A PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF THE POISONOUS PRINCIPLE(S) OF ELAEODENDRON BUCHANANII (LOES.) LOES

By

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted for a degree in any other University.

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CANDIDATE

This thesis has been submitted for examination with our approval as University Supervisors.

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A C K N O W L E D G E M E N T S

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D E D I C A T I O N

This thesis is dedicated to my wife and son.

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SUMMARY

A pharmacognostical investigation of Elaeodendron buchananii (Loes.) Loes. has been undertaken. Phytochemical and pharmacological properties of the active (poisonous) principles of the plant have also been studied.

The pharmacognostical investigation of the plant involved identifying features of the different parts of the plant using photographic and macroscopic methods.

Results of the screening tests of the different parts of the plant for the active constituents indicated the presence of chemical compounds with α, β- unsaturated δ - lactone ring, possibly cardiac glycosides. Investigation of a suitable solvent system for the extraction of these compounds was undertaken. Of the different parts of the plant examined for active principles, the leaves were found to contain the highest percentage of the chemical compounds with α, β- unsaturated δ- lactone ring. Isolation and purification of the active principle(s) from the original crude plant extracts involving the removal of pigments, tannins, resins and excess lead has been described. Crystallisation of the isolated glycoside from a suitable solvent system and the subsequent study of some of the physical and chemical properties of the isolated compound has been described.

From the elemental analysis and the molecular weight of the isolated compound the molecular formula of the compound has been determined as $C_{32}H_{47}O_{11}$. Using the infra-red, ultraviolet, nuclear

magnetic resonance and mass spectra, a partial molecular structure has been suggested.

The isolated compound has been reacted with Kedde reagent and the resulting coloured complex has been examined to see whether it obeys Beer - Lambert law. The calibration curve obtained has been used to determine the percentage recovery of the isolated compound in the leaves of the plant.

The pharmacological study of the isolated compound has also been undertaken. This study involved the investigation of the effects of the isolated compound on the blood pressure of anaesthetised rat and the effect of the compound on the isolated perfused rabbit heart.

Suggestions for further work as regards pharmacognostical investigation of the plant together with ascertaining the exact structural formula of the compound has been proposed.

INTRODUCTION

In his intensive search among the plants for food, man found that certain plants were not only edible but had poisonous and/or medicinal value as well. As time passed the interest in the medicinal properties of plants grew and the findings were passed down from generation to generation, at first orally and then in writing. This interest continues today despite the vast range of synthetic drugs. The majority of synthetic drugs are expensive to manufacture, and consequently, in developing countries extensive research is being carried out into old herbal remedies and also other indigenous plants in an attempt to discover new, better and cheaper sources of drugs.

Use of plants as poisons and medicines dates back to the dawn of recorded history. For example, the Greeks are known to have used poisonous plants to condemn criminals and philosophers. A case in print is Socrates who was forced to drink "oil of hemlock" now known to contain a deadly alkaloid, coniine. As early as five thousand years ago, the Chinese used the plant Ma Huang, (Ephedra species) but it was not until 1887 that the active constituent, ephedrine, was isolated. Since then ephedrine has been used as a reliever of asthma and hay fever (Waller 1970, Bowman et al, 1970, Trease and Evans, 1972). In India, the usefulness of Serpentine rauwolfia root (snake root) in treating snake bites dates back several centuries. The alkaloid, reserpine, whose present day medicinal use is in treatment of essential hypertension, and in certain neuropschiactric disorders, was isolated from the plant in 1952. Similarly the use of

opium dates back to time immemorial even though the tincture of opium was introduced in official monograph in 1670. Meconic acid and morphine (some of the constituents of opium) were isolated in 1806 and 1816 respectively. Cinchona bark had been and is still used by the indigenous people of South America for cure of malaria. Its alkaloids, quinine and cinchonine which are used for the same purpose today were isolated in 1820 and 1821 respectively. Another of its alkaloid, quinidine was isolated in 1833. Other plants, for example, digitalis species have a long traditional use as herbal medicines and are presently used as sources of cardiac glycosides (Bailey 1976). The use of arrow poisons by Africans for hunting and in tribal wars is a legend. Presently, these arrow poisons have been shown to contain cardiac glycosides (Mines, 1908; Laidlaw, 1908; Raymond, 1936; Watt et al, 1962). Such cardiac glycosides like ouabain have been isolated from ouabio tree (Arnand 1888).

Despite the interest shown by numerous workers in medicinal and poisonous plants, there are still many other plant drugs which have not been investigated and the properties of their chemical constituents still remain unknown. Research organisations all over the world are now becoming interested in screening plants of East Africa for possible useful products and the result of such researches have been encouranging. Anti - cancer drugs have been isolated from Catharanthus roseus, from which the alkaloids viniblistine and vincristine have been isolated. A cardiac glycoside whose structure is yet to be elucidated has been isolated from Ajuga remota Benth (K. A. M. Kuria, M.Sc. thesis 1976, University of Nairobi).

The poisonous plants of East Africa have been and are still used in small quantities for medicinal purpose by the indigenous people with varying degrees of efficacy probably due to variation in the amount of active principles in these plants and also due to varying dosages. Therefore, it is often impossible, without additional evidence, to state that death was not due to error of dosage or circumstances over which the person administering the medicine had control. The matter is often greatly complicated by the fact that as many as a dozen plants may be used in the mixture. In other cases, plants have been reported to be poisonous or of medicinal value, but when analysed, the plant have been found to be devoid of any known active principles. In some other cases the active principles present have been found to have pharmacological actions which have no relation to the medicinal uses the plants are put to.

Plant poisoning is also a frustrating problem. Quite often, the amount of toxic principle and even its presence or absence depends on climatic and soil conditions. A plant may be poisonous at one stage and not at another. A human or animal in poor health may succumb to a certain mild plant poison whereas a healthy one could tolerate the effect. Therefore, results of screening of such plants have to be viewed critically. The screening results of many of those poisonous plants have indicated that those plants could be sources of:-

a) Rat poisons, for example, sodium fluoroacetate and strychnine from the genus dichapetalum and strychnos species respectively.
b) Important drugs being used in the treatment of such diseases as cancer, hypertension, and cardiac failure.
c) Raw materials from which other drugs, for example, anti - inflam-

matory agents and anti - fertility drugs can be prepared, for instance, steroids from sisal.

The screening of plants for their active (poisonous) principles is time consuming, expensive and requires painstaking patience, but still there are plants with valuable medicinal properties whose chemical constituents remain unknown. It is, therefore, imperative to screen them for possible chemicals which could prove beneficial to the suffering humanity.

Knowledge of poisonous plants is also important espesially in developing countries, where the imported pedigree cattle have been known to die after ingesting the poisonous plants. The indigenous cattle seem to have a sense of caution and instinctly avoids grazing areas where poisonous plants occur. Imported stock, which is often more valuable, does not seem to possess this sense of caution and succumbs more readily. Indigenous stock moved to a completely different area is also sometimes unable to distinguish poisonous plants. Therefore, it would well pay a farmer if he has prior information of areas where poisonous plants grow. This would enable him to seek pastures and brownsing grounds free from the same.

Presently, persistent failures to develop new synthetic drugs for the treatment of diseases like cancer, hypertension and asthma have given a new impetus to search for new drugs from the plant kingdom. Numerous attempts are now being made to discover the cures from the plants. As a result emphasis on research in this field is great and has been intensified in the last few years.

The plant, Elaeodendron buchananii (Loes.) Loes, is found widely distributed in East Africa and has been responsible for several livestock deaths. There is no evidence to show that it has been used as a herbal medicine or as an arrow poison. Literature survey showed that no work had been undertaken to elucidate the poisonous principles of this plant. It was therefore, decided to carry out a detailed research on this plant in an attempt to ascertain the nature of all or some of the active (poisonous) principles present in the plant. It was also the intention of the author to do preliminary pharmacological work on the isolated compound(s) to determine if such compounds would have some medicinal properties which could be usefully exploited.

LITERATURE REVIEW

Elaeodendron buchananii (Loes.) Loes is a very common and poisonous plant in East Africa. Verdcourt et al (1969) has reported that ingestion of the leaves of the plant has resulted in many deaths to livestock, especially sheep. In one case a large number of rabbits died on being fed the leaves of the plant. Information obtained from many indigenous people of Kenya (Kamba, Kikuyu, Kisii etc) indicate that sheep and cows die when they eat the small shoots growing from the bases of tree trunks. Verdcourt et al (1969) report that poisoning following ingestion of the leaves results in sudden death and the post-mortem findings after such deaths reveal congestion of the liver, oedema of the lungs and haemorrhages of the heart.

Mugera (1970) has reported similar observations when he experimented with the leaves of the plant on sheep, goats and cows. He showed that 5g of dried leaves would kill a goat or sheep in two days and 25g of leaves would kill a cow in five days. He has also shown that poisoning by Elaeodendron may be paracute or acute. In both cases the symptoms are the same except that symptoms in acute poisoning appear in 2 - 3 days after eating the leaves of the plant while in paracute the symptoms appear in about two hours after eating the leaves of the plant. However, Mugera (1970) has not reported what the poisonous principles might be.

Elaeodendron buchananii (Loes.) Loes. has not been included by Watt et al (1962) in their book "Medicinal and Poisonous Plants of Southern and Eastern Africa", but these authors have included

other species of Elaeodendron and have indicated that these might be poisonous.

Literature survey did not show any contradiction with respect to symptoms observed following poisoning of animals by the plant. Verdcourt et al (1969) suggest that the poisonous principles of Elaeodendron might be a resin, but none of the other workers have suggested what the poisonous principles might be. Literature survey also showed that Elaeodendron buchananii has not been used by Africans for medicinal purposes. A related plant, Elaeodendron stuhlmanii (Loes.) is a Tanzanian ankylostomicide and the active principle of this plant has been suggested to be a glucoside (Watt et al 1962). In Rhodesia, there is a species of Elaeodendron which is also known to be poisonous and the Africans use it for trial by ordeal. The symptoms of poisoning of this plant are instant unconsciousness and vomiting.

Present work

The present work was undertaken partly to elucidate diagnostic features of Elaeodendron buchananii (Loes.) Loes. which would help to identify the plant in the field. For this purpose, photographic and macroscopical methods have been employed. Similarly standard methods of extraction, purification and identification of naturally occuring active compounds were used in an attempt to study the active constituents of Elaeodendron buchananii. The physical, chemical and pharmacological properties of the isolated active (poisonous) principle present in this plant have also been investigated.

CHAPTER I

A PHARMACOGNOSTICAL INVESTIGATION OF ELAEODENDRON BUCHANANII LOES.

Elaeodendron buchananii (Loes) Loes. (Synonyms :- Cassine buchanani. loes., Elaeodendron Keniense loes.) belongs to the plant family Celastraceae. The plant was identified by the Department of Botany , University of Nairobi, and a reference sample is preserved in the Department of Pharmacy, University of Nairobi. .The plant is widely distributed in Kenya and is known by the following tribal names:- enkanda(Kisii) murunda (Kikamba),Mutanga (Kikuyu), mutimweru (Meru), sawanet (Kipsigis), sunwa (Sebei). The plant is very common in East and Central Africa and can be encoutered as a tree of different sizes depending on soil and climatic conditions. The height ranges from 7 to 80ft but the normal height is about 35 ft with a compact crown (Plate I).

Description of the leaves- the leaves are moslly opposite and the lamina of the mature leaves are 2-7 inches long and 1-4 inches wide. They are ovate to ovate lanceolate in shape. The apex varies in different leaves being rounded to acute (Fig. 1 d). The margin is dentate and the base is obtuse to acute, sometimes unequal. The upper surface is conaceous glabrous and dark green. The midrib on the upper surface is more prominent on the basal half. The lower surface is pale green, slightly conaceous and glabrous and the midrib is more prominent on the basal half. The side veins are more conspicuous on the lower surface than on the upper surface and they leave the midrib at varying angles showing anastomosis near the margin. The petiole is short and rounded and up to 1 cm long. At the growing point of some braches, the young leaves are bright green on both surfaces and the

leaves are linear lanceolate in shape and up to 1 cm long. (Fig. 1 d).

The flowers are small with five yellowish green petals and five conspicuous stamens (Fig. 1c). They occur in short stalked clusters in the axils of the leaves (Plate IV). The fruits are green rounded berries when young and orange when ripe.

ELAEODENDRON BUCHANANII (LOES.) LOES. AS ENCOUNTERED IN THE FIELD

Plate I: Photograph of the tree (about 15ft high) growing among aloes and grass

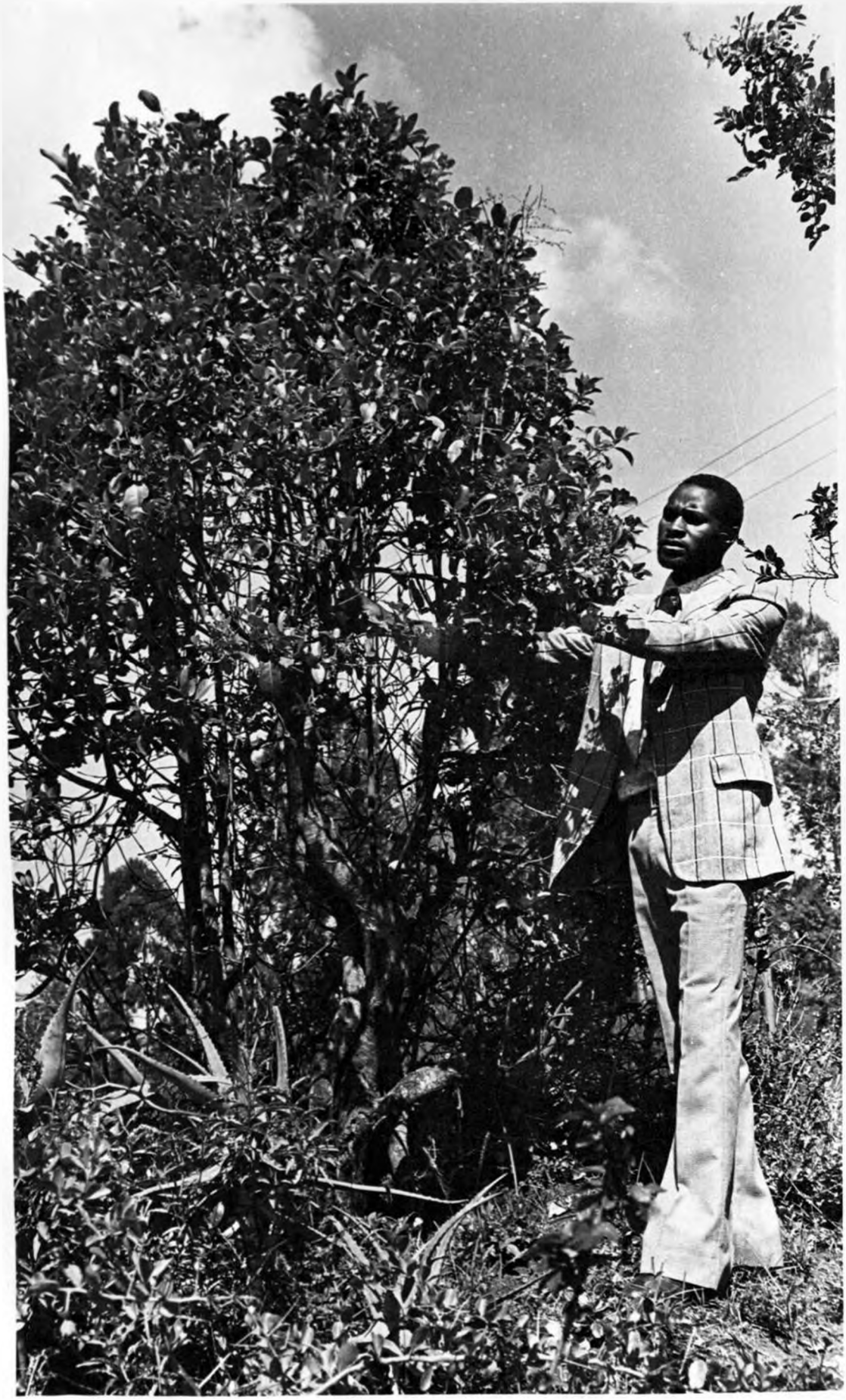


Plate II: A photograph of the tree which has coppiced readily from a cut stump. The tree is growing singly in dry land.

NB: It has very large and dark green leaves.

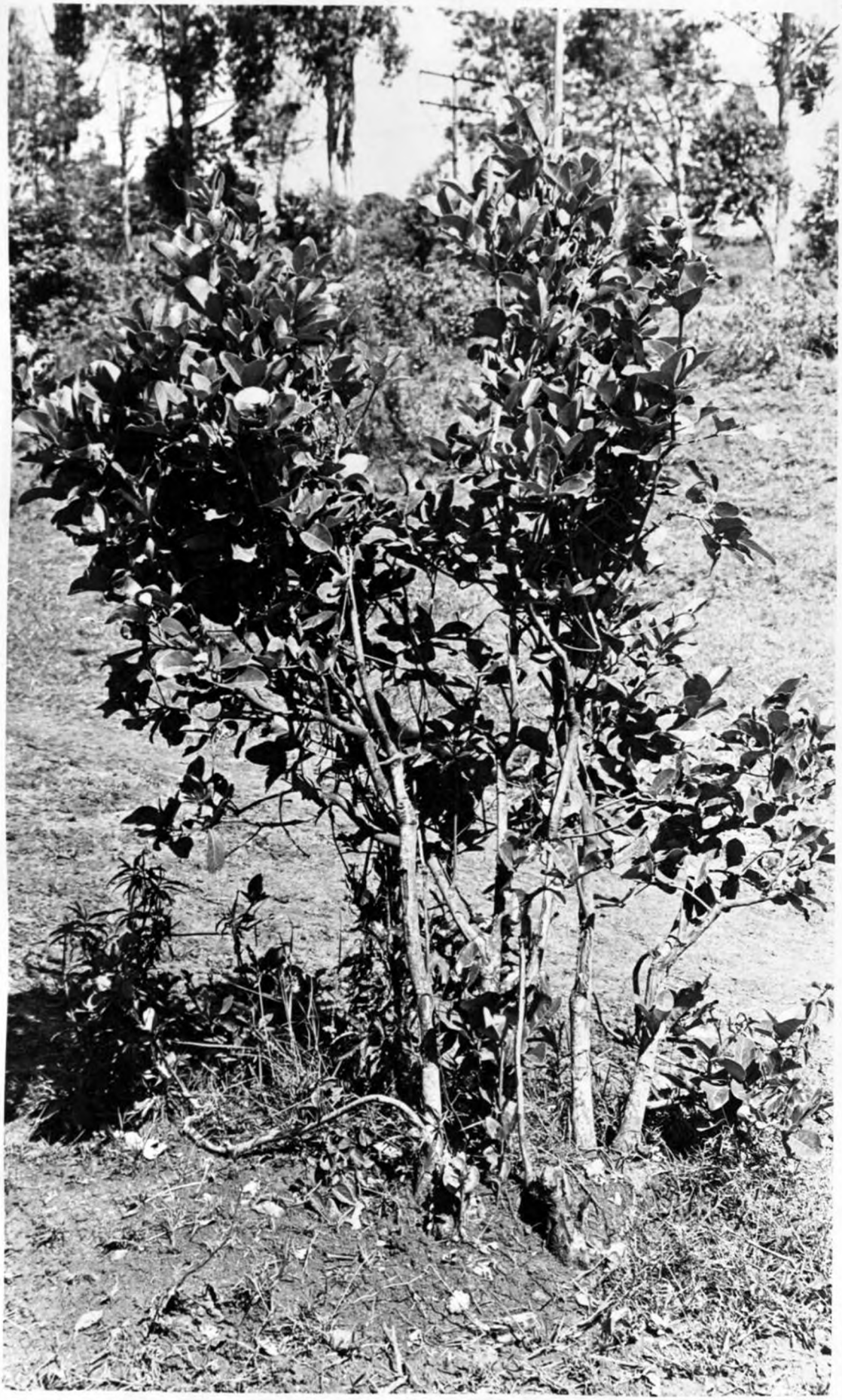


Plate III: A photograph of the tree growing in a bush among other foliage. The tree is in form of a small shrubby growth.

Plave IV: A photograph of a flowering branch of Elaeodendron showing the distribution of the leaves and flowers on the tree.

CHAPTER 2

EXPERIMENTAL WORK

1. Reagents
2. Preparation of reagents
3. Apparatus
4. Collection of plant material for investigation
5. Preliminary investigations
6. Extraction and purification of the active (poisonous) principle from the leaves of Elaeodendron buchananii (Loes.) Loes.
7. Characterisation of the isolated compound(s)
8. Estimation of the total content of cardiotonic compound(s) from the leaves.
9. Preliminary pharmacological investigaiion of the isolated compound

REAGENTS

The reagents used for the present work were obtained from British Drug House (BDH), May and Baker (M & B), and Imperial Chemical Industries (ICI).

Reagent	Grade	Brand
Acetic acid (Glacial)	Lab. reagent	BDH
Acetone	" "	"
Benzene	" "	"
Chloroform	" " and Analar	"
Decolourising charcoal	Lab. Chemical	M & B
m - dinitrobenzene	Lab. reagent	BDH
3:5 dinitrobenzoic acid	" "	"
Ethanol absolute	" " and Analar	ICI
Ethyl acetate	" "	BDH
Ferric chloride	" "	"
Formamide	" "	"
Lead acetate	Lab. Chemical	M & B
Petroleum spirit	Lab. reagent	BDH
Potassium hydroxide	" "	"
Proplylene glycol	" "	"
Silica gel (160 - 120 mesh)	" "	"
Sodium hydroxide	" "	"
Sodium sulphate (anhydrous)	" "	"
Sulphuric acid (conc)	Lab. Chemicals	M & B
Digoxin		BDH
Ouabain (G - strophanthin)		BDH

PREPARATION OF REAGENTS

1. 3:5 dinitrobenzene solution

A 1% w/v solution of 3:5 dinitrobenzene in absolute alcohol was prepared by weighing the required amount of 3:5 dinitrobenzene and dissolving it in absolute alcohol. The solution was warmed to enhance dissolution process. The reagent was stored in amber coloured bottles until required.

2. 3:5 dinitrobenzoic acid (alkaline) Kedde reagent

This reagent was prepared by mixing equal quantities of a 2% solution of 3:5 dinitrobenzoic acid in methanol and a 5.7% w/v aqueous solution of potassium hydroxide.

3. Sodium hydroxide solution

Sodium hydroxide pellets were weighed accurately and dissolved in freshly distilled water to make the required strength.

4. Keller - Killiani reagent

A 5% w/v solution of ferric sulphate was prepared by dissolving the appropriate weight of ferric sulphate in distilled water. One volume of the ferric sulphate solution was added to 99 volumes of glacial acetic acid. This reagent was used with concentrated sulphuric acid to test for glycosides with 2 - desoxy sugars.

INSTRUMENTS

1. The UV Spectra Pye Unicam SP 800 spectrophotometer

2. The Ir Spectra Pye Unicam SP 1000 IR spectrophotometer

3. The Mass Spectra were run by Butterworth Microanalytical Consultancy Ltd. (BMAC), 41 High Street, Teddington - Middlessex, UK) and Dr. F. Pascher (Microanalytical Laboratory 5300 Boon 1, W. Germany)

4. Nuclear Magnetic Resonance Spectra run by Drs. Pascher (address above) and Prof. Dr. Speiser (Pharmaceutic Inst. Galenishe, Abterlung, Switzerland) and BMAC (address above)

5. The ^{13}C NMR was run by Dr. Pascher (address above)

6. Elemental Analysis this was carried out by Drs. F. Pascher and Prof. Speiser.

7. Langendorrfs Heart Perfusion Apparatus from Scientific Research Instruments was used to study the effect of the isolated compound on the heart

8. A Condon Blood Pressure Manometer was used to study the effects of the isolated compound on the blood pressure of anaesthetised rat

CHEMICAL INVESTIGATION OF THE ACTIVE PRINCIPLES IN THE LEAVES OF ELAEODENDRON BUCHANANII (LOES) LOES.

A. Collection of plant material for investigation

The plant material was collected around Dagoretti, Riruta Satellite, and Uthiru on the outskirts of City of Nairobi. All the morphological parts of the plant (i.e. roots, bark and leaves) were separately cut and collected in separate plastic bags. The plant material collected were cut into small pieces and dried in the oven at 60°C for approximately 48 hours.

The dried materials were ground to powder using a Willey Mill and stored in plastic bags until required.

B. Preliminary investigation of the active principles of Elaeodendron buchananii Loes.

The different morphological parts (about 3 gm. each) of the plant were separately screened for the following active principles, alkaloids, cyanogenetic glycosides, cardiac glycosides, anthroquoinones and their derivatives and saponins as follows-:

Alkaloids - The powdered material was extracted with ethanol and to the extract was added dragendorff's reagent, the presence of alkaloids being indicated by an orange precipitate.

Cyanogenetic glycosides- Fresh plant material was moistened in a test tube. A sodium picric acid paper was trapped in the mouth of the test tube and test tube was placed in a warm place. Presence of cyanogenetic glycosides is indicated by the formation of a purple colour on the paper.

Cardiac glycosides- To alcoholic extracts of the plant materials, Keede and Raymond reagents were added, the presence of cardiac glycosides being indicated by the formation of pink and blue-violet in the extracts respectively.

Anthroquinones and their derivatives- The plant material was hydrolysed with hydrochloric acid and extracted with toluene. To the toluene extract was added ammonium hydroxide, the presence of anthroquinones and their derivatives being indicated by the formation of a pink colour.

Saponins- The plant material was mixed with water in a test tube and shaken vigorously to find out if any persistent frothing occured.

The results obtained are presented in Table I.

TABLE I

Results of tests for chemical constituents likely to be present in different morphological parts of Elaeodendron buchananii Loes.

Active principle	Morphological part	Result
a. Alkaloids	Bark, leaves and root	no alkaloids
b. Cyanogenetic glycosides	Bark, leaves and root	no cyanogenetic glycosides
c. Cardiac glycosides	Bark	no cardiac glycosides
	Leaves	cardiac glycosides present
	Root	no cardiac glycosides
d. Anthroquinones and their derivatives	Bark, leaves and root	no anthroquinones and their derivatives
e. Saponins	Bark, leaves and root	no saponins

The results of preliminary investigation of active principle in Elaeodendron buchananii for different morphological parts of the plant indicated that the plant contains substances with α, β- unsaturated γ- lactone ring probably cardiac glycosides. The test for cardiac glycosides were only positive for extractions of the leaves showing that only the leaves contain cardiac glycosides in significant amount. The other active constituents tested for were not detected in any part of the plant.

Investigation of suitable extracting solvents for cardiac glycosides from the leaves of Elaeodendron buchananii

The leaves were shown to contain cardiac glycosides (page 011) in the preliminary investigation of active principles of Elaeodendron. The other parts of the plant were found to contain negligible amount of these compounds. Therefore, investigation for suitable solvents or mixtures of solvents for extraction of cardiac glycosides from the leaves was carried out.

A comparative study of extraction efficiency of various solvent systems was done by placing equal quantities of dried powdered leaves (about 250g.) in 500 ml flasks containing different solvent systems. The extraction was carried out using the following solvent systems:-

i) Ether
ii) Methanol
iii) Chloroform
iv) 95% alcohol
v) 70% alcohol
vi) 50% alcohol/chloroform (1:1)
vii) Chloroform/absolute alcohol (1:1)

To each flask containing 250g of powdered leaves was added 200 ml of

each of the above solvent system and allowed to macerate on a water - bath at 30°C overnight except for ether, where the maceration was carried out at room temperature.

In case of 50% alcohol/chloroform system, adequate water was added to the macerate to effect the separation of the mixture into aqueous and organic layers. The mixture was transferred to a separating funnel and the two layers were then separated. Each of these two layers was treated as an extract on its own right.

Each of the extract from the above solvent systems was filtered and decolourised using charcoal and evaporated at 45 - 50°C to dryness under reduced pressure. The residue from each extract was dissolved in 80 percent alcohol and treated with saturated lead subacetate solution to precipitate out tannins. The precipitated tannis were filtered off and the tannin - free extracts treated with a 10% solution of sodium sulphate to remove excess lead. The contents were filtered and decolourised further by passing through a bed of charcoal held in a filter paper. The extracts were reduced to 15 ml at 45 - 50°C under reduced pressure.

In each case, the extract obtained was slightly yellow in colour, but sufficiently clear to allow measurement of absorbance of the coloured complex formed when reacted with Kedde reagent.

To 5 ml of each extract was added 2 ml of Kedde reagent and the intensity of the coloured complex formed was measured by a UV spectrophotometer. The results obtained are presented in Table II.

From these results it was found that powdered leaves macerated with 50% alcohol/chloroform solvent system gave the highest absorbance reading for the chloroform portion of the extract. The aqueous portion contained little or none of cardiac glycosides. The efficiency of extraction was found to decrease in the order:- 50% alcohol/chloroform > chloroform/absolute alcohol > chloroform > 95% alcohol > 70% of alcohol >> methanol > ether. Therefore, it was concluded that 50% alcohol/chloroform system was the best solvent system for the extraction of cardiac glycoside(s) using the above procedure. An added advantage of the above solvent system over the others is that most of the tannins were left in the aqueous layer, thus giving a relatively tannin - free extract.

Extraction and purification of cardiac glycosides from the leaves of Elaeodendron buchananii

Material and Methods

Approximately 2 kg of ground dry leaves were weighed and put into a 12 litre flask. Adequate 50% alcohol was added until the plant material was completely covered with the solvent. The material was left to macerate overnight on a water - bath at 30^{o}C. Enough chloroform (3 litres) was then added to the material and the extraction was allowed to continue for another 24 hours at 30^{o}C with occasional shaking. The material was then removed from the water bath and allowed to stand for four days at room temperature with occasional shaking. Adequate water was added to the material to effect the separation of chloroform and aqueous layers. The mixture was filtered by passing through cotton wool held in a filter funnel after which it was transferred to a separating funnel and left to separate. The chloroform layer (at the bottom) was run off and the aqueous layer was returned

to the plant material and the extraction was repeated twice, each time with 4 litres of chloroform. The chloroform extracts were combined (about 11 litres) and distilled under reduced pressure at 45 - 50°C using a rotatory evaporator. The residue obtained (about 15g) was a dark green resinous material.

Purification of the extract

The dark green resinous material was defatted by washing several times with petroleum spirit which removed most of the green colour along with most of the fatty material. The residue obtained was a dry crumbly material. This was dissolved in alcohol (95%) and treated with lead subacetate strong solution to precipitate out tannins (Canback, 1949). The alcoholic extract was then filtered and the precipitate of tannins was washed with alcohol (3 x 100 cc) so as to recover as much as possible any glycosides which might have co-precipitated with tannins (Canback, 1949, Rowson, 1952). The alcoholic washings were added to the mother liquor. The filtrate (combined alcoholic extracts) was treated with a 10% solution of sodium sulphate to remove excess lead (Sehandri and Subramanian 1952, Rowson 1952, Rangaswami and Subramanian, 1955). The contents were again filtered and the lead precipitate was washed with alcohol (2 x 100 cc), each time adding the washings to the filtrate.

The above process not only removed tannins and excess lead but also most of the pigments. The final alcoholic extract (900 cc) had a light green colouration.

The alcoholic extract was evaporated to dryness under reduced pressure at 45 - 50°C and the residue was dissolved in 350 ml of chloroform. This helped to remove any excess sodium sulphate present in the extract. The chloroform extract was further decolourised by adding charcoal to the extract and shaking the mixture vigorously and finally filtering the extract. The charcoal was washed with chloroform (300 cc) until the washings were negative for cardenolides when tested with Raymond reagent. The washings were added to the filtrate. The charcoal removed most of the pigments. However, the solution obtained was yellow in colour and gave positive test for cardenolides when tested with Kedde and Raymond reagents. The volume of glycosidal solution obtained (about 650 cc) was found to be too large to work on. Consequently, it was concentrated to 50 ml under reduced pressure at 45 - 50°C, transferred to a petri - dish and evaporated to dryness by blowing hot air current over the surface of the dish. The residue obtained had a syrupy consistency and was brown in colour. The residue indicated the presence of cardiac glycosides when tested with Keller - Killiani reagent.

The residue was then dissolved in minimum amount of chloroform and run down a column of neutral active alumina (2 ft long). Elution was first carried out using alcohol (95%) and ethyl acetate (200 cc). Alcohol and Ethyl acetate eluates were collected into 100 ml portions. When chloroform was used, the eluate collected (first 100 ml and then 50 ml portions) contained the glycoside(s) and little of the coloured material. The results of the column purification are presented in Table III.

The positive eluates were combined and evaporated to dryness under reduced pressure at 45 - 50°C. The residue obtained was yellow

in colour and was dissolved in the minimum amount of ether. Most of the yellow coloured material did not dissolve in ether. The ethereal solution was filtered and both the filtrate and the precipitate (yellow coloured material) were tested for cardiac glycosides with Kedde reagent. The precipitate gave a negative test while the ethereal solution was positive for cardenolides. The glycosidal solution of ether was evaporated to dryness on a water - bath at 30°C and a glycosidal residue which was slightly yellow coloured and amorphous was obtained.

To effect further purification of the amorphous glycoside(s), the glycosidal residue was dissolved in minimum amount of chloroform and rerun down a column of neutral active alumina (1ft long). Chloroform was used as the eluant and the eluate was collected in 5 ml portions, each fraction being tested for cardenolides with Kedde reagent.

It was found that the first few portions were coloured but negative for cardenolides. The rest of the eluates were clear and positive for cardenolides. Elution from the column was carried out until the eluate was negative with Kedde reagent.

The eluate (230 ml) was distilled to 20 ml under reduced pressure at 45 - 50°C. The concentrated extract was transferred to a petri - dish and the chloroform was evaporated on the water - bath at 45°C. A slightly yellow coloured crystalline residue was obtained.

The residue was positive to the glycosidal test with Keller - Killiani reagent and with Kedde and Raymond for cardenolides. The glycosidal residue was redissolved in minimum amount of ether and the

solution was filtered through a bed of charcoal held in a filter paper. A clear glycosidal solution of ether was obtained.

C. Crystallisation of the isolated glycoside(s)

The glycosidal solution of ether was warmed on the water - bath at 30°C to evaporate the ether. As the ether evaporated a white amorphous precipitate was observed to form. The precipitate was allowed to form until no further precipitation took place. The precipitate was filtered off and tested for cardiac glycosides with Kedde, Raymond and Keller - Killiani reagents. The results were positive after which attempts were made to obtain the amorphous glycoside(s) in crystalline form.

The amorphous glycoside(s) was sampled into different peteri - dishes so that different solvents or solvent mixtures could be tried to find a suitable solvent system for crystallisation. The following solvent systems were tried:-

i) chloroform
ii) chloroform - ethanol (9:1)
iii) chloroform - ethanol (3:2)
iv) chloroform - ethanol (2:1)

These solvent systems were chosen because they have been used by other workers (Rangaswani and Subramanian 1955) with success to crystallise cardiac glycosides from squill (Indiana maritima).

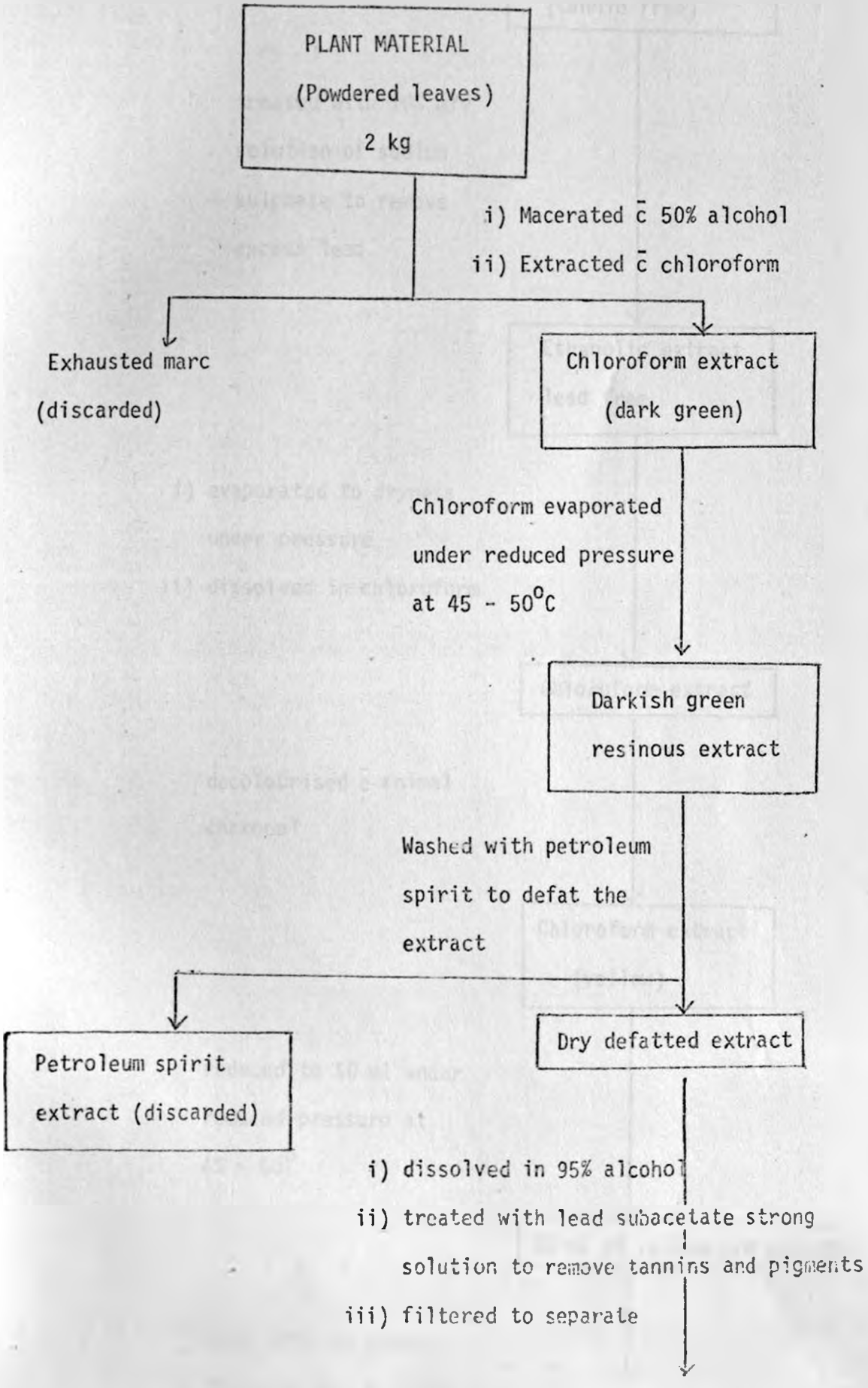
Attempts to crystallise the glycoside(s) was made by dissolving the amorphous glycoside(s) in minimum amount of each of the above solvent system and allowing the glycosidal solutions to cool and evaporate at 30°C.

It was found that the best solvent system for crystallisation of the isolated glycoside(s) was chloroform - ethanol (9:1) system The rate of appearance of crystals was faster than in the rest of the solvent systems. The rate of appearance of crystals was observed to occur in this order:- chloroform - ethanol (9:1) > chloroform - ethanol (2:1) > chloroform - ethanol (3:2). The crystals produced by the three solvent systems (ii), (iii), (iv) above were the same. The chloroform system (i) above also produced crystals but they were smaller in size than those formed from the other solvent systems. So for further work chloroform - ethanol (9:1) system was used to crystallise the isolated amorphous glycoside(s).

The 2 kg of the dried leaves of Elaeodendron buchananii (Loes.) Loes yielded about 300 mg of crystals.

The extraction and purification techniques described above are summarised in form a flow chart on the next page.

FLOW CHART FOR THE EXTRACTION AND PURIFICATION OF THE ACTIVE PRINCIPLE OF ELAEODENDRON BUCHANANII



Ethanolic extract
(tannin free)

treated with 10% w/v solution of sodium sulphate to remove excess lead

Ethanolic extract
lead free

i) evaporated to dryness under pressure
ii) dissolved in chloroform

Chloroform extract

decolourised c̄ animal charcoal

Chloroform extract
(yellow)

reduced to 50 ml under reduced pressure at 45 - 50^{o}

50 ml of chloroform extract

evaporated to dryness using hot air current

resinous extract

i) Dissolved in the minimum amount of chloroform
ii) Run down on an alumina (neutral) column

chloroform eluate (slightly yellow)

i) evaporated to dryness under reduced pressure at 45 - 50°C
ii) residue dissolved in the minimum amount of ether

ethereal extract

evaporated to dryness at 30°C

amorphous glycosidal residue

i) dissolved in the minimum amount of chloroform
ii) run down an alumina (neutral) column
iii) eluated c chloroform

a clear eluate of chloroform

evaporated to dryness

A glycosidal residue (slightly yellow

dissolved in the minimum amount of ether

An ethereal glycosidal solution

ether evaporated off

A white amorphous glycosidal precipitate

i) dissolved in the minimum amount of chloroform/ethanol 9:1

ii) cooled and allowed to evaporate at $30^{o}C$

CHARACTERISATION OF THE ISOLATED GLYCOSIDES

A. Investigation of the general physical properties

i) Solubility - the solubility of the isolated glycoside(s) was investigated by attempting to dissolve the compound in various solvents at room temperature (21^{o}C). About 10 mg of the compound was placed in a micro - test tube and to it was added the respective solvent using a dropper pipette. The degree of solubility of the isolated glycoside(s) was determined by the number of drops of the solvent required to dissolve the compound. The following solvents were tried:-

i) Ether
ii) Methanol
iii) Acetone
iv) Ethyl acetate
v) Absolute alcohol
vi) Chloroform

The results of solubility are presented in Table IV.

ii) Melting point - the melting point of the isolated glycoside(s) was determined using capillary melting point apparatus and the hot stage microscope. It was observed that the compound melted at 110 - 111^{o}C. The narrow melting range indicated that the compound was pure and probably only one glycoside.

B. Detection and identification

i) Colour reactions - Concentrated sulphuric acid - two drops of concentrated sulphuric acid were added to a few crystals of the isolated compound. The crystals dissolved and the solution went

brown in colour. When the same test was repeated with digoxin and ouabain, similar results were obtained.

ii) Reaction with m - dinitrobenzene (Raymond reagent) - A few crystals were dissolved in 10 ml of alcohol (80%). To 1 ml of this solution was added 1 ml of Raymond reagent followed by a few drops of a 20% w/v solution of sodium hydroxide. A blue - violet colour developed and this was observed to fade fairly rapidly to a pale greenish colour. Similar results were observed with digoxin and ouabain. The above test have been used to test for cardenolides and a positive test with Raymond reagent indicated that the isolated compound is a cardenolide.

iii) Reaction with 3:5 dinitrobenzoic acid (3:3 DNBA) Kedde reagent
The Kedde reagent is also used to test for cardenolides. The isolated compound, having been positive with Raymond reagent, it was tested with Kedde reagent. 1 ml of the ethanolic solution containing the isolated compound (approx. 0.1% w/v) was reacted with Kedde reagent. A purplish colour developed, and unlike with Raymond reagent, the colour was observed to fade very slowly.

iv) Reaction with sodium nitroprusside (Legal's reagent) - A few crystals of the isolated compound were dissolved in pyridine. To this was added a drop of a 2% w/v solution of sodium nitroprusside, followed by a drop of a 2% w/v solution of sodium hydroxide solution. A deep red colour was produced indicating that the isolated compound is a cardiac glycoside.

v) Reaction of Keller - Killiani reagent - A few crystals of the isolated compound were dissolved in 1 ml of glacial acetic acid.

Two drops of a 5% w/v solution of ferric chloride were added and the contents were carefully transferred onto the surface of concentrated sulphuric acid (1 ml) in a test tube. A brown ring developed at the junction of the two liquids and the upper layer slowly acquired a bluish green colour after about 10 minutes. This constitutes a positive test for cardiac glycosides containing 2 - desoxy sugars (Fieser, 1959).

C. Chromatographic analysis of the isolated glycoside(s)

Chromatographic analysis of the purified glycoside(s) was carried out to determine the possible number of cardiac glycosides from the material isolated from the leaves of Elaeodendron buchananii (Loes.).

Various solvent systems were tried in an attempt to effect the resolution of the isolated glycoside(s). In all cases some reference glycosides (digoxin and ouabain) were run against the isolated glycoside(s) of Elaeodendron.

THIN - LAYER CHROMATOGRAPHIC ANALYSIS OF THE ISOLATED GLYCOSIDE(S)

Experimental procedure - TLC plates (20 x 20 cm) were prepared according to the method described by Stahl, (1965) using Silica gel G. The plates were air - dried by leaving them exposed to air at room temperature overnight. Several chromatographic tanks were prepared using different solvent systems and the tanks were saturated with each solvent system. To the silica gel plates about 5 µl of the test and reference glycosidal solutions were spotted on the plates which were developed in the tanks. The solvent system was left to travel to approximately 13 cm after which the plates were

removed and dried in a current of hot air. The plates were sprayed with Kedde reagent and visualised in daylight. The results obtained are presented in Table V.

The TLC results show that both the isolated glycosidal fraction and a crude extract of the leaves of the plant produced one spot with the same Rf value and same colour for all the solvent systems tried. The Rf value of the spot produced by the isolated glycoside and crude extract of the plant was different from that of ouabain and digoxin. Literature survey also showed that no known cardiac glycoside has similar Rf values in the solvent systems used. The colour, on spraying with Kedde reagent was the same for the isolated glycoside, crude extract of the leaves, ouabain and digoxin and for unknown glycoside from Ajuga remota Benth.

D. Spectroscopy of the isolated compound

i) Examination of UV absorption spectrum of the isolated compound

Method: Approximately 100 mg of the isolated crystals were accurately weighed and dissolved in 100 ml of absolute alcohol in a volumetric flask. This solution was used as a stock solution for the study of UV absorption spectra of the isolated compound.

2 ml of the stock solution were transferred to a 10 ml volumetric flask and made up to volume with absolute alcohol. This solution was used for UV spectrophometric analysis of the isolated compound.

The isolated compound was found to have an absorption maximum at 208 nm. The spectrum is presented in Figure 2.

ii) Examination of the absorption spectrum of the by the isolated compound and 3:5 dinitrobenzoic acid (Kedde reagent)

One millilitre of a 100mg % solution (stock solution) was added to 1 ml of a 2%w/v solution of dinitrobenzoic acid (3:5 DNBA) and 1 ml of 1 N sodium hydroxide solution and the volume was made up to 5 ml with ethanol in a volumetric flask. The mixture was allowed to stand at room temperature for four minutes. A blank was prepared in the same way as the reaction mixture except that 1 ml of alcohol was substituted for the test solution.

The maximum intensity of the purple colour which developed in the reaction mixture was not attained immediately. The purple colour has a steady value approximately 8 minutes after the addition of 1 N sodium hydroxide solution (C.K. Maitai, Msc thesis, Otago University, New Zealand). This time interval was employed in the intial spectroscopic studies of the isolated compound.

After the full purple colour had developed in the reaction mixture, the absorption spectrum (190-700 nm) was determined. The absorption spectrum showed two maxima, a sharp peak at 355 nm and a broad peak at 540 nm (Figure 3).

The change in colour intensity of the reaction mixture was investigated over a period by running spectra of the reaction mixture after 15, 20, 30 and 45 minutes. No change could be detected at the absorption maximum at 355 nm wavelength but because of the broad nature of the peak at 540 nm it was impossible to discern the change in the absorption maximum. The optical density at both wavelengths decreased. This indicated that the two wavelengths can be used to

plot a graph of absobance against concentration to find out if Beer-Lambert law is obeyed under specified experimental conditions.

iii) Examination of the absorption spectrum of the coloured complex formed by the isolated compound and m-dinitrobenzene (Raymond reagent)

The coloured complex of the reaction mixture of the isolated compound and 1, 3 DNB was developed by taking 1 ml of 100mg % solution of the isolated compound, 1 ml of 1, 3 DNB and 1 ml of 1 N sodium hydroxide. The solution was made up to 5ml in a volumetric flask. A blank was prepared in the same way except that 1 ml of absolute alcohol was substituted for the test solution.

The violet colour which appeared on adding sodium hydroxide was found to change to blue and eventually to light brown. This change in colour has been found not only to affect the height of the peak but also the position of the absorption maximum (C.K. Maitai, Msc. thesis, Otago University, New Zealand). Therefore, it was decided to examine the absorption spectrum in the blue colour of the reaction mixture because the colour is intense and the peak height is at its maximum (Hassel et al 1953 thro' C.K. Maitai, MSc. thesis, Otago University, New Zealand). The spectrum is presented in Figure 4.

The spectrum was found to have a broad peak with maximum at 590 nm. The spectrum is disorted due to colour change during the time of run.

iv) Examination of the infra-red spectrum of the isolated compound

A potassium bromide disc of the isolated compound was prepared using the hydraulic press. A whole spectrum of the compound was run and the main peaks noted.

The spectrum obtained is presented in Figure 5 and the main peaks are presented in Table VI where those of digitoxigenin have been included for comparison purposes only.

v) Elemental analysis of the isolated compound - An elemental analysis of the isolated compound was done by two independent laboratories:-

1) Dr. F. Pascher, Microanalytical Laboratory, Boon, West Germany

2) Prof. Speiser, Pharmaceutical Institute, Abteilung, Switzerland

The results are presented in Table VII.

TABLE VII

RESULTS OF ELEMENTAL ANALYSIS

Element	Dr. Pascher's laboratory	Prof. Speiser's laboratory
Carbon	63.67	63.9
Hydrogen	7.87	5.32
Oxygen	28.01	28.3

From these elemental analysis the empirical formula was determined. The molecular weight of the compound was determined as 603 and from the empirical formula and the molecular weight, the molecular formula was calculated. The results are presented in Table below.

TABLE VIII

EMPIRICAL FORMULA AND MOLECULAR FORMULA OF THE ISOLATED GLYCOSIDE AS CALCULATED FROM THE ELEMENTAL ANALYSIS AND MOLECULAR WEIGHT

Laboratory	Empirical formula	Molecular weight	Molecular formula
Dr. Pascher's	$C_3H_{4.5}O_1$	603*	$C_{32}H_{47}O_{11}$
Prof. Speiser's	$C_3H_{4.43}O_1$		$C_{32}H_{47}O_{11}$

* Molecular weight was determined by Dr. Pascher.

vi) Examination of the ^{13}C NMR spectrum of the isolated compound

The ^{13}C NMR of the isolated compound is presented in Figure 7 and the data is presented in Table IX. $CDCl_3$ was used as the solvent and TMS as the internal standard.

Only a partial interpretation of the spectrum has been carried out by attempting to estimate the number of carbon atoms in the isolated compound. This has been done using the intensity of the peaks and with reference to the internal standard (TMS).

vii) Examination of the proton NMR spectrum of the isolated compound

The proton NMR spectrum of the isolated compound is presented in Fig. 8. The spectrum was not properly integrated and so it was found difficult to determine the number of protons in the compound.

viii) Examination of the mass spectrum of the isolated compound

The mass spectrum of the isolated compound is presented in Fig. 9 and the m/e values of its fragment ions are presented in Table X. For comparison purposes, the m/e values of two steroids, somalin and digitoxigenin are included in the same table. The m/e values of somalin and digitoxigenin was obtained from Ardene (1964).

SPECTROPHOTOMETRIC DETERMINATION OF THE PERCENTAGE RECOVERY OF THE ISOLATED GLYCOSIDES

To determine the percentage recovery of the isolated glycoside, the relationship between absorbance of the coloured complex formed by reacting the isolated glycoside with Kedde reagent and the concentration of the drug was investigated first.

A 0.1% w/v solution of the isolated glycoside was made by dissolving 100 mg of the glycoside in 95% alcohol and making the solution to 100 ml in a volumetric flask. From this solution, a series of dilutions were made to give the following standard concentrations:- 0.06, 0.048, 0.04, 0.032, 0.020, 0.012 and 0.004% w/v. To 2 ml of each of these concentrations was added 1 ml of 2% solution of dinitrobenzoic acid (3:5 DNBA) and 1 ml of sodium hydroxide. The volume was made up to 5 ml in a volumetric flask and the mixture was allowed to stand at room temperature for four minutes.

The abosrbance of the coloured complex from each standard concentration was determined and a calibration curve of absorbance against concentration was plotted. The results obtained are presented in Table XI and Fig. 10. A straight line passing through the origin was obtained indicating that Beer - Lambert law was obeyed under the prevailing conditions.

DETERMINATION OF THE PERCENTAGE RECOVERY

Two samples of 50 g of dry powdered leaves were accurately weighed into 1 litre flasks and extracted with a mixture of 50% alcohol and chloroform as described previously. After the decolourising stage, the chloroform extracts were adjusted to 250 ml. From each of the 250 ml extract, 10 ml were evaporated to dryness. Each of the residue was dissolved in 3 ml of alcohol (95%) and 1 ml of a 2% solution of 3:5 DNBA and 1 ml of 1 N. Sodium hydroxide solution were added.

A blank was prepared in the same way except the extract was omitted.

The absorbances of the coloured complexes formed from each extract was read from the calibration curve (Fig. 10).

CALCULATION OF THE PERCENTAGE RECOVERY

From the absorbances of the two extracts (referred to as T_1 and T_2) their concentrations from the calibration curve were found to be 26×10^{-3}% and 29.5×10^{-3}% respectively.

i) 10 ml of the extract were found to contain 26×10^{-3}g.

∴ 250 ml of the extract contain:-

$$\frac{26 \times 10^{-3} \times 250}{10}$$

$$= 0.65 \text{ g.}$$

∴ The percentage recovery is

$$\frac{0.65 \times 100}{250}$$

$$= \underline{0.26\%}$$

ii) Using the second extract, the percentage recovery was found to be 0.295%.

A PRELIMINARY INVESTIGATION OF THE PHARMACOLOGICAL EFFECTS OF THE ISOLATED COMPOUND ON INTACT AND ISOLATED MAMMALIAN PREPARATIONS

The chemical screening of the plant for active principles showed the presence of cardiac glycoside(s) in the leaves of the plant. Subsequent extraction, isolation and purification gave a chemical compound which on investigation proved to be a cardiac glycoside.

Cardiac glycosides have a direct stimulating effect on the myocardium with consequent increase in the force of contraction and hence an increase in efficiency of a failing heart. This effect of cardiac glycosides has secondary effects such as diuresis due to improved circulation. Cardiac glycosides may also have a direct effect on renal tubules (Sim, 1967). Digoxin, for example is used to treat oedema in addition to its use to treat heart diseases (Bowman et al, 1970).

Since the isolated compound was shown to be a cardiac glycoside, pharmacological investigations were carried out to find out whether the isolated compound has any effects on the blood pressure of an intact rat and its effects on an isolated perfused rabbit heart. The above two pharmacological effects would help to confirm the chemical and spectroscopical findings which indicated that the isolated compound is a cardiac glycoside.

INVESTIGATION OF THE EFFECTS OF THE ISOLATED COMPOUND ON THE BLOOD PRESSURE OF AN ANAESTHETISED RAT

Material and methods

Adult rats (200 - 300g) were anaesthetised with a 25% w/v aqueous solution of urethane by injecting 0.3 ml/1 kg of the anaesthetic intraperitoneally. The trachea, common carotid artery and external jugular vein were exposed and cannulated according to the method described by Mcleod L. J. et al 1970 in his book "Pharmacological experiments on intact preprations". The arterial cannula was connected to a condon blood pressure manometer and the drug injections were given through the venous cannula. The condon blood pressure manometer was in turn connected to a kymograph (see Fig. 11).

Preparation of the drug mixture

100 mg of the isolated compound were dissolved in 10 ml of propylene glycol by warming on a water - bath until all the compound dissolved. The solution was cooled and made to 100 ml with normal saline. A control was also prepared containing a 10% w/v propylene glycol in normal saline.

Injections of adrenalin 100 mg/ml and digoxin 1 mg/ml were also prepared for comparison.

Test with the drug

0.1 ml of the control was injected at point A and the effect of the control is represented by section AB (Fig. 12). The blood pressure was allowed to settle and adrenalin (10 µg) was injected at point C. After the effect of adrenalin had subsided and the blood pressure was back to normal the test material (0.4 mg) was injected at point E after mean blood pressure had returned to normal.

RESULTS

The results (Fig. 12 and Table XII) show that the control caused a transient fall in blood pressure and that adrenalin had an immediate increase in blood pressure with a quick recovery. The drug mixture first showed a fall in blood pressure due to propylene glycol then a gradual rise above the mean blood pressure. The effect was sustained. Digoxin was also found to have a sustained pressor effect. The response of the isolated compound compared very well with that of digoxin (Fig. 12).

INVESTIGATION OF THE EFFECTS OF THE ISOLATED COMPOUND ON THE ISOLATED PERFUSED RABBIT HEART

The isolated perfused rabbit heart preparation was carried out as described by Mcleod et al, 1970. The heart response i.e. both chronotropic and inotropic effects, were recorded in a kymograph and also by a Harvard smooth muscle/heart recorder. Solutions of the isolated glycoside, adrenalin, control and digoxin were used on the isolated heart each at a time.

RESULTS

The results (Fig. 13 and 14) show that propylene glycol did not have any significant effects on the isolated perfused heart. Adrenalin had an immediate increase in heart rate and force of contraction while the isolated compound increased the force of contraction while lowering the heart rate (Fig. 14). Digoxin had an effect similar to that of the isolated compound (Fig. 13). They both had a gradual increase in force of contraction, by far the most important property of cardiac glycosides on the heart.

CHAPTER 3

DISCUSSION

A literature survey of Elaeodendron buchananii (Loes.) Loes. showed that there is no record of the plant having been used as a medicinal herb by the Africans. The plant, however, is known to have caused deaths to stock. Most of the poisonous plants of East Africa are used in small doses either in the treatment of various ailments in form of decoctions prepared by herbalists or for trial by ordeal or as arrow poisons by poachers.

Pharmacognostical investigation on Elaeodendron showed that the plant grows in different habitats and the height of the tree varies depending on soil and climatic conditions. The plant can always be distinguished by the characteristic features of the leaves and flowers. No other plant growing in the same habitats was found to have similar features. Even those growing among grass and aloes (Plate I) can easily be distinguished. Animals hardly have any access to the leaves and consequently few deaths have been reported. Where a tree has coppiced from a cut stump (Plate II) young shoots appear around the tree trunk and these shoots have been responsible for numerous deaths that have been reported. Where the tree is growing among other foliage in form of a shrubby growth (Plate III) it is difficult even for man to distinguish it from other trees. Such shrubby growths of Elaeodendron have been responsible for deaths to stock, especially goats which feed very much on twigs. Human fatalities have not been reported although the tree is used as a firewood in the homes in rural areas. This would probably be due to the

fact that is the dry trunk which is used and not the leaves. Giraffes have been known to brownse on the tree with punitive immunity.

Preliminary work using standard chemical tests on ethanolic extracts of the leaves of Elaeodendron buchananii indicated the presence of compounds α, β- unsaturated δ- lactone ring which were shown to be cardiac glycosides by Keller - Killiani reagent. Investigation of other active principles, for example, alkaloids, cyanogenetic glycosides et centra in different morphological parts of the plant revealed that only the leaves contain cardiac glycosides in significant amount. Test on different morphological parts of the plant for the active principles gave negative result.

Investigation for the best extracting solvent for the cardiac glycoside(s) present in the leaves of the plant showed that the suitable solvent and conditions under which the extraction of the glycoside(s) could be carried out was as follows:- first, digesting the powdered leaves with 50% alcohol overnight at $50^{o}C$ and then adding chloroform to the macerate and adding enough water to effect the separation of the aqueous and chloroform layers. The chloroform was found to extract most of the cardiac glycoside(s) and after exhausting the aqueous layer of the glycoside(s) by washing several times with small portions of chloroform the aqueous layer was discarded. The solvent systems chosen for this investigation were those which have been used by other workers (Rangaswani and Subramanian, 1955, Canback 1949, Raymond 1932, Rowson 1952) with success to extract different cardiac glycosides from different plant sources. Also these solvent systems have been given as general solvent systems for extraction of medicinal plant glycosides (S. K. Sim, 1967).

Chloroform and alcohol (absolute alcohol), 95%, 70%) were found to extract the glycoside(s) from the plant and the differences in the amount extracted could be due to differences in the degree of selectivity and solubility of each solvent system. Alcohol being non - selective extracted a lot of other unwanted material (e.g. pigments, tannins etc) than chloroform. The differences in the amount of glycoside extracted by different alcohol percentages could also be due to differences in selectivity and differences in the degree of solubility of the cardiac glycoside(s). From the results, it seems that the cardiac glycoside(s) of Elaeodendron buchananii are more soluble in chloroform than in absolute alcohol. The solubility in alcohol decreases with decrease in the percentage of alcohol. This fact was confirmed when the solubility of the isolated glycoside was investigated. The advantage of the above extraction procedure over the other solvent systems is that almost all the tannins were extracted in the aqueous layer and this on its own was a stage of purification in the isolation process. Maceration by 50% alcohol seemed to help the extraction of the glycoside(s) by chloroform probably by removing a lot of tannins, pigments and resins which would otherwise have interferred.

Von Eew et al (1951), Stoll and Kreis (1951), Rangaswani and Subramanian (1955), Fieser and Fieser (1959), and many other workers have indicated the use of petroleum spirit to remove resinous fatty materials from plant extracts. In this case too the unwanted material was removed by washing with petroleum spirit the syrupy residue obtained on evaporating off the chloroform.

The petroleum spirit only removed part of the unwanted material. Canback (1949), Sehandri and Subramanian (1950), Rowson (1952), Maitai (1969) and many other workers have shown that plant tannins

(and pigments) could be removed successfully from alcoholic extracts of the plant by use of lead subacetate solution. Canback (1949) and Rowson (1952) also have shown that during the precipitation of tannins co-precipitation of active principles occurs. Therefore, it was necessary to dissolve the glycosidal residue in alcohol (95%) and on precipitation of tannins, to wash the precipitate with ethanol several times and to add the ethanolic washings to the mother liquor to recover as much of the co-precipitated glycosides as possible. The tannin - free extract contained excess lead which needed to be removed before further purification was attempted. A 10% solution of sodium sulphate was used to remove excess lead (Rangaswani and Subramanian, 1955). The precipitation of tannins enhanced the decolourisation of the extract but still the extract was green in colour and had excess sodium sulphate. By evaporating the alcoholic extract to dryness and redissolving the residue in chloroform, excess sodium sulphate was got rid off. Animal charcoal was used to decolourise the extract and by first saturating the charcoal with chloroform the loss of the glycoside(s) was minimised. Also saturation of the charcoal hastened the filtration.

In the present work, charcoal failed to remove the coloured material completely. This probably may be due to the fact that the yellow colouring matter of the extract was not adsorbed to the charcoal or if any adsorption occured, the desorption in the chloroform was favoured. The alumina column was found to be successful in removing most of the coloured matter. Choice of the eluent was an important factor. When alcohol and ethyl acetate were used as eluants, both the glycoside(s) and a lot of the colouring matter were eluated simultaneously from the column but when non-polar solvents, for example, chloroform was used most of the colouring matter was

left adsorbed to the column. The fact that the first column did not decolourise the extract completely could be attributed to the short length of the column used.

Final purification of the isolated glycoside(s) was done by crystallising the glycoside from chloroform/ethanol (9:1), a system which was found to give pure crystals at a reasonably good time as compared to the other solvent systems tried.

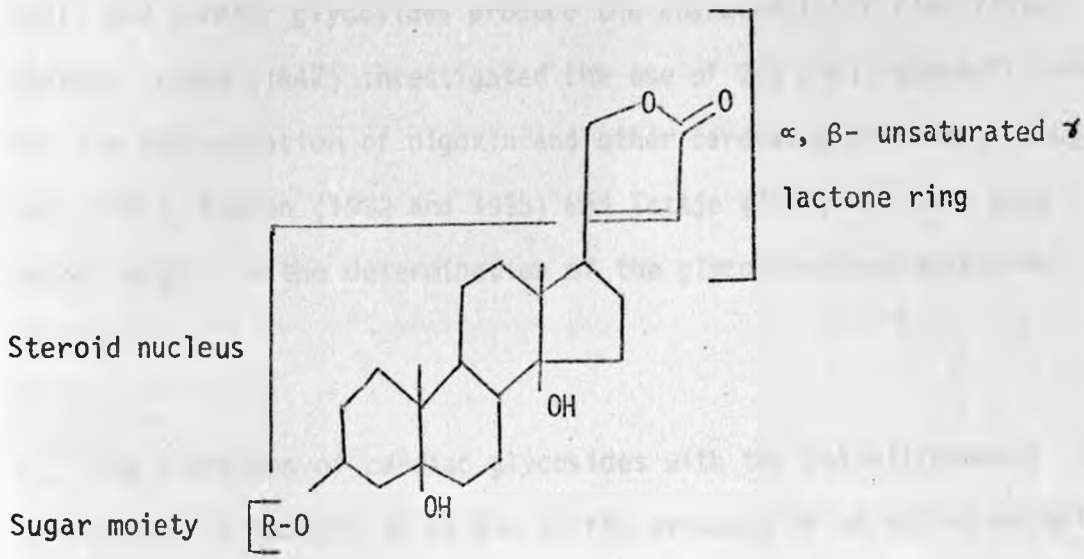
CHARACTERISATION AND IDENTIFICATION OF THE ISOLATED GLYCOSIDE

The solubility and the melting point of the isolated glycoside were determined as described under physical properties (page 024). The compound was found to be insoluble in water but soluble in organic solvents. However, the degree of solubility in different organic solvents was found to vary, being extremely soluble in chloroform, soluble in alcohol and fairly soluble in the others. The isolated glycoside melted at 110 - 111^{o}C which indicated that the compound was pure. Literature survey showed that no known cardiac glycoside melts within this range, an indication that the isolated glycoside could be a new cardiac glycoside.

The isolated glycoside was reacted with various colour reagents to determine the best chemical tests for the purpose of detection. The colour reactions (given by the isolated glycoside when reacted with various colour reagents) while not being specific for cardiac glycosides and their aglycones, are nevertheless characteristic of the compounds having a cardenolide type of aglycones. Such reagents included concentrated sulphuric, Raymond, Kedde and Keller - Killiani reagents. When the isolated glycoside gave these characteristic colour

reactions, they constituted a positive test for cardiac glycosides indicating that the isolated compound is a cardiac glycoside.

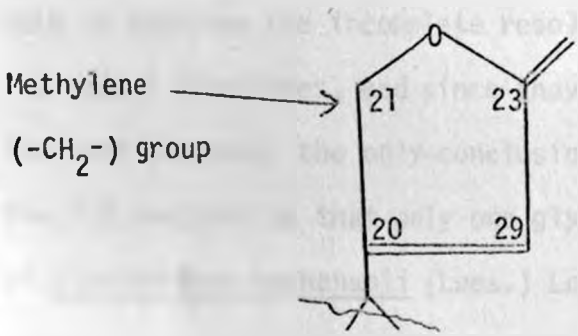
Cardiac glycosides undergo colour reactions with the above reagents based on the different parts of the molecule.



The α, β- unsaturated lactone ring gives reactions with polynitrophenyl derivatives (e.g. Kedde and Raymond reagents), the steroid nucleus reacts with concentrated acids and oxidising agents, while the sugar moiety reacts with ferric salts. When the isolated compound was reacted with concentrated sulphuric acid, a reddish brown colour was produced. This colour with concentrated sulphuric acid is known to be produced by cardiac glycosides like ouabain, digoxin, strophanthidin etc. and can be attributed to the steroidal nucleus. Not all cardiac glycosides give this colour and impurities too could give a positive test. However, since other chemical tests with the isolated compound were positive for cardiac glycosides, this test could be considered positive for a cardiac glycoside for the isolated compound.

Testing with polynitrophenyl derivatives, the isolated compound gave positive colour reactions as would be expected with other cardiac glycosides. The observed colour complexes with Kedde and Raymod reagents for the isolated compound are given by cardenolides. Buch and Taylor (1952), Zoller and Tamm (1953), Shoppe (1964), Mathound (1935) pointed out that m - dinitrobenzene (Raymond reagent) and cardiac glycosides produce the characteristic blue-violet colour. Kedde (1947) investigated the use of 3:5 dinitrobenzoic acid for the determination of digoxin and other cardiac glycosides. Langejam (1947), Rowson (1952 and 1955) and Tattje (1957) all have used Kedde reagent in the determination of the glycosides and aglycones of digitalis.

The reactions of cardiac glycosides with the polynitrophenyl derivatives is thought to be due to the presence of an active methylene ($-CH_2$) groups C - 21. These colour reactions are given by other



compounds e.g. acetone and the 17 keto-stroids will give a positive Raymond test (Stahl, 1969). So these tests are not on their own conclusive for the butenolide group. The Keller - Killiani test for 2 - desoxy sugars was positive indicating that the isolated compound was a glycoside and not an aglycone. The identity of the sugar is not known and whether the glycoside has one or more sugar units has as yet to be determined.

The results of the TLC analysis showed that the isolated compound was one glycoside. The solvent systems used produced one spot for a crude extract of the leaves whose Rf value was the same as that of the isolated glycoside. This showed that no other glycoside was lost during the extraction and purification process. The solvent system, chloroform - methanol - formamide (80:19:1) has been used with success (Planta Medica, 1973) to resolve the glycosides and aglycones of squill (Indiana maritima) which has over 14 glycosides and has been indicated to be a good solvent system for TLC analysis of other plant glycosides. These results indicate that there is one glycoside in the plant. The Rf values of the isolated compound for the solvent systems and adsorbent used do not compare with any known cardiac glycoside.

In general very rarely do cardiac glycosides occur singly in plants but the TLC analysis show that only one glycoside is present in Elaeodendron. Use of more than one solvent system would normally help to overcome the incomplete resolution of several compounds into individual glycosides, and since they all produced one spot for the isolated compound, the only conclusion which can be arrived at from the TLC analysis is that only one glycoside is present in the leaves of Elaeodendron buchananii (Loes.) Loes.

Since the TLC analysis showed the presence of only one glycoside in the plant, further analytical techniques were employed to confirm this.

The ultraviolet absorption of the isolated glycoside in alcohol showed an absorption maximum at 208 nm. This absorption maximum was not in the normal range 211 - 220 nm (Canback 1949) which is characte-

ristic for compounds with α, β- unsaturated carbonyl group. The absorption spectrum (Fig. 2) gave a symmetrical peak and the absence of other absorption peaks in both the UV and visible regions showed absence of impurities.

The absorption spectrum of the isolated compound for the colour reaction with Kedde reagent was observed to have two absorption maxima, one at 540 nm and the other at 360 nm. The 540 nm peak was broad and less intense and symmetrical whereas the 360 nm peak was sharp and unsymmetrical. The 540 nm peak would be recommended for assay of the isolated compound. With Raymond reagent the isolated compound was observed to have a broad absorption maximum at 590 nm. The spectrum is slightly distorted and this could be due to the fact that the blue colour produced with Raymond reagent was fading rapidly while the spectrum was being recorded. Repeated determination of the absorption spectrum while the colour was rapidly fading revealed that the wavelength of maximum absorption at 590 nm remained unaffected.

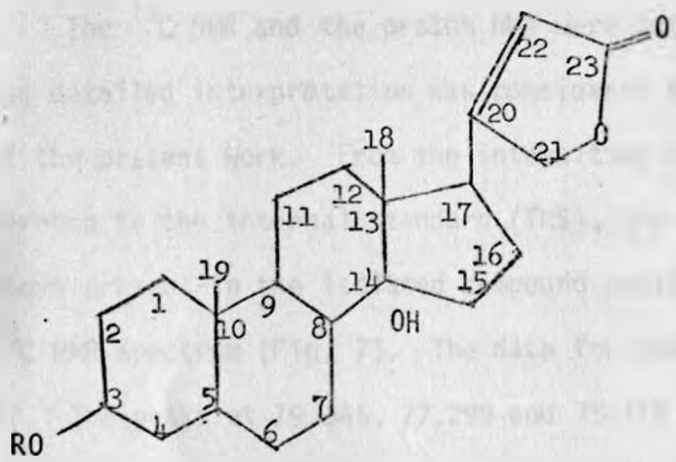
Apart from the colour reactions produced by the polynitrophenyl derivatives, the physical and chemical properties of the isolated compound indicate that it is a cardiac glycoside. Examination of the infra-red spectrum (Fig. 5) show features characteristic to cardiac glycosides. The spectrum shows good resolved peaks indicating that the compound was pure.

A broad peak at 3450 cm^{-1} indicates the presence of associated hydroxyl (OH) groups. A strong absorption at 2950 cm^{-1} suggests C-H stretch of a liphatic features. Strong absorption at 1750 cm^{-1} indicates a stretch vibration of an α, β- unsaturated ketone (C=O). A weak absorption at 1630 cm^{-1} suggests a stretch vibration of an

olefinic (C=C). A peak at 1760 cm^{-1} and 1790 cm^{-1} indicates the presence of a five membered ring compound having a carbonyl i.e. a lactone. A strong absorption band at 1260 cm^{-1} indicates the presence of a C-O link, the carbon atom of which is unsaturated.

The infra-red spectrum of the isolated compound compares very well with that of digitoxigenin in the finger-print region (CF Fig. 5 C Fig. 6). The principal peaks of both the isolated compound and digitoxigenin have been compared and are shown in Table V.

The above main peaks noted are those which might be expected from an i.r. spectrum of a typical cardiac glycoside because cardiac glycosides have all these features which contribute to the above peaks from their general structure.



The finger-print region (1400 - 600 cm^{-1}) of the isolated compound is complicated and no attempt was made to assign the absorption peaks to functional groups. The finger-print region is characteristic of each molecular species and is commonly used for identifying unknown compounds by matching the i.r. spectra of the unknown compounds with those of known compounds. An attempt was made to com-

pare the infra-red spectrum of the isolated compound with those of digoxin, somalin, echujin and ouabain but the spectrum of the isolated glycoside did not resemble any of them.

The elemental analysis from two laboratories (Micro Analytical Laboratory, Boon, West Germany and Pharm. Inst. Ethz. Switzerland) were in close agreement. From the molecular weight (mwt - 603) the molecular formula of the compound was worked out to be $C_{32}H_{47}O_{11}$. This molecular formula indicates that the isolated compound is a glycoside and not an aglycone. The aglycones of cardiac glycosides have the molecular formula $C_{23}H_{34}O_{(4-8)}$. It is difficult to state whether the isolated glycoside is found in the same form in the plant because one or more sugars might have split off during the isolation procedure.

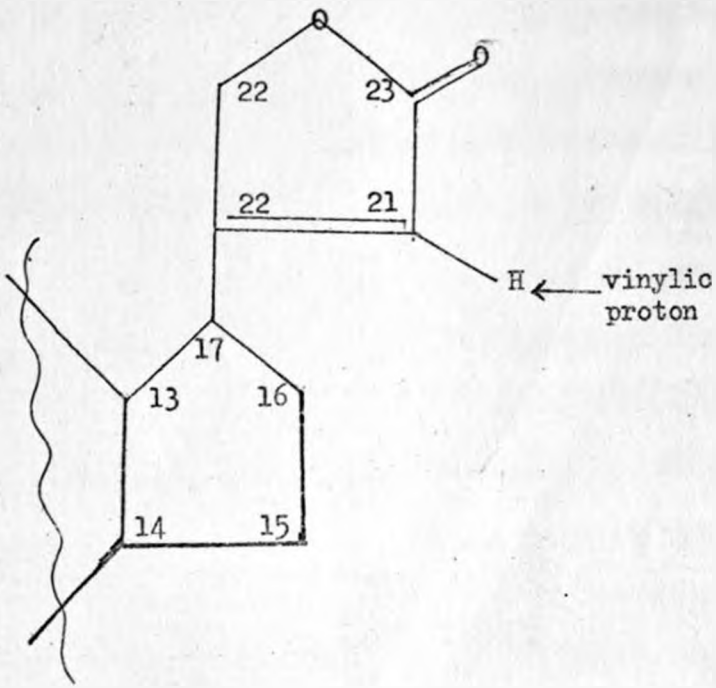
The ^{13}C NMR and the proton NMR were only partially interpreted and detailed interpretation was considered to be beyond the scope of the present work. From the intensities of the peaks and with reference to the internal standard (TMS), the total number of carbon atoms present in the isolated compound could be estimated from the ^{13}C NMR spectrum (Fig. 7). The data for same is presented in Table VI. The peaks at 79.345, 77.299 and 75.113 parts per million are due to the solvent $CDCl_3$ and the intensity at 0.000 ppm is due to the internal standard. The rest of the peak intensities are due to the carbon atoms in the molecule. When these intensities due to carbon atoms in the isolated glycoside are added up they come to 10387. Assuming there is no spin - spin coupling, the following peaks would be considered to be due to one carbon atom.

Delta/ppm	Intensity
16.601	371
18.228	252
45.328	307
51.106	398
52.571	281
62.662	346
66.243	445
68.359	355
73.892	452
96.028	465
121.171	273
140.136	377
174.641	382

The total intensity of the peaks due to single carbon atoms is 4330 which gives an average of 333% intensity. So assuming that one carbon atom in isolated glycoside has an average intensity of 333% and the total intensity of all the carbon atoms in the compound is 10387, the number of carbon atoms in the isolated compound is 31.

The number of carbon atoms in isolated glycoside as calculated from the molecular weight and elemental analysis is 32. So the number of carbon atoms in the isolated compound as calculated from the ^{13}C NMR spectrum would be considered reasonable as the calculation of such number of carbon atoms has been based on the assumption that there is no spin - spin coupling. In the presence of spin - spin coupling, not all the peak intensities would be due to one carbon atom but some intensities would be due to part of a carbon atom.

The proton nuclear magnetic resonance of the isolated compound (Figure 8) is not properly integrated and so it was found difficult to determine the number of protons in the compound. The only peak distinguishable from the nuclear magnetic resonce spectrum is the peak due to vinylic proton. The peak occurs at τ5.92 and this supports the presence of α, β, unsaturated $-\gamma$ lactone ring in the isolated glycoside.



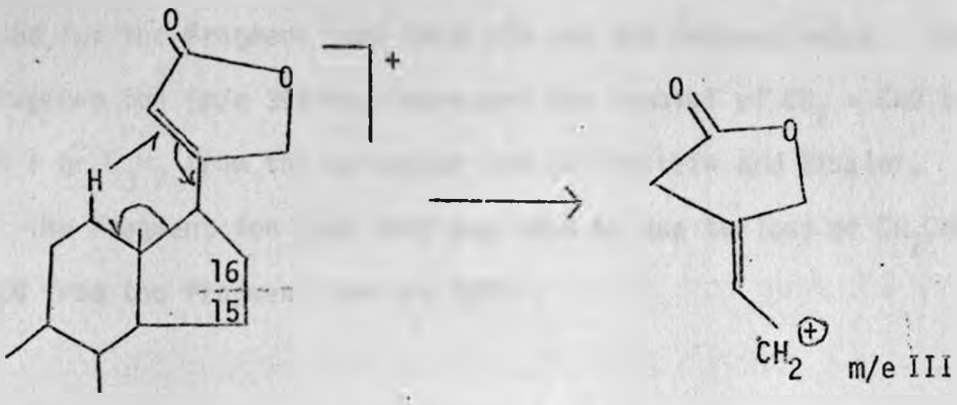
Steroids usually have complex proton nuclear magnetic resonance spectra due to too many protons in the molecule. Again these protons are too close together and the transfer fo these usually occur making the nuclear magnetic resonance spectra of steroids complex and difficult to determine the number of protons from such spectra. This seems to be the case with the isolated glycoside.

The mass spectrum of the isolated compound (Fig. 9) showed features very characteristic of cardiac glycosides and helped to confirm the chemical tests on the isolated compound. For comparison purposes the m/e values of the fragment ions of the isolated compound were compared with the m/e values of somalin and digitoxigenin (Table VII). The m/e values of the fragment ions of somalin and digitoxigenin were obtained from spectra of Ardene, 1964. The parent peak of the isolated compound was lacking from the mass spectrum and the disappearance of the molecular ion could be due to various reasons. In mass spectrometry, the molecular ion formed upon electron impact (due to 70 ev energy) bears a localised charge or radical. The molecular ion is more stable if there is a centre in the molecule with comparatively little energy which can lose an electron readily upon impact, for example, aromatic groups or some heteroatoms like nitrogen. In such cases, the fragments resulting from the molecular ion are few and their formation is usually predictable. In steroids, however, a centre of comparatively weak energy for molecular ion formation is lacking. The tertiary and quaternary carbon atoms and oxygen containing groups all ionize readily upon electron impact. This results in a large number of degradation processes with concommittant rearrangements. As a result the parent peak may not appear or could be very weak. In other cases, especially with polyhydroxy steroids, they lose a molecule of water (presumably the 14 α - hydroxy) and this peak is recorded instead of the molecular peak. Use of a large sample of the isolated glycoside might have produced the parent peak or preparation of a suitable derivative of the compound.

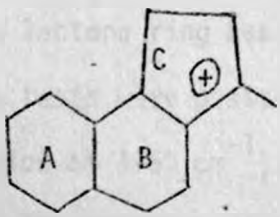
To identify the isolated compound, its mass spectrum was compared with a large number of reference spectra currently available

at United Kingdon Chemical Information Service at Nottingham with no success. The mass spectrum (Fig. 9) shows that the spectrum of the isolated compound is complex. However, by comparing the m/e values of the fragment ions of the compound with those of somalin and digitoxigenin (see Table VII) and the fragmentation pattern of digitoxigenin, a few deductions could be made:-

The fragment ion with m/e 111 has been known to be formed by McLafferty rearrangement and cleavage of the 15, 16 - bond.



The presence of this fragment ion in the mass spectrum of the isolated compound indicates the presence of α, β- unsaturated δ- lactone ring and hence possibly lactone ring in cardiac glycosides or their aglycones. The same m/e value was observed to occur in somalin (a cardiac glycoside and digitoxigenin (an aglycone). Steroids also undergo a mass fragmentation to give the fragment ion $C_{15}H_{23}$ ⊕ and this is indicated by the fragment ion with m/e 203 and represents parts of the rings A, B and C (Spiteller, 1963).



The presence of this fragment ion indicates the presence of the steroidal skeleton. The isolated compound had a fragment ion with m/e 205 probably due to cleavage of the ring system requiring breaking at least two bonds giving a fragment ion $C_{15}H_{25}O$. This process is frequently accompanied by the rearrangement of a hydrogen atom.

Usually loss of two methyl groups from steroids is usually indicated by a fragment ion (m/e 404) and the removal of the side chain at one of the methyl groups by the fragment ion (m/e 203). Rearrangement of hydrogen atoms in the isolated compound may have accounted for the fragment ions (m/e 405 and 205 respectively). The mass fragment ion (m/e 393 may represent the removal of $CH_2 = CHO$ or $CH_3 \equiv O +$ or C_3H_7 from the molecular ion (Silvestein and Bassler, 1967). The fragment ion (m/e 364) may also be due to loss of CH_2CHO or CH_3CO from the fragment ion m/e 405.

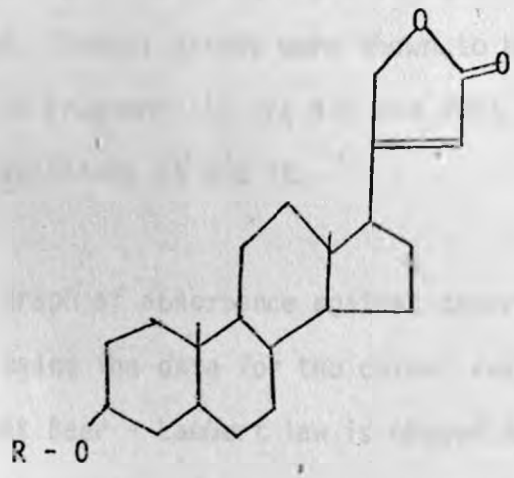
The fragment ion with m/e 405, 393, and 364 point strongly to the presence of oxygenated functions in the isolated compound, at least two hydroxyl (OH) groups and some carbonyl (aldehyde or ketone groups). The fragment ions (m/e 71 and 43) may indicate the loss of $C_2H_5.CO\text{-}CH_3$ which further suggests some oxygenated functions in the compound.

The hydroxyl (OH) functional groups in the compound are confirmed by the infra-red spectrum of the isolated compound (Fig. 5 broad peak at 3450 cm^{-1}). The presence of ketone group in the α, β- unsaturated lactone ring has been shown by chemical tests (Raymond and Kedde tests were positive) and by infra-red spectrum (a strong absorption at 1750 cm^{-1}) and by the mass spectrum (fragment ion m/e 111). The presence of such other groups as ketone (C=O) or

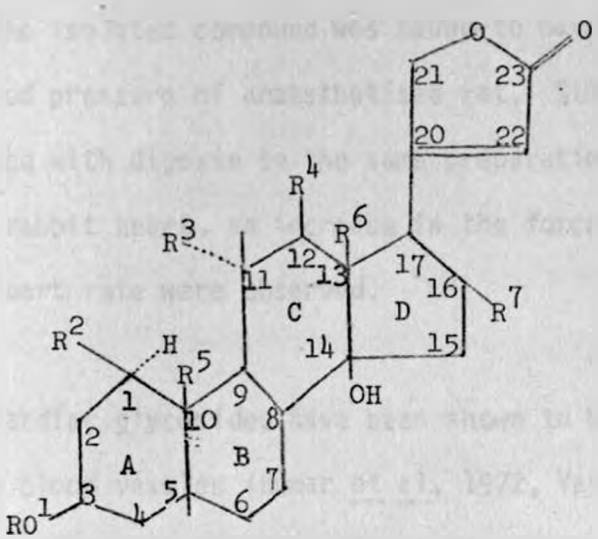
aldehyde (CHO) in the isolated compound are yet to be determined although there are indications that such groups may be present (fragment ion m/e 71, 51 and 43).

When the mass spectrum of the isolated compound was compared with those of steroids available in literature, especially with those of somalin and digitoxigenin, it was found that a lot of the major peaks present in the isolated compound are also present in these steroids. This further suggests that the isolated compound is a steroid.

Prof. Speiser of Pharmaceutical Institute, Abterlung, Switzerland analysed the compound for elements and for protons (NMR) and helped to confirm the heart glycoside structure of the isolated compound. He assigned the following structure to the compound.



From the chemical and spectroscopical analysis of the isolated compound the following structure can be arrived at so far:-



The Keller - Killiani reagent indicated that the isolated compound is a cardiac glycoside with a desoxy sugar. Therefore R^1 is a sugar residue. An attempt to hydrolyse the glycoside and identify the sugar did not succeed. Whether some other hydroxyl (OH) groups, aldehyde (C(=O)H) or ketone (C=O) groups occur in the isolated compound at any other position (R^2, R^3, R^4, R^5, R^6 and R^7) has as yet to be determined. Methyl groups were shown to be present in the compound (removal of fragment ion m/e 405 and 205). Such methyl groups could occur at positions 18 and 19.

The graph of absorbance against concentration of the isolated compound using the data for the colour reaction with Kedde reagent showed that Beer - Lambert law is obeyed and so the graph could be used for quantation of the percentage yield of glycoside from the leaves of Elaeodendron buchananii. A low yield of the glycoside was obtained and this was about 0.29%. This low yield would be probably due to significant losses of material during the lengthy extraction and purification process.

PHARMACOLOGICAL EFFECTS OF THE ISOLATED COMPOUND

The isolated compound was found to have a sustained increase in blood pressure of anaesthetised rat. Similar effects were observed with digoxin on the same preparation. With isolated perfused rabbit heart, an increase in the force of contraction and a slow heart rate were observed.

Cardiac glycosides have been shown to have a pressor effect on the blood vessles (Kumar et al, 1972, Vatner et al, 1971). The pressor effect is due to a direct vasoconstrictor action of the cardiac glycosides on blood vessles. Also cardiac glycosides have been shown to increase the peripheral resistance by other workers (Higgins et al, 1972, Goodman and Gilman, 1971). The increase in force of contraction of the heart is due to a direct stimulation by the isolated compound on the myocardium. These pharmacological effects of the isolated compound are typical of cardiac glycosides and these effects compare very well with those of digoxin used as a refcrence.

The pharmacological investigation carried out on the isolated compound are only preliminary but support the chemical findings that the isolated compound is a cardiac glycoside.

CHAPTER 4

CONCLUSION

The aim of the present work was to investigate the nature of the active (poisonous) principles in Elaeodendron buchananii (Loes.) Loes., a plant very well known because of its poisonous nature especially to livestock. Investigation of the nature of the poisonous principles led to the extraction, purification, characterisation and partial identification of the isolated active principles. Preliminary pharmacological effects of the isolated compound were carried out to find out the pharmacological properties of the compound.

The physical and chemical properties of the compound indicated that the compound was a cardiac glycoside. The thin-layer chromatographic analysis supported by other analytical techniques showed that the isolated compound was one glycoside. The fact that the isolated compound and the crude extract of the plant gave one spot with same Rf value with same solvent systems and adsorbent suggest that the isolated glycoside was the only one present in the plant. This glycoside is probably a new one as indicated from literature survey. The infra-red, ^{13}C NMR, mass and proton NMR spectra of the isolated compound are not identical with any of the known cardiac glycosides and the conclusion that can be reached is that this is probably a new cardiac glycoside. All these spectra are extremely complex which suggest that the structure of the glycoside is of considerable complexity and to assign a molecular structure could possibly be a major project as regards time and money.

The isolated glycoside was found to have an absorption maximum at 203 nm and had a narrow melting range 110 -111 ^{o}C.

Elaeodendron buchananii (Loes.) Loes needs further investigation and such investigation could include:-

i) Complete structural elucidation of the cardiac glycoside:- hydrolysis of the glycoside to its respective aglycone and glycone and analysing them individually.

ii) Acetylation of the glycoside would also help in structural elucidation.

iii) Preparation of suitable derivatives of the compound and study of their pharmacological properties.

iv) The pharmacology of the cardiac glycoside should also be investigated to establish the toxicity, absorption, distribution and metabolism and suitable level doses.

Elaeodendron buchananii (Loes.) Loes. grows wildly in many parts of Kenya. So the variation of glycosidal content with different regions and seasons could also be investigated. The plant would be easy to cultivate. So if further research on the plant showed that the glycoside and its derivatives could be put into medicinal use e.g. treatment of congestive heart failure, the plant could be exploited.

TABLE 3.

Investigation of the best solvent for extraction of the active principles from the leaves of Elaeodendron xylocarpum (Loes.) Loes.

Extracting solvent	Absorbance of the coloured complex (residue + Sodd reagent)
1. Chloroform	0.30
2. Methanol	0.13
3. 95% alcohol	0.26
4. 70% alcohol	0.20
5. 50% alcohol + chloroform	
i) alcoholic extract	
ii) chloroform extract	0.30
6. Ethanolic/chloroform	0.29
7. Ether	0.11

TABLES

TABLE II

Investigation of the best solvent for extraction of the active principles from the leaves of Elaeodendron buchananii (Loes.) Loes.

Extracting solvent	Absorbance of the coloured complex (residue + Kedde reagent)
1. Chloroform	0.29
2. Methanol	0.13
3. 95% alcohol	0.26
4. 70% alcohol	0.20
5. 50% alcohol + chloroform	
i) alcoholic extract	-
ii) chloroform extract	0.30
6. Ethanolic/chloroform	0.29
7. Ether	0.11

TABLE III

Results of purification of the glycoside of Elaeodendron from chloroform extract using an alumina column

Eluant	Fraction	Volume (ml)	Kedde reaction	Comment on eluant
Alcohol (95%)	1	100	Positive	yellow coloured
	2	100	+ve	yellow coloured
Ethyl acetate	3	100	+ve	yellow coloured
	4	100	+ve	yellow coloured
Chloroform	5	100	+ve	Slightly yellow
	6	50	+ve	coloured
	7	50	+ve	"
	8	50	+ve	"
	9	50	+ve	"
	10	50	-ve	clear eluate
	11	50	-ve	clear eluate

TABLE IV

Solubility of the isolated compound in different solvents

Solvent	Comments on solubility
Ether	Fairly soluble
Methanol	Fairly soluble
Acetone	Fairly soluble
Ethyl acetate	Fairly soluble
Absolute alcohol	Very soluble
Chloroform	Extremely soluble

TABLE V

ILC analysis of the isolated glycoside(s) of Elaeodendron compared with digoxin and ouabain

Solvent system	Sample	No. of spots	Distance moved	Solvent front	Rf	Colour after spray
Chloroform methanol formamide 80:19:1	Isolated glycoside	one	10.9	13.0	0.84	
	Ouabain	one	-	13.0		pink
	chloro-form crude extract	one	10.85	13.0	0.84	pink
	Digoxin	one	10.0	13.0	0.76	pink
Ethyl acetate-methanol (90:10)	Isolated glycoside	one	8.7	10.8	0.80	pink
	Digoxin	one	7.7	10.8	0.71	pink
	Chloro-form crude extract	one	8.7	10.8	0.80	pink
	Ouabain	one	-	10.8	-	pink
	Isolated					

	glycoside from *Ajuga remota*	one	8.9	10.8	0.82	pink
Chloroform Ethanol (7:3)	Isolated glycoside	one	6.9	11	0.63	pink
	Chloroform crude extract	one	6.9	11	0.63	pink
	Digoxin	one	5	11	0.45	pink
	Ouabain	one	-	11	-	pink
Chloroform methanol	Isolated glycoside	one	10.5	13.0	0.80	pink
	Chloroform crude extract	one	10.6	13.0	0.81	pink
	Digoxin	one	9.8	13.0	0.75	pink

TABLE VI

Comparison of infra-red spectra of isolated glycoside with that of digitoxigenin

Isolated compound Note worthy peaks (cm^{-1})	Digitoxigenin Note worthy peaks (cm^{-1})
3520 (Broad)	3440 (Broad)
2930	2940
1790	1780
1735	1760
1630	1645
1450	1465
1380	1390
1260	1260
	1100

TABLE IX

^{13}C NMR spectrum data for the isolated glycoside from the leaves of Elaeodendron buchananii (Loes.) Loes with reference to spectrum Fig. 7

Serial number of peak	Dealta/Hz	Delta/ppm	Intensity
1	2619.629	174.641	382
2	2564.697	170.976	621
3	2102.050	140.136	377
4	1817.626	121.171	273
5	1440.429	96.028	465
6	1357.421	90.494	710
7	1204.833	80.322	1085
8	1190.185	79.345	7072*
9	1158.447	77.229	7188*
10	1126.708	75.113	6567*
11	1108.398	73.892	452
12	1025.390	68.359	355
13	993.652	66.243	445
14	939.941	62.662	346
15	864.257	57.616	731
16	788.574	52.571	281
17	766.601	51.106	398
18	697.931	45.328	307
19	603.027	40.201	712
20	445.556	29.703	689
21	313.720	20.914	1134
22	273.437	18.228	253
23	249.023	16.601	371
24	0.000	0.000	@ 1984

TABLE X

Comparison of mass spectrum of the isolated glycoside from Elaeodendron buchananii with the m/e values of digitoxigenin and somalin

m/e values of the isolated compound	m/e values of digitoxigenin	m/e values of somalin
-	-	519
-	-	500
-	-	474
455	-	456
436	-	-
-	-	430
426	-	-
417	-	417
405	-	-
393	-	-
381	-	-
-	374	374
367	-	-
364	-	-
355	356	356
343	-	-
-	-	339
-	338	338
336	-	-
331	-	-
-	323	323
319	-	-

305	-	-
-	-	297
293	-	-
69	67	-
-	-	59
-	55	-
51	-	-
43	41	41
-	-	31
-	29	29
	18	-
		15
281	284	284
-	-	275
269	-	-
255	-	257
243	246	246
231	228	231
-	213	213
219	-	-
218	-	-
205	203	203
193	195	195
189	-	-
181	-	181
-	174	175
169	-	-
162	162	163
151	-	-

	147	145
143	-	145
131	133	-
125	124	-
119	-	119
113	-	113
111	111	111
100	-	-
97	-	95
93	93	95
85	-	84
83	-	84
-	79	-
-	-	74
71	-	71

TABLE XI

Data for the calibration curve of absorbance of the glycoside of Elaeodendron reacted with Kedde reagent against concentration of the glycoside

Concentration %	Absorbance
0.004	0.05
0.012	0.15
0.020	0.23
0.032	0.35
0.040	0.45
0.048	0.55
0.06	0.66
T_1 (unknown)	0.33
T_2 (unknown)	0.29

TABLE XII

Effects of the isolated compound, adrenalin, and digoxin on the blood pressure of anaesthetised rat

Injection	Observation	Remarks
Control - 1 ml of 10% w/v solution of propylene glycol in normal saline	A transient fall in blood pressure. Blood pressure rose from 10.6 mm Hg to 10.3 mm Hg. Returned to 10.6 mm Hg after about 2 minutes.	This fall could be due to vasodilation
10 mg/Adrenalin	An immediate rise in blood pressure. The blood pressure rose from 106 mm Hg to 133 mm Hg.	This rise is probably due to the vasoconstrictor effect of adrenalin
1 mg of the isolated compound	A very slight and immediate fall in blood pressure and then a gradual rise above the mean blood pressure. The blood pressure fell from 106 to 104 then rose to 109.	The fall could be due to vasodilation

FIGURES

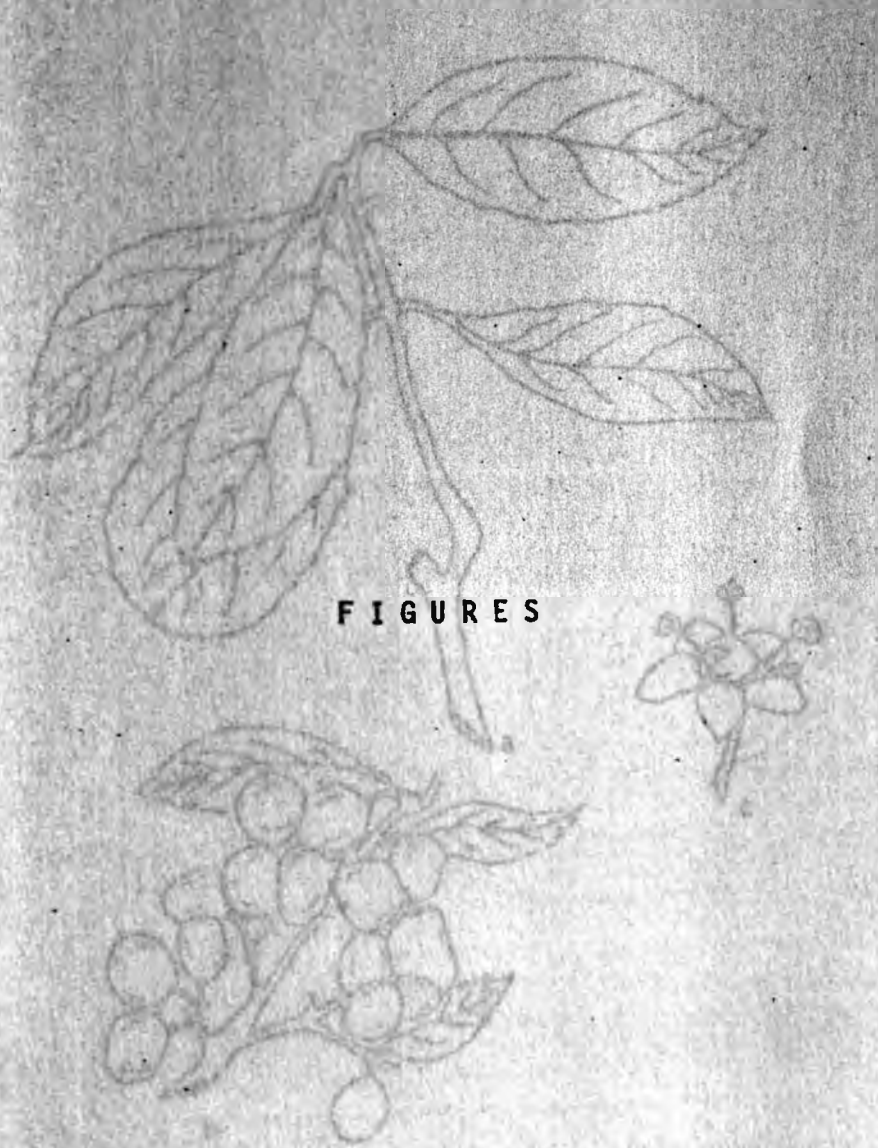
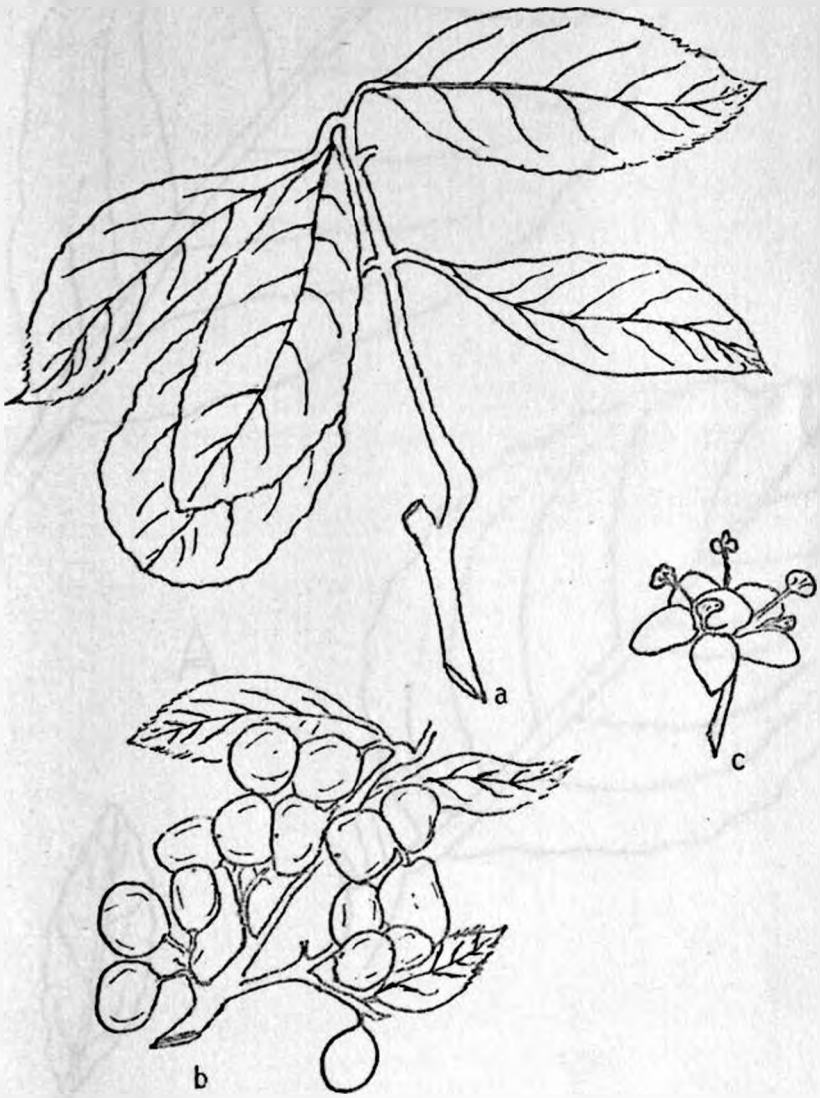


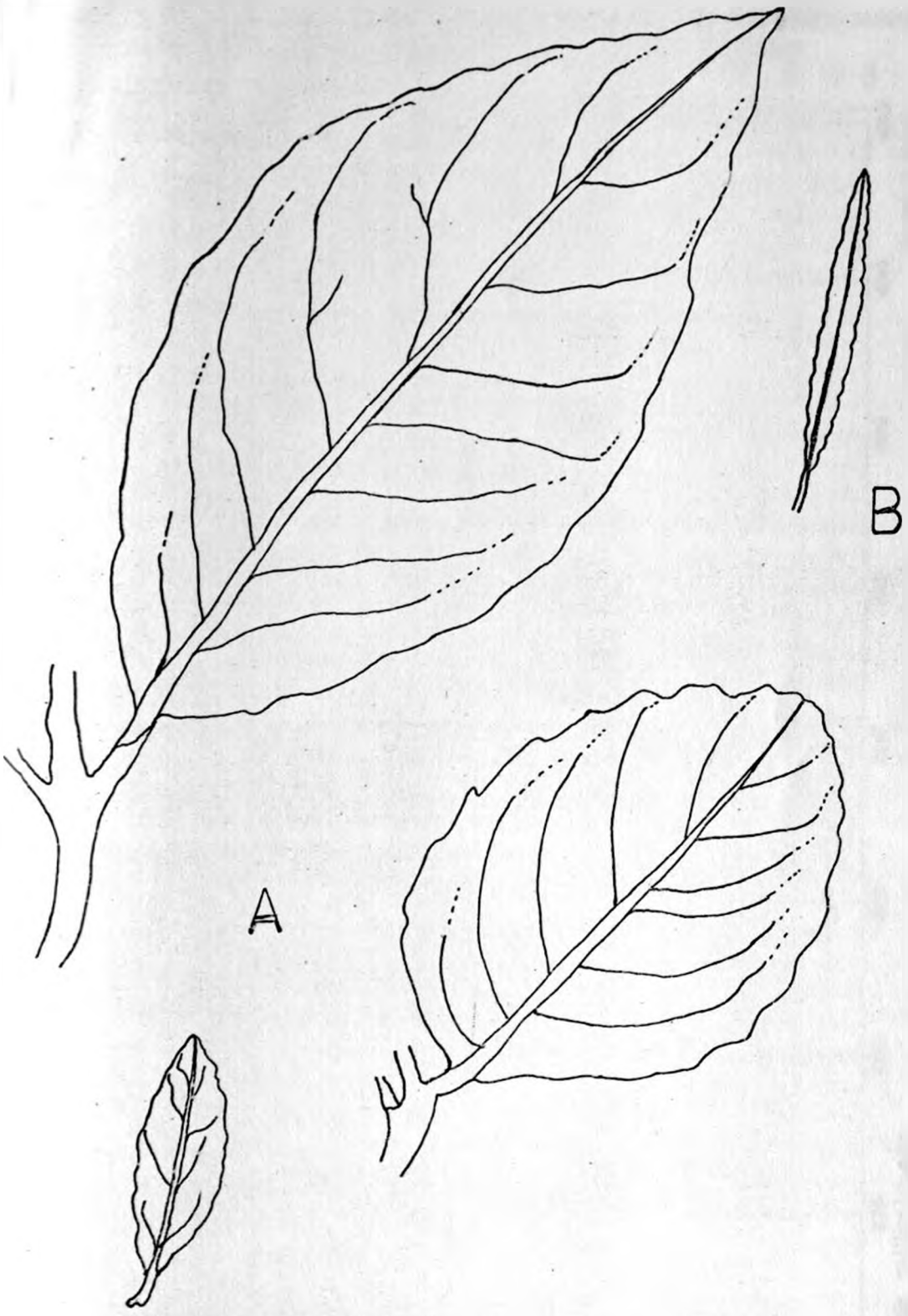
FIG. 1: ELAEODENDRON BUCHANANII (LOES.) LOES



a A branchlet (x 0.5)

b Fruiting branchlet (x 0.5)

c Flower (x 5)



A. Leaves of Elaeodendron buchananii (Loes) Loes. Natural size.

B. Young leaves of Elaeodendron buchananii (Loes) Loes.

FIG. 2: ULTRAVIOLET ABSORPTION SPECTRUM OF THE ISOLATED GLYCOSIDE IN ALCOHOL

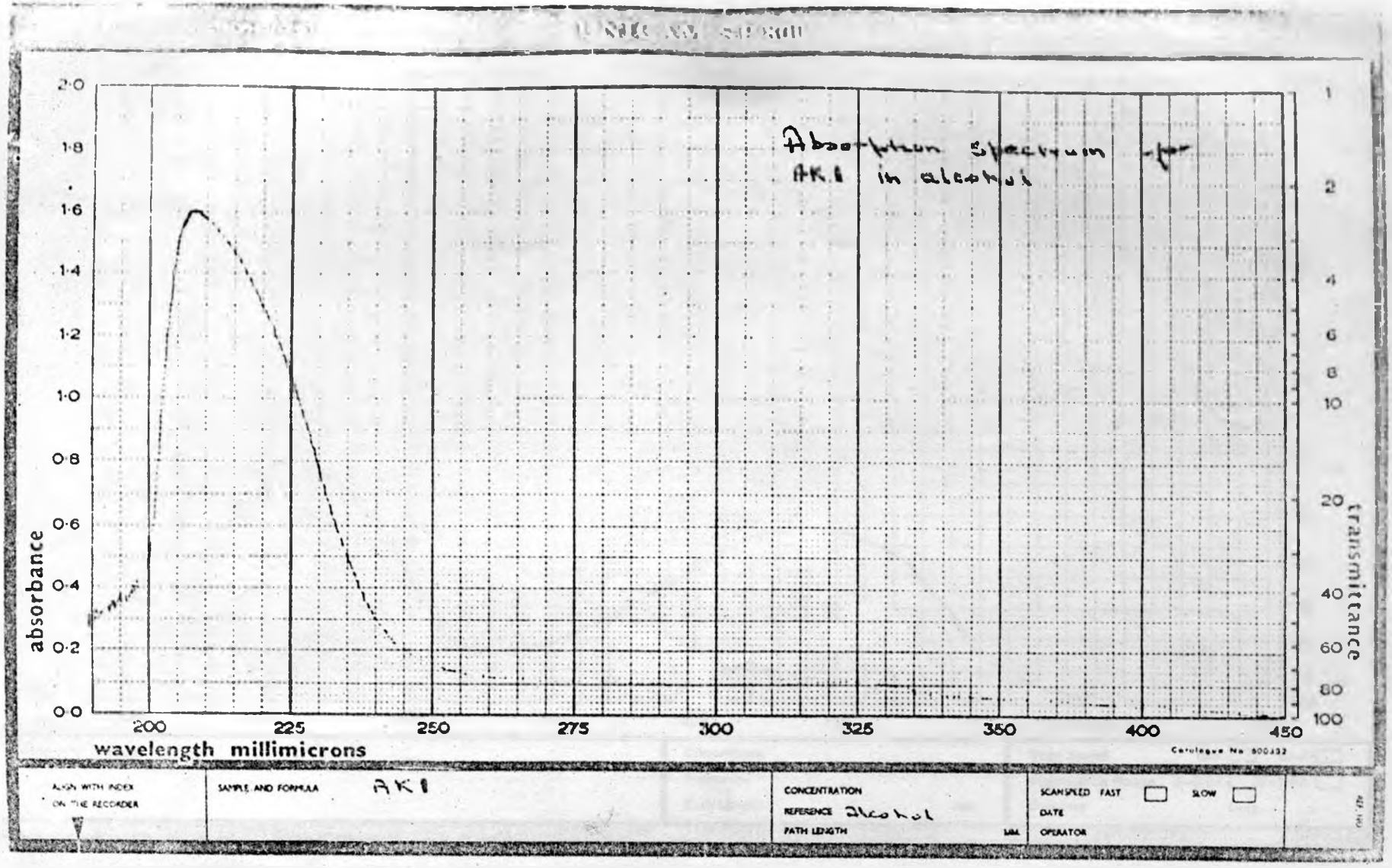


FIG. 3: ULTRAVIOLET ABSORPTION SPECTRUM OF THE ISOLATED GLYCOSIDE FOR THE COLOUR REACTION WITH 3:5 DINITROBENZOIC ACID (3:5 DNBA)

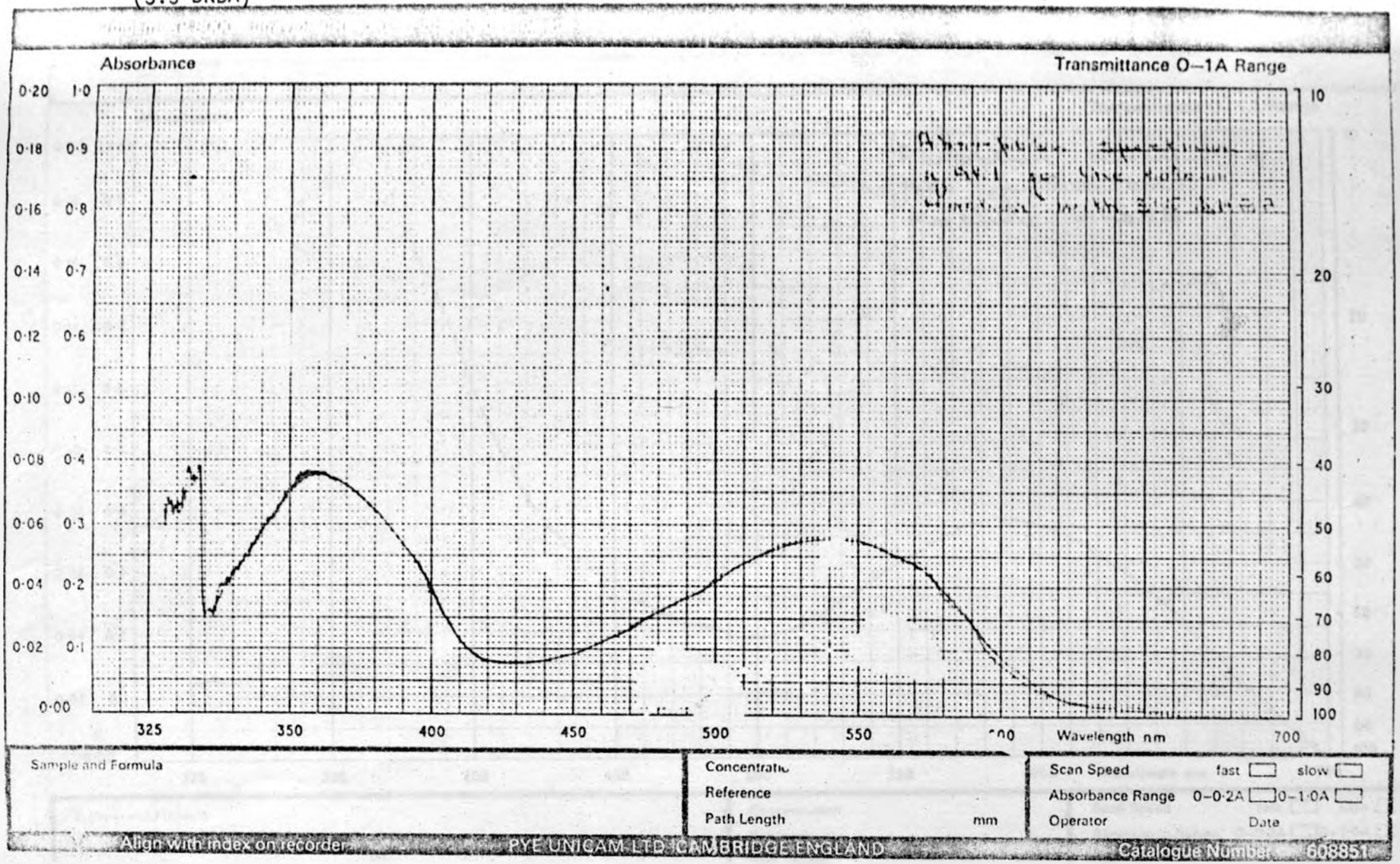


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FIG. 4: ULTRAVIOLET ABSORPTION SPECTRUM OF THE ISOLATED GLYCOSIDE FOR THE COLOUR REACTION WITH 1,3 DINITROBENZENE (*m* - DNB)

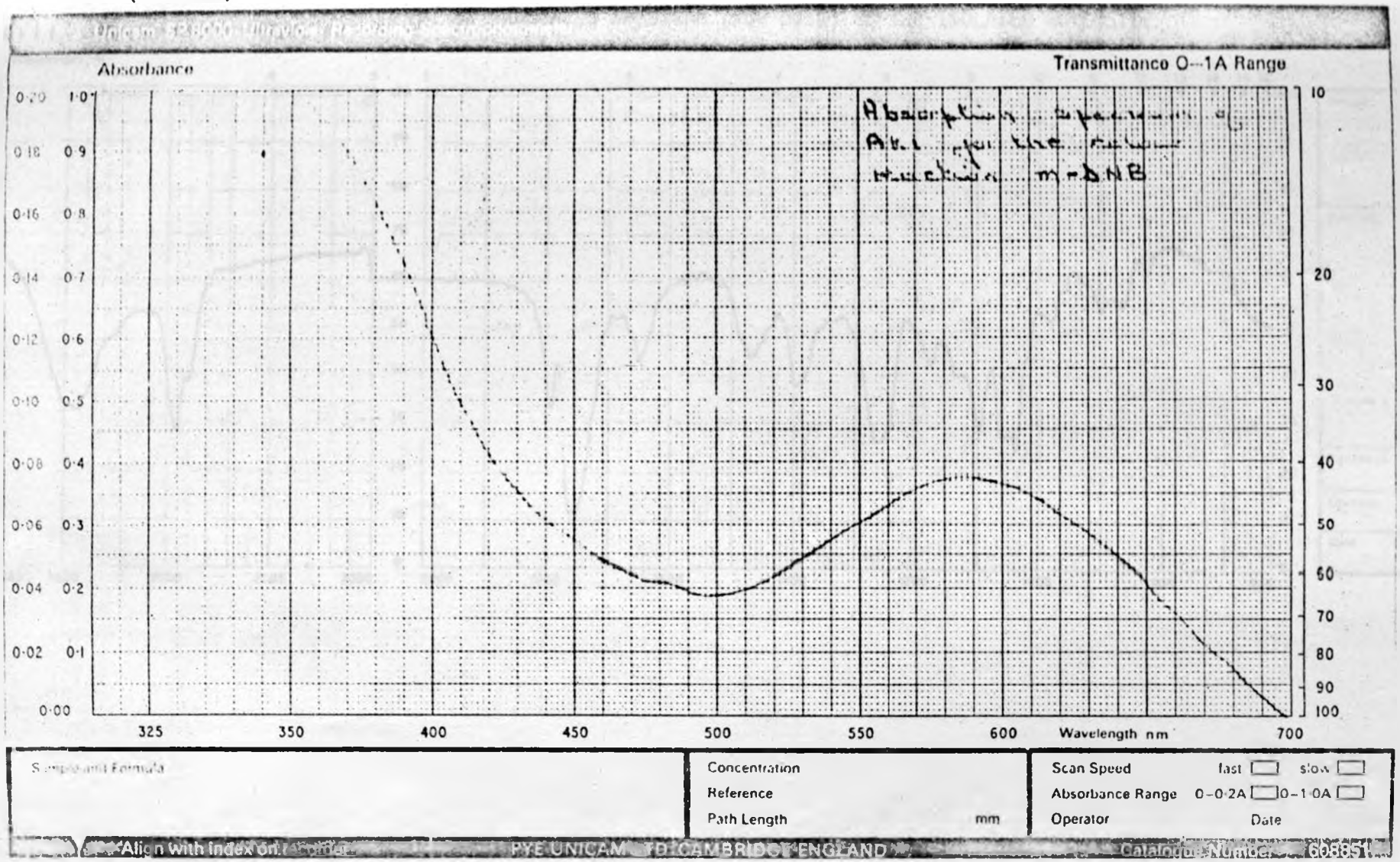


FIG. 5: INFRA-RED SPECTRUM (KBr DISC) OF THE ISOLATED GLYCOSIDE

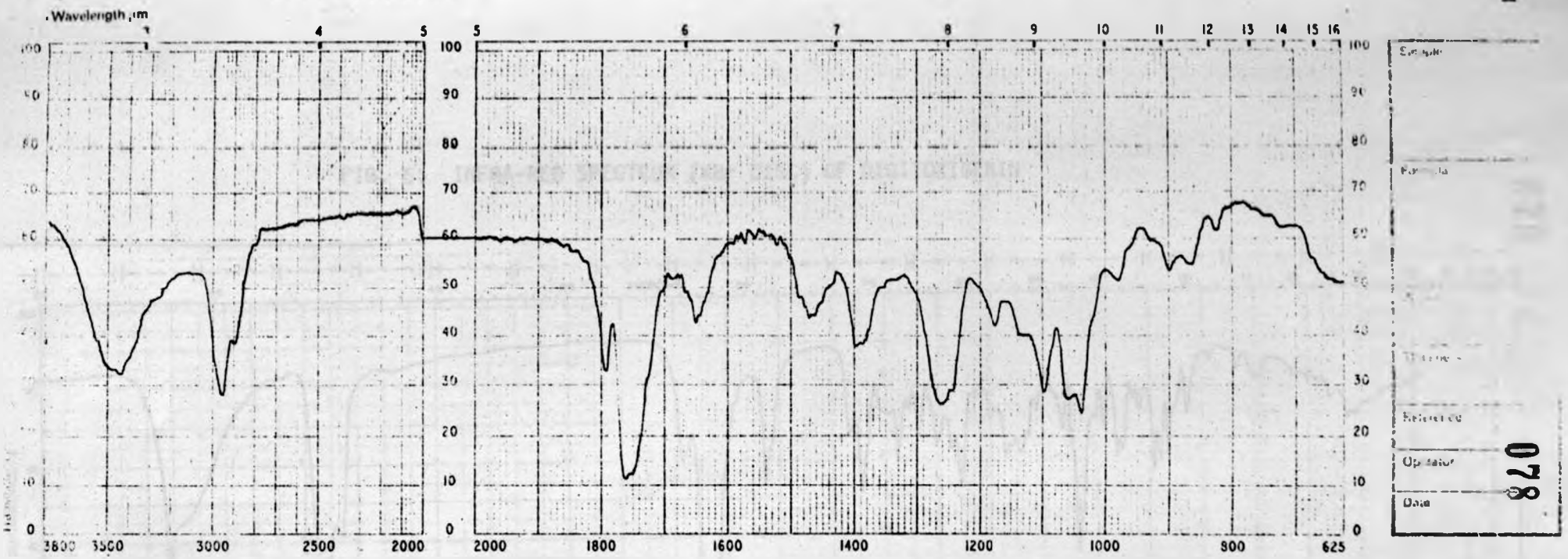


FIG. 6: INFRA-RED SPECTRUM (KBr DISC) OF DIGITOXIGENIN

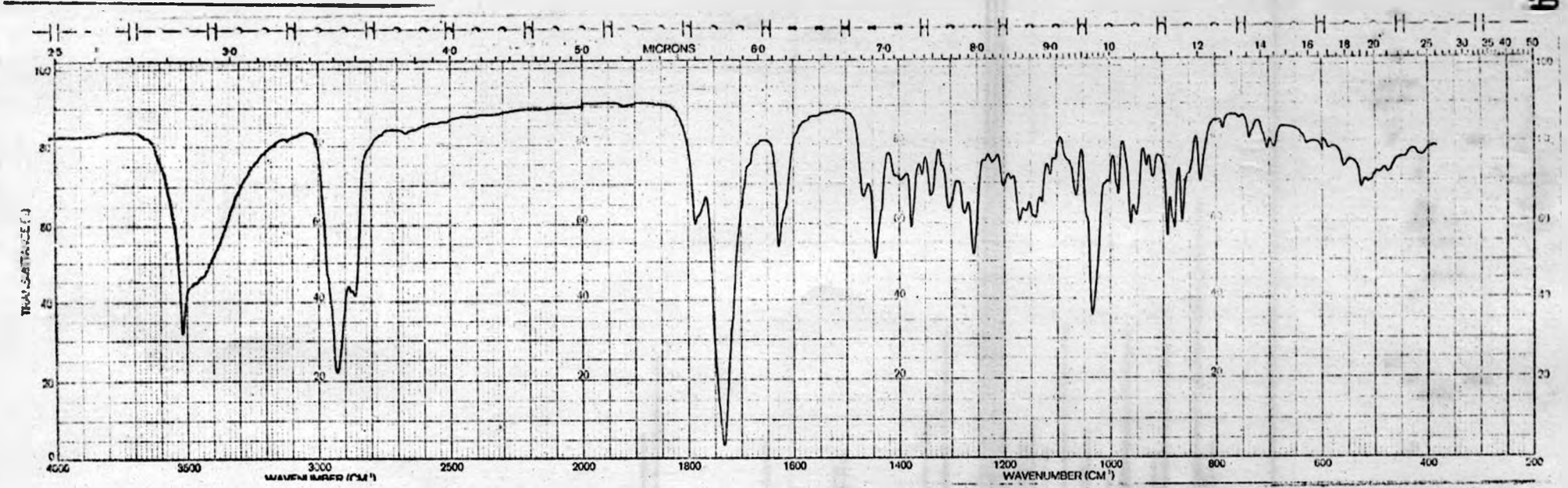
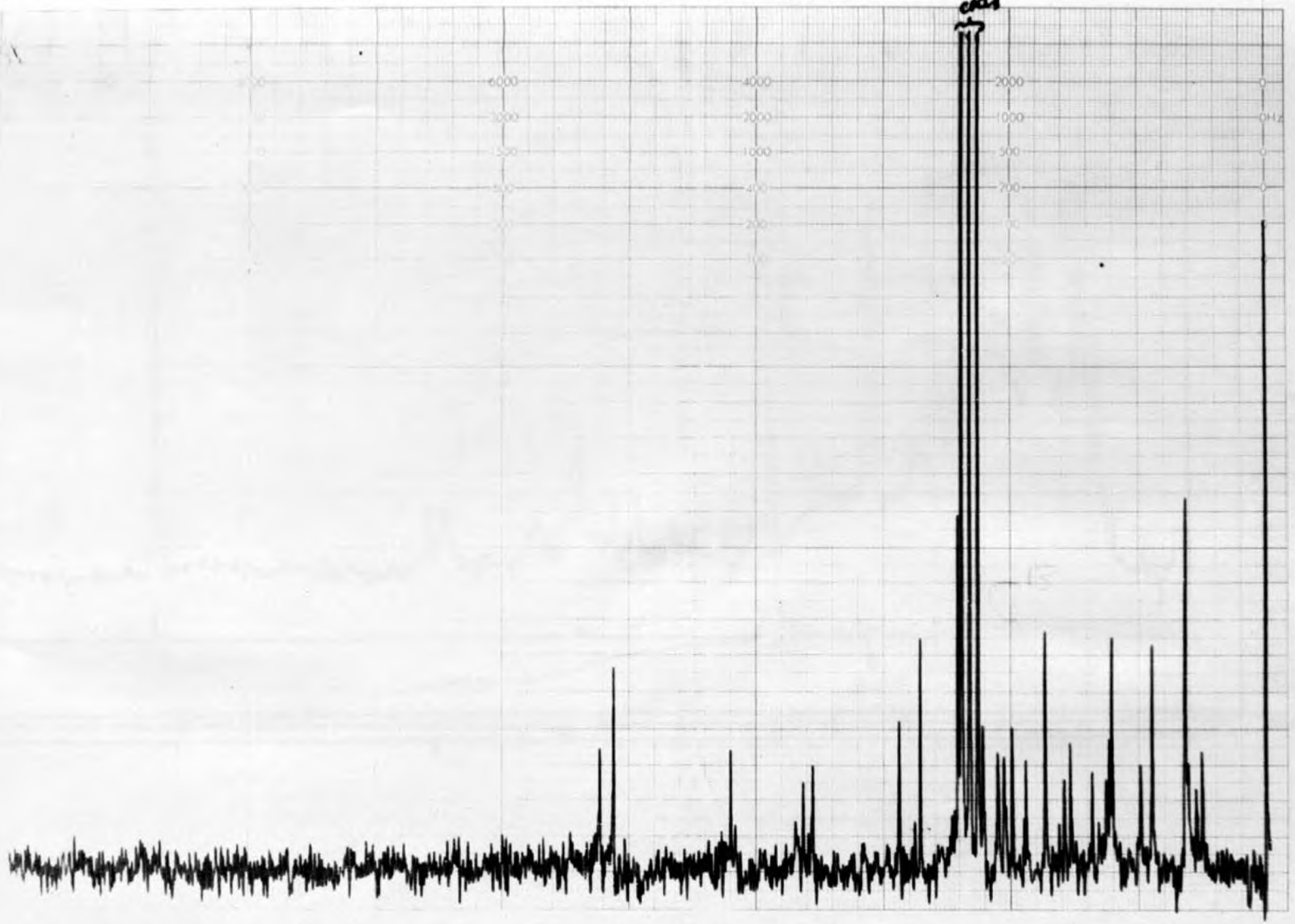


FIG. 7: ^{13}C NMR SPECTRUM OF THE ISOLATED GLYCOSIDE



080

FIG. 8: PROTON NMR OF THE ISOLATED GLYCOSIDE

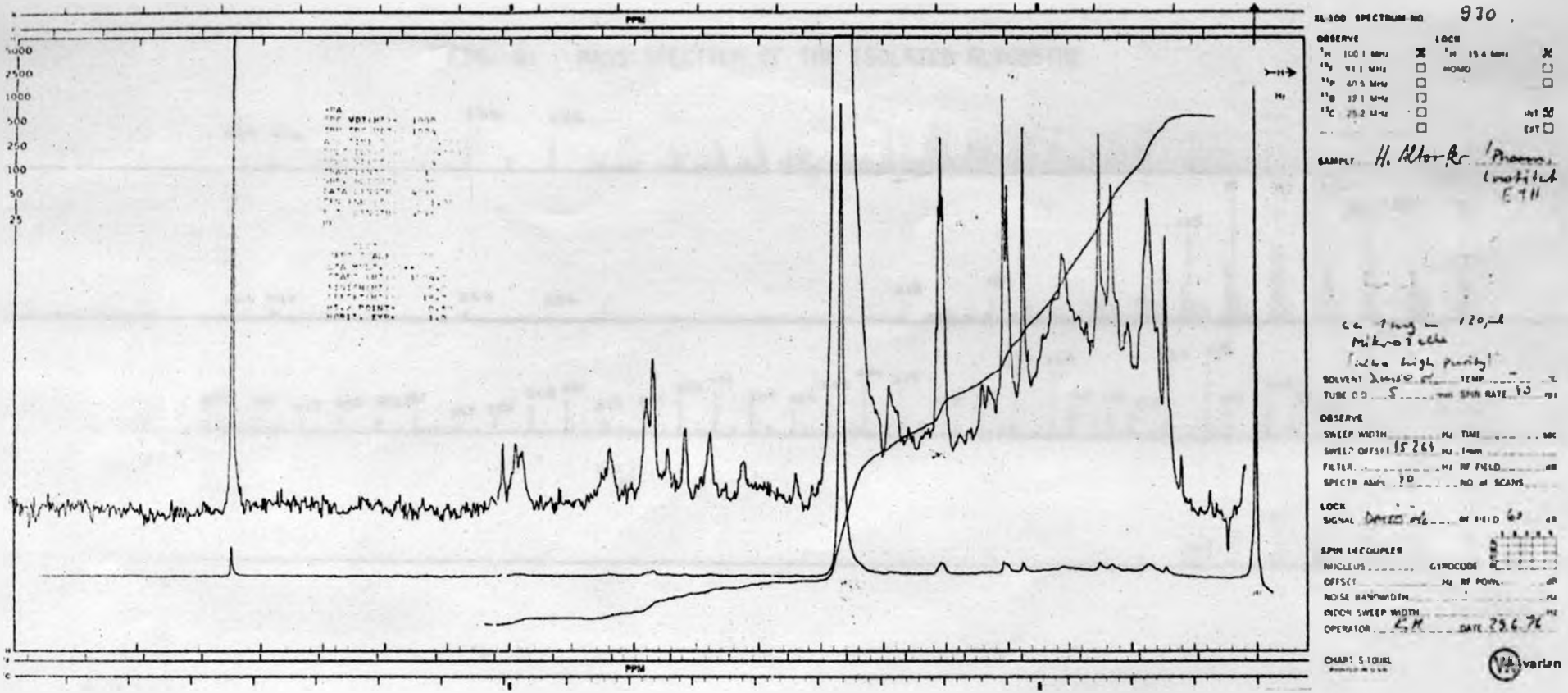
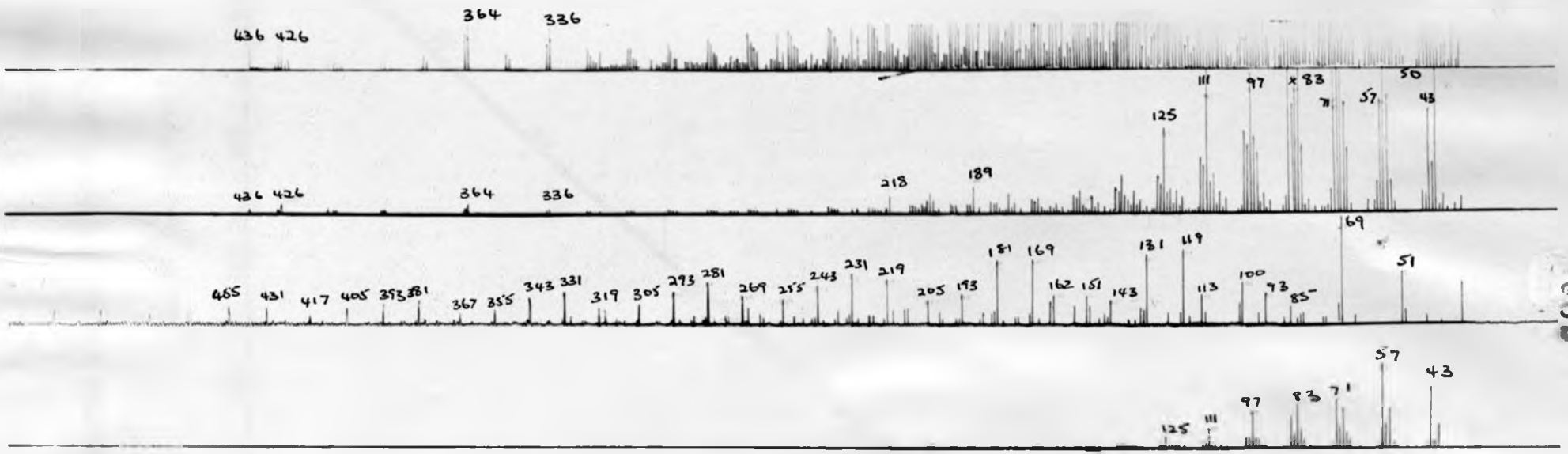


FIG. 9: MASS SPECTRUM OF THE ISOLATED GLYCOSIDE



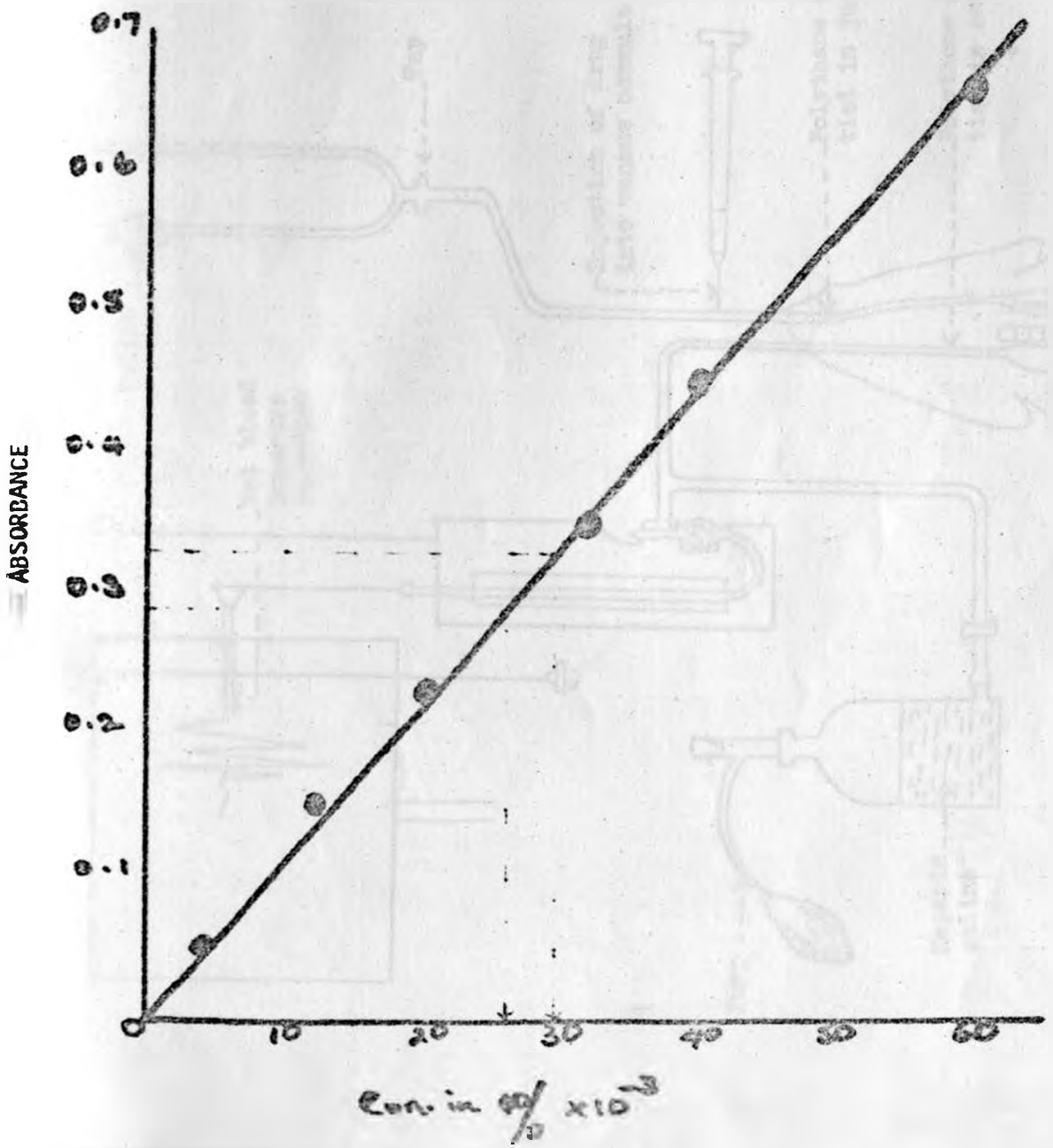


FIG. 10: A GRAPH OF ABSORBANCE AGAINST CONCENTRATION OF THE ISOLATED GLYCOSIDE

FIG. 11: A CONDON PRESSURE MANOMETER FOR THE STUDY OF THE EFFECTS OF THE ISOLATED GLYCOSIDE ON THE BLOOD PRESSURE OF ANAESTHETISED RAT

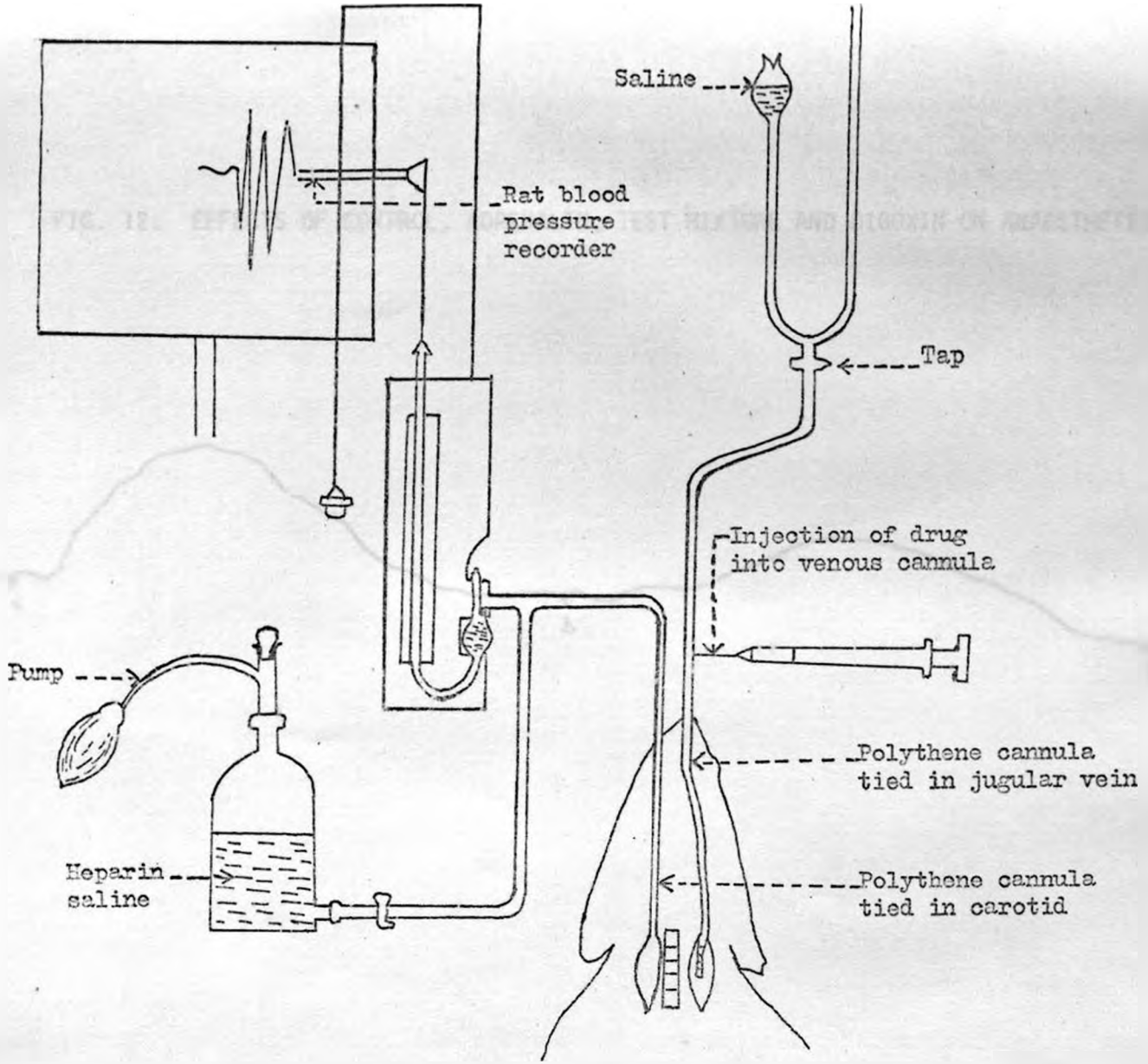


FIG. 12: EFFECTS OF CONTROL, ADRENALIN, TEST MIXTURE AND DIGOXIN ON ANAESTHETISED RAT

FIG. 13: EFFECTS OF ADRENALIN, ISOLATED GLYCOSIDE AND DIGOXIN ON ISOLATED PERFUSED RABBIT HEART ON A KYMOGRAPH

FIG. 14: EFFECTS OF CONTROL, ADRENALIN, ISOLATED GLYCOSIDE ON ISOLATED PERFUSED RABBIT HEART USING A HARVARD SMOOTH MUSCLE/HEART TRANSDUCER

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