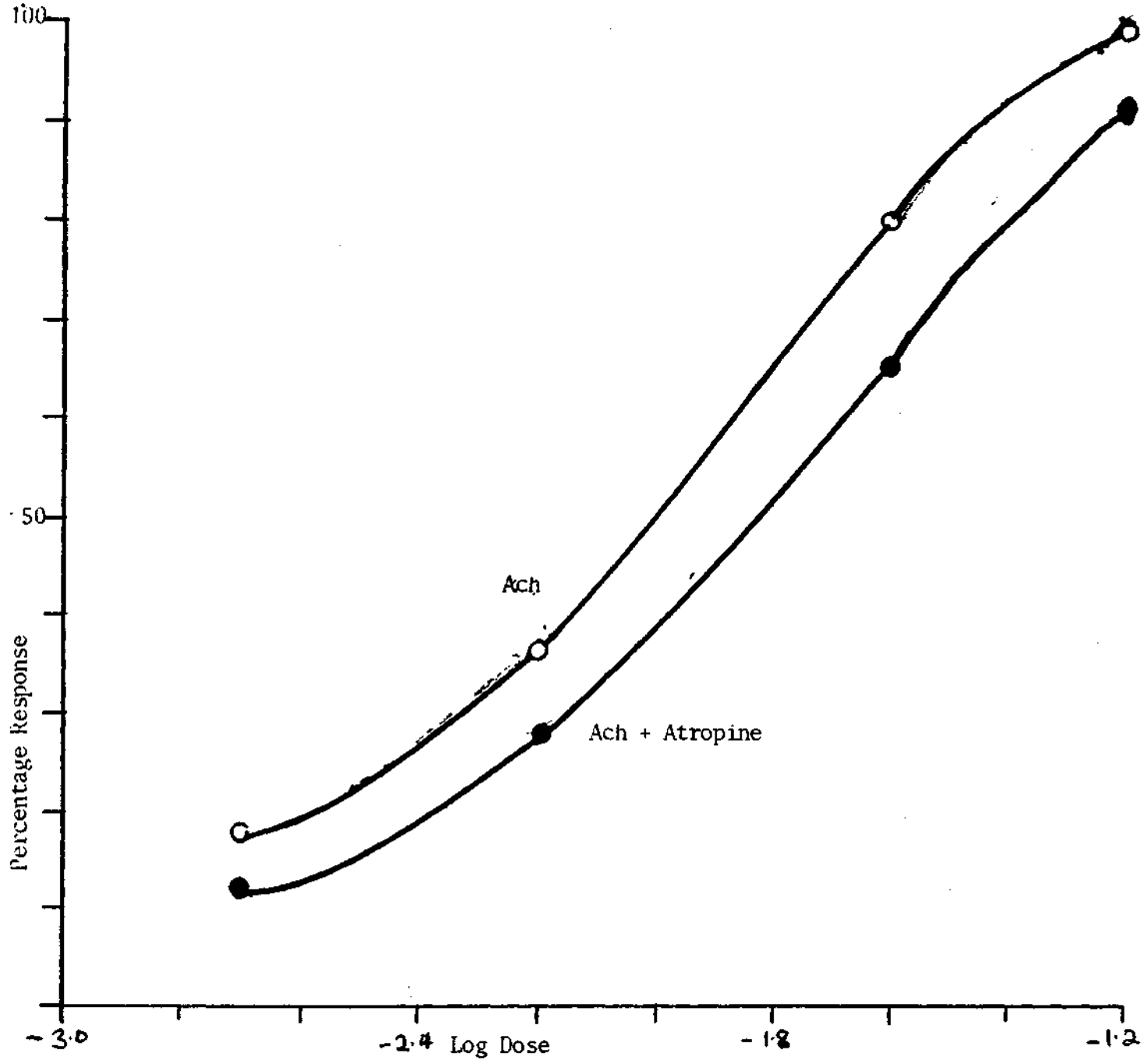


Figure 13: Dose Response Curve of Ach on Guinea Pig Ileum.

Table 35(a): Effect of Methanolic Extract of Bark on Guinea Pig Ileum. Organ Bath = 50ml

1.6×10^{3} µg/ml

Volume Admin. (ml)	Response (cm)	Conc. Admin. (µg/ml)	Log dose	Percentage Response (%)
0.5	1.0	1.6	1.20	33
1.0	1.5	32	1.50	50
3.0	2.5	96	2.0	83
10	3	320	2.5	100

Table 35(b): Effect of Methanolic Extract of Bark In presence of Atropine.

1.6×10^{3} µg/ml

Volume Amin. (ml)	Response (cm)	Conc. Admin. (µg/ml)	Log dose	Percentage Response (%)
0.5	0.02	16	1.20	2
1.0	0.1	32	1.50	10
3.0	0.4	96	2.0	40
10	1.0	320	2.5	60

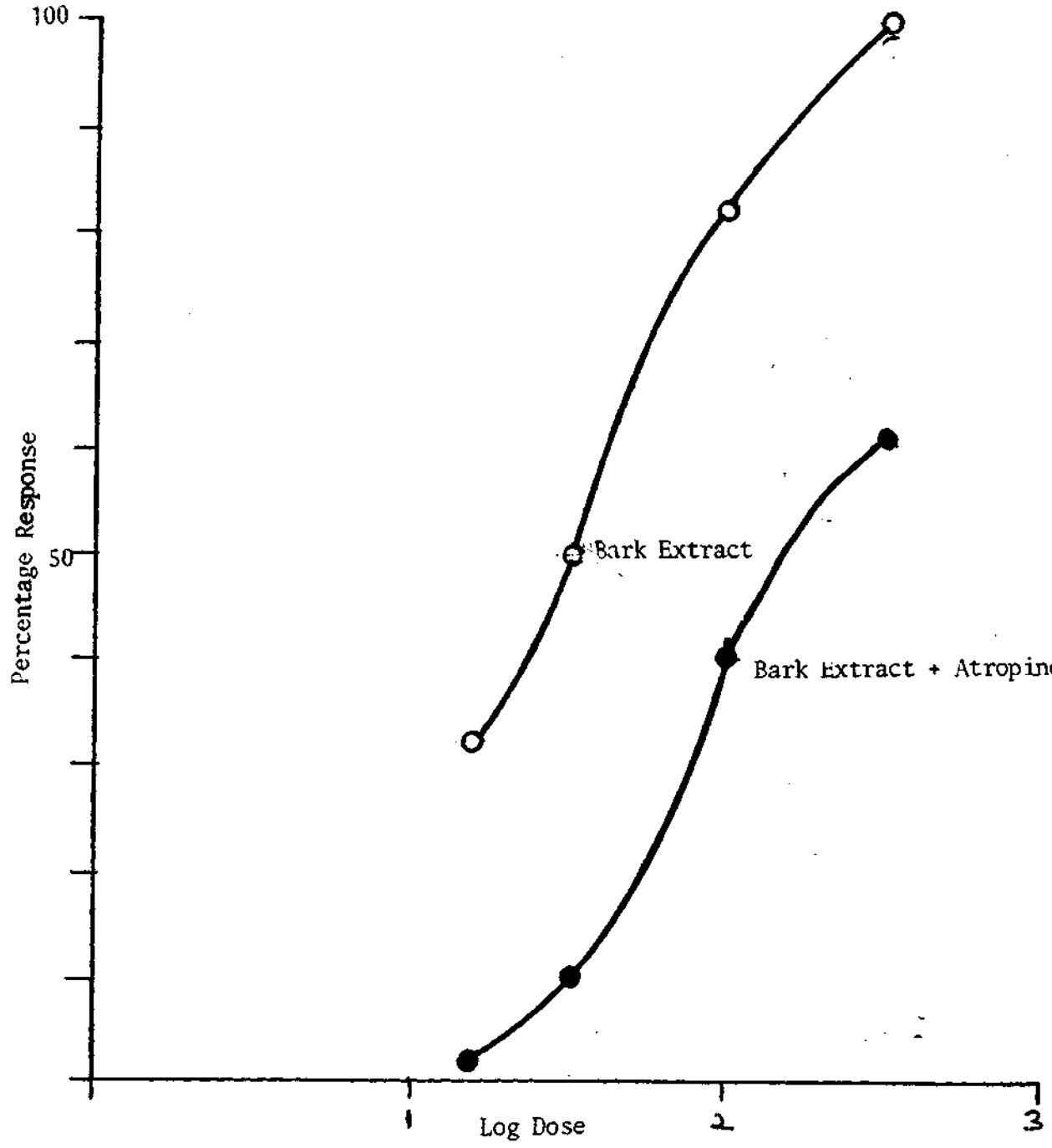


Figure 14: Dose Response Curve of Methanolic Bark Extract on Guinea Pig Ileum.

Table 36(a): Effect of Ach Only on Guinea Pig Ileum

Volume Admin. (ml)		Response (cm)	Conc. Admin. (µg/ml)	Log dose	Percentage Response (%)
0.1	1µg/ml	2	0.002	-2.7	25
0.3	1µg/ml	5.1	0.006	-2.2	62
0.1	10µg/ml	7	0.02	-1.7	85
0.3	10µg/ml	8.2	0.06	-1.22	100

Table 36(b): Effect of Ach In Presence of Atropine

Organ Bath = 50ml

Volume Admin. (ml)		Response (cm)	Conc. Admin. (µg/ml)	Log dose	Percentage Response (%)
0.1	1µg/ml	0.2	0.002	-2.7	3
0.3	1µg/ml	1.38	0.006	-2.2	23
0.1	10µg/ml	3.2	0.02	-1.7	52
0.3	10µg/ml	6	0.06	-1.22	68

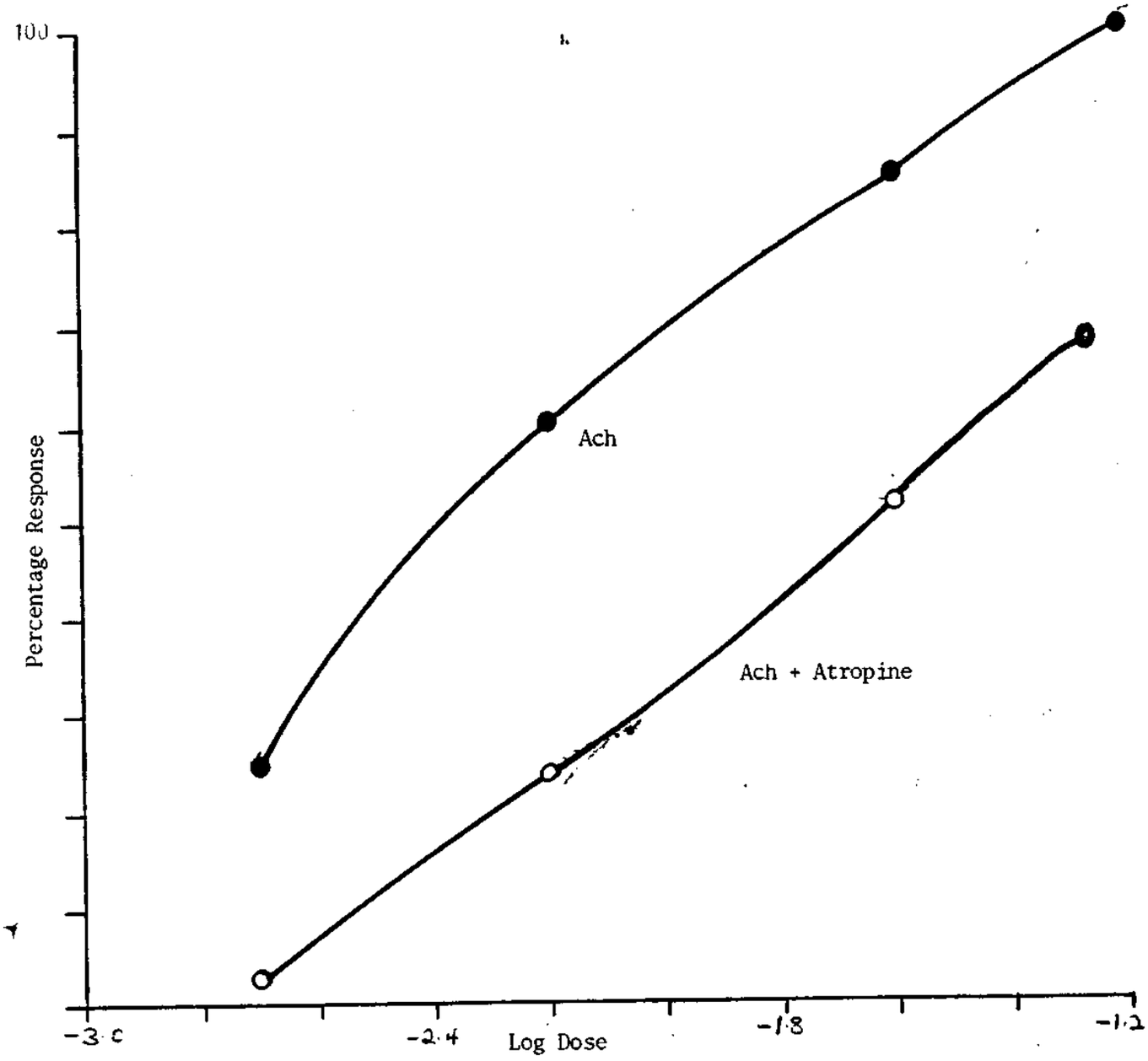


Figure 15: Dose Response Curve of Ach on Guinea Pig Ileum.

CHAPTER FOUR

DISCUSSION

4. DISCUSSION OF RESULTS.

4.1. THE PHYTOCHEMICAL SCREENING:

Chemical constituents of plants are known to be influenced by season, age and geographical location (Watt 1962). Variations also occur in the distribution of constituents in different organs of plants. Such variations may be both qualitative and quantitative . Subject to these factors the phytochemical screening of the fruit, bark and leaves of Erythrina senegalensis clearly showed the presence of alkaloids in high quantities. Alkaloids have been shown to be present in some other members of the family (Games et al 1974) while glycosides, saponins and anthraquinon were seen in moderate quantities . However, of the 108 species representing this family most have been shown to contain erythrina-type alkaloids. Interesting enough cocculus species family Menispermaceae also produce closely related alkaloids (Masouda et a 1991).

The weak reaction to the shinoda reduction test is suggestive of trace amounts of flavonoids. However, the positive Keller-Killiani test is indicative of steroids of cardiac glycosides type and the result of Borntrager's test is indicative of presence of only little amount of glycosidic anthraquinones. Negative results were obtained for tanins quinoline alkaloids and Indole alkaloids. It was also observed that alkaloids were highly present in the methanolic extract of bark of the plant than in the fruit and leaves while the petroleum spirit extract of Fruit and leaves also contained more alkaloids than the petroleum spirit of the bark.

4.2. METHANOLIC EXTRACT OF BARK

(a) Preliminary Separation -

The methanolic extract of the bark consist of a higher proportion of the plant material judging from the weights obtained - 205g to 160g and 120g of leaves and fruits respectively.

The methanolic extract of the bark was blue to litmus paper indicating that it is basic in nature. However, separation of the extract into fractions A, B, C, D and E showed that a high proportion of the extract was unrecovered from the chloroform soluble fractions A and the water soluble medium fraction C (Scheme 1).

Fraction D showed the presence of mainly phenolic bases as confirmed by simple chemical tests and TLC.

The non-phenolic bases fraction E had almost the same properties as those of petroleum spirit extract of leaves and fruits of the plant as can be seen later.

TLC examination of the fraction D of the methanolic extract of bark revealed the presence of at least five phenolic bases or alkaloids. These are spots that reacted with Dragendoff's reagent giving R_f values of 0.04, 0.14, 0.71, 0.82 and 0.93 as can be seen on table 10 (Stahl 1964).

A close look at the table showed that the least polar came out as oils while most polar came out as solids.

(b) Isolated Compounds:

Four compounds were isolated from the methanolic extract of the bark mainly $ESMB_1$, $ESMB_2$, $ESMB_3$ and $ESMB_4$ which

correspond to the spots giving orange with Dragendoff's and purple with Anisaldehyde in H_2SO_4. Their R_f values were 0.28, 0.41, 0.55 and 0.71 in n-butanol-acetic acid-water (4:1:5) and 0.14, 0.71, 0.82 and 0.93 in Benzene-ethylacetate-ammonia (20:10:2) as seen in tables 16 and 20 respectively. However, attempted separation and purification of $ESMB_1$ and $ESMB_2$ which came out as oils using distillation methods proved abortive.

Compound $ESMB_4$

The IR spectrum indicates a terminal methyl group with absorption at 1200cm^{-1} CH_3-bending, the methylene group - CH_2 - gave absorption at 1000cm^{-1} CH_2 rocking typical of methylene groups attached to ring structures with two or three bands , 1550cm^{-1} of C=C stretching of a benzene ring and C-H stretching of an aromatic ring structure with absorption at 2900cm^{-1}. The IR spectrum of $ESMB_4$ is seen in appendix (I) (William Kemp 1975).

The mass spectrum shows ions at m/z 256 as the highest mass (m^+) which is of relatively low intensity with a base peak of m/z 227 indicating loss of ethyl ion $C_2H_5^{\bullet}$ from the molecular ion . Other salient peaks are those of ($m^+ - CH_3$) with fragment ion at m/z 241 (Fig. 16). However, the base peaks in the mass spectra of the 11-methoxy and 11-trimethylsilyloxy - alkaloids are due to the characteristic losses of the CH_3O and CH_3OH or Me_3S_1O and Me_3S_1OH/ions respectively whilst other substantial ions are

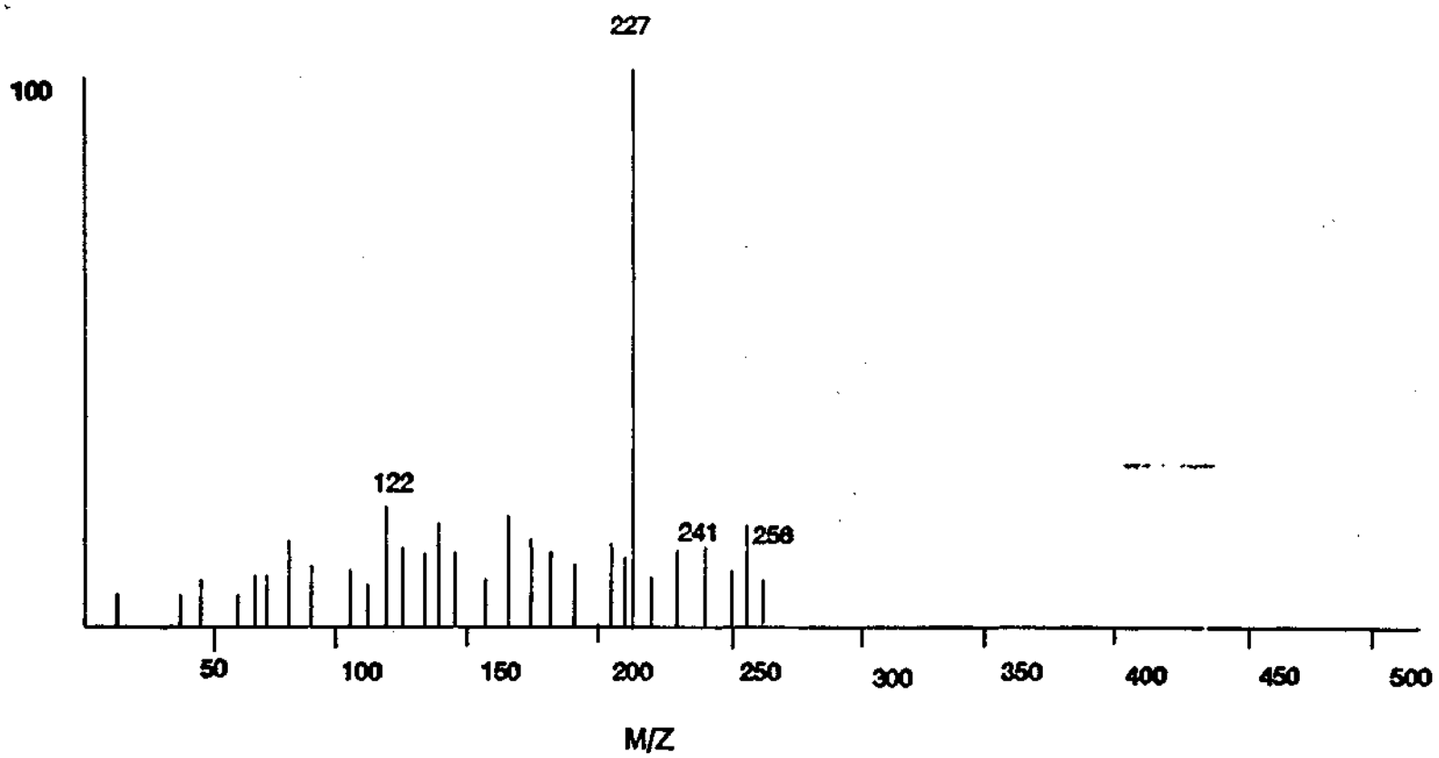
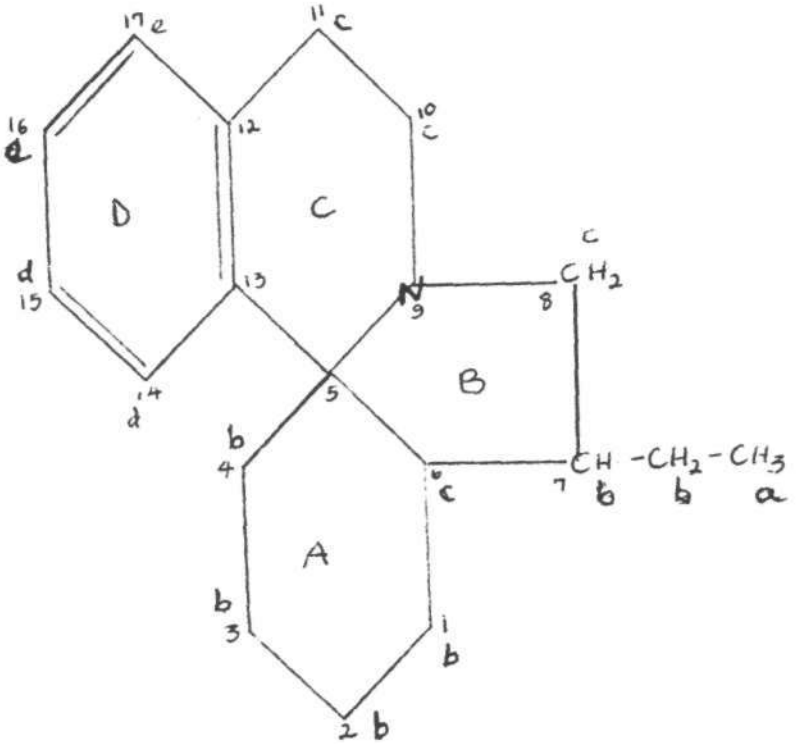


Fig. 16 : Mass Spectrum of ($ESMB_4$) 7-ethylerythrina

due to the cleavage of the methoxyl substituent in the ring A (Games et al 1974).

The nmr spectrum of erythrina alkaloid contained protons in at least five different electronic environments as can be seen in their chemical shifts in compound [10] Figure 17:

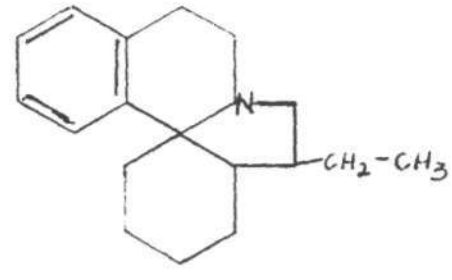


[10] $ESMB_4$

The triplet at δ 0.88(3H) is typical of a terminal methyl group adjacent to a methylene group as in (a). The multiplets at δ 1.26ppm (11 H) is obviously of the methylene groups as in (b) while the multiplets at δ1.9 (7H) are likely to be the methylene group at positions 8, 10 and 11 and probably due to steric hindrance especially at position 6 and the

van der vaals deshielding effect of the adjacent atoms and also the effect of $-CH_2-$ methylene group attached to Nitrogen atom, inductive effect of the nitrogen atom making the chemical shift resonate at a lower field.

The doublet at δ 6.50(2H) is suggestive of an aromatic ring structure as in ring D with protons in C_{14} and C_{15} coupling with each other as seen in Figure 17. Also the multiplets at 7.1(2H) is indicative of aromatic protons as in (e) . Hence both protons have the same electronic environment and would resonate at a lower field because of the anisotropic effect of the chemical bonds of the benzene ring. On the bases of the presented spectral information from the IR. NMR and MS compound $ESMB_4$ is an Erythrina alkaloid, hence compound $ESMB_4$ is assigned structure [10]



7-ethyl erythrina [10]

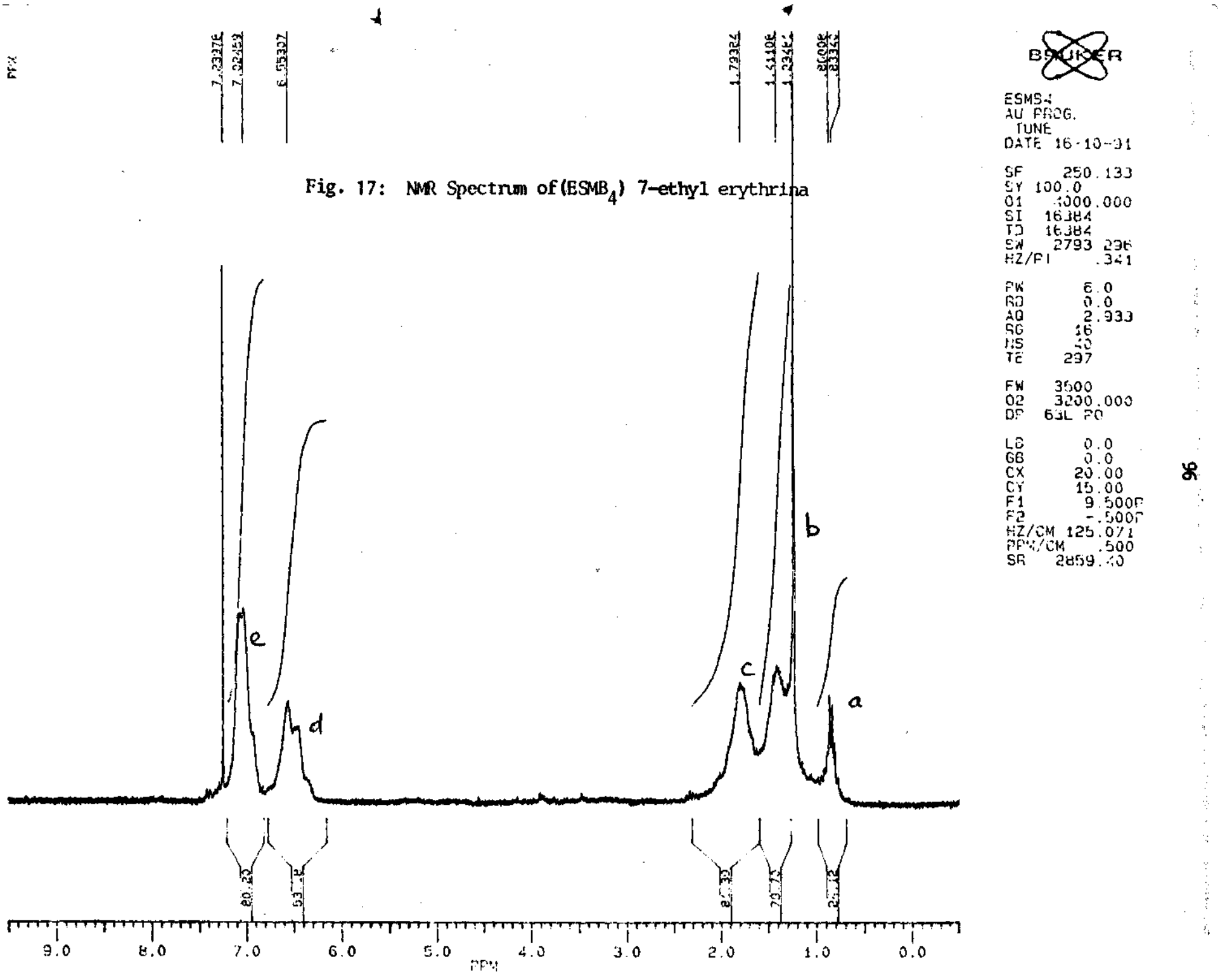


Fig. 17: NMR Spectrum of($ESMB_4$) 7-ethyl erythrina

NMR Spectrum of 7-ethylerythrina

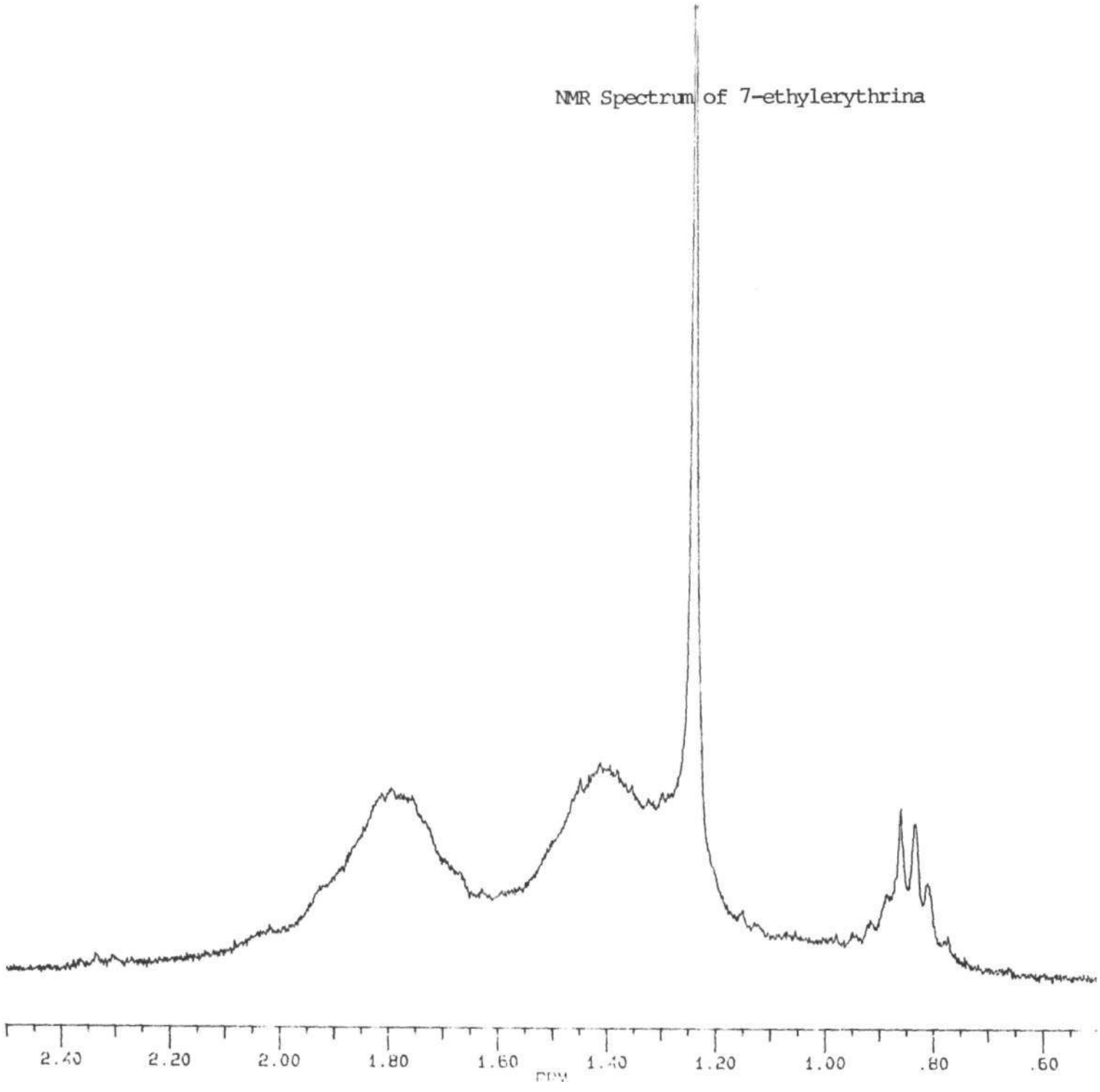


Fig. 17(b)

$ESMB_4$ is thus concluded to be 7-ethyl erythrina.

The spectra obtained compared favourably with literature (Masouda et al 1991) spectra of other related alkaloids of erythrina . Erythrina alkaloids are generally dextrorotatory and based on that, the optical rotation was found to be $[\alpha]_D^{25}$ + 119 ($CHCl_3$) with a chiral centre at C-5 and a melting point of 148 - 150°C.

Compound (10) $ESMB_4$ appears to be a relatively new compound isolated from the bark of this species of Erythrina alkaloids as no such compounds have been reported in literature to-date.

4.3. PETROLEUM SPIRIT EXTRACT:

(a) Preliminary Separation of Petroleum Spirit of the Fruit Extract -

The petroleum spirit of the fruit extract gave a large proportion of the yield 202g compared to 120g of the methanolic fruit extract with a brown colour and slightly basic in nature. Separation of the extracts into various fractions as seen in scheme 2 showed that a high porportion of the extract was unrecovered in the petroleum spirit extract and hence was not further processed, while the aqueous acidic fraction F contained most of this extract. The fraction F was further processed as in scheme 2 to yield the non-phenolic bases fraction of I (5.08g) and phenolic bases as in fraction H.

The phenolic bases gave almost the same properties as the methanolic extract of the bark and hence no further investigation was carried out. The TLC analysis of fraction I table 13 revealed the presence of at least four compounds

with R_f values of 0.21, 0.32, 0.50 and 0.93 with Anisaldehyde and Dragendoff's reagents for staining purposes (Egon Stahl 1969).

(b) Isolated Compound -

Two compounds were isolated from the petroleum spirit extract of the fruit namely $ESFP_1$ and $ESFP_2$ (as oils) which corresponds to the spots giving purple with Anisaldehyde, brown with Cerric ammonium sulphate and no reaction with Dragendoff's reagent (Table 24). Their R_f values were 0.82 and 0.73 in petroleum spirit 60 - 80°C benzene 9:1 respectively, while their R_f values using benzene ethyl acetate (7:3) were 0.88 and 0.69 respectively (Table 25). While $ESFP_1$ had no reaction with Dragendoff's reagent, $ESFP_2$ reacted with Dragendoff's as well as Anisaldehyde and Cerric ammonium sulphate reagents. The non reaction of $ESFP_1$ to Dragendoff's reagent was suggestive that the compound may not be an alkaloid while its reaction to Molybdophosphoric acid is suggestive that compound $ESFP_1$ may be a steroid (Egon Stahl 1969).

Compound $ESFP_1$:

The (IR) spectrum of the compound indicates a saturated fatty acid ester with absorptions at $730cm^{-1}$ - $(CH_2)n$-rocking, $1735cm^{-1}$ C=O stretching of COOR, $1179cm^{-1}$ C - O stretching and $2950cm^{-1}$ C-H stretching . The IR spectrum of $ESFP_1$, is shown in Appendix II (Freeman 1964, Gross 1964).

The mass spectrum shows ions at m/z 806 as the highest mass (m^+) which is of low intensity compared to other peaks as seen in figure 18.

The most abundant ions are m/z 522, 550, 578 which corresponds to the loss of acid groups in the molecule i.e. (m^+ - RCOOH) ion which are peaks for the ion of stearic acid molecule/(m^+ - 284), loss of palmitic acid (m^+ - 256) and loss of myristic acid (m^+ - 228). Mechanically this (m^+ - RCOOH) loss is the precursor of many of the fragment peaks of lower mass. This characteristic fragmentation was first noted by Ryhage . A set of peaks were also observed with 74 and 128 mass numbers higher than each acyl ion peak. These are peaks at m/z 285, 313 and 341 for (RCO + 74) ions and m/z 339, 367 and 395 for (RCO + 128) ions. These peaks are quite characteristic of acid being attached directly to the glycerol skeleton as can be seen in Reaction I (Ryhage 1960 and Borber 1964).

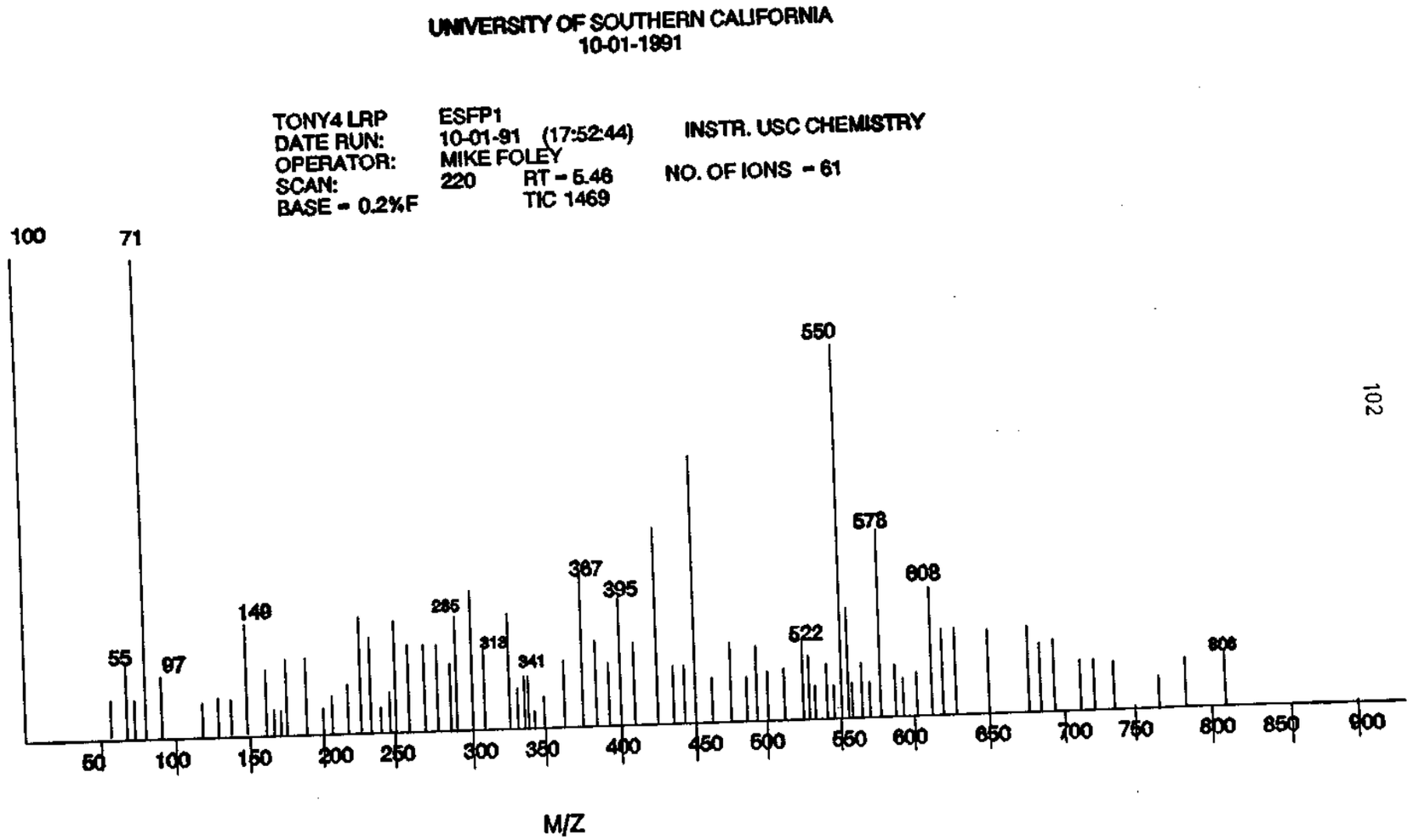
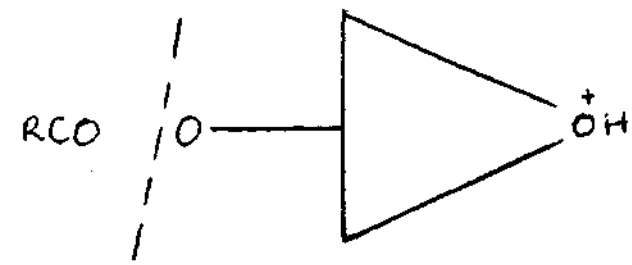
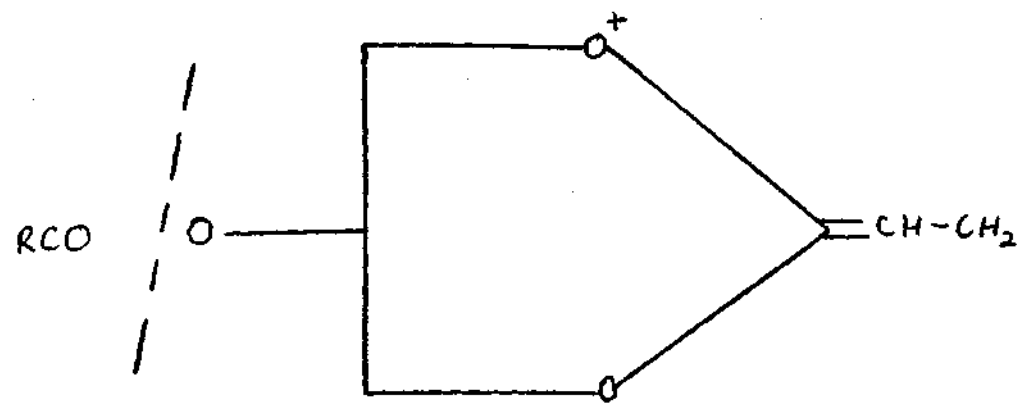


Fig. 18: Mass Spectrum of ($ESFP_1$) 1 myristo-2-stero-3-palmitin



(RCO + 74)



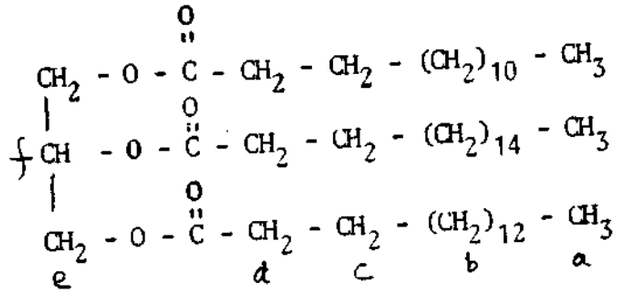
(RCO + 128)

REACTION (I)

The peak at m/z 788 is due to the loss of one molecule of water ($m^+ - 18$) indicating this to be due to ionic fragmentation and not thermal cracking. The other salient peaks in the spectrum of the triglyceride molecule are the hydrocarbon chains. The series $C_nH_{2n+1}^+$ ($C_4H_9^+$ to $C_6H_{13}^+$) and the series $C_nH_{2n-1}^+$ with m/z 71 as the base peak.

The nmr spectrum of $ESFP_1$ contained protons in at least five different electronic environments as can be seen from their chemical shifts in Figure 19. The triplet at δ 0.88 (9H) is typical of the terminal methyl groups of the different fatty acid because they all have the same electronic environment as in Compound (Mullen 1974) (11)

The singlet at δ 1.25 (74H) is typical of normal alkyl methylene groups as in (b). The broad singlet (triplet at



Compound (11)

δ 1.60 (6H) is obviously of the adjacent methylene group as in (c). The triplet at δ 2.30 (6H) is typical of a methylene groups

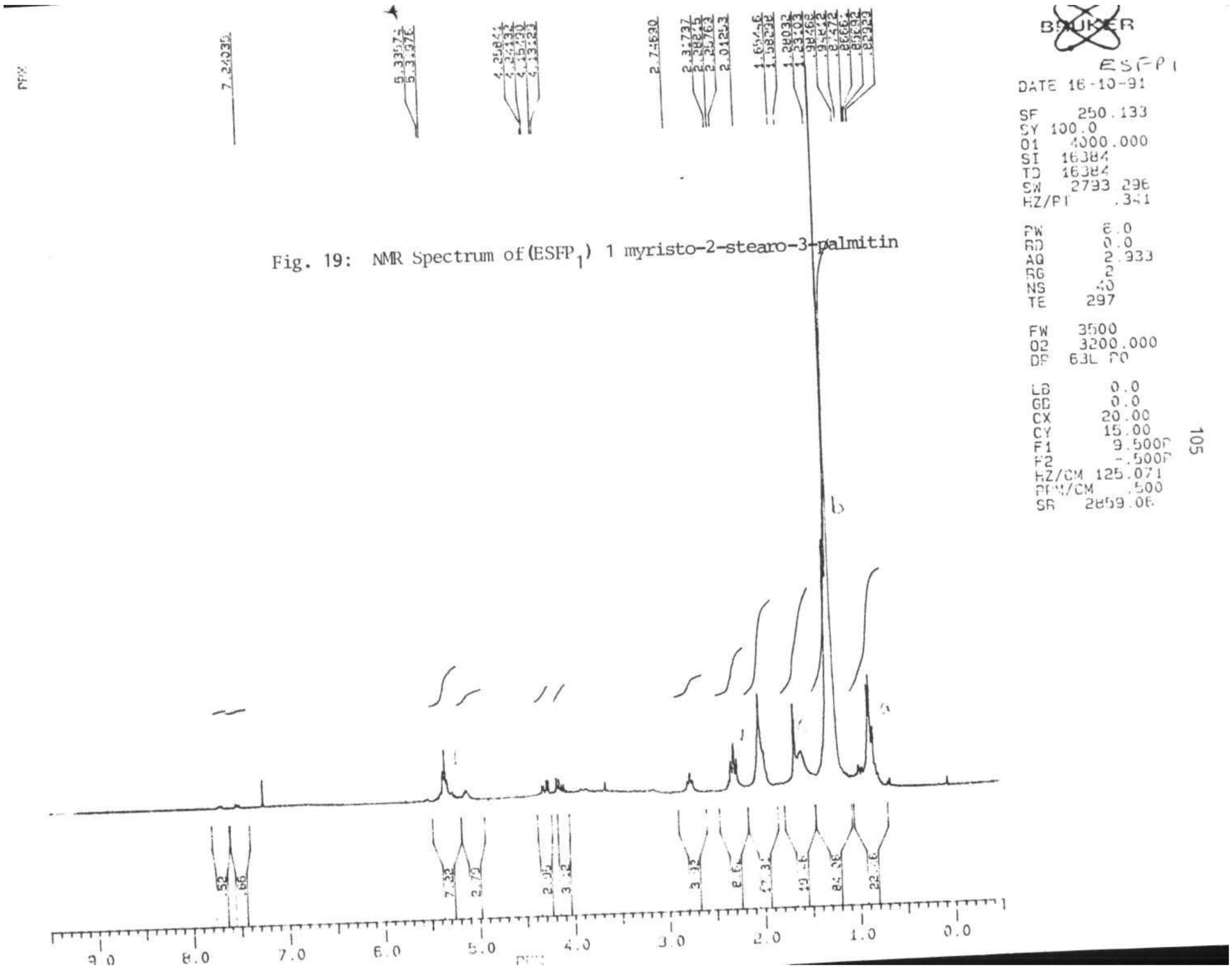


Fig. 19: NMR Spectrum of ($ESFP_1$) 1 myristo-2-stearo-3-palmitin

adjacent to a carbonyl as in (d). The double duplets at δ 4.10 (4H) indicates that the protons must be diamagnetically deshielded by the oxygen with some paramagnetic deshielding from the carbonyl function. The multiplicity of the signals shows that the two methylene protons couple to each other as well as couple with the adjacent methylene proton as in (e).

The multiplets at δ 5.26 ppm (1H) is typical of a proton attached to a tertiary carbon adjacent to an oxygen atom which indicates that the proton must possess some Van der Waals deshielding effect together with the diamagnetically deshielding effect of the oxygen and the paramagnetic deshielding from the carbonyl function. The multiplicity of the signals shows that the proton is adjacent to two methylene protons as in (f).

On the basis of the presented spectral information from the IR, NMR and MS the ester is a triglyceride. Hence compound $ESFP_1$ has been assigned structure [II].

Compound $ESFP_1$ is thus concluded to be 1 myristo-2-steoro-3-palmitin. The spectra data obtained compared favourably with the literature spectra (Barber 1964).

The melting point value obtained compared favourably with the literature value (Chapman 1962).

Value obtained	57° - 59°C
Literature value	58° - 60°C

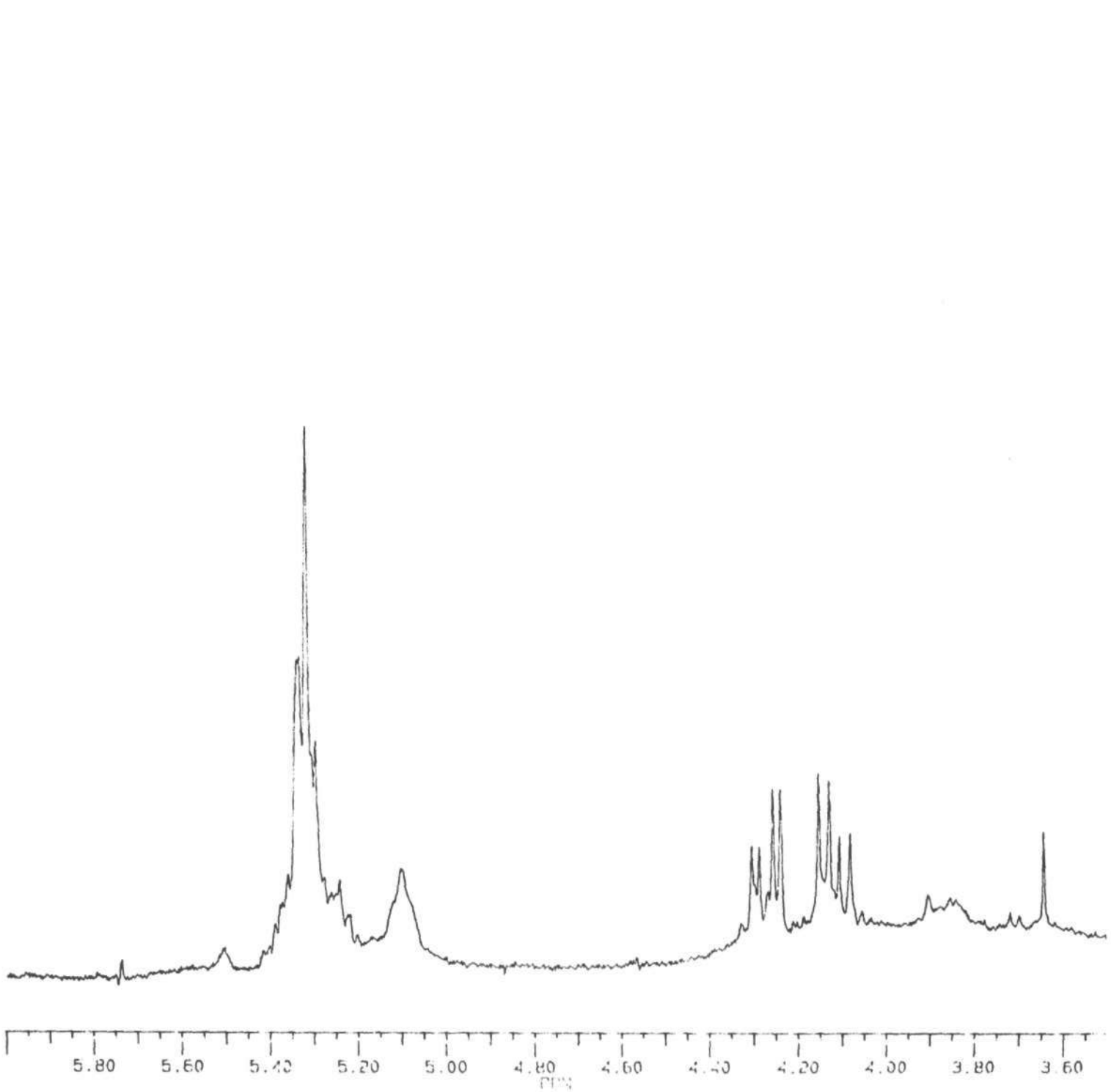

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RG 2
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FW 3500
O2 3200.000
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LB 0.0
GB 0.0
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CY 10.00
F1 6.0010
F2 3.5010
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PPM/CM .125
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5.60
5.40
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5.00
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4.60
4.40
4.20
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3.80
3.60
PPM

Fig. 19a.

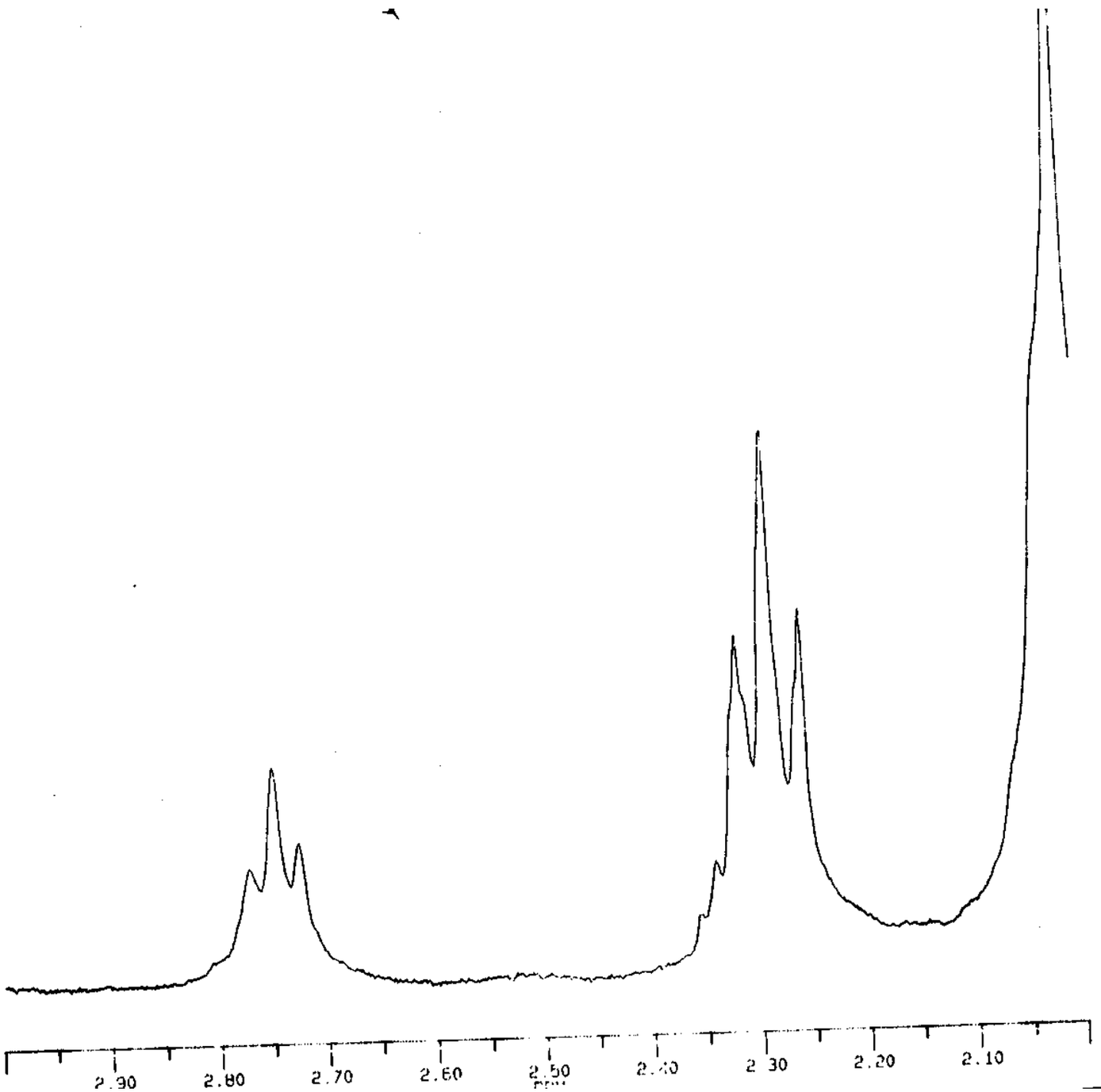

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Fig. 19b.

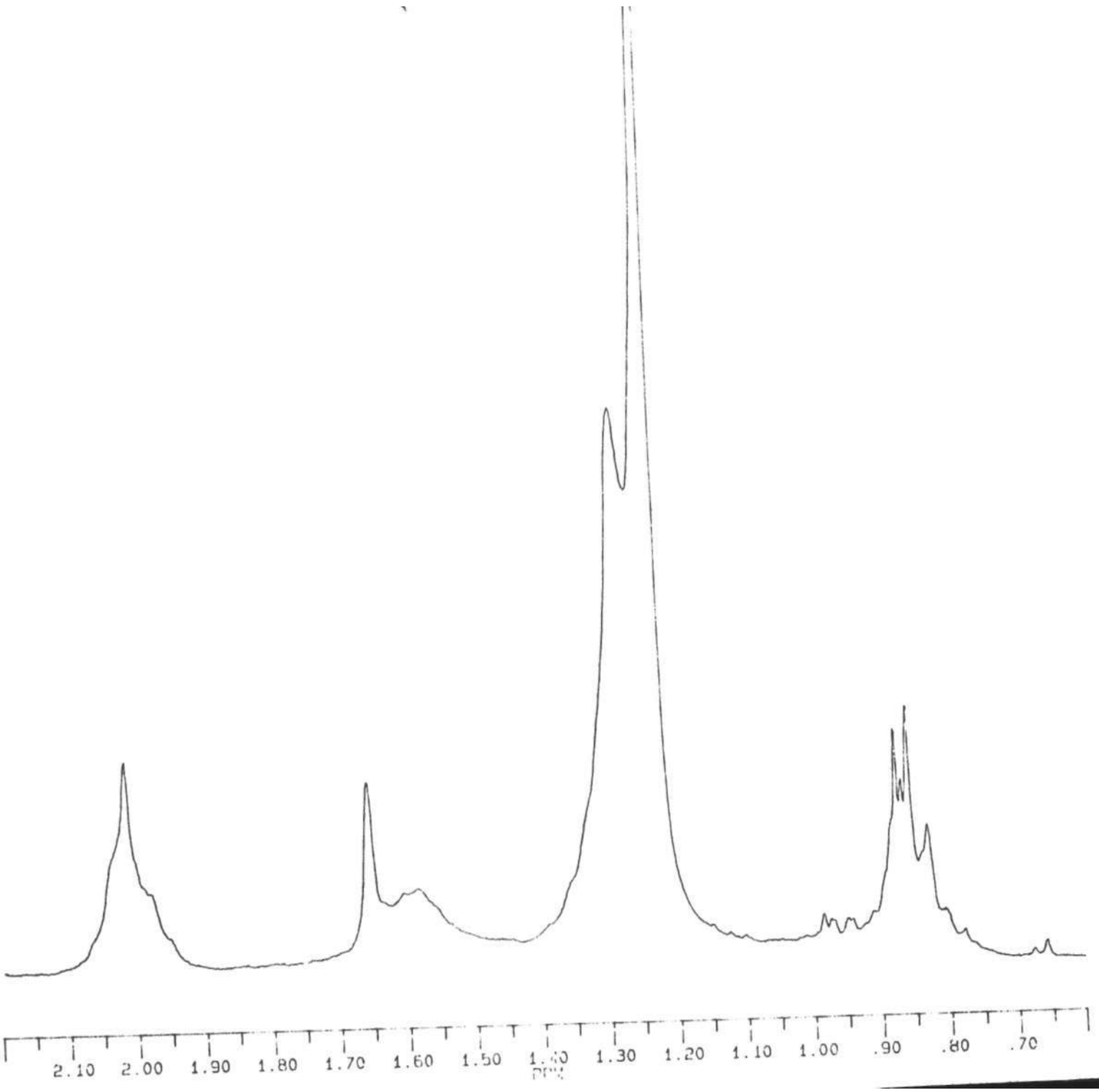

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PPM

Fig. 19c.



```
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HZ/PT      .341

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AQ        2.933
RG          2
NS         40
TE        297

FW     3500
O2     3200.000
DE    6U  PO

LB        0.0
GB        0.0
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F2        7.300P
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PPM/CM     .025
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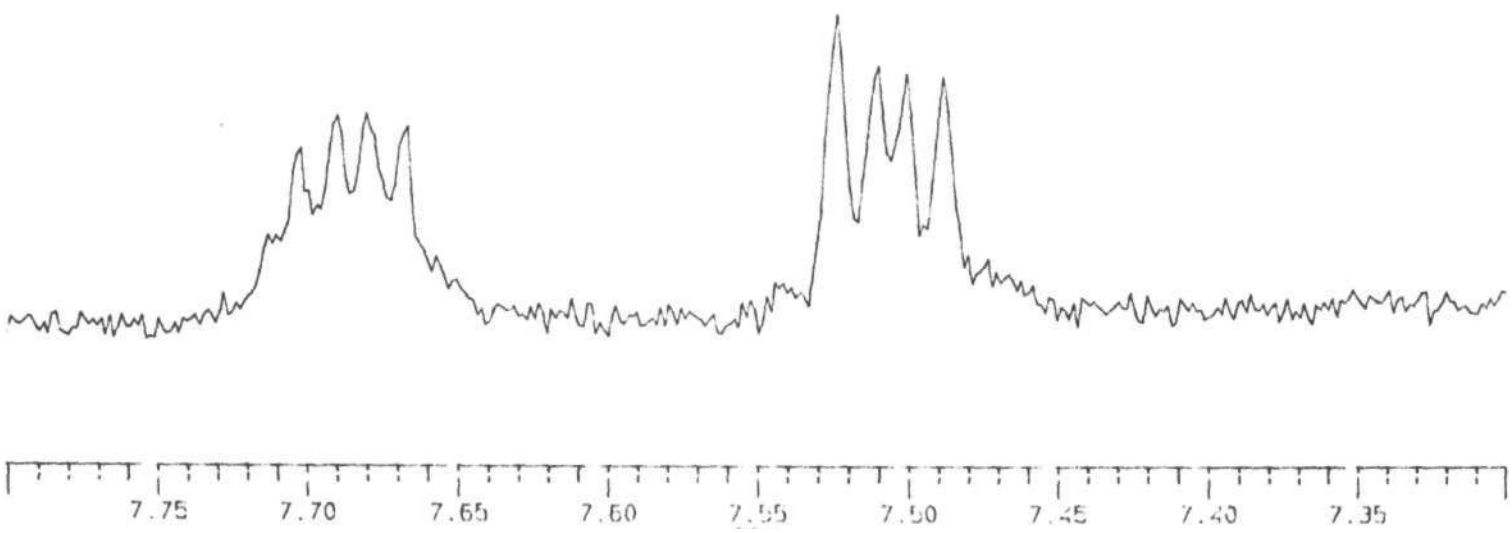


Fig. 19d.

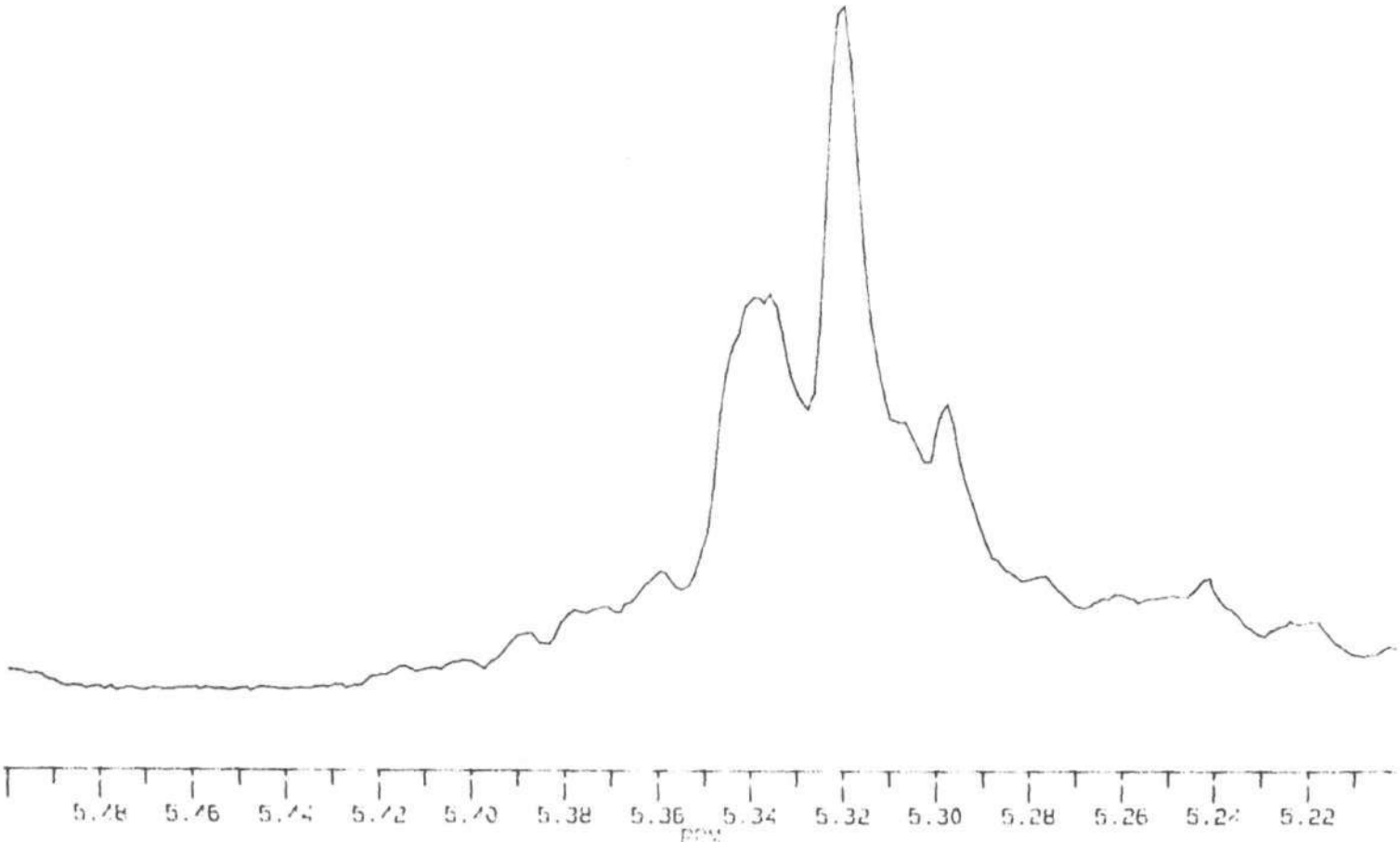

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RG 2
NS 32
TE 297
FW 3500
O2 3200.000
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LB 0.0
GB 0.0
CX 20.00
CY 10.00
F1 5.501
F2 5.201
HZ/CM 3.751
PPM/CM .015
SR 2859.06
5.48 5.46 5.44 5.42 5.40 5.38 5.36 5.34 5.32 5.30 5.28 5.26 5.24 5.22
PPM

Fig. 19e.

(c) Preliminary Separation of Petroleum Spirit Extract of the Leave:

The petroleum spirit extract of the leaves gave a high proportion of the yield 265g compared to its methanolic extract of 160g revealing that more of the constituents are present in the leaves.

The leave extract of the petroleum spirit was found to be slightly acidic in nature and gave a brown colour.

Preliminary separation into various fractions as can be seen in scheme 2 gave largely the non-phenolic fraction I of the leaves with 7.04g. TLC analysis of fraction I table 15 revealed the presence of at least six different components judging from their R_f values of 0.01, 0.02, 0.25, 0.50, 0.71 and 0.86 using benzene-ethylacetate (7 : 3) as solvent system while Dragendorff's was used for staining purposes.

Fraction I was further subjected to column chromatography for further separation to give three major components of $ESPL_1$, $ESPL_2$ and $ESPL_3$ as can be seen in table 23 and table 24. $ESPL_1$ and $ESPL_2$ came out as oils and had R_f values of 0.25 and 0.45 using benzene : ethylacetate (7 : 3) and both reacted with cerric ammonium sulphate,

anisaldehyde and Dragendorff's reagent. However, no further characterisation was carried out as purification with distillation method proved abortive.

$ESPL_2$ gave an R_f values of 0.61 using benzene : ethyl-acetate (7 : 3) and 0.75 with petroleum spirit 60-80°C : benzene (9 : 1) Tables 25 and 26.

$ESPL_3$ reacted with anisaldehyde, cerric ammonium sulphate and molybdophosphoric acid but did not react with dragendorff's reagent revealing that compound $ESPL_3$ may not be an alkaloid.

4.4. PHARMACOLOGICAL SCREENING:

4.4.1. Effect of Methanolic Extract on Rabbit Jejunum:

Methanolic extracts of E. senegalensis on smooth muscle of rabbit jejunum shows that the fruit and leaves methanolic extract relax rhythmic contraction or pendular movement of the smooth muscle (Ogbeche et al 1993).

This relaxation of the pendular movement is similar to the response produced by administration of adrenaline (a sympathomimetic drug) at almost same concentration. The acetylcholine used however produced its characteristic contractions on the intestinal smooth muscle and when compared to the response of the crude methanolic extracts of the fruit and leaves (Figures 22 and 23). It also showed that the crude extract had no parasympathomimetic action on the smooth muscle Adrenaline relaxes the smooth muscle but stimulates the sphincters of the gastrointestinal tract (James 1971).

The crude drug therefore has sympathomimetic effects as it also relaxes the smooth muscle. These effects resemble those of activity in the sympathetic nervous system. The term includes drugs that act directly on adrenoceptors in tissue (Directly acting sympathomimetic) as well as those that act indirectly by evoking the release of noraldrenaline from sympathetic nerve terminals (Indirectly acting sympathomimetic drugs). From the chemistry and Structural-Activity

Relationship of the directly acting sympathomimetic to produce its action, it must have an aromatic ring, α-and β-carbon atoms and a terminal amino group all linked together (Doerge 198 These structural features may be present in Erythrina alkaloids which are the main constituent present in Erythrina senegalensis . It also means that the crude extract can also produce its pharmacological action by indirect means or by other mechanism of action.

There are three main mechanism by which drugs can cause smooth muscle relaxation (Rand 1987).

(i) By interfering with calcium ion (Ca^{++}) entry.

(ii) By increasing intracellular cAMP (Adrenosine 3',5' - monophosphate) concentration.

(iii) By increasing intracellular cGMP (cyclic guanine monophosphate) concentration.

Drugs that interfere with Ca^{++} entry may do so directly (e.g. Ca antagonists) by blocking receptor-operated calcium channels (ROCs) or potential-operated calcium channels (POGs), Papaverine [13], owes part of its action to a block of Ca channels. Alternatively, drugs may cause hyperpolarization of the cells, which tends to switch off POCs and secondarily reduce Ca^{++} entry. This occurs in visceral, but not in vascular, smooth muscle. Drugs that work by increasing intracellular cAMP concentration include adrenoceptor agonists,

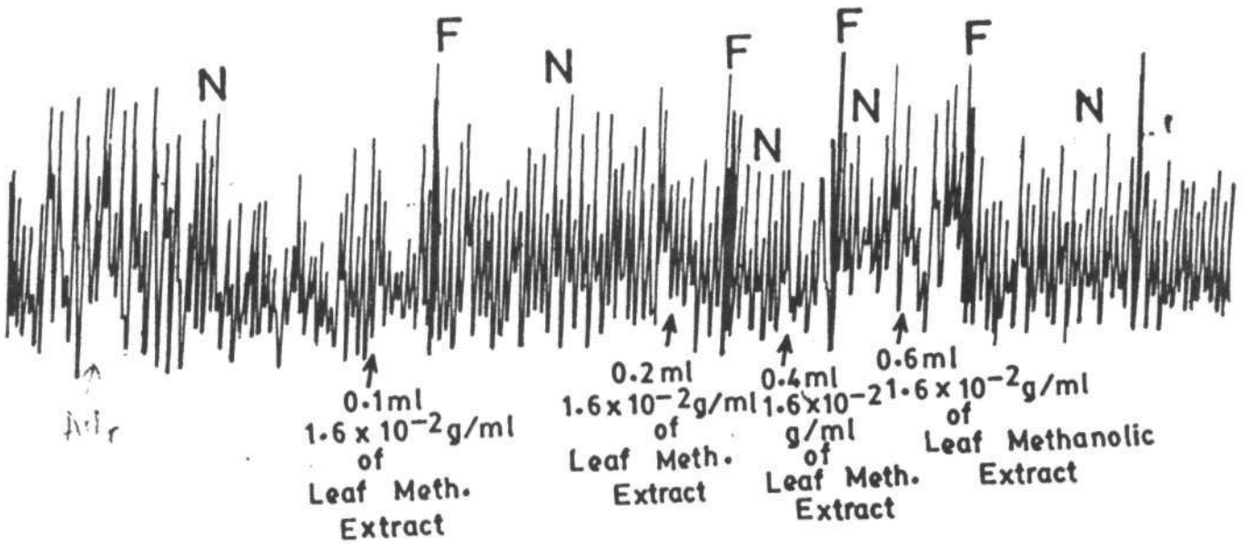


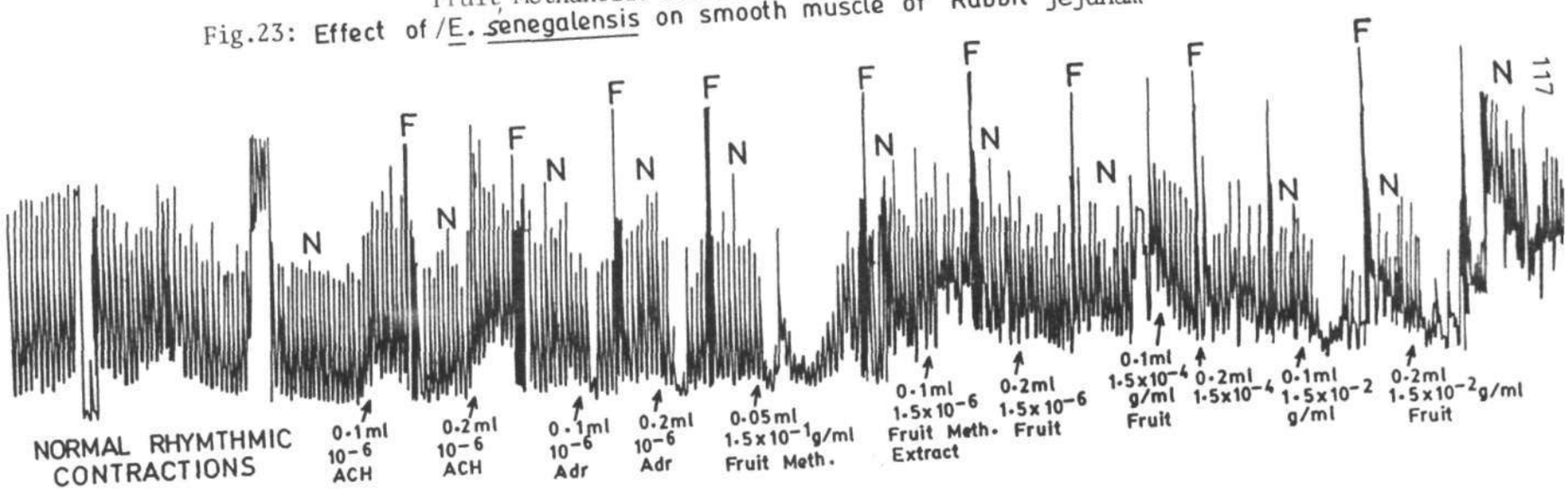
Fig. 22: Effect of E. senegalensis on smooth muscle of Rabbit jejunum

Key:

N = Normal rythymic contractions

F = Flush

Fig.23: Effect of Fruit Methanolic Extract of /E. senegalensis on smooth muscle of Rabbit jejunum

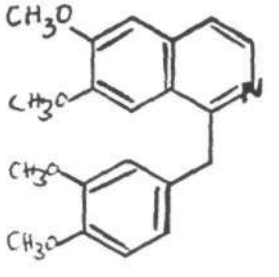


Key:

N = Normal rythymic contractions

F = Flush

methylxanthines , adenosine and dopamine. Organic nitrates and nitroprusside relax smooth muscle by increasing intracellular cGMP. It is noted that of the drugs listed above papaverine [13], have an isoquinoline structure similar to Erythrina alkaloids which (Folkers 1937)



Papaverine [13]

is very close to N-Nororientalline, and N-Norprotosinomenine. Papaverine is a drug closely related to morphine. Pharmacologically it is quite unlike morphine, it relax all smooth muscle including that of the arterioles . The mechanism of action by which papaverine acts is probably similar to that of the crude extract since they may be structurally related. This mechanism of action involves a combination of phosphodiesterase inhibition (as with methylxanthine) - increasing cAMP concentration and blockade of calcium channels (Schild 1980).

4.4.2. Effect of Methanolic Extract on Guinea Pig Ileum:

In contrast to the effect of methanolic extract of leaves and fruits of Erythrina senegalensis on the smooth muscle of rabbit jejunum, the methanolic extract of the bark and leaves of E. senegalensis contracted the isolated guinea pig ileum just like Acetylcholine (Figs. 24 and 25). The contraction produced by the crude methanolic extract of the bark and the leaves were reduced by antagonist atropine. The quantitative aspects of the drug Ach and crude extracts antagonism were readily analysed from the log dose-effect curves in figures 12, 13, 14, and 15 respectively.

The contractile responses of the isolated guinea pig ileum to leaves extract and acetylcholine as standard in the absence and presence of 10μg/ml of competitive antagonist, atropine in the medium bathing of the isolated tissue (Figs. 12 and 13) revealed that the two curves are identical in shape and attain the same maximum but when atropine is present it is as if the leaves extract and acetylcholine had become less potent, since much more was required to produce responses equal in magnitude to those elicited before the addition of the antagonist.

The contractile responses of the isolated guinea pig ileum to the methanolic bark extract and Acetylcholine (Figs. 14 and 15) also had similar effects as their actions were blocked by a competitive antagonist atropine. Atropine has affinity

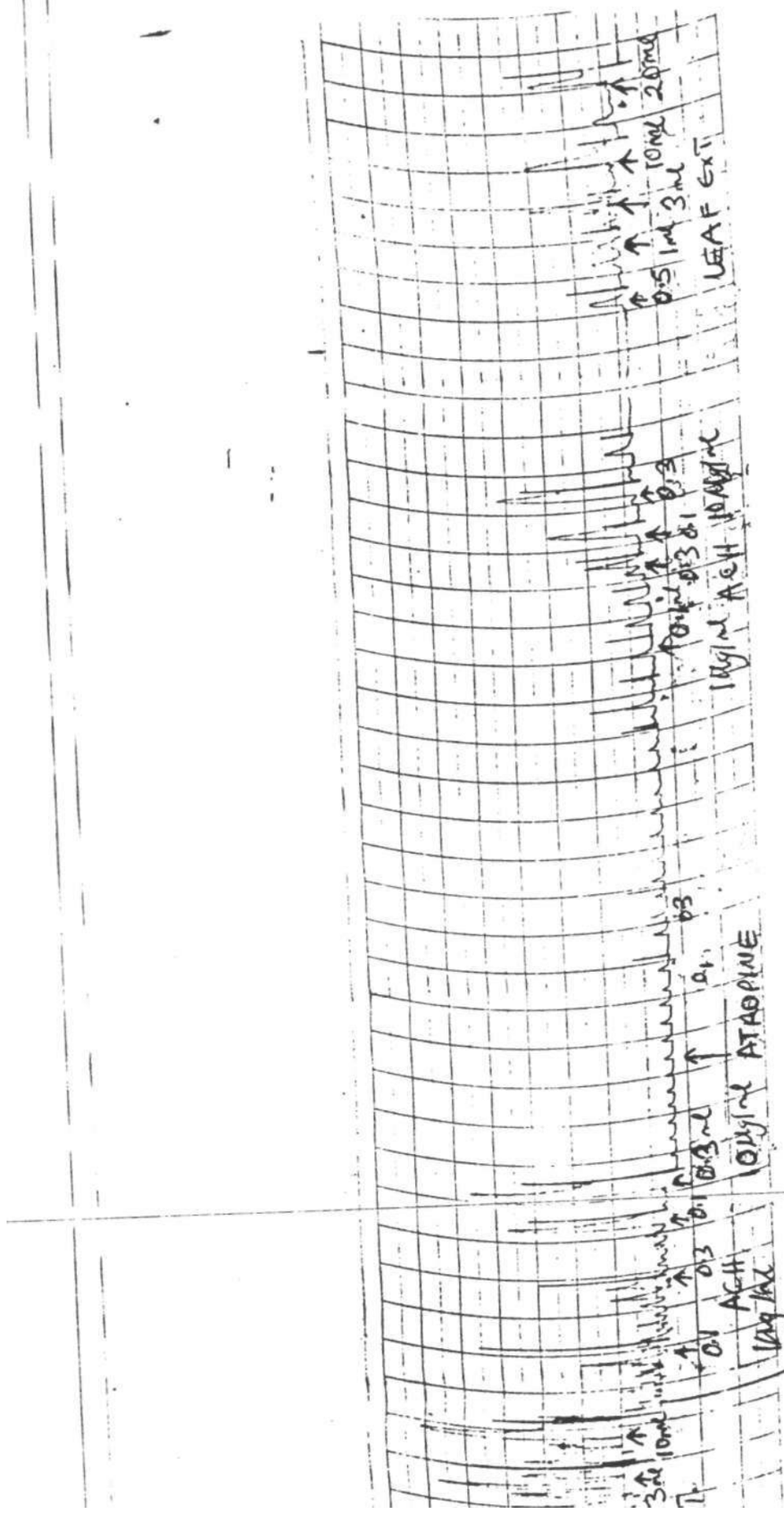


Fig. 24: Effect of Methanolic Extract of Leaf on Guinea Pig Ileum

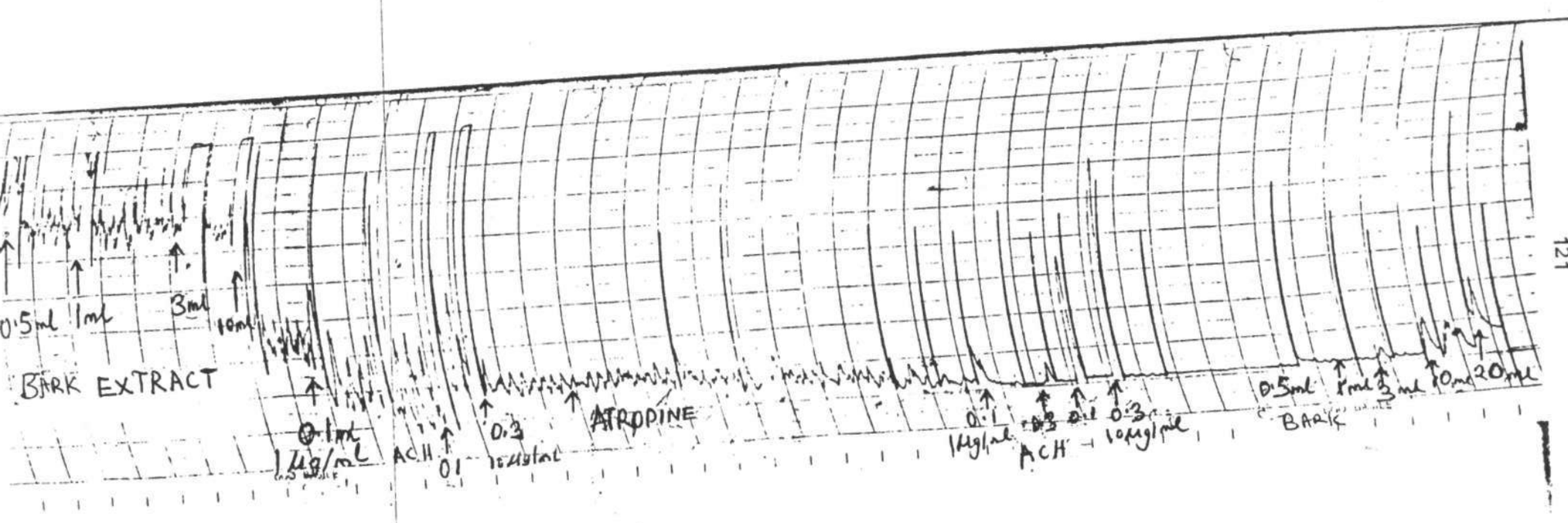


Fig. 25: Effect of Methanolic Extract of bark on Guinea Pig Ileum

for the acetylcholine receptor and it is able to combine with it . But the atropine receptor combination by itself produces no biologic response, atropine merely decreases the number of receptors available for occupancy by acetylcholine and the crude methanolic extracts hence the response to a particular dose of acetylcholine or the crude methanolic extracts were reduced as seen in Tables 33, 34, 35 and 36.

As the dose of the methanolic leaves and fruit extracts with acetylcholine was increased the agonist displaced atropine from the receptors (Figs 24 and 25) until all the receptors were occupied and a maximal contractile response obtained.

The log dose-effect curve for methanolic leave and bark extracts and also that of acetylcholine in the presence of a competitive antagonist atropine were shifted to the right indicating an alteration in the effective affinity of the agonist for its receptors. However, non-competitive antagonists may also produce its effect by combining either with the same sites as the active drug or with different sites in a manner that alters the agonist capacity to combine with its own receptor sites. Eventhough non-competitive antagonists cannot be influenced by increasing concentrations of the agonist, the effect of the antagonist may be reversible in reversible non-competitive antagonism (Ruth 1990).

Atropine is effective in antagonising the muscarinic effects of acetylcholine accumulating after administration of an anticholinesterase as it is in preventing the action of exogenous acetylcholine; It also blocks the muscarinic actions of other choline esters and the response to stimulation of parasympathetic nerves (James 1971).

4.5. EFFECT OF METHANOLIC EXTRACTS ON SKELETAL MUSCLE OF FROG RECTUS ABDOMINIS:

For bioassay of the leaves, fruits and bark of methanolic extract of Erythrina senegalensis shows a gradual increase in neuromuscular blockade as the dose of the extract was increased (Figs. 26, 27 and 28). This blockade occurs at the application of acetylcholine (which produces contraction) and methanolic extract of the leaves and fruits to the skeletal muscle of a frog (Ogbeche 1993).

Drugs can block neuromuscular transmission in three main ways:

i) By interfering with acetylcholine (Ach) synthesis;

ii) By interfering with acetylcholine (Ach) release.

iii) By interfering with the post-synaptic action of acetylcholine. This category include all of the clinical useful drugs and may be further subdivided into:

(a) Non-depolarizing (competitive) blocking agents, which act by blocking acetylcholine nicotinic receptors (and, in some cases, also by blocking ion channels).

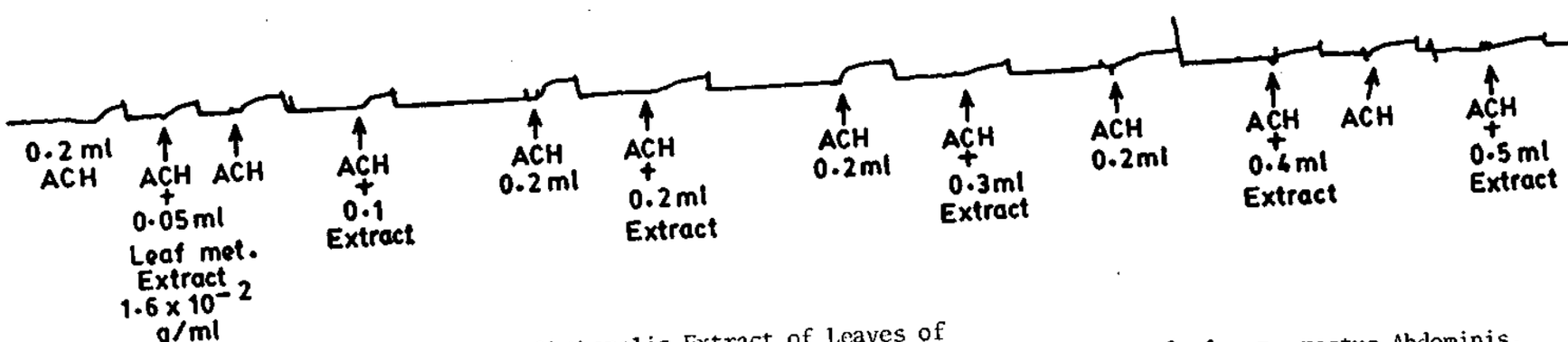


Fig. 26: Effect of /E. Senegalensis on skeletal muscle of frog Methanolic Extract of Leaves of rectus Abdominis.

(b) Depolarising blocking agents, which are agonists at acetylcholine receptors (nicotinic).

The crude extracts specifically blocked acetylcholine induced spasms. The spasmolytic ED_{50} against acetylcholine for methanolic leave extract was 1.26×10^{-4}g/ml, for fruits extract was 6.61×10^{-4}g/ml and for bark extract was 2.85×10^{-7}g/ml (Figs. 9, 10 and 11;

The potency ratio relative to methanolic fruit extract was 3.46×10^{-4}g/ml while that of leave extract was 1.82×10^{-3}g/ml and the bark extract was 8.035×10^{-1}g/ml. Since the potency ratio was less than 1 tubocurarine was found to be more potent than the crude methanolic extract of fruit and leaves of E. senegalensis, while the bark extract had similar potency to D-Tubocurarine.

The relatively high potency of the bark extract may be due to the high content of alkaloid obtained during extraction process. (Hargreaves 1974).

It has been shown that Erythrina alkaloids have curare-like activity Hence the crude extract must have produced its curariform action by diminishing progressively the amplitude of the End-plate potentials (EPP) as the concentration was increasing (James 1971).

Nerve Action potential (AP) ——> Acetylcholine release ——> Depolarization (EPP) (Increased permeability to Na and K)

↑ ↑ ↑

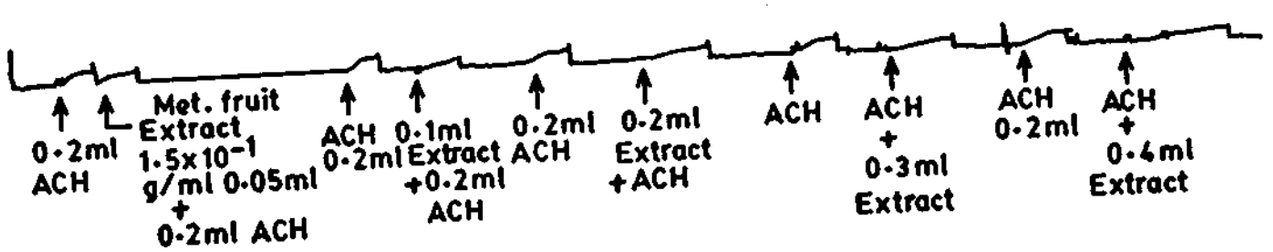
Blockade by Curare and alkaloid.

——>muscle action potential (MAP) ——> Spread of excitation in muscle ——> Muscle contraction via Transverse tubular system (TTS)

Scheme 3 - Mechanism for neuromuscular blockade.

Methanolic Extract of Leaves of

Fig.27: **Effect of /E. senegalensis on skeletal muscle of frog** rectus abdominis;



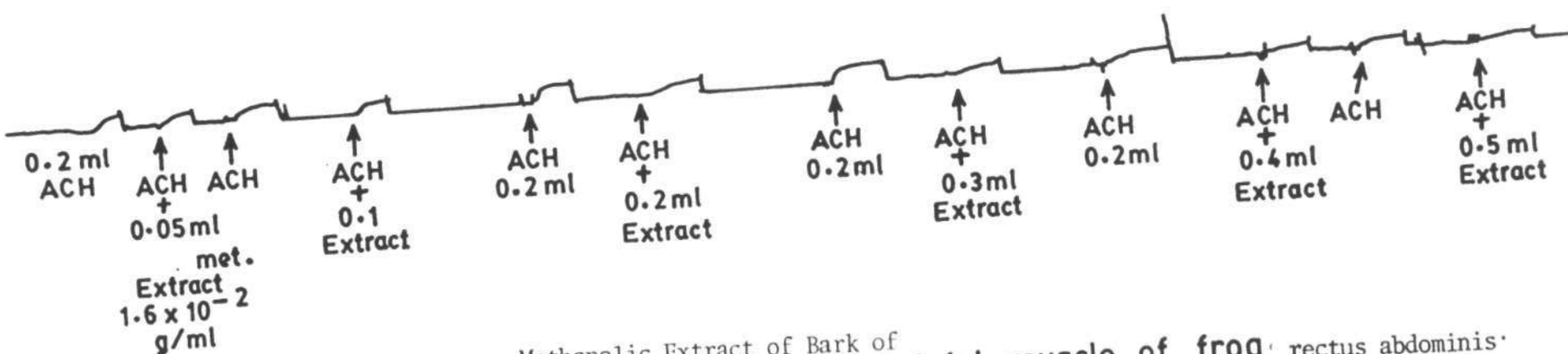


Fig.28: Effect of Methanolic Extract of Bark of *E. senegalensis* on skeletal muscle of frog rectus abdominis.

Contraction of the crude extract may be by blockade of ion channels. At higher concentration curare and other competitive antagonist will block the channel directly in a fashion that is non-competitive with agonist like acetylcholine. The magnitude of this inhibition is dependent on membrane potential (Colquohoun et al, 1979) .

The structural activity relationship of competitive agents shows that they are mostly relatively bulky, rigid molecules (for example, tubocurarine, gallamine ɣ-β erythroidine), whereas the depolarizing agents (for example, decamethonium, succinylcholine) generally have a more flexible structure that enables free rotation .

The functional relationship of curare to acetylcholine focuses attention on the role of quaternary ammonium groups; Many well known drugs (for example, atropine, quinine) show a marked increase in neuromuscular blocking potency when their nitrogen atom is quaternized. On the other hand, many non-quaternary ammonium compounds block the neuromuscular junction (for example, nicotine, ɑ- erythroidine) . The neuromuscular blocking activity of ɑ- erythroidine is actually abolished by quaterization of the nitrogen atom (Alfred Goodman 1990)

In traditional medicine practice Erythrina senegalensis is said to be used to cure jaundice, malaria, dysentery, colitis, gonorrhoea and also as a diuretic. The presence of secondary metabolites particularly of the alkaloid type is probably responsible for the smooth muscle relaxant properties of the

extract. Thus Erythrina alkaloids as probably responsible for relieving dysentery and colitis.

CHAPTER FIVE

CONCLUSION

5.1. CONCLUSION:

Preliminary phytochemical screening carried out on the crude extracts of the fruits, leaves and bark of Erythrina senegalensis revealed the presence of alkaloid in high quantities and glycoside, saponins, steroidal substances in moderate quantities.

Much work has been done on Erythrina senegalensis showing mainly alkaloidal constituents called erythrina alkaloid (Masouda 1991). A number of these alkaloids are to be phenolic in nature some in form of Sulpho-acetic ester or of glycosides. This probably explains why it was positive to general glycoside test and also to borntragers test for anthraquinones as most antraquinones are phenolic in nature.

Based on this information the bark of Erythrina senegalensis contained among others 7-ethyl erythrina while the fruit contained 1-myristo-2-stearo-3-palmitin and the leaves contained n-octa decanoic acid;. 1 myristo-2-stearo-3-palmitin and stearic acid have also been found in other plant families such as Cnestis ferruginea D.C (Ogbeche 1987).

In traditional medicine Erythrina senegalensis is said to cure jaundice, malaria, dysentery, gonorrhoea and also used as diuretic. The diuretic action could be attributed to the steroidal resemblance of the isolated compound 7-ethyl erythrina as it is a tetracyclic alkaloid.

General bioactive agents are mostly phenolic substances, terpenoids, alkaloids or acidic in nature, this might also explain why the isolated compound 7-ethyl erythrina 1-myristo-2-stearo-3-palmitin and stearic acid could be a precursor used in treating infections such as gonorrhoea as mentioned earlier.

Alkaloids are basic nitrogenous compounds of plant origin that have a physiological activity. The total amount and relative proportion of the alkaloid changing greatly as condition of cultivation and season changes.

However, the above preliminary description of the chemical components of the plant of Erythrina senegalensis is by no means exhaustive and only represents a small fraction towards the correlation and evaluation of the medicinal significance of the plant species.

The pharmacological screening revealed that the methanolic extract of fruits and leaves of Erythrina senegalensis relaxed smooth muscle of rabbit jejunum and the skeletal muscle of the frog rectus abdominis; The bark and fruit infusion with spices and honey is widely used as muscle relaxant (Dalziel 1955).

The relaxation of the pendular movement caused by the crude extracts on rabbit jejunum is similar to the response produced by the administration of adrenaline a sympathomimetic drug; The acetylcholine used however, produced its characteristic contractions on the intestinal smooth muscle and when compared to the response of the crude methanolic extract of

the fruit, and leaves showed that the crude extracts had no parasympathomimetic effects on the smooth muscle.

However, the fruits, leaves and bark of the methanolic extract of E. senegalensis showed a gradual increase in neuromuscular blockade as the dose of the crude extract increased on frog rectus abdominis (Ogbeche 1993). This blockade occurs at the application of acetylcholine which produces contraction and the methanolic extracts of the plant materials on skeletal muscle of a frog using D-tubocurarine as reference standard. Erythrina alkaloids have been shown to contain curariform activity and the first curarizing erythrina alkaloid was obtained from the seeds of E. americana (Folkers 1941).

Tubocurarine has curarizing activities and its structure is similar to erythrina alkaloids which are bases, hence the crude extract must have produced its curariform action by diminishing progressively the amplitude of the end plate potential.

The crude extracts specifically blocked acetylcholine induced spasms. The spamolytic ED_{50} against acetylcholine for leave extract was 1.26×10^{-4}g/ml, for fruits extract 6.61×10^{-4}g/ml and for bark extract 2.85×10^{-7} as against 2.29×10^{-7}g/ml for tubocurarine. The potency ratios relative to methanolic fruit extract was 3.64×10^{-4} while that of leave extract was 1.82×10^{-3} and the bark extract was 8.035×10^{-1}

From this one can conclude that the methanolic extract of the bark is more potent than the crude extracts of the fruits and leaves of E. senegalensis and has almost the same potency as tubocurarine. This could be attributed to the high content of alkaloid in the methanolic extract.

The effect of crude methanolic extract of leaves and bark of E. senegalensis on guinea pig ileum showed that the crude extract had muscarinic effect on the smooth muscle just like acetylcholine as it contracted the smooth muscle. However, atropine antagonised the muscarinic action of acetylcholine and the crude extracts. Thus the log dose-effect curve for crude extracts of bark and leaves with acetylcholine in the presence of atropine was shifted to the right indicating an alteration in the effective affinity of the agonist for its receptors and also that the effect of atropine on the crude extracts is a competitive antagonism.

CHAPTER SIX

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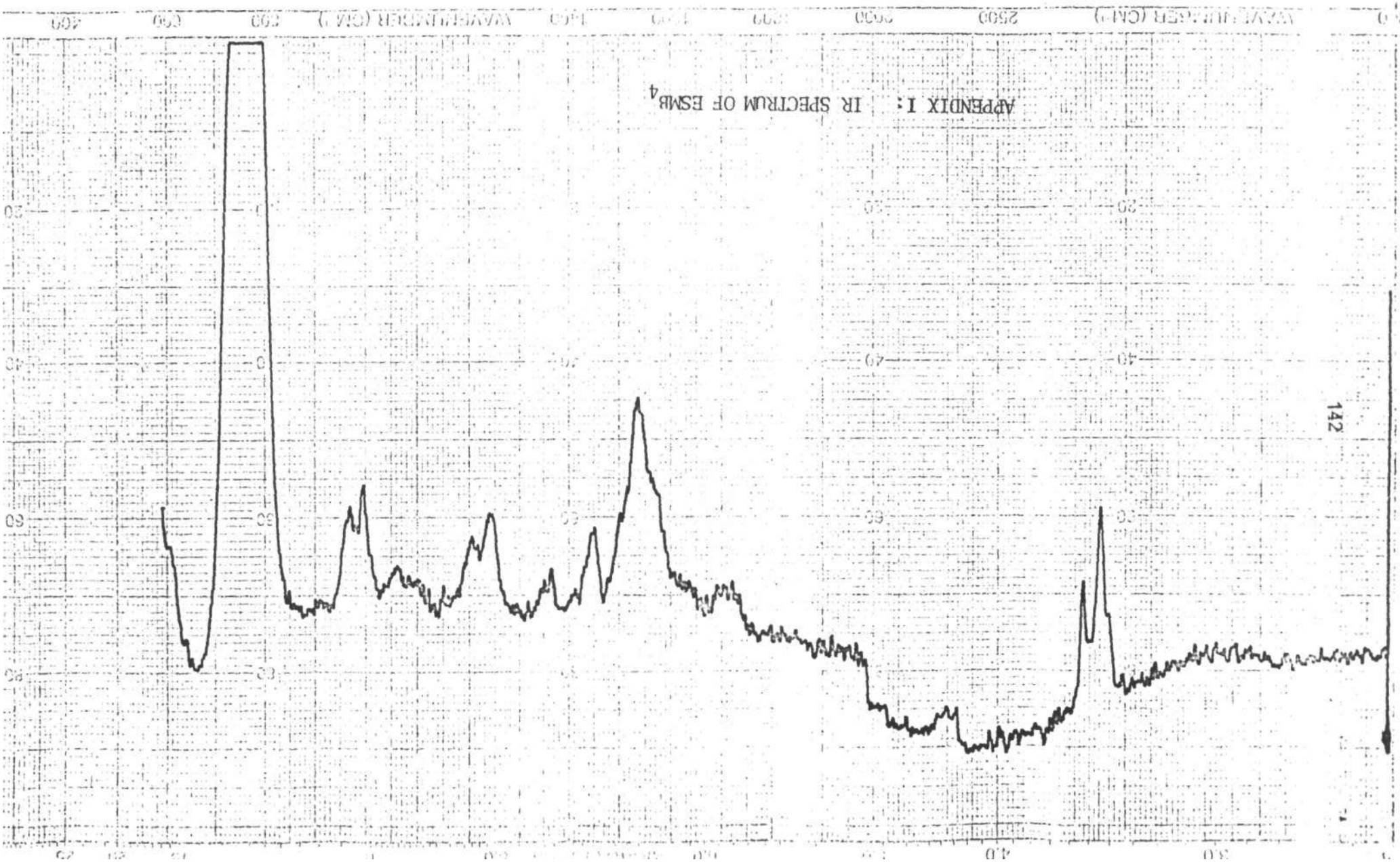
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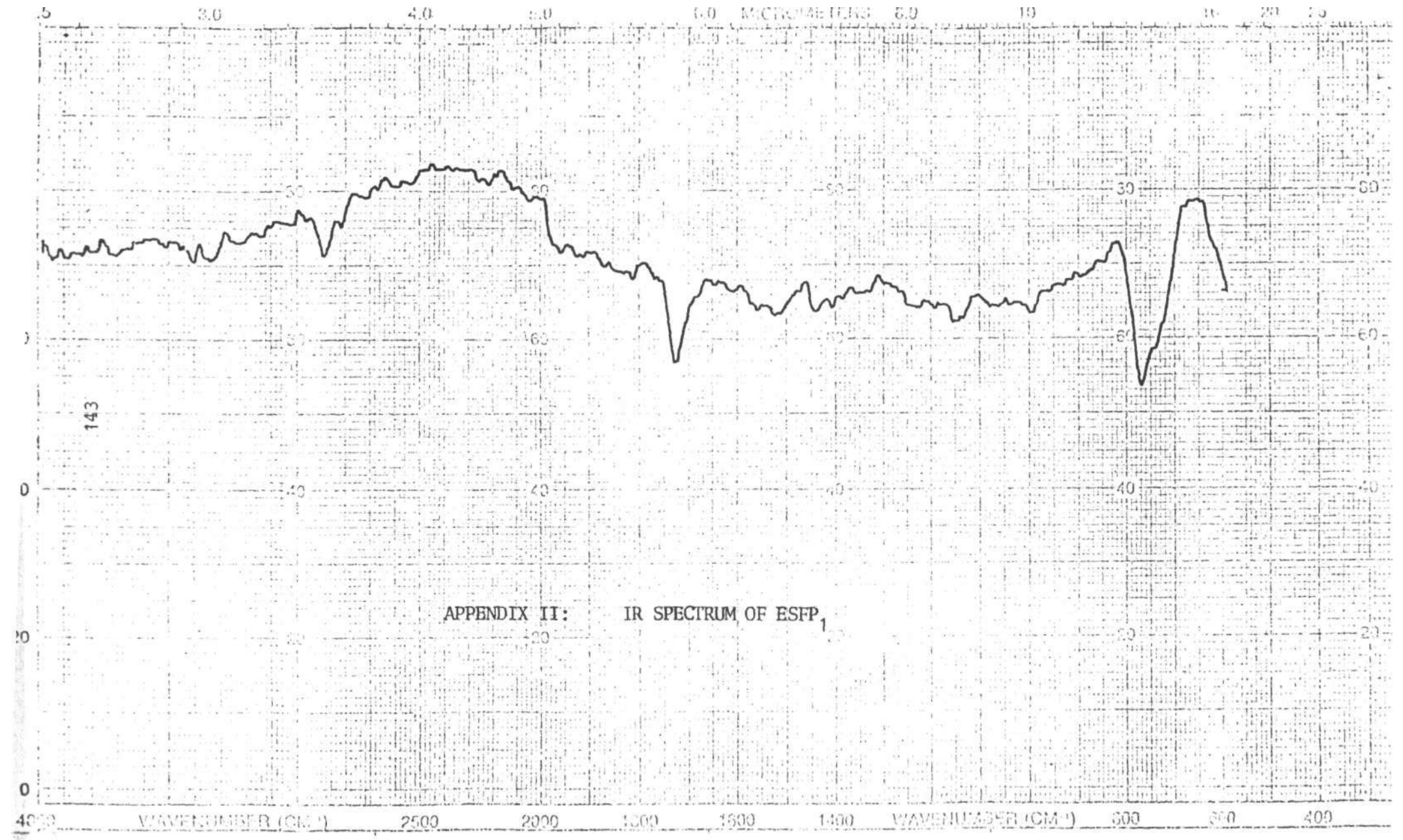
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APPENDIX

APPENDIX I: IR SPECTRUM OF $ESMB_4$





APPENDIX II: IR SPECTRUM OF ESFP$_1$

TLC Chromatogram of Fraction D

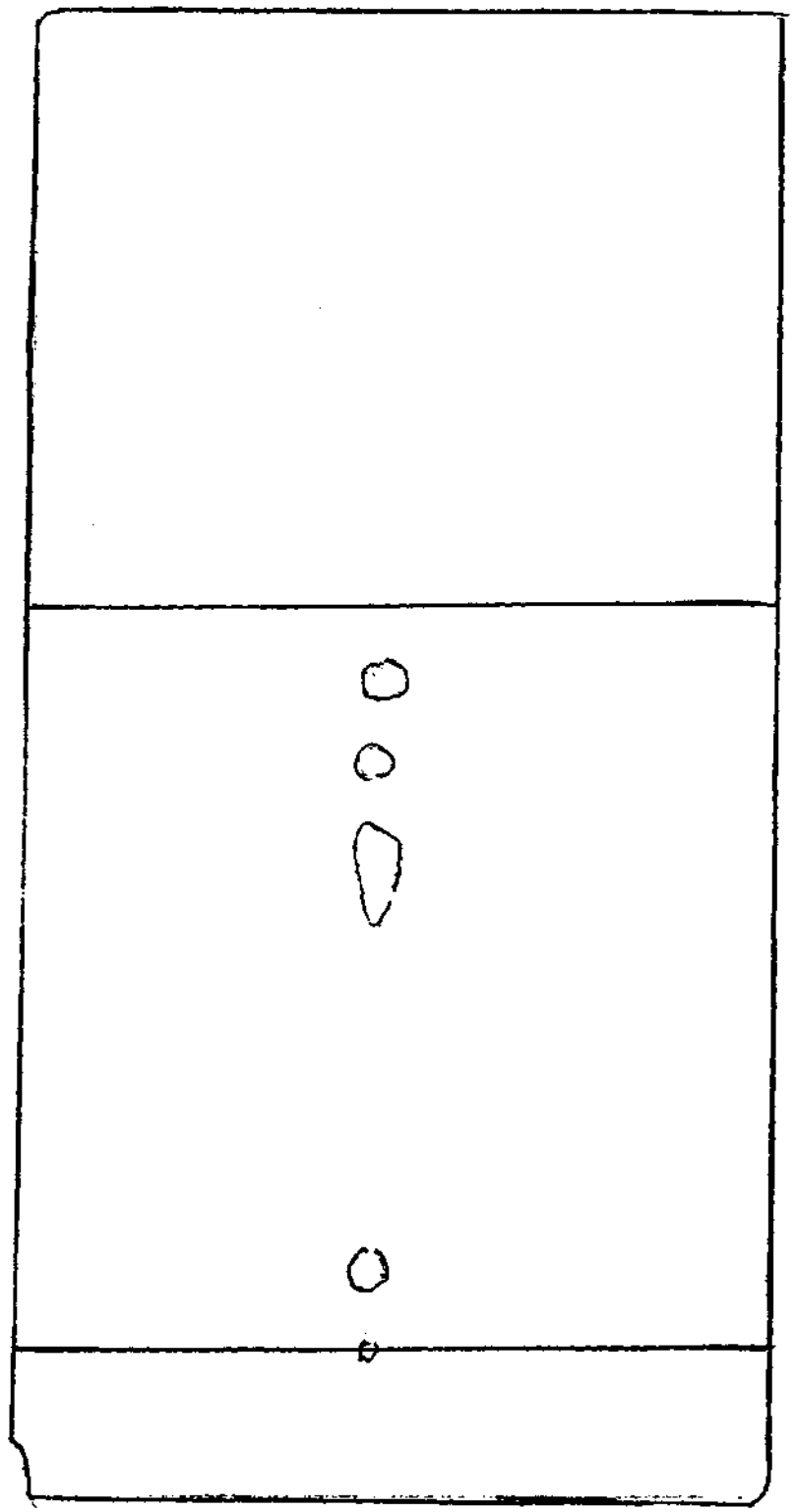


Fig.3· Plate under U.V. after spraying.

TLC Chromatogram of Franction E

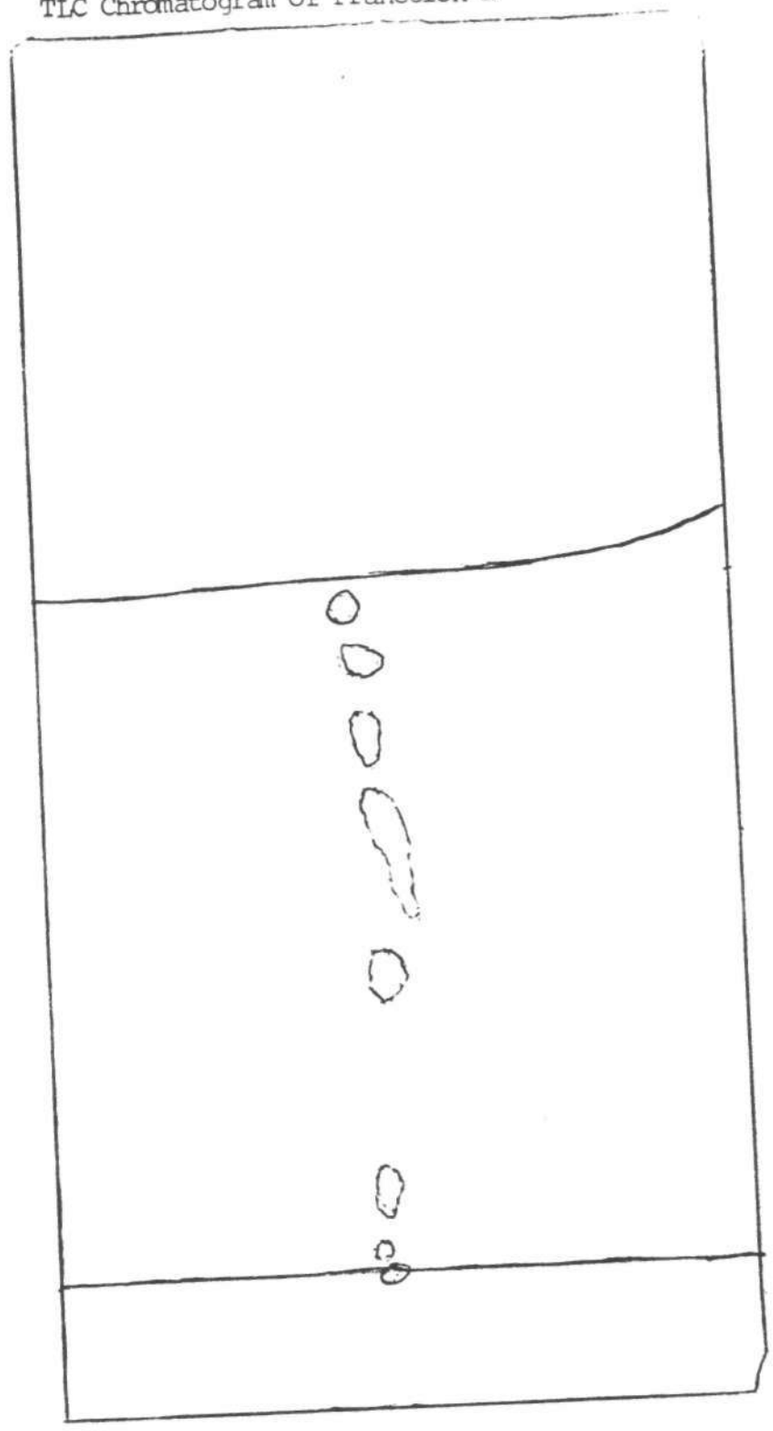


Fig.4: Plate under U.V. after Spraying

TLC Chromatogram of Fraction H

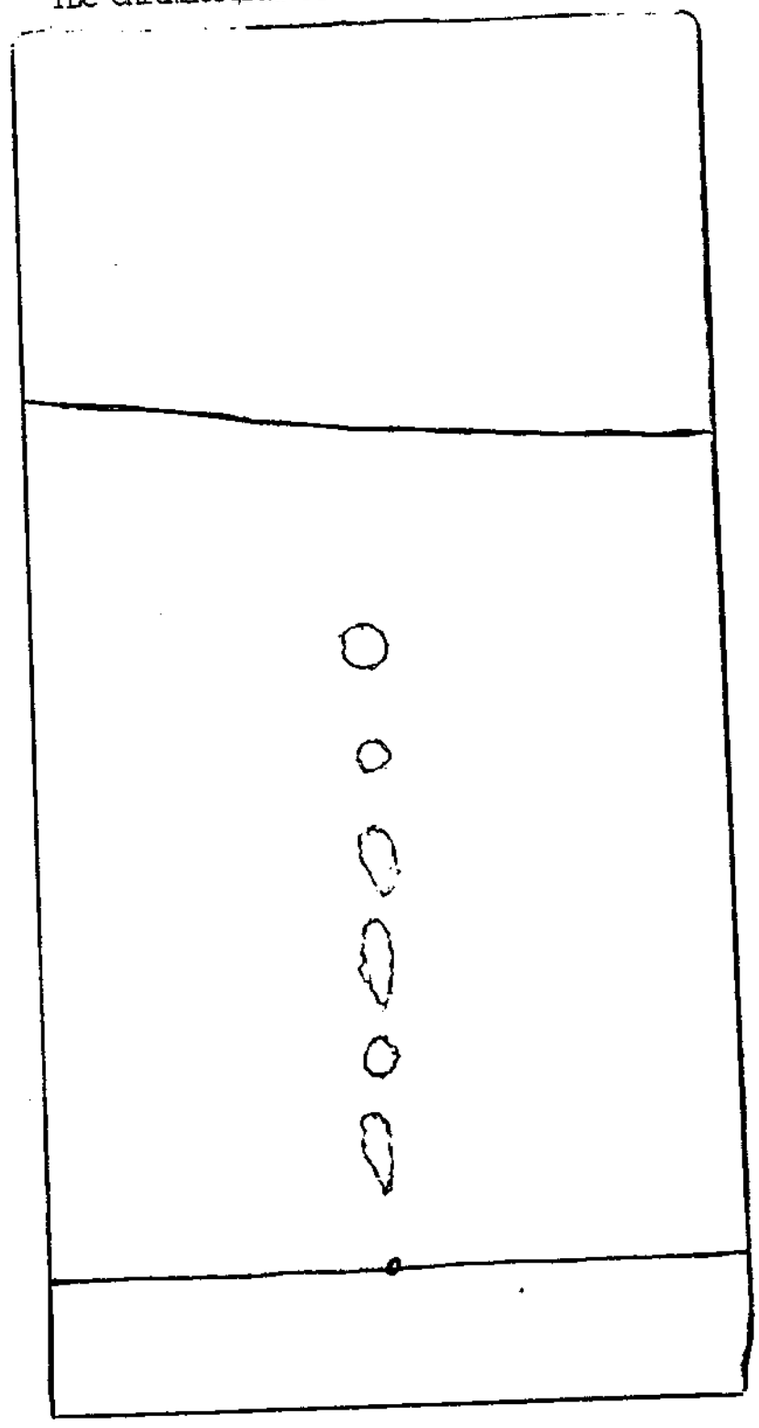


Fig.5: Plate under U.V. after Spraying

TLC Chromatogram of Fraction I

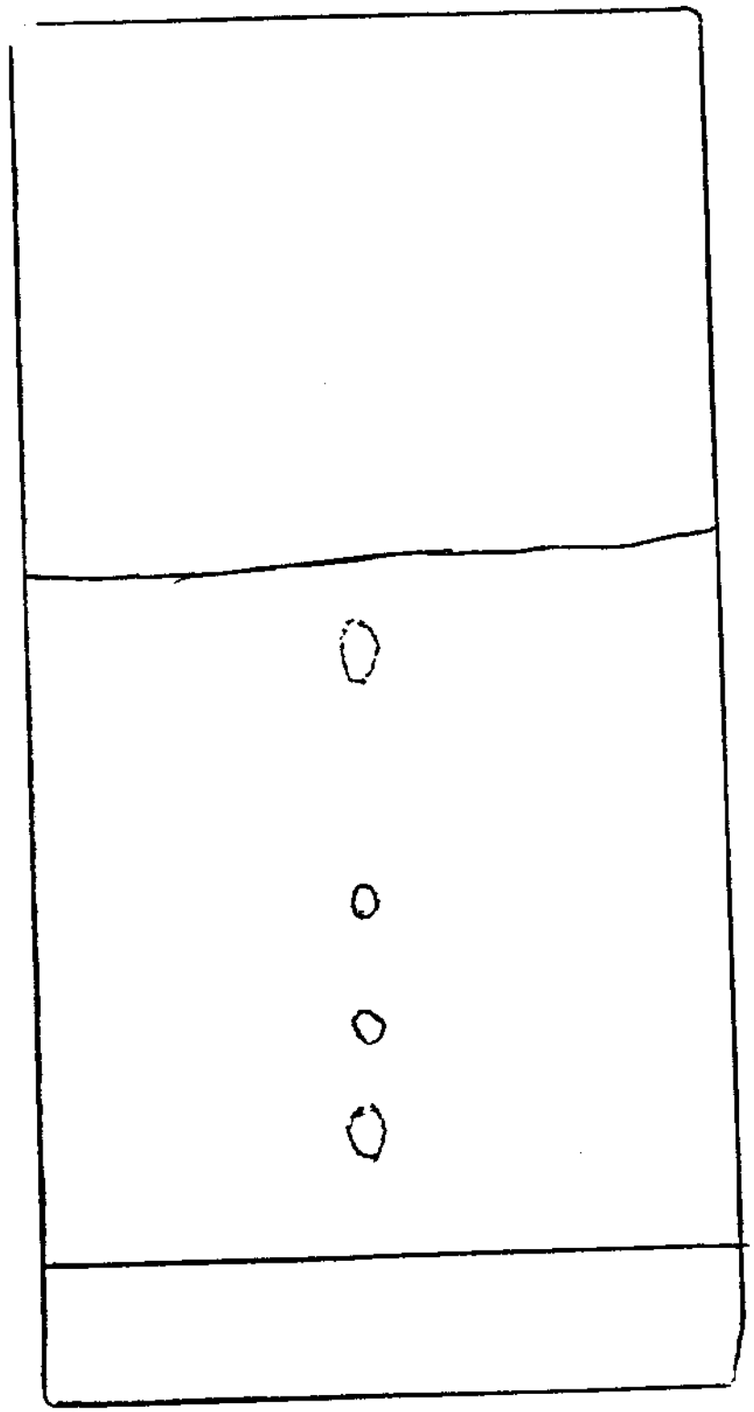


Fig.6: Plate under U.V. after spraying

TLC Chromatogram of Fraction H

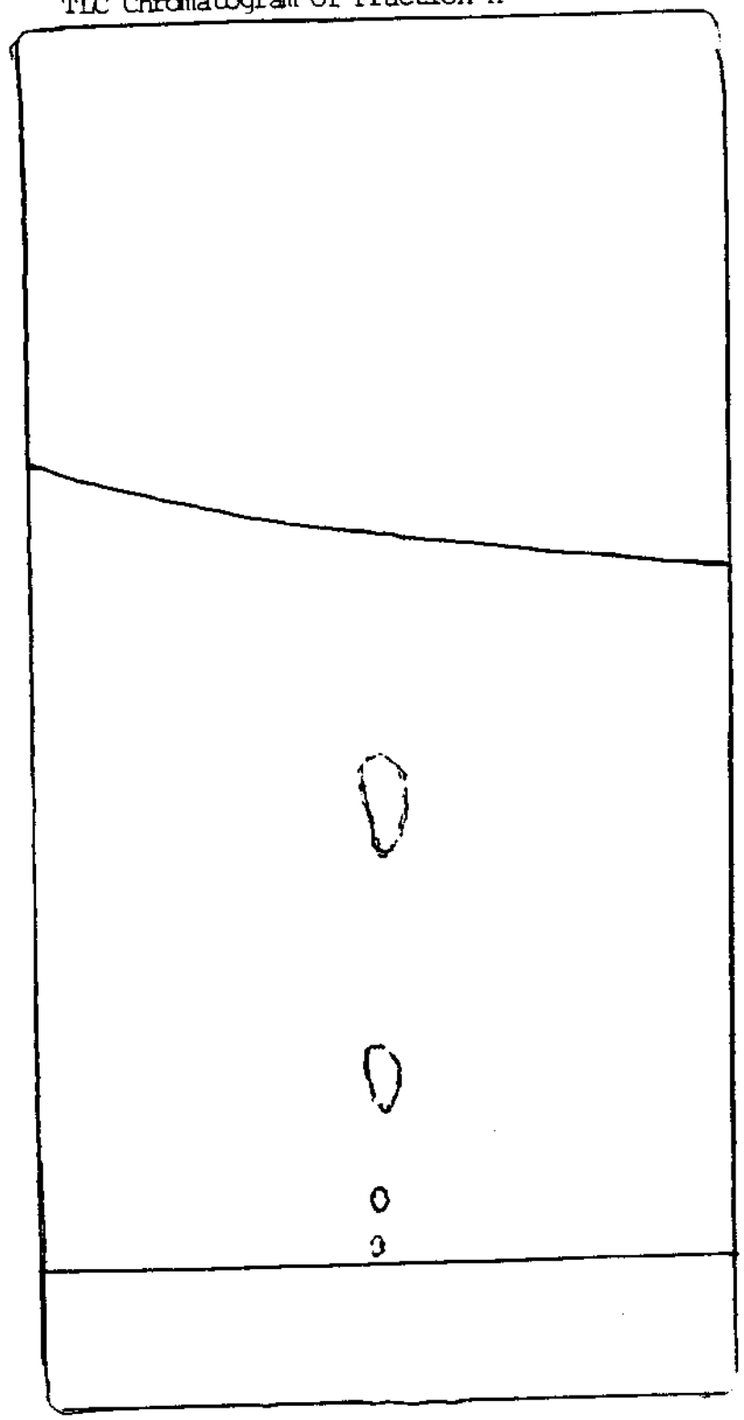


Fig.7: Plate under UV after spraying

TLC Chromatogram of Fraction I

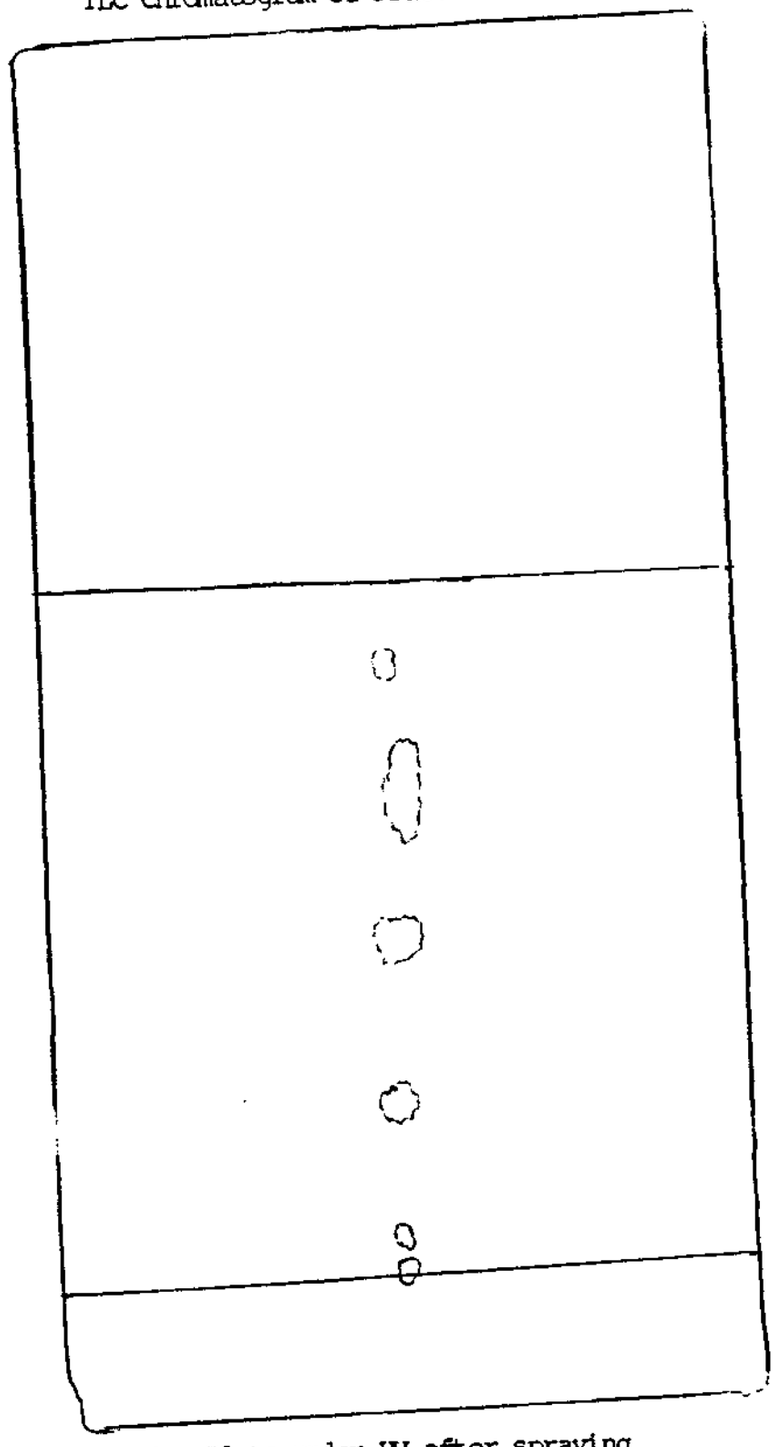


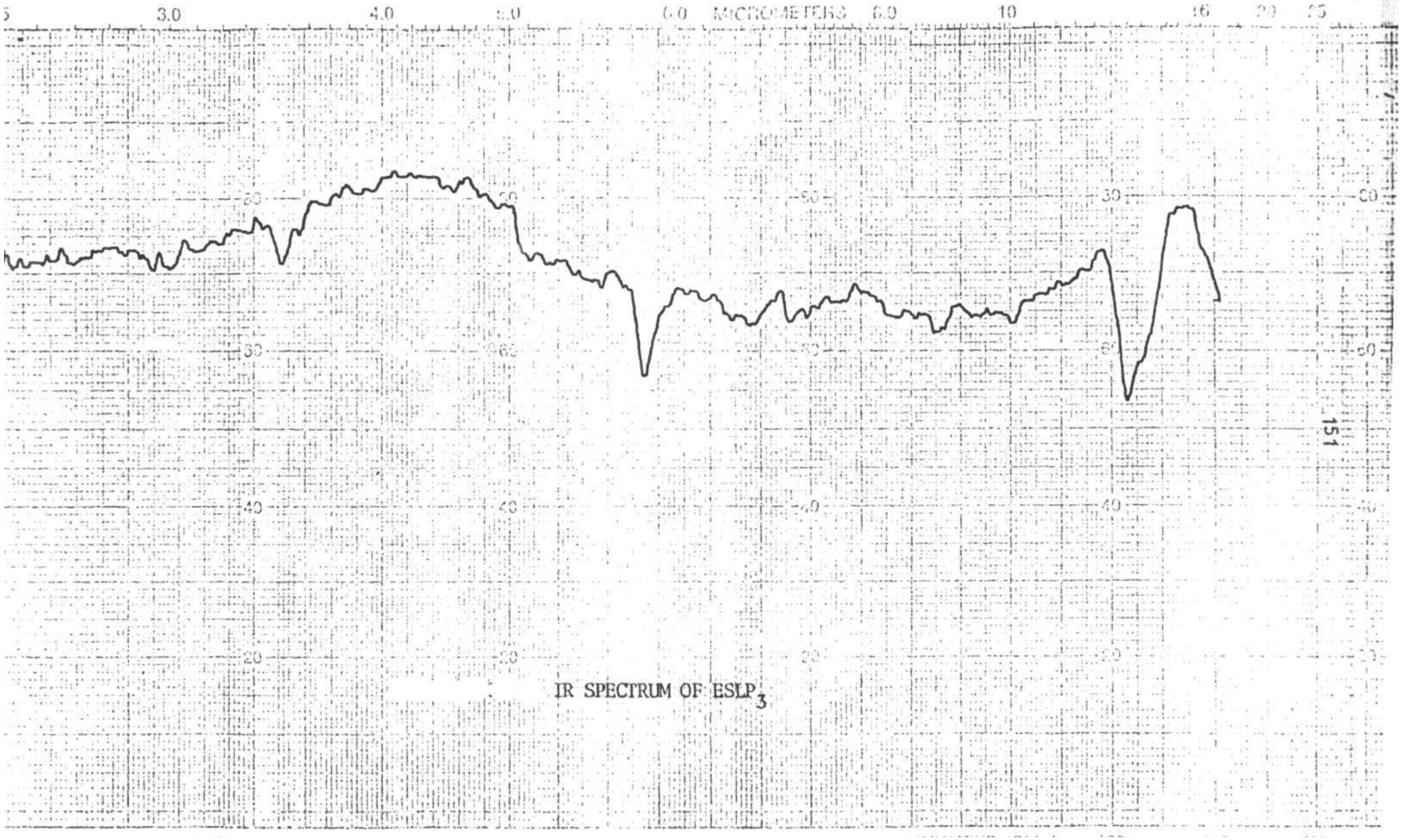
Fig. 8: Plate under UV after spraying

Compound $ESPL_3$:

The IR spectrum indicates a saturated long chain fatty acid 730cm^{-1} -$(CH_2)n$- rocking, 1710cm^{-1} C=O stretch of an acid (next page).

The mass spectrum shows ions at m/z 284 as the highest mass which is of low intensity as seen in Figure 20 with m/z 73 as the base peak.

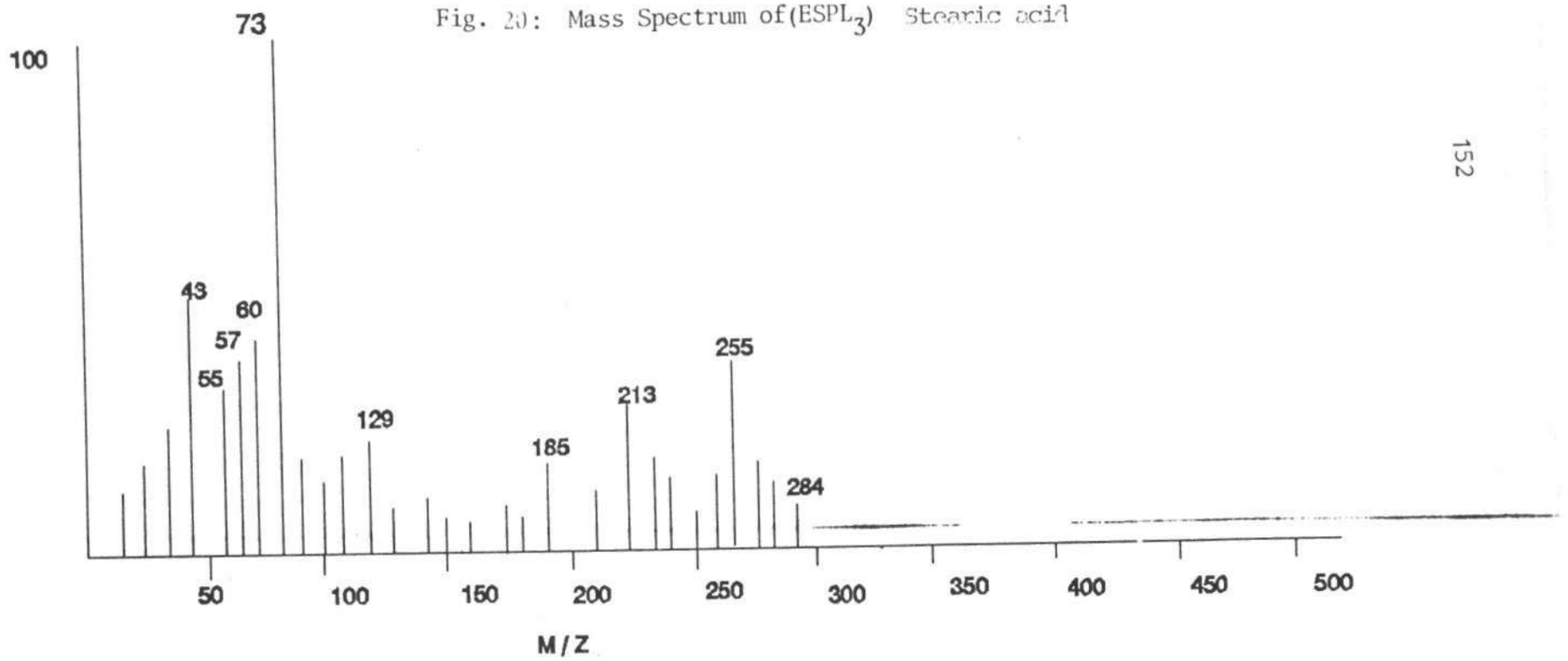
The more abundant ions from this straight chain aliphatic acid are the unusual periodicity in the $CnH_{2n}COOH^+$ ion series that is quite different from the regular abundance decrease with increasing molecular weight found for the CnH_{2n+1}^+ series of n-alkanes . These are the peaks at m/z 60 which shows the γ - hydrogen rearrangement $CRR'C(OH)_2$+ indicating the α- branching for R=R'=H i.e. $CH_2C(OH)_2^+$ as shown in reaction (II) (Stenhagen 1972) .



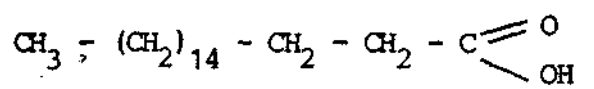
IR SPECTRUM OF $ESLP_3$

TONY2 LRP ESPL3
DATE RUN 10-01-1991 (17:52:44) INSTR:. USC CHEMISTRY
OPERATOR: MIKE FOLEY SCAN 220 RT = 5.46 NO. OF IONS = 29
BASE 0.2%F TIC 1469

Fig. 20: Mass Spectrum of($ESPL_3$) Stearic acid



as in (a) . The singlet at δ 1.25 (28H) is typical of a normal alkyl methylene group as in (b) and the broad triplet at δ 1.63 (2H) is obviously of the adjacent methylene group as in (c) while the triplet at δ 2.34 (2H) is typical of a methylene group adjacent to a carbonyl function as in (d).



(a) (b) (c) (d)

(12)

On the basis of the presented spectral information from the IR, NMR and MS the fatty acid compound $ESPL_3$ has been assigned structure (12).

$ESPL_3$ is thus concluded to be n-octadecanoic acid or stearic acid.

The mass spectrum obtained compared favourably with literature spectra . Also the melting point value obtained compared favourably with literature value

Value obtained 68 - 70°C

Literature Value 69 - 70°C

(McLafferty 1973 and Merck Index 1976).

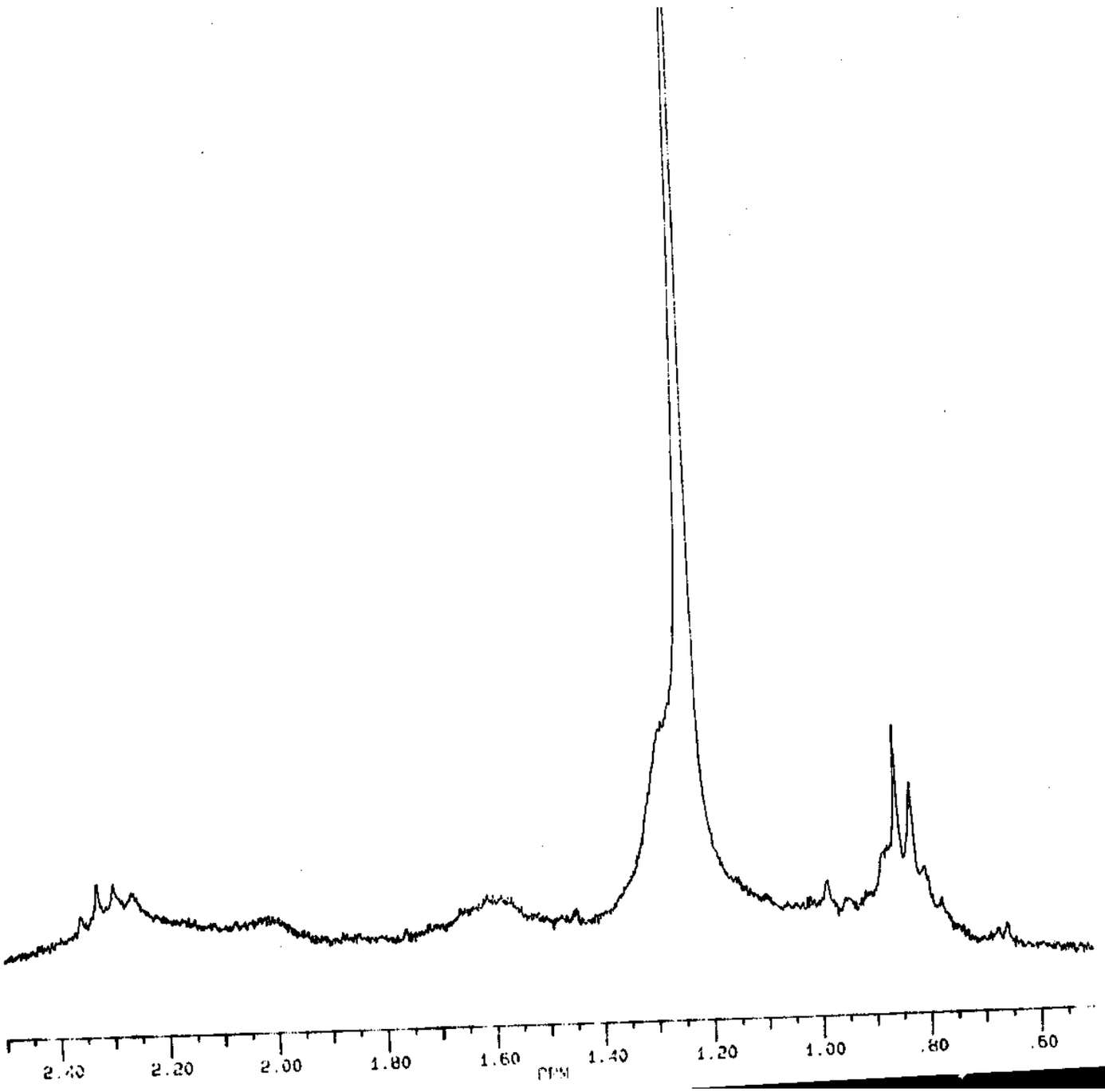

BRUKER
ESPL3
AU PROG.
TUNE
DATE 16-10-91
SF 250.133
SY 100.0
O1 4000.000
SI 16384
TD 16384
SW 2793.296
HZ/PT .341
PW 3.0
RD 0.0
AQ 2.933
RG 40
NS 40
TE 297
FW 3500
O2 3200.000
DP 68L PO
LB 0.0
GB 0.0
CX 20.00
CY 30.00
F1 2.500P
F2 .500P
HZ/CM 25.011
PPM/CM .100
SR 2859.00
2.40
2.20
2.00
1.80
1.60
PPM
1.40
1.20
1.00
.80
.60

Fig. 21a.

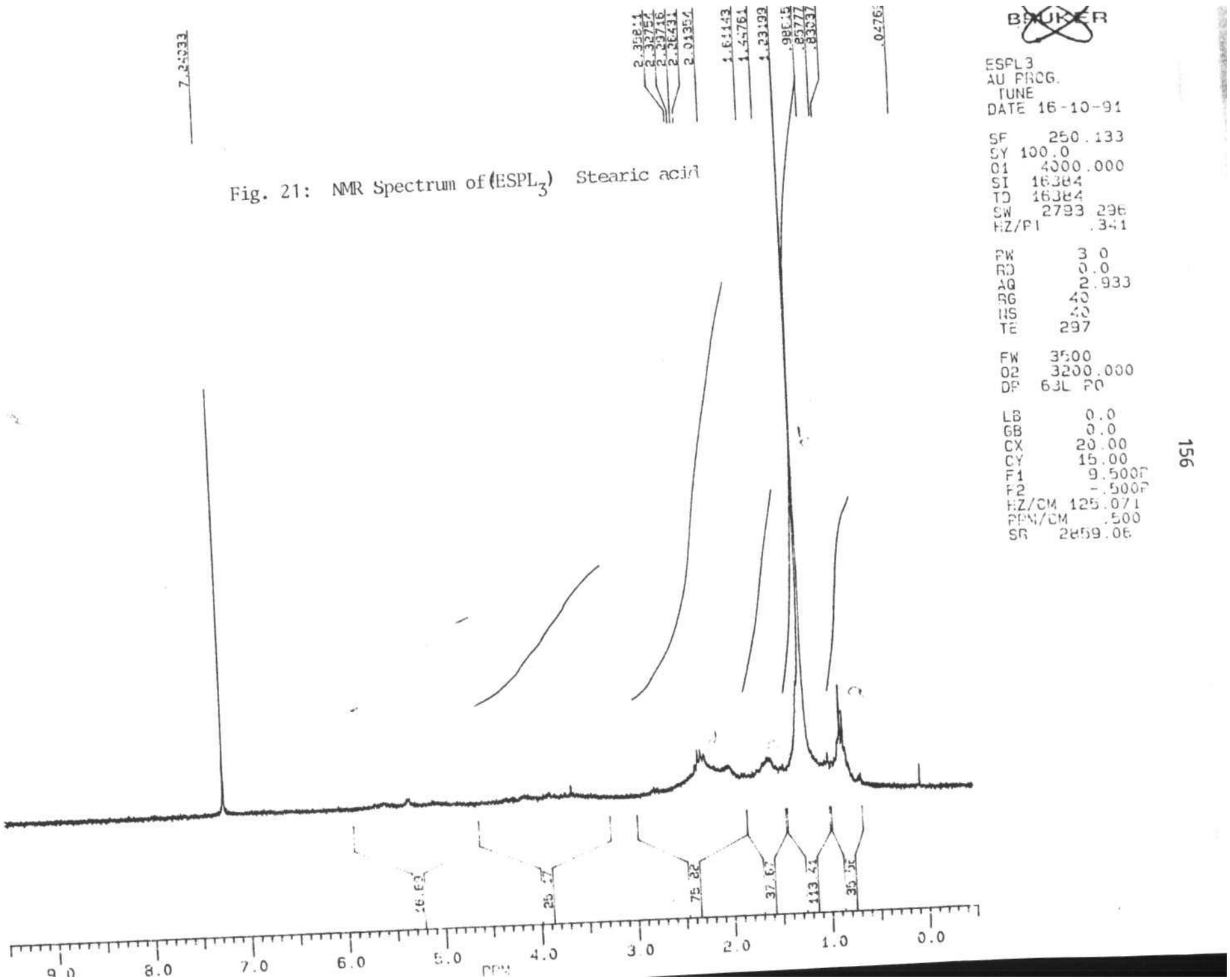
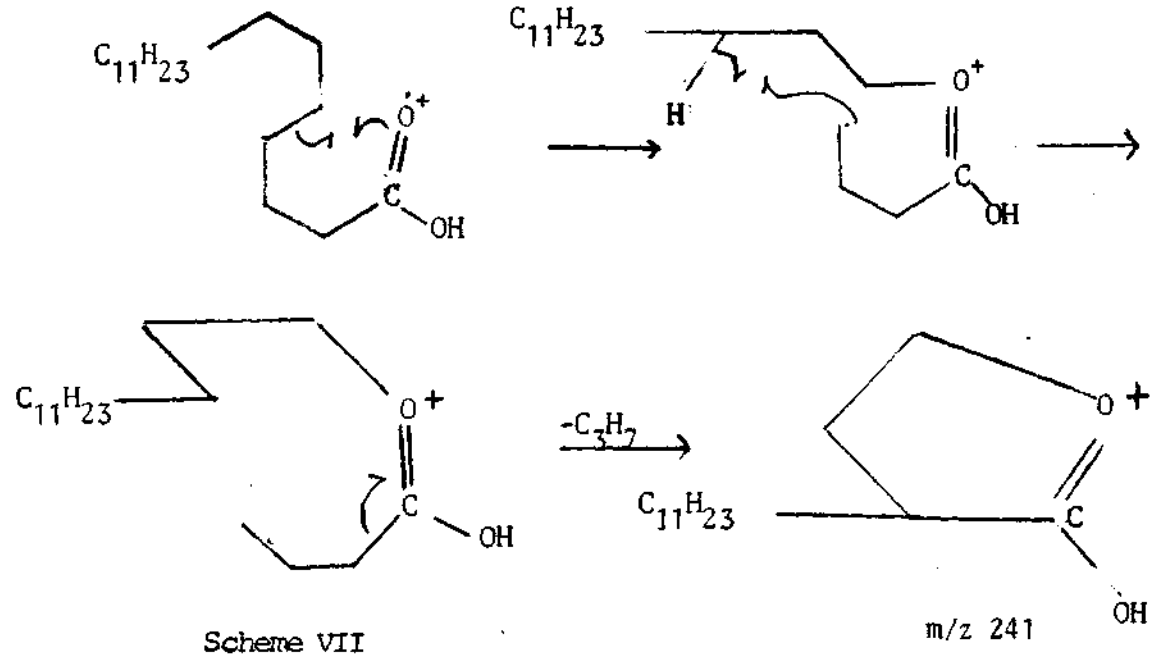


Fig. 21: NMR Spectrum of ($ESPL_3$) Stearic acid

The ions of m/z 241 may be due to the rearrangement yielding $C_nH_{2n+1}CO^+$ ions identified by Stenhagen and his co-workers[57] which involves the formation of $(M - C_3H_7)^+$ possibly through two displacement reactions as seen in Scheme VII .

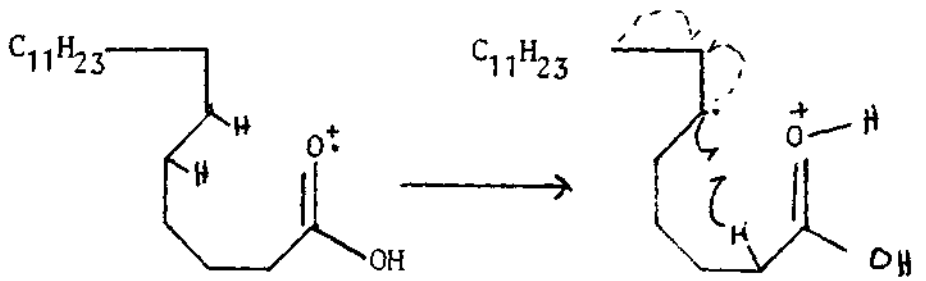


Scheme VII

The other salient peaks are due to the decomposition of the alkyl moiety to produce the characteristic $C_nH_{2n+1}^+$ ion series $C_3H_7^+$ to $C_5H_{11}^+$ and the $C_nH_{2n-1}^+$ ion series $C_4H_7^+$ and $C_5H_7^+$

The nmr spectrum figure 21 of the fatty acid contained protons in at least four different electronic environments as can be seen from their chemical shifts in compound (12). The triplet at δ 0.88 (3H) is of the terminal methyl group

The ions at m/z 73 and 129 arises through initial migration of δ or ε- hydrogen with reciprocal hydrogen transfer to produce the radical site on the α-carbon Schemes IV and V (McLafferty 1973).

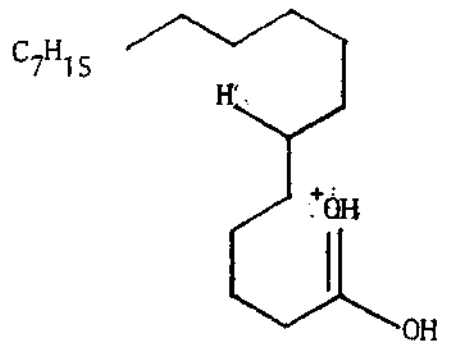


$$\xrightarrow[\alpha]{-\,C_{11}H_{23}} \quad CH_2 = CH(CH_2)_4 \; C(=\overset{+}{O}H)OH$$

m/z 129

Scheme V Mclafferty Rearrangement

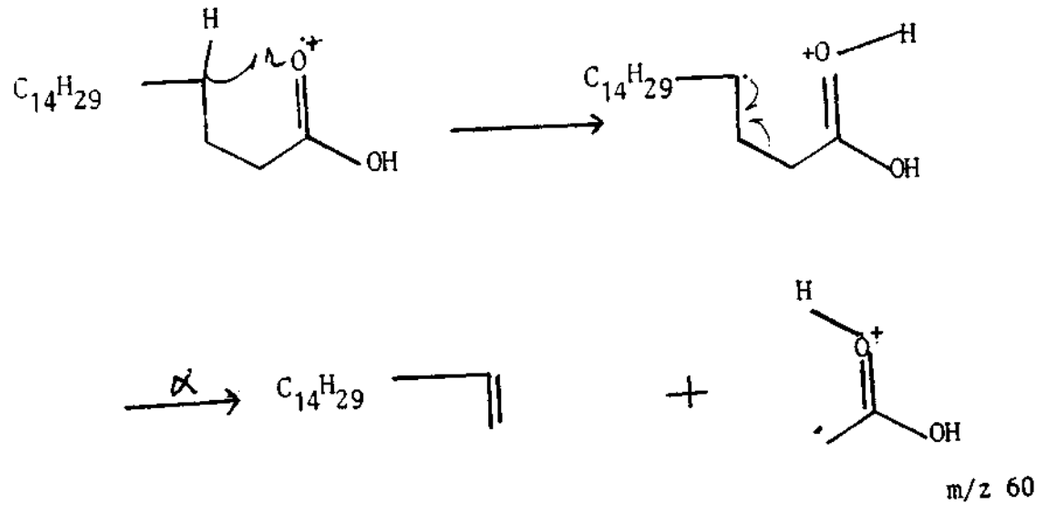
The ion at m/z 185 may be due to the favoured loss of the allylic δ- hydrogen and the favoured six membered ring transition states as in Scheme VI.



$$\xrightarrow[-\,C_7H_{15}]{\delta} \quad CH_2{=}CH(CH_2)_8 \; C(=\overset{+}{O}H)OH$$

m/z 185

Scheme VI

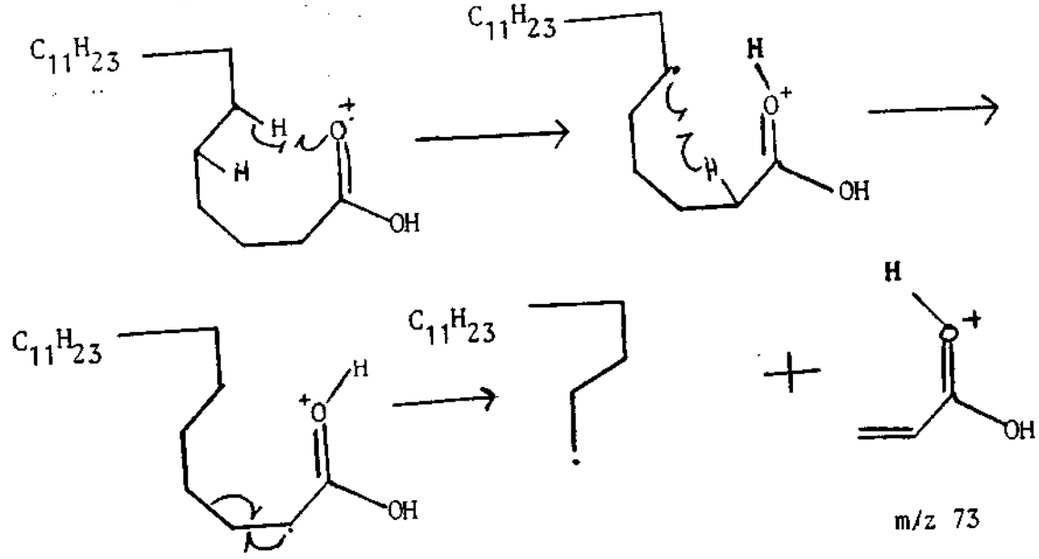


m/z 60

Scheme III

The other peaks are the ions with m/z 73, 129, 241 and 185.

These $C_nH_{2n}COOH^+$ ion series are characteristic of chain branching and arises through mechanism analogous to the Scheme (III) (McLafferty 1973).



m/z 73

Scheme IV