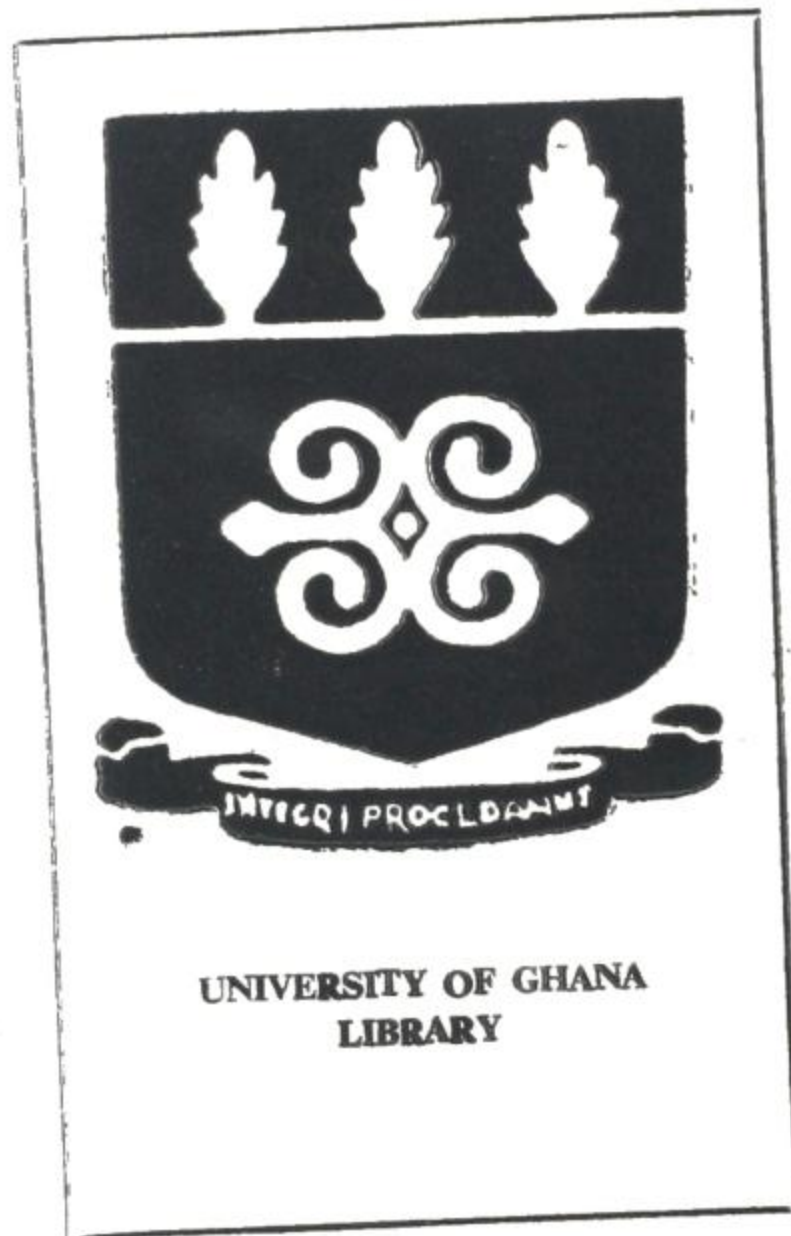


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INVESTIGATION OF THE ALKALOIDS AND
OTHER CONSTITUENTS OF
PIPER NIGRUM AND PEPEROMIA PELLUCIDA



A Thesis submitted to the
University of Ghana, Legon,
in fulfilment of the requirements
for the degree of Master of Science

by

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OCTOBER 1982



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TO: Oboshie, Mousy and Rita.

D E C L A R A T I O N

It is hereby declared that the following is the result of two years* research project undertaken by the author under supervision, and that it has neither wholly nor partly been presented elsewhere for another degree.


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* A substantial part of the first year was taken up by attendance at courses for the written examinations.

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(iv)

A B S T R A C T

The amide alkaloids, steroids and lactone - like constituents of the fruits and roots of a locally cultivated Piper nigrum variety and the whole plant of Peperomia pellucida have been investigated. Of the constituents, Piperine, Piperettine, Stigmasta -4, 22-dien -3-ol and β -sitosterol were isolated, purified and characterised as fully as possible. Piperine, stigmasta -4, 22-dien -3 -ol and β -sitosterol were isolated from both plants whilst Piperettine was obtained from only the fruits of Piper nigrum. The chemotaxonomic potential of these compounds and other partially characterised minor constituents as applied to the two species which belong to the same family but different genera has been considered.

I N T R O D U C T I O N

The genera Piper (Linn) and Peperomia (Ruiz and Pav.) are two widely distributed West African plants which belong to the family Piperaceae⁽¹⁾.

Unlike Peperomia, Piper species have been intensively investigated both in West Africa and abroad. Investigations in West Africa have been centred on Piper guineense (Schum and Thonn), a common wild growing species which has been reported to contain both alkaloids and non-alkaloidal compounds⁽²⁻⁵⁾. Piper nigrum which has also been investigated, is a native of India⁽⁶⁻⁸⁾. In Ghana it is locally cultivated and known among the Akans as Nsesa.

It is a perennial glabrous woody climber reaching up to 10 m but in cultivation is usually kept as a bushy plant to 4 m high with some support. The plant has clasping roots with some adventitious roots near the base of the stem; the leaves are up to 5-nerved base, the fruits are in racemes and are red or red-brown turning black when dry.

The fruit has a wide range of medicinal and culinary uses. It is considered a stimulant in weakness following fevers, vertigo and coma. Also as stomachic in flatulence, as antiper in malarial fever and as an alter in paraplegia and arthritic diseases⁽⁹⁾. It is externally used as a rubefacient and as a local application for relaxed sore-throat, piles and skin diseases. Its numerous culinary uses include seasoning and flavouring of soups, meats, poultry, fish, eggs, vegetable, salads, sauces and gravies⁽¹⁰⁾.

Peperomia pellucida (Linn) H.B. & K., known in Creole as 'Cow's foot'⁽¹¹⁾ and Mi>Mi> among the Gas⁽¹²⁾ in Ghana, is common in moist shady places in the tropics. Unlike most Peperomia species which are grown in gardens for decorative purposes, Peperomia pellucida occurs as a common weed in flower-pots, gutters, on river banks and in Cocoa farms. It is distributed from Senegal to Cameroon in West Africa. It occurs also in Fernando Po.

The plant is an erect fleshy herb up to 30cm high with a pale-pink square stem. The leaves are rounded cordate, pale green, glabrous and shiny with 5-7 nerved base; inflorescence axillary, up to 6cm long; flowers small, scattered, pale green and sessile. The fruits are dark green but turn black when dry.

Peperomia pellucida also has a wide range of medicinal and culinary uses. In Ghana a poultice of the whole plant mixed with the juice of limes is drunk for stomach-ache. The poultice is locally applied to boils. It is grown in gardens for use as a vegetable.

The broad uses of the plant in other parts of West Africa are exemplified by the following. It is cultivated as a vegetable and its infusion is used in treating convulsions in children and adults in Nigeria⁽¹¹⁾. In Sierra Leone, the leaves are warmed and put on boils in children's feet. The leaves are used in Nigeria for cancer of the breast. In Togo it is eaten in salad as a remedy for hypertension⁽¹²⁾. Finally, a personal observation has shown that it is eaten by the millipede.

Oppong in investigating the alkaloids of the fruits of Piper guineense⁽¹³⁾ reported the isolation and characterisation of all alkaloids from various Piper species between the period 1908 - 1975.



The report showed that only the fruits of Piper nigrum had been investigated. Four amide alkaloids were isolated viz: Piperine, Piperettine which were solids, and two oils, Piperoleine A and B. A second survey covering the same period has shown that Richard and Jennings had also investigated the fruits and had detected the presence of some hydrocarbons and oxygenated terpenes by gas chromatography⁽¹⁴⁾.

A brief review of the work done on the fruits and other parts of the plant after this period is given in a separate chapter.

On the other hand, a survey from 1908 - 1980 showed that no chemical work has been done on Peperomia species. The present investigation therefore is aimed at:

- (a) Isolation and characterisation of the constituents of the fruits and roots of locally cultivated Piper nigrum (Balamkotta variety) and that of Peperomia pellucida. It is worthwhile to mention here that there are five varieties of Piper nigrum; Balamkotta, Kallavalli, Karinotta, Kalbalacotta and Chariakodi.
- (b) To find any evidence for a chemotaxonomical classification of the two plants as species belonging to the same family but to different genera. A further point of interest is raised by the initial naming of Peperomia pellucida as Piper pellucida by Linnaeus⁽¹⁾. He later changed it to the present name which now identifies it as a member of the genus Peperomia.

The fruits and roots of Piper nigrum were collected from Kade in the Eastern Region (Agricultural Research Station) on 13th March 1981 and the Peperomia pellucida plant, on the University Campus between October 1980-June 1981. An attempt has been made to accomplish as fully as possible the aims of this project.

CHAPTER ONE

CHEMOTAXONOMIC RELATIONSHIPS

Taxonomy is defined as the theory and practice of classification and chemotaxonomy as classification which incorporates principles and procedures involving the use of chemical evidence⁽¹⁵⁾.

Chemotaxonomy, which is apparently a recently developed field, originated from primitive plant classification. Primitive chemotaxonomy, which was an applied science, had practical aims and benefits. Thus early men simply classified plants as

- (i) herbs of healing where plants with similar medicinal properties might have been grouped together,
- (ii) plants of no medicinal use,
- (iii) Poisonous and non-poisonous categories.

Several decades passed before there was a real start to the chemical approach to plant classification, beginning around 1960 with a stream of publications.

Results obtained from investigations into the chemical variation of plant taxa serve two main purposes. First, to provide taxonomic characters which may improve upon existing plant classification and secondly, to add to knowledge of relationships between all plants. The latter is known as evolutionary or phylogenetic relationship. Two major groups of compounds are applied to taxonomic problems⁽¹⁶⁾. These are macromolecules, in particular proteins, and micromolecules, mostly secondary metabolic compounds such as terpenes, flavonoids, alkaloids, cyanogenetic and other glycosides, amino-acids and lipids of various types.

The first method of classification, which utilizes macromolecules, involves the examination of the primary products of plant DNA (deoxyribonucleic acid) and changes in amino-acids within the protein. Results obtained from such studies provide valuable information about phylogeny and relationships at the higher taxonomic categorial levels viz; families, orders, classes.

The information content of an organism is encoded exclusively in its DNA. It is supposed, therefore that organisms which were closely related would have DNAs whose structures(base sequences) were more similar to each other than to those of organisms less closely related. Since not all DNA is informational, attempts to find relationships between plants from the characterisation of their DNAs has not at all been simple. A short-cut technique developed to study the polynucleotide variation in bacteria and higher plants involves hybridisation of DNA. Its usefulness in bacterial taxonomy has been well established but higher plants studies are few and the true value of the method is yet to be assessed.

The method as applied to bacteria is exemplified by the work of Priest et al to determine the taxonomic position of a common brewers' yeast contaminant Obesumbacterium proteus (Achromobacteriaceae)⁽¹⁷⁾. They initially determined the taxonomic position of the bacteria by using conventional biochemical tests which revealed that O. proteus satisfied the requirements for membership of the Enterobacteriaceae. In the DNA hybridization tests, O. proteus single-stranded, labelled DNA was made to compete for filter bound single-stranded O. proteus DNA with DNA from other bacteria.



Nineteen isolates of O. proteus and eighteen strains of enterobacteria were compared and the results for some bacteria are shown in Table I

Table I

Competitor	Percent Competition with <u>O. proteus</u> DNA	
D N A	<u>O. proteus</u> 502	<u>O. proteus</u> 511
<u>O. proteus</u> 511	100	100
<u>O. proteus</u> 500	100	100
<u>O. proteus</u> 510	100	95
<u>O. proteus</u> 501	77	85
<u>O. proteus</u> 530	65	20
<u>Hafnia alvei</u>	70	82
<u>Salmonella gallinarum</u>	17	0
<u>Escherichia coli</u>	53	21
<u>Proteus mirabilis</u>	0	0

They attributed high competition to similarity of the heterologous DNA with O. proteus DNA. The results showed that DNAs from Escherichia coli and Hafnia alvei had high ability to compete with O. proteus DNA. This they suggested indicated a high level of sequence homology. They finally demonstrated that two strains of Hafnia alvei could grow with yeast in simulated brewery conditions. This satisfied the criterion for Obesum-bacterium.

Concluding from the nucleotide similarity, they recommended that O. proteus be renamed Hafnia protea, and that the genus Obesumbacterium be abolished.

In protein sequencing data one of the techniques widely used is electrophoresis. Boulter et al applying this method to the albumins of various species of the genus Baptisia and Vicia (family Leguminosae) observed that species from the same genus showed very similar, sometimes identical, band patterns. However, with the genus Phaseolus, they observed that it could be resolved into several groups with different patterns, which did not entirely agree with the existing sub-divisions within the genus. No generalization was made from the findings because the species examined were too few. On the other hand, on examining thirty-six species of Crotalaria, they obtained band patterns which when split into two groups, demonstrated a reasonable correlation with the existing generic sub-divisions.^(18a)

The second method which involves micromolecular chemical data has been used widely by many researchers to classify bacteria and higher plants but at the lower taxonomic categories viz; the species and genus levels, though this may also be value at the higher taxonomic levels. In the case of bacteria which have so few structural features, even when viewed with the electron microscope, their chemical properties have provided much of the evidence for characterisation. For example, the tribe Nitrobacterieae is defined largely on the ability of the members to gain energy from the oxidation of ammonia to nitrite or from nitrite to nitrate^(18b). Some other chemical characters used for classification are the metabolic products of the bacteria. These include acids, gases or complex products.

Classification may also be assessed on the range of possible respiratory substrates such as carbohydrates and gelatin.

The work by Ikawa on cell wall chemistry in Lactic acid bacteria is a typical example⁽¹⁹⁾. He reported that the Lactobacilli and Streptococoid members of the tribe all had cell walls containing glutamic acid, alanine, lysine, glucosamine and muramic acid, hence their close relationship. On the other hand, aspartic acid which was normally absent from streptococcus cell walls, occurred wherever lysine was present in the walls of lactobacilli. The work was expanded by investigating the distribution of sugar alcohols and reducing sugars in the two groups of bacteria. He found them to parallel the amino-acid differences. These findings were of great taxonomic significance in distinguishing the two groups of lactic acid bacteria, but when compared to higher plants they were united into one group. The comparison revealed that, unlike the amino-acids of higher plants those found in the bacterial cell walls were of the uncommon D-configuration. He speculated on this as of possible phylogenetic significance, attributing association of these D - acids in lactic acid bacteria to the prokaryotic condition, a classificatory system applied to less advanced organisms.

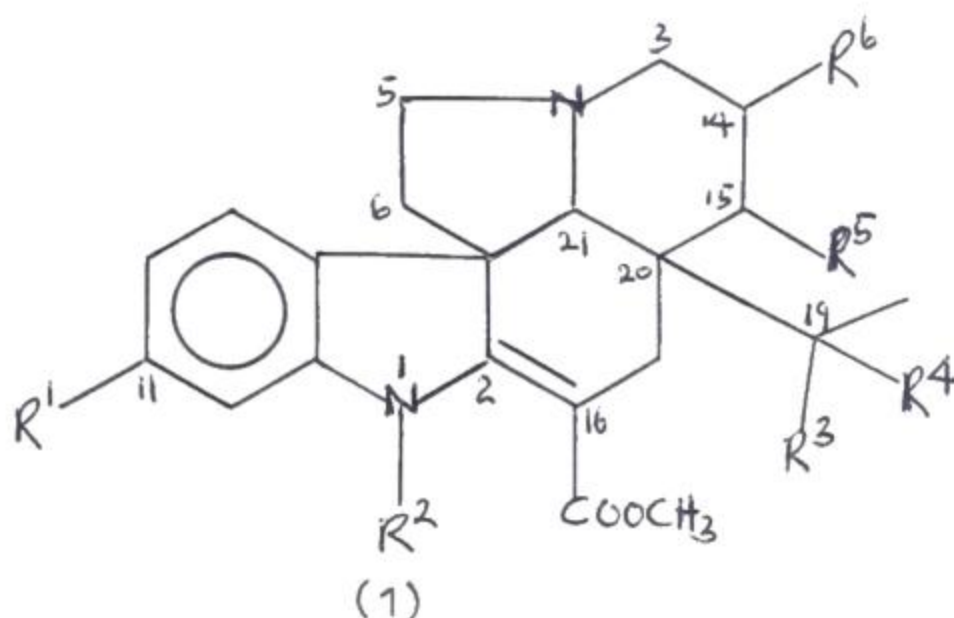
Another method which has been used in bacterial taxonomy, is the electrophoresis of enzyme extracts, followed by staining the separated enzymes with dyes to produce a zymogram. Lund, using this method produced evidence of esterase variation and variation in other proteins in strains of streptococcus. From the results he split the strains in a Streptococcus faecalis group and S. faecium / durans. He compared the results to patterns of variation established in the metabolic properties of these bacteria studied by Whittenbury and found them to agree⁽²⁰⁾.

For higher plants, studies of the alkaloid content and biosynthetic pathways of the secondary compounds have provided valuable complementary information about the placement of groups within a taxonomic system. Takhtajan at a Nobel symposium, presented a paper showing how the alkaloids of the benzyloquinoline type could be used as a classificatory tool⁽¹⁶⁾. He pointed out that berberine, palmitine and related alkaloids have been found in the genera Berberis, Mahonia, Epimedium, Bongardia, Caulophyllum, Leontice and Gymnospermium which all belong to the family Berberidaceae. Alkaloids of the berberine type have also been found in the genus Enantia (palmitine) which belongs to the family Annonaceae. The same observation has been made in other families such as the Hydrastidaceae, Menispermaceae, Ranunculaceae, Papaveraceae and Fumariaceae. On morphological grounds, these families belong to three related orders and the occurrence of berberine type alkaloids in their members lends support to their taxonomic positions.

The distribution of alkaloids in plants have also been used in determining the taxonomic positions of some species. This is exemplified by the work done by Farnsworth et al in determining the taxonomic position of Vinca libanotica, a member of the family Apocynaceae⁽²¹⁾. Several classificatory systems were proposed for the taxonomic differentiation of Vinca species. Pichon, classified Vinca libanotica as a variety of Vinca herbecea whereas Stearn considered it as a distinct species belonging to one of the six main sections, Herbacea. A similar classification was proposed by Trojanek et al. On the basis of the above classifications Farnsworth et al investigated the alkaloid contents of the two plants, Vinca libanotica and Vinca herbecea to determine whether the two species were the same or distinct from each other.

Results obtained from the studies indicated some common features and some differences between the two species which were of taxonomic significance.

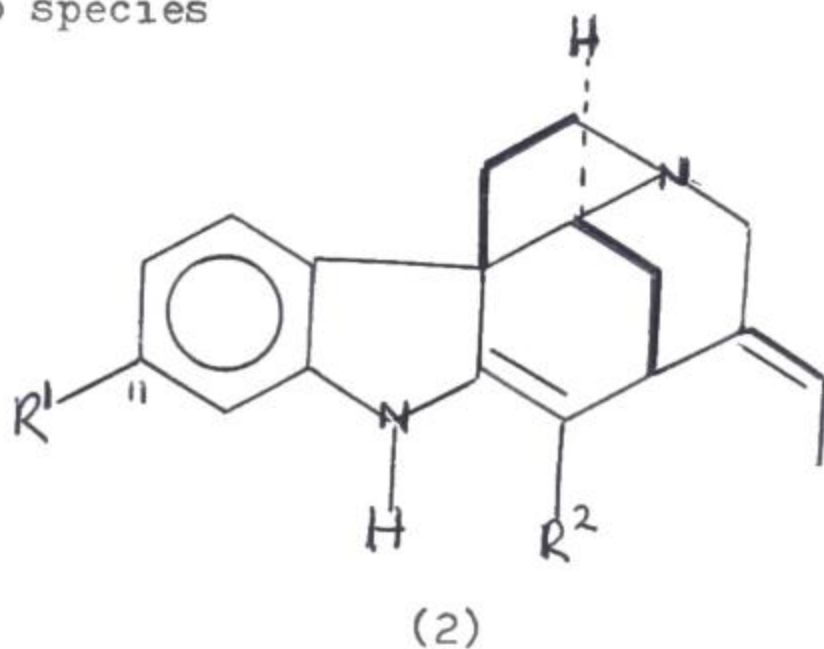
The two species were found to contain α - methylene indoline type alkaloids of the skeleton (1)



e.g.

	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶
(-) - Tabernosine	H	H	H	H		
Lochnerinine	OCH ₃	H	H	H	— O —	

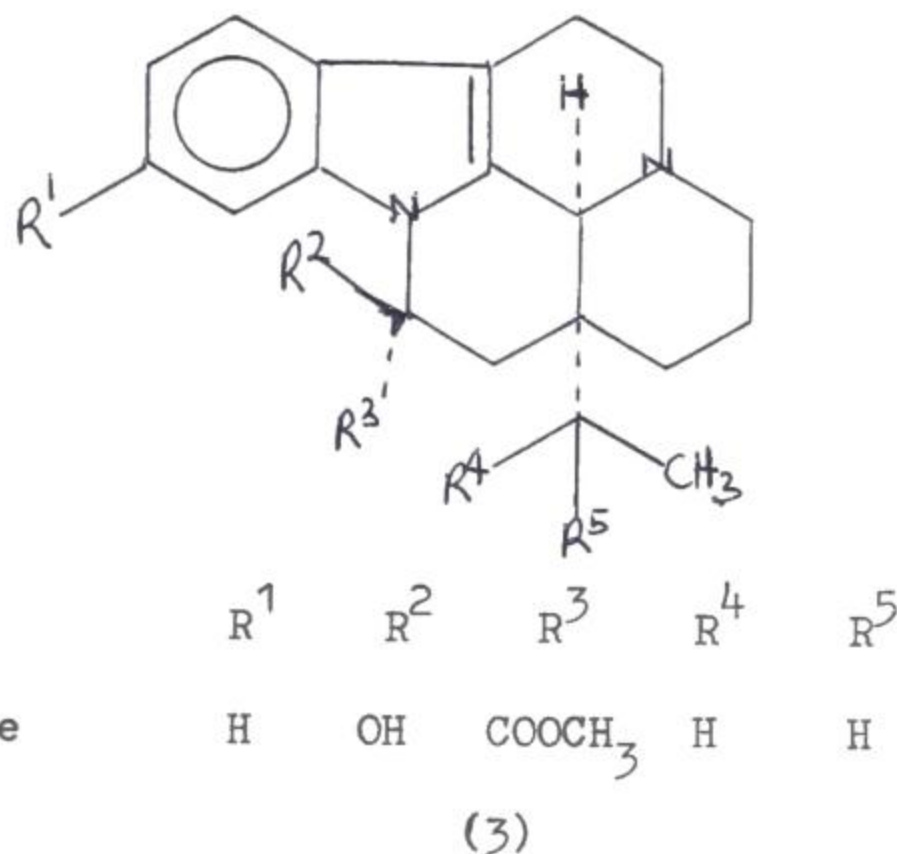
Alkaloids of the akuammicine family with the skeleton (2) were also isolated from the two species



e.g.

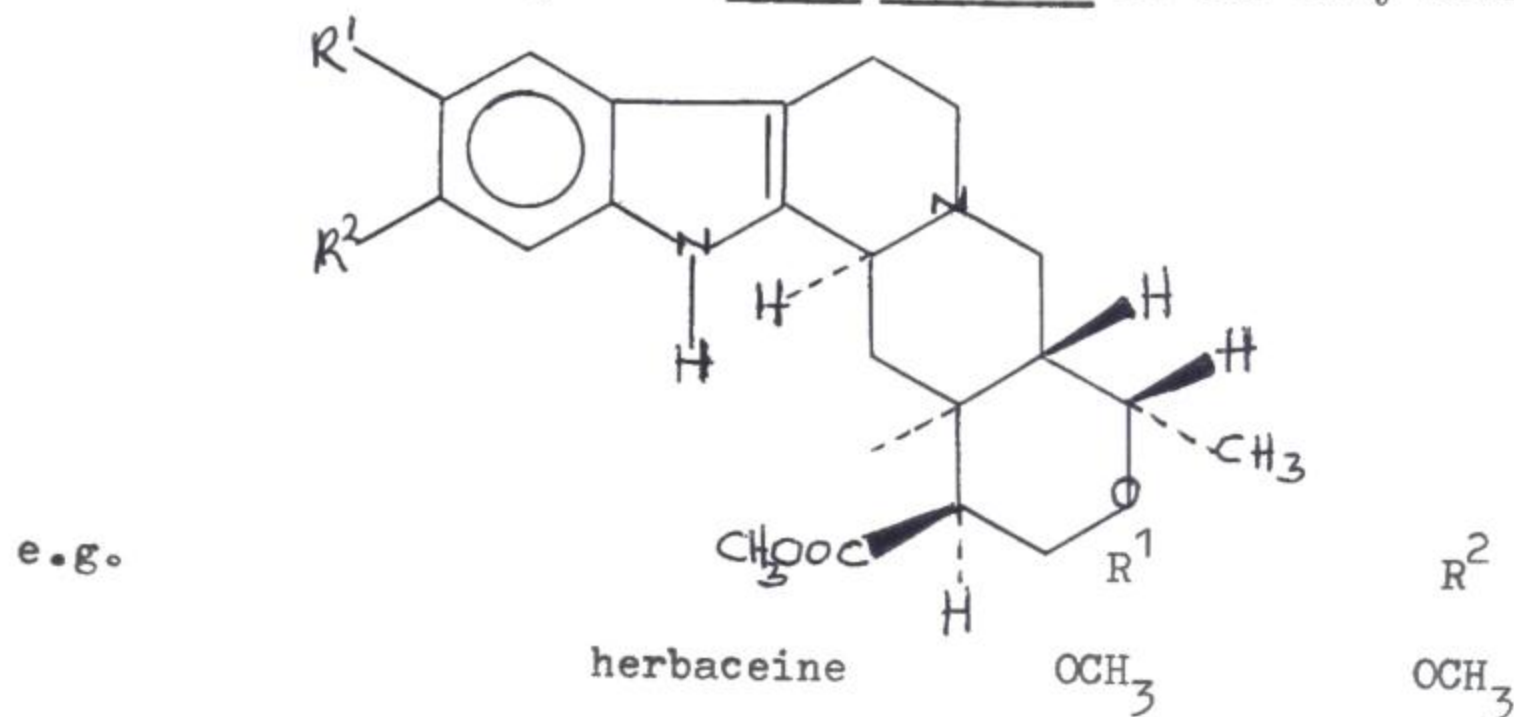
	R ¹	R ²
Vincanine	H	CHO
Akuammicine	H	COOCH ₃

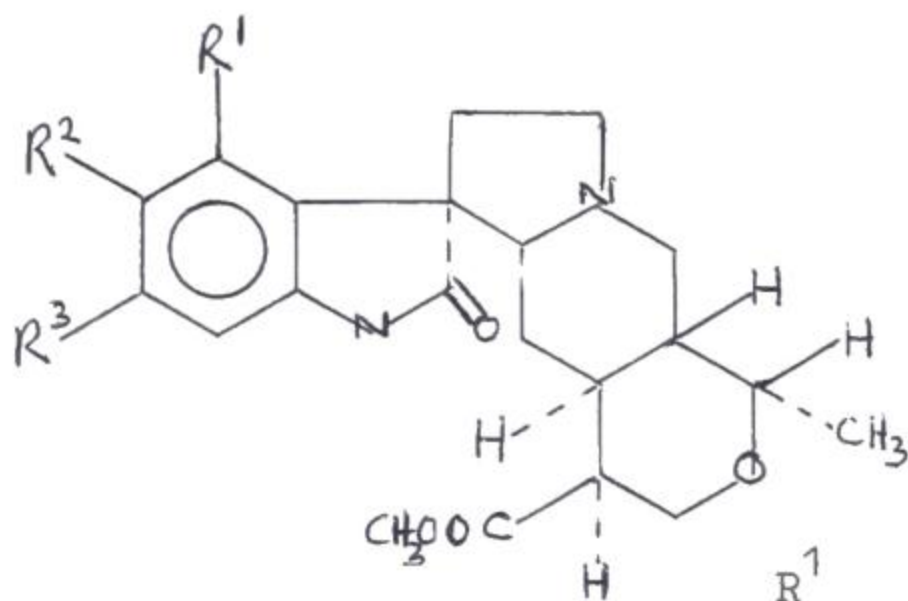
It was observed that there was a low incidence of vincamine (3) and vincamine-type alkaloids in Vinca herbecea.



On the other hand, vincamine which has been isolated from all other Vinca species was found to be absent in Vinca libanotica. The vincamine-type alkaloids were also absent.

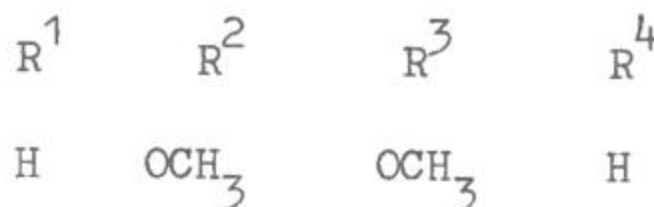
Major differences were found between the two species. Vinca herbecea was found to contain a high percentage of alkaloids containing a hydrogenated heterocyclic E-ring (4). Vinca herbecea is the only source of these alkaloids.





e.g.

herbaline



(4)

Vinca libanotica was also found to contain two alkaloids which had not been reported from any Vinca species. These were quebrachidine and rauniticine. However, an alkaloid named ervine isolated from Vinca erecta was reported to be identical with rauniticine. Farnsworth et al, concluded that Vinca libanotica represented a distinct species and therefore suggested the separation of it from the section Herbacea.

A similar work was done by Harbone, who investigated the flavonoid pigment of some angiosperm families in the order Parietales ⁽²²⁾. Several classificatory systems have been proposed for the Parietales. Table II shows the classificatory systems of Cronquist and Takhtajan.

Table II

<u>Cronquist</u>	<u>Takhtajan</u>
<u>Violales</u> <u>Flacourtiaceae</u>	<u>Violales</u> <u>Flacourtiaceae</u>
<u>Violaceae</u>	<u>Violaceae</u>
<u>Turneraceae</u>	<u>Bixaceae</u>
<u>Passifloraceae</u>	<u>Cochlospermaceae</u>
<u>Bixaceae</u>	<u>Cistaleae</u>
<u>Cistaceae</u>	<u>Tamaricales</u> <u>Tamaricaceae</u>
<u>Tamaricaceae</u>	<u>Frankeniaceae</u>
<u>Frankeniaceae</u>	
<u>Theales</u> <u>Guttiferae</u>	<u>Theales</u> <u>Guttiferae</u>

Harbone's analysis of the leaves of most of the species of the families listed above, except the Passifloraceae, indicated the presence of some flavonoid bisulphates. The Passifloraceae had already been investigated by Bate-Smith, and his results showed that it lacked recognisable constituents. Results obtained from the survey of the bisulphates indicated that they were uniformly present in Bixaceae, Tamaricaceae, Frankeniaceae, and occasionally present in Cistaceae and Guttiferae. These families were also found to contain ellagic acid, which demonstrated a close relationship between them and the bisulphates. The only exception was the detection of ellagic acid in three species of the Cochlospermaceae. Comparing his results to the classificatory systems proposed by Cronquist, Takhtajan and Hutchinson, he found a reasonable agreement with Cronquist's arrangement of the Parietales but observed that they fitted less with Takhtajan's, which as in the table shows the grouping of the Tamaricaceae and Frankeniaceae into a separate order, the Tamaricales. He found no fitting at all with Hutchinson's system.

The classification of the families in the order Centrospermae is rather interesting. Mabry et al proposed to define the order on the presence of betalains commonly called nitrogenous anthocyanins⁽²³⁾. On this basis, they added two families Cactaceae and Didiereaceae which were previously not in the group. On the other hand, they excluded the Caryophyllaceae and Molluginaceae which had usually been considered members of the order because they lacked betalains.



Their results provided the additional evidence that was needed to clarify the taxonomic positions of the Cactaceae and Didiereaceae. The Cactaceae had for a long time been a puzzling group of plants to taxonomists.

These findings also demonstrated that betalain containing plants completely lacked anthocyanins, even though other types of flavonoids were present.

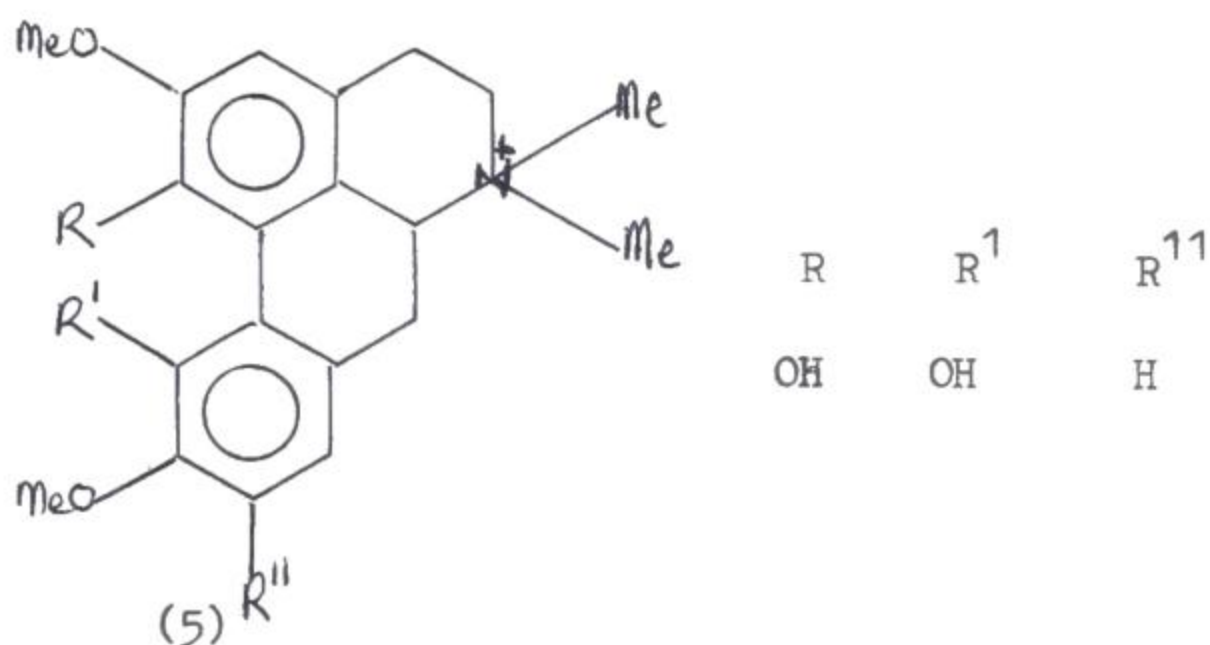
The results were of great taxonomic significance because of the completely different nature of the two sets of pigments. They attributed the production of betalains to a correlated set of genes and concluded that the two groups of pigments were mutually exclusive, hence the exclusion from Centrospermae of all non-betalain families. This sparked off a long debate among taxonomists, in which Cronquist and Takhtajan were strong opposers to Mabry's conclusion. Cronquist's views would be used to illustrate this. (236)

Cronquist's arguments could be traced along genetic, biosynthetic and morphological lines. Genetically, he pointed out that the absence of anthocyanin from betalain-containing families agreed with Ownbey and Aase's genetic principles. The principles are, first an individual's phenotypic characters were governed by a complex system of genes, rather than by a single gene unaffected by others. Also, the appearance of a particular character in the phenotype required the whole genetic system. Thus, if two individuals or allied taxa shared the same structure or complex chemical substance then they probably should share the same genetic system that determined its development. Finally, in the formation of a compound, if one of the essential genes in the system was lost, then the compound should fail to develop. These principles perfectly explained the mutual exclusiveness of the anthocyanins and betalains.

The observations made by other researchers like Alston and subsequently Kimler et al laid grounds for the disagreements of Cronquist and others. These men observed that anthoxanthins and betaxanthins frequently occurred together, and in the Centrospermae as noted by Cronquist, the anthoxanthins commonly included flavonols. Using Grisebach's biogenetic scheme of flavonoids, where it is postulated that both flavonols and anthocyanins were all derived directly from a dihydroflavonol, which is considered to be a compound pool in the flavonoid biosynthetic chain, he pointed out that, the Centrospermae in general only lacked the terminal link in the biosynthetic chain for the production of anthocyanin. This he explained, could only be a matter of a single gene. He also argued that since anthoxanthins and betaxanthins occurred together, and since anthocyanins were chemically so similar to anthoxanthins, then a complete mutual exclusion seemed inherently unlikely. He also cited several morphological and anatomical features which demonstrated the close relationship between the families in the order Centrospermae.

In concluding, though he agreed that the betalains had been very useful to taxonomists in helping to show the relationship of the Cactaceae and Didiereaceae to the traditional Centrospermae, as regards Caryophyllaceae and the Molluginaceae their exclusion from the order was contrary to the bulk of evidence available. He maintained that, if even the complete mutual exclusion of anthocyanins and betalains were eventually demonstrated, he would consider it merely an interesting anomaly rather than a matter of major taxonomic importance. A similar argument was made by Takhtajan⁽¹⁶⁾ who with Thorne and Cronquist in their separate classificatory systems have placed the two families in the Centrospermae.

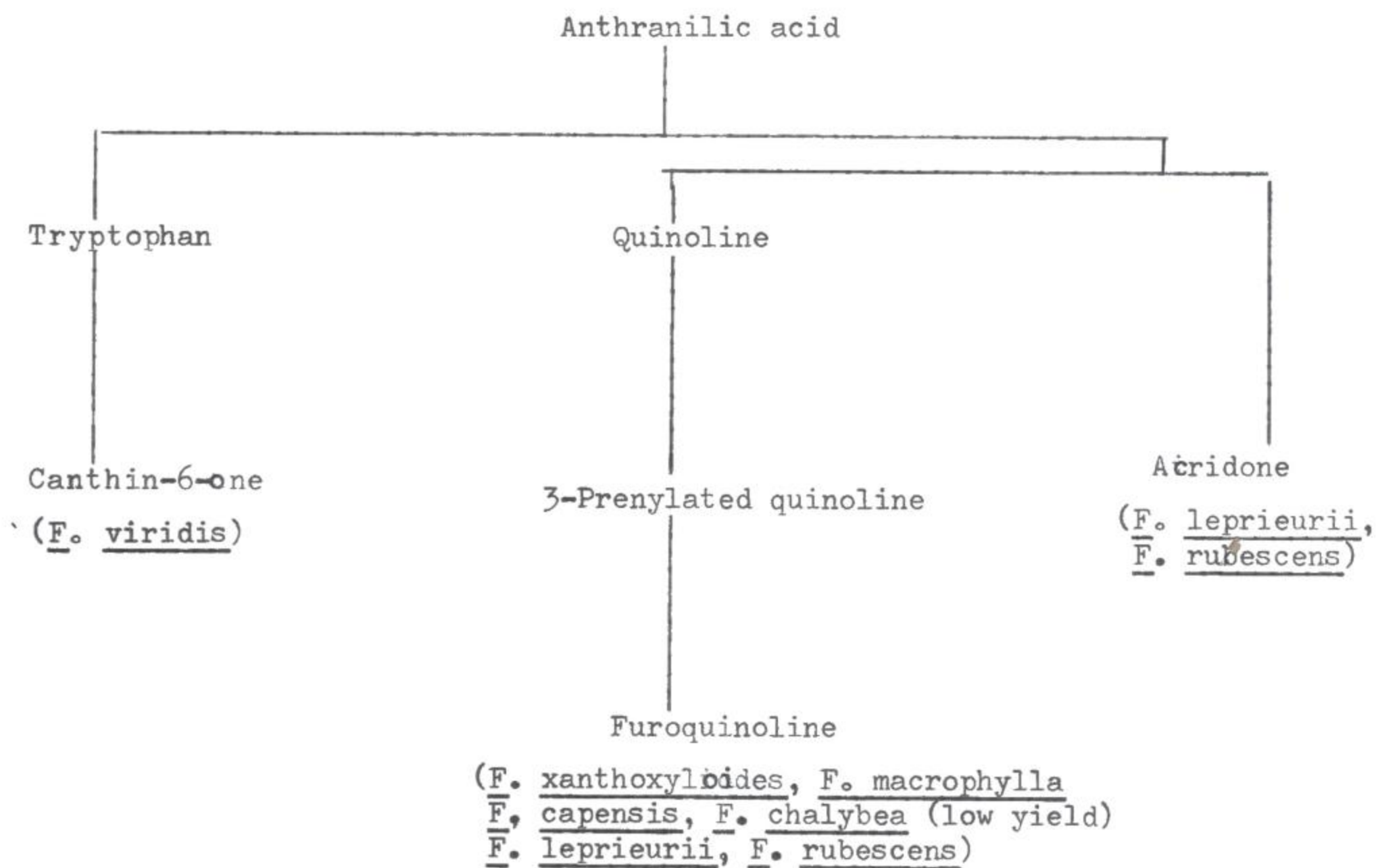
A taxonomic confusion between the genus Fagara (Linn) and its closely allied Zanthoxylum (Linn) led Fish and Waterman to investigate the chemosystematics of the genus Fagara⁽²⁴⁾. They examined the quaternary alkaloids of seven African species of Fagara which included F. leprourii, F. rubescens, F. xanthoxyloides, F. macrophylla, F. capensis, F. chalybea and F. viridis. The chemotaxonomic studies of the seven species involved several quaternary alkaloids, some of which were identified by chromatographic and electrophoretic techniques whilst others were isolated in sufficient yields, including magnoflorine, tembetarine and candicine. They found magnoflorine (5) to be present in all the species they investigated and observed that with the exception of F. macrophylla and F. chalybea it appeared to be the major quaternary base.



Their results also indicated that one or both of the monomethyl ethers of (5) were present as minor constituents.

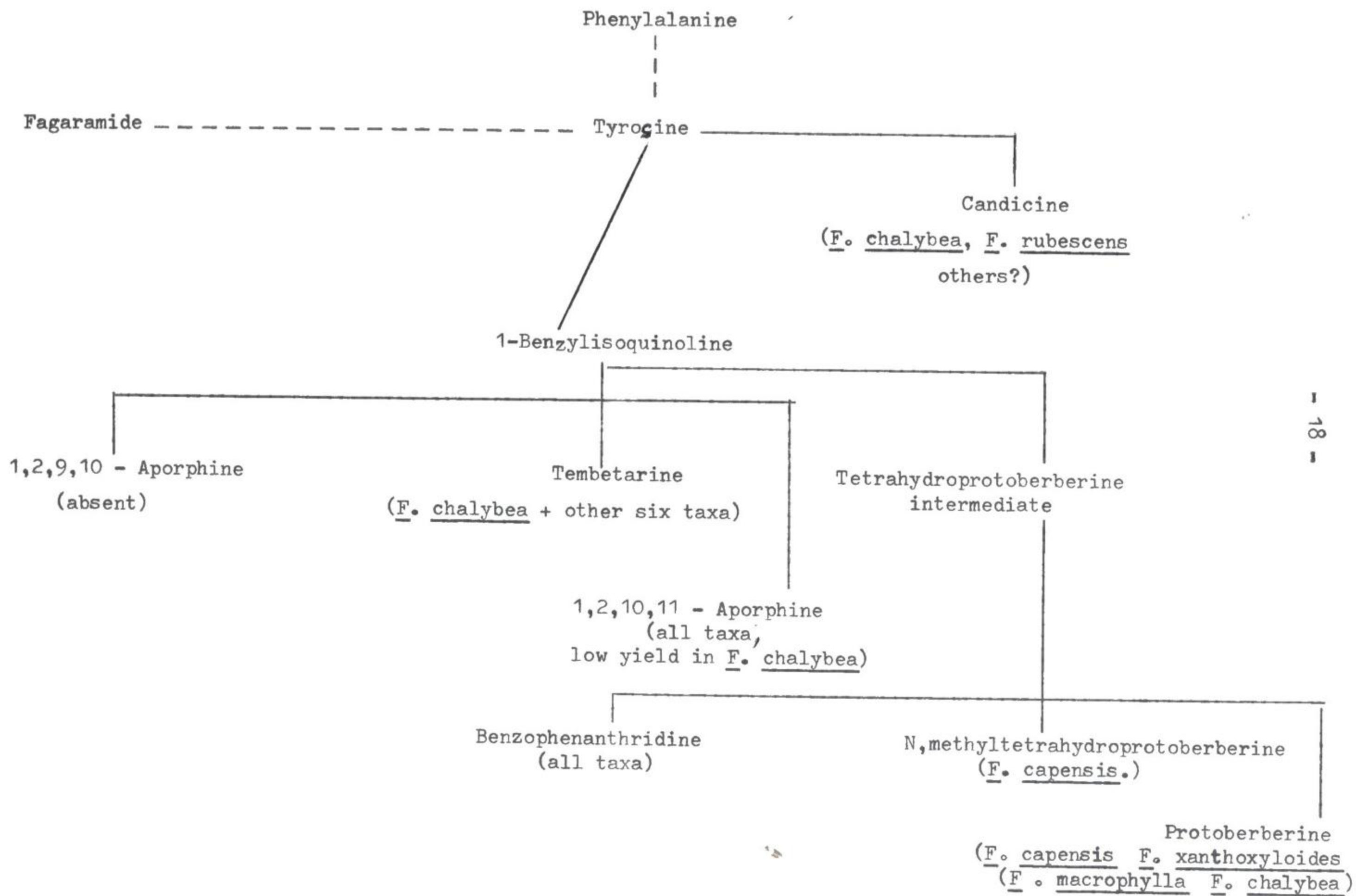
The striking feature of their results was that the alkaloid patterns were similar in all the seven species examined. They showed the inter-relationship between the species by breaking down the alkaloids into anthranilic acid derived alkaloids, having the nuclei cathin-6-one, furoquinoline and acridone, and the phenylalanine/benzylisoquinoline derived bases containing the benzophenanthridine or protoberberine nucleus.

Scheme (1) summarizes the anthranilic acid biosynthetic pathway.



Scheme(1)

With regard to phenylalanine/benzylisoquinoline metabolism, two pathways were considered to occur in all seven species leading to the aporphine and benzophenanthridine nuclei. Both of these nuclei required a 1-benzylisoquinoline intermediate. Scheme (2) summarizes the pathways.



SCHEME (2)

From the two biosynthetic schemes, they proposed a chemotaxonomic grouping for the seven African species. The groupings were as follows:

A) F. viridis

B) F. leprieurii

C) (i) F. macrophylla

F. xanthoxyloides

(F. laurentii)

(ii) F. capensis

(iii) F. chalybea

F. viridis was separated into one group because it was unique in containing canthin-6-one as in Scheme (1). Group B species were separated from the furoquinoline-producing taxa into another group because of their distinct property to synthesise acridone alkaloids (Scheme (2)). The four remaining taxa under the furoquinoline were grouped after examining the phenylalanine/benzylisoquinoline pathway. From this pathway, they observed that F. viridis, F. leprieurii and F. rubescens were similar. They also found F. xanthoxyloides and F. macrophylla to be similar due to the isolation of fagaramide and some benzophenanthridines from the two species.

They proposed that fagaramide which had also been isolated from F. laurentii was derived from an early modification of phenylalanine or a derivative involving the initial loss of nitrogen, then followed by condensation with an amino-acid which could be valine to yield the amide. As regard the benzophenanthridines, they observed an interesting feature, in that F. xanthoxyloides contained chelerythrine whereas F. macrophylla contained nitidine.

They suggested that in the biosynthesis of chelerythrine an ortho coupling of the 1-benzylisoquinoline precursor was required for the production of the tetrahydroprotoberberine intermediate whereas a para coupling leads to the production of nitidine. The relationship between F. macrophylla, F. xanthoxyloides on the one hand and F. laurentii has not yet been established.

The groupings in C show F. capensis and F. chalybea to be different. This they suggested was due to the uniqueness of F. capensis to be the only taxon to contain an N-methyltetrahydroprotoberberine, while F. chalybea was also distinct in its ability to produce large quantities of phenylethylamine, benzylisoquinoline and benzophenanthridine alkaloids in favour of the usual aporphines and furoquinolines. Finally, the general grouping of the seven species was supported by the detection of berberine in the four species in C and its absence in A and B.



CHAPTER TWO

PREVIOUS WORK ON PIPER SPECIES

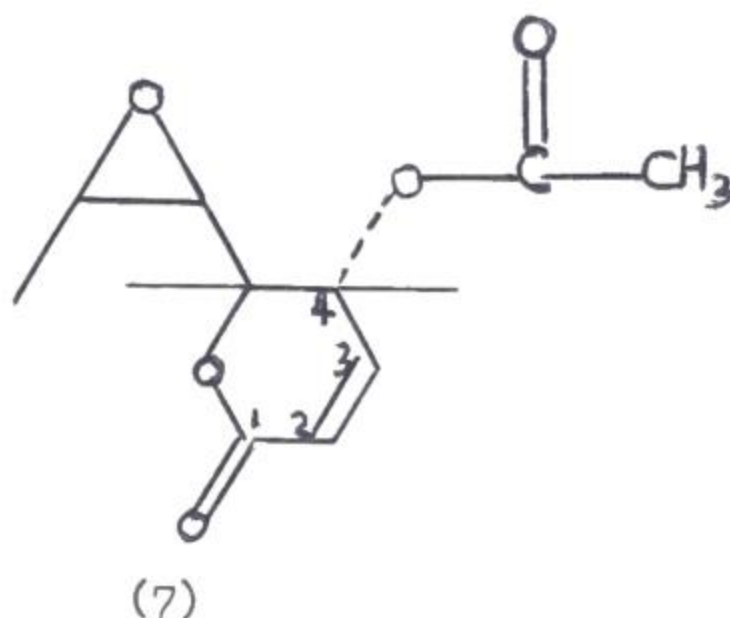
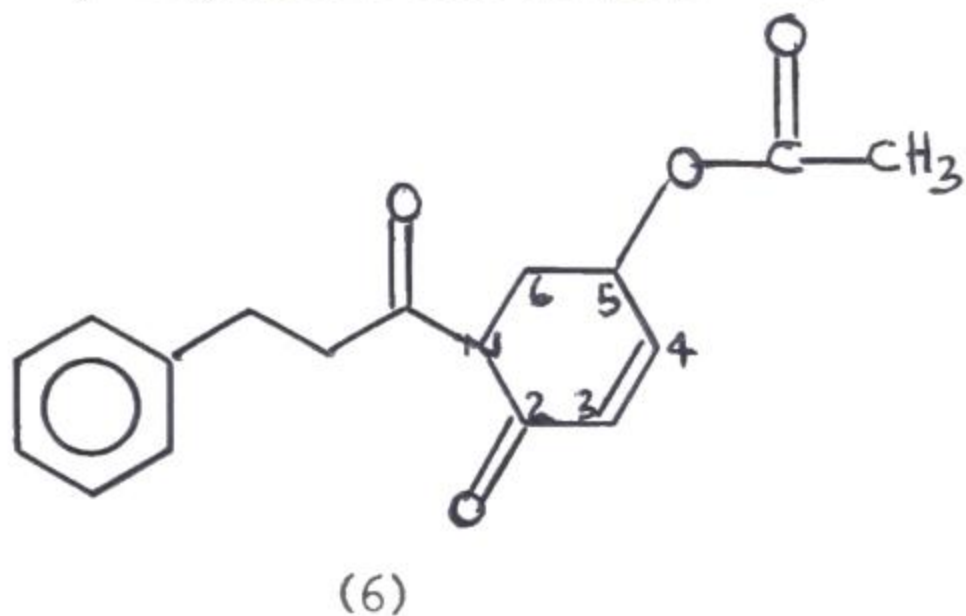
In this chapter, a summary of all the work done on Piper species between 1979 and 1980 is given. The areas covered include method of isolation, structural elucidation of compounds and wherever possible, pharmacology. Some earlier workers in this Department have already reviewed the work done on Piper species before this period (13, 25, 26). Each compound is mentioned once and the first chemical approach used in elucidating the structure is discussed.

Pipermethystine

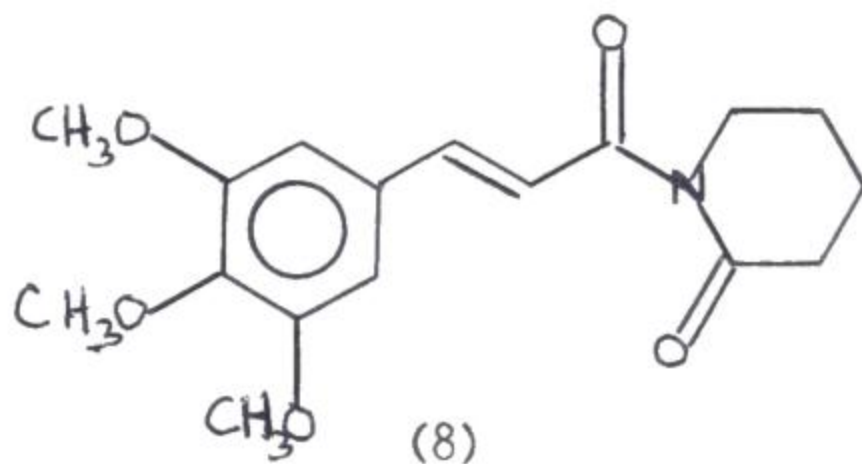
Roger Smith,⁽²⁷⁾ reported the isolation of a novel pyridone alkaloid, pipermethystine (6) from the leaves of Piper methysticum. The compound was isolated by tlc as an oil from the ethyl acetate extract. The UV spectrum showed peaks at λ_{max} 210, 243 nm, which indicated the presence of a conjugated system. The IR spectrum contained bands at 3070, 3035 which were assigned to (Ar-H), an ester carbonyl at 1740, a C = O amide group at 1700 and 1690 and a double bond system at 1630 cm^{-1} .

The PMR spectrum was found to contain signals for an acetyl group (δ 2.06, 3H, s), and an aromatic ring (δ 7.2, 5H, bs). A series of coupled signals were assigned to the pyridone ring C - 3H (δ 6.15, d, $J_{3,4} = 10 \text{ Hz}$ broadened slightly by long range coupling), C - 4H (δ 6.84, ddd, $J_{3,4} = 10$, $J_{4,5} = 5$, $J_{4,6} = 1 \text{ Hz}$), C - 5H (δ 5.42, q, $J_{4,5} = J_{5,6} = 5 \text{ Hz}$), C - 6H₂ (A-B system 3.86, dd, $J_{AB} = 15$, $J_{5,6} = 5 \text{ Hz}$ and 4.32, ddd, $J_{AB} = 15$, $J_{5,6} = 5$, $J_{4,6} = 1 \text{ Hz}$).

The chemical shifts and couplings were found to be similar to those of the acetyl- δ -lactone group of asperline (7) reported by Argoudelis and Zieserl⁽²⁸⁾.



The signals for this group were C-2H (δ 6.21, d, $J_{2,3} = 10$ Hz), C-3H (δ 7.08, dd, $J_{2,3} = 10$, $J_{4,5} = 5.5$ Hz), and C-4H (δ 5.33, dd, $J_{3,4} = 5.5$, $J_{4,5} = 3$ Hz). The C-3H and C-4H peaks were also similar to those of the alkaloid piperlonguminine (8) isolated from Piper longum⁽²⁹⁾.



The assignments in the spectrum of (6) were confirmed by double irradiation at 5.30 ppm (C-5H) when the signals at 3.86 and 4.32 ppm collapsed to an AB quartet ($J_{AB} = 15$ Hz) and the signal at 6.77 ppm collapsed to a doublet ($J = 10$ Hz). Irradiation of the (C-6H) signal at 4.30 ppm caused a partial collapse of the signal at 5.30 ppm (C-5H). The remaining signals which appeared as an A_2B_2 system were assigned to C-8H₂.

(δ 3.01, slightly broadened and C-9H₂ (δ 3.38), which was found to agree with the -CH₂CH₂ - system in 3-phenylpropanoic acid, (δ 2.80, 4H, A₂B₂ multiplet). The long range coupling observed between C-4H and one of the C-6 protons suggested that the two protons were in a coplanar W - configuration.

An attempt was made to confirm the relationships between the protons using Eu(fod)₃ shift reagent. This was unsuccessful as there was no specific interaction of (6) with the reagent. Smith observed no clear differences in contact shifts. The C M R spectrum (Table III) was in agreement with the proposed structure.

Table III

CMR Spectrum of Pipermethystine (6) ppm (relative to TMS)

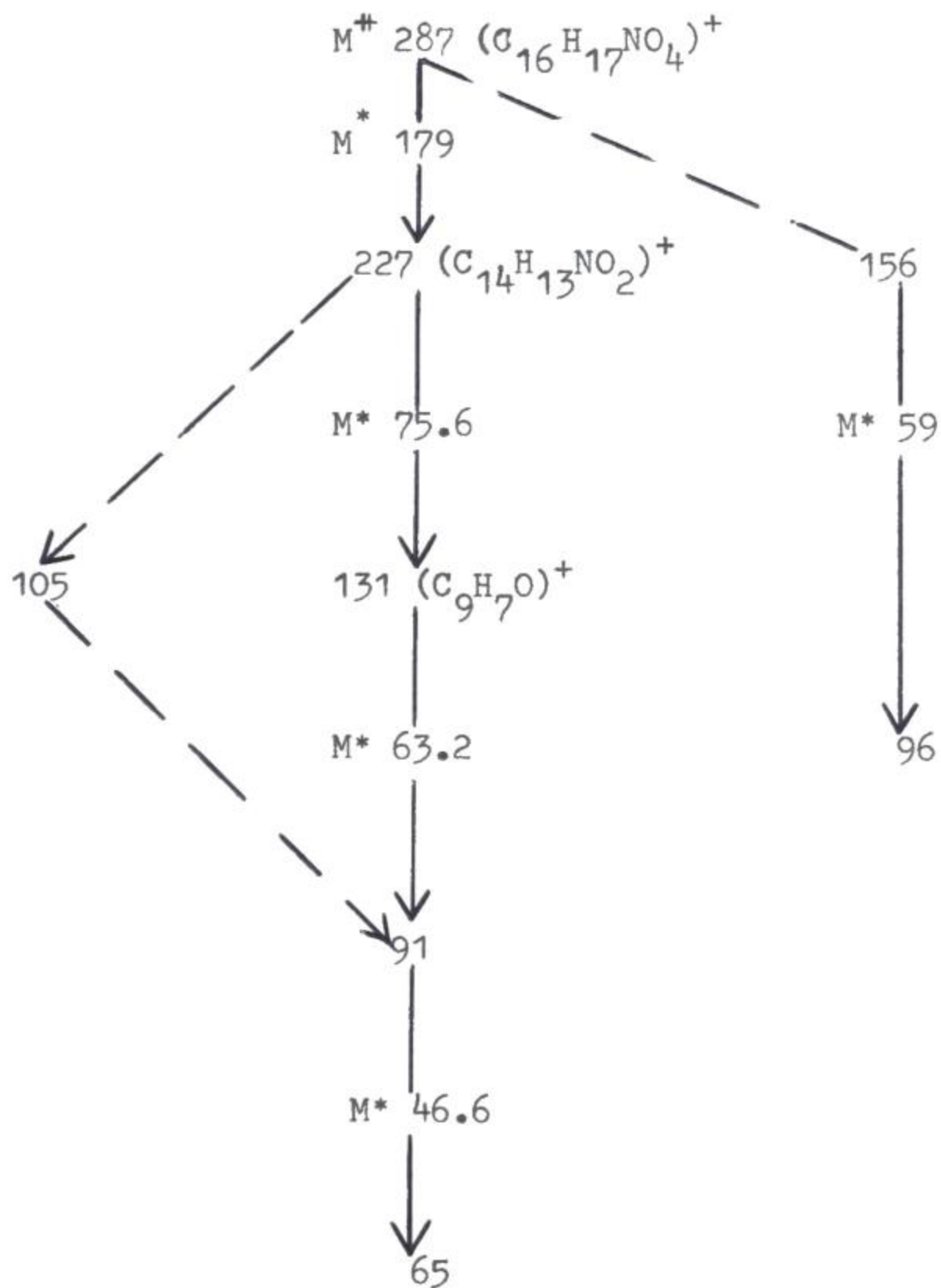
	<u>Splitting</u>	
20.8	q	$\text{CH}_3 - \text{C} = \text{O}$
30.9	t	C - 9
40.9	t	C - 8
45.1	t	C - 6
63.4	d	C - 5
126.1	d)	
127.6	d)	C = C and
128.4	d)	Ar C = C
128.8	d)	
140.2	d)	
140.9	s	C - 1
163.8	s	C = O
169.9	s	C = O
175.3	s	C = O

The C - 8 (40.9, t) and C - 9 (30.9 ppm, t) signals were found to be similar to those of C - 2 (35.5) and C - 3 (30.5 ppm) in 3-phenylpropanoic acid.



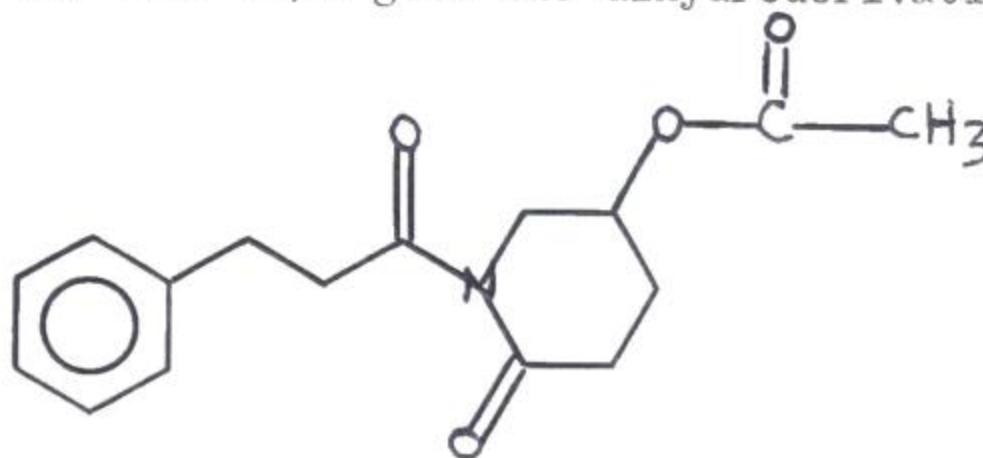
The mass spectrum of (6) indicated a molecular ion at m/e 287, $C_{16}H_{17}NO_4$, and a strong fragment ion at m/e 227, $C_{14}H_{13}NO_2$ which suggested the presence of an acetoxyl group.

The fragmentation pattern is illustrated by Scheme 3.



Scheme (3)

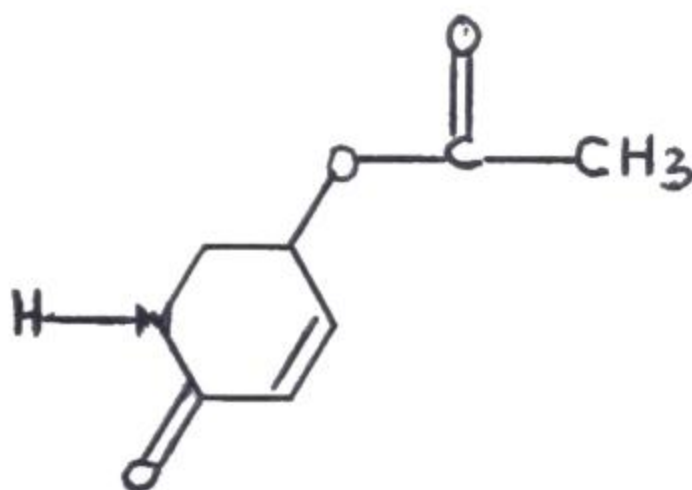
Hydrogenation of (6) over Pd/C gave the dihydroderivative (9) as an oil.



(9)

The UV spectrum showed an aromatic band at 213 mμ. The IR spectrum indicated peaks at 1740 and 1690 cm⁻¹ for the ester and amide groups respectively but observed that the olefinic band at 1630 cm⁻¹ which was present in the spectrum of (6) was absent. The PMR spectrum also contained signals for the acetyl (δ 2.03 s) and aromatic groups (δ 7.23 s). The signal at δ 5.25, m was assigned to the C-5H. It was also observed that there were no olefinic signals in the P.M.R. spectrum. The remaining protons were assigned to a complex series of multiplets which appeared between δ 3.7 - 1.9 ppm.

The compound (6) could not be separated by t l c or column chromatography on activated alumina due to decomposition. A similar degradation was observed on allowing (6) to stand for some months. The two principal degradative products isolated and identified were 3-phenylpropanoic acid and the dihydropyridone (10) mp. 130 - 131.5, C₇H₉NO₃.



(10)

The UV spectrum of (10) $\lambda_{\max}$ 207, 241 nm ($\log \epsilon$ 4.10, 3.30) was found to be similar to that of 5,6-dihydro-2 (1H)-pyridone, $\lambda_{\max}$ 204, 240 nm ($\log \epsilon$ 3.92, 3.08)⁽³⁰⁾. The following peaks were contained in the IR spectrum. Peaks for a secondary amide (3420 NH and 1680 C = O cm^{-1}), an ester (1732 cm^{-1}) and an olefinic group (1615 cm^{-1}).

The PMR spectrum contained a signal for an acetyl group (δ 2.10 s) and a series of coupled signals were assigned to C-3H (δ 6.13, dd, $J_{3,4} = 10$, $J_{3,6} = 2$ Hz) C-4H (6.66, dd, $J_{3,4} = 10$, $J_{4,5} = 5$ Hz), C-5H (5.38, q, $J_{4,5} = 5$ Hz) C-6H₂ (2H, 3.70, m). These signals were found to be similar to those in the spectrum of (6). These assignments suggested that C-3H signal was coupled across the carbonyl group to the NH group (δ 6.42, bs). The assignments were confirmed by double irradiation studies.

Irradiation at 6.12 ppm. collapsed the signal at 6.66 ppm to a doublet, at 6.66 ppm collapsed 6.13 ppm and 5.38 ppm to multiplets, at 3.70 ppm collapsed 5.38 ppm to a multiplet, and at 5.38 ppm collapsed the signal at 3.70 ppm.

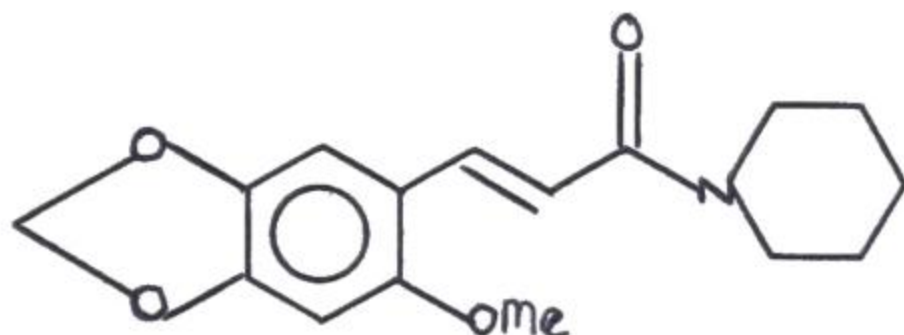
There were some differences observed in the mass spectrum of (6) (Scheme 3) and (9). An unexpected ion at m/e 131, C₉H₇O, and a corresponding M⁺ - 131 ion at m/e 156, were found to be absent from the spectrum of (9). The fragmentation pattern proposed for (9) involved a cleavage of the amide - CO - N bond with double hydrogen transfer to the pyridone ring, to give (Ph - CH₂ - C = C = O)^{•+} or (Ph - CH = CH - C = O)^{•+}

Pipermethystine (6) was also found to be present in small amounts in the stems and roots of P. methysticum.

Smith, concluded that the instability of (6) on standing or on alumina chromatography probably accounted for no previous report on its isolation.

2-methoxy-4,5-methylenedioxy-cis-cinnamoylpiperidide

Gupta et al⁽³¹⁾ reported the presence of a piperidine alkaloid which was characterised as 2-methoxy-4,5-methylenedioxy-trans-cinnamoyl-piperidide (11) on the basis of spectral data. Sehgal, Kachroo et al⁽³²⁾ upon studying the PMR spectrum of this alkaloid disagreed with the configuration of the protons at $\Delta^{7,8}$ in the proposed structure. These protons, they noticed, were a little upfield from their expected positions. Gupta et al found the coupling constant of the $\Delta^{7,8}$ protons to be 14 Hz whereas Sehgal et al found it to be 12.3 Hz. Secondly, the earlier workers assigned two multiplets to the methylene protons adjacent to the nitrogen in the piperidine moiety instead of an expected broad singlet. These facts raised doubts regarding the configuration at $\Delta^{7,8}$. Sehgal et al decided to synthesise the cis and trans geometrical isomers to confirm the structure of the compound.



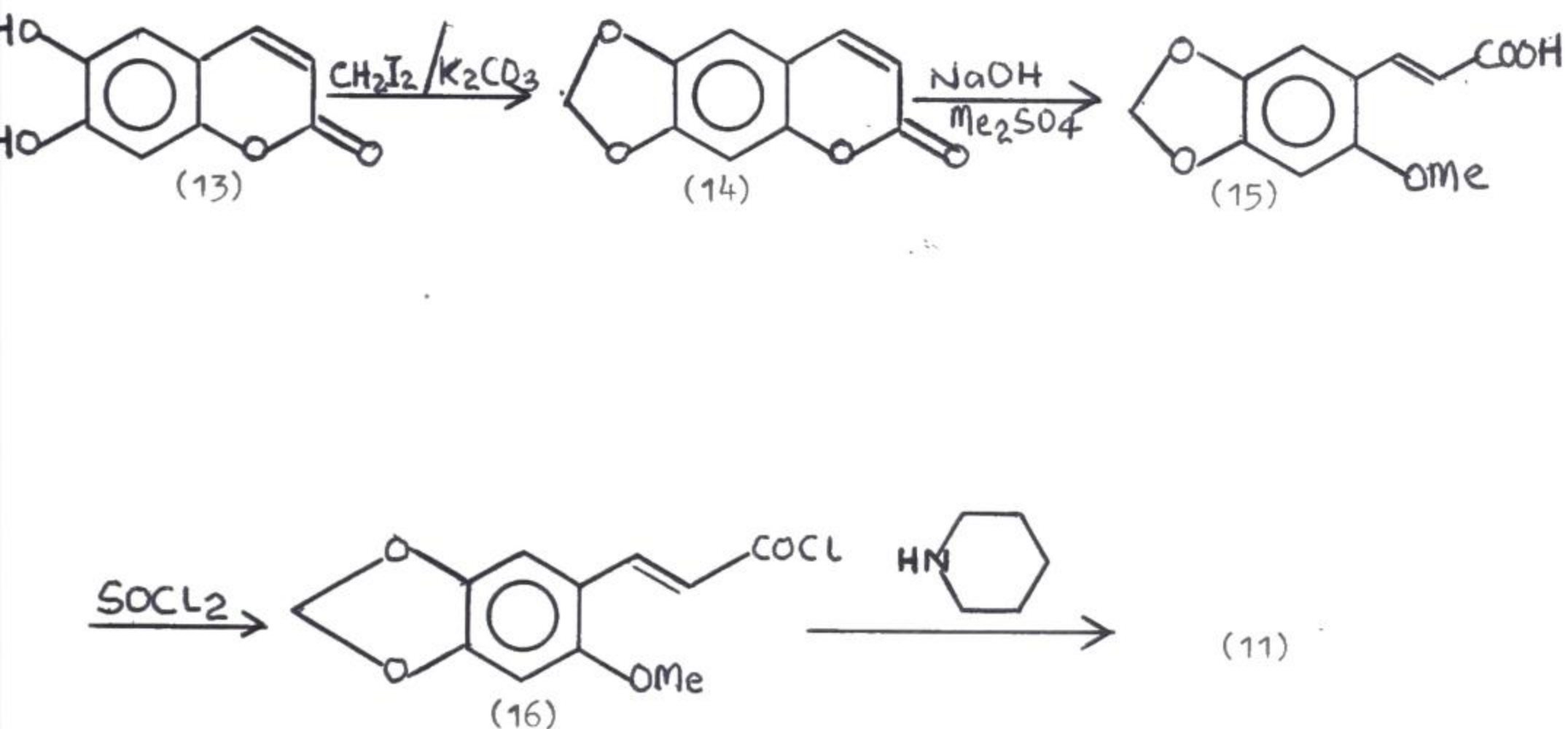
11(7,8) trans

12(7,8) cis



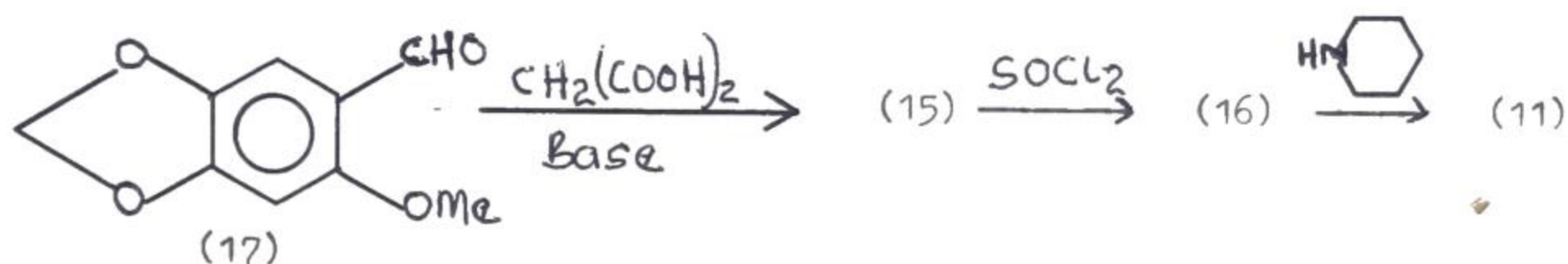
The trans isomer was synthesised by two different methods.

Method one involved, initially, the conversion of 6,7-dihydroxy-coumarin (esculetin) (13) into 6,7-methylenedioxy-coumarin (14) using di-iodomethane in the presence of base. (14) was converted to 2-methoxy-4,5-methylenedioxy-trans-cinnamic acid (15) by simultaneous ring opening and methylation according to the method of Rao et al (33). They used dimethylsulphate and sodium hydroxide. The trans acid chloride (16) obtained from (15) was then subjected to condensation with piperidine to give (11). Scheme (4) outlines the synthesis.



Scheme (4)

In the second method for the synthesis of the trans isomer 2-methoxy-4,5-methylenedioxybenzaldehyde (17) was used as the starting material. The compound was subjected to Knoevenagel condensation in the presence of malonic acid, pyridine and piperidine. The trans acid (15) was then converted to trans amide (11) as in Scheme (4). The second synthetic method is outlined in Scheme (5)



Scheme (5)

In both cases the product was found to be similar in all respects. However, a comparison of the physical constants and spectral data of the synthetic and natural alkaloid showed a marked difference between the two.

The synthetic trans compound melted at 117°C , whereas the natural product has mp 99°C .

The UV spectrum (MeOH) of the compound reported by Gupta et al showed absorption bands at 333, 275 and 240 nm whereas in the synthetic compound the absorption bands were at 360, 290 and 235 nm respectively.

In the PMR spectra (CDCl_3), the $\Delta^{7,8}$ trans protons were located at δ 7.85 and 6.70, respectively, as doublets ($\Delta\delta = 1.15$, $J = 15.8$ Hz), whereas in the case of the natural alkaloid these protons were located at δ 6.86 and 5.95 respectively ($\Delta\delta = 0.91$, $J = 12.3$ Hz). These were compared with the reported values $\Delta\delta$ for the cis and trans olefinic protons in cinnamic acids. The cis values were $\Delta\delta$ 0.95 $J = 12.3$ and the trans $\Delta\delta$ 1.15, $J = 15.8$ Hz. A broad signal centred at δ 3.66 was assigned to the 4 protons ($-\text{N}(\text{CH}_2)_2$) of the piperidine moiety. In the case of the reported amide by Gupta et al, two clear multiplets at δ 3.30 and 3.55, respectively were assigned to these protons. The synthetic trans compound also exhibited a red shift, which was expected. From these observations Sehgal et al concluded that the reported alkaloid had a cis rather than a trans configuration at $\Delta^{7,8}$.

To confirm the structure, the cis isomer was synthesised. A novel method involving the opening of the 6,7-methylenedioxcoumarin(14) was achieved by using NaH and MeI in T H F at room temperature. At the end of the reaction, a mixture of the cis - and trans-2-methoxy-4,5-methylenedioxycinnamic acids 15a and 15b was obtained in the ratio 4:1, which was converted to the amide by the method already outlined. The resultant amide was a mixture of the cis and trans in the ratio 3:1. The cis isomer was separated by repeated crystallization.

When the spectral data of the synthesised compound was compared with that of the natural amide, it was found to agree in all respects.

The structure was further confirmed by hydrogenating separately both isomers in the presence of Pd/C. Both isomers gave the dihydro-derivative, the IR's and PMR's of which were found to be superimposable.

These findings gave the first confirmed report of a piperamide with a cis configuration at a double bond in conjugation with the ~~carbonyl~~ carbonyl. All previous reports indicated a trans configuration of double bonds in the side chain of the amides. Finally, Sehgal et al gave an explanation to the presence of a multiplet instead of a singlet for the two methylene protons adjacent to the nitrogen in the cis isomer. They attributed this to the restricted free rotation of the N - C = O bond, which makes the two methylenes non equivalent.

Aurantiamide acetate

Banerji, Ray et al⁽³⁴⁾ isolated aurantiamide acetate from the seeds of Piper sylvaticum. Sesamin was also isolated from the seeds.

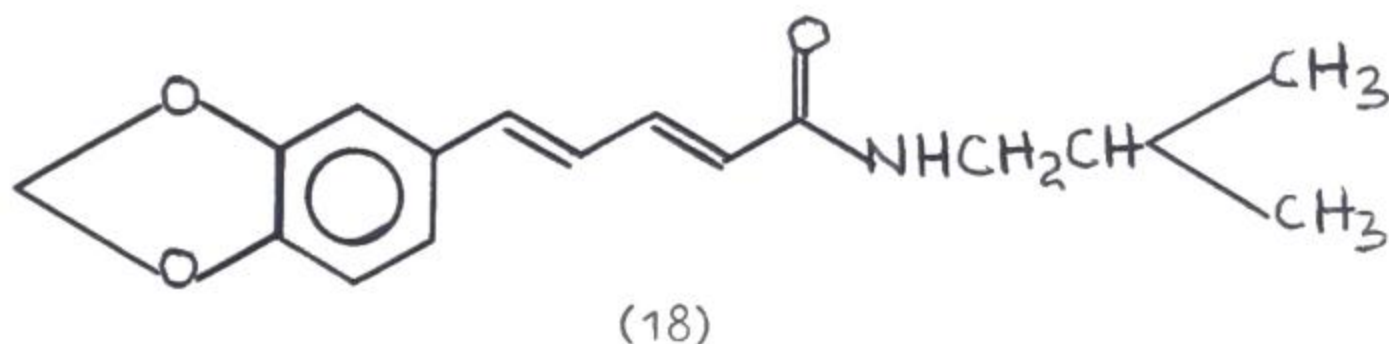
Piperine

Dasgupta and Ray⁽³⁵⁾ reported the isolation of piperine from the roots of Piper attennatum.

Shin et al⁽³⁶⁾ isolated, from the methanol extract of the seeds of Piper refractum, piperine which was found to exert a potent anticonvulsant effect in mice.

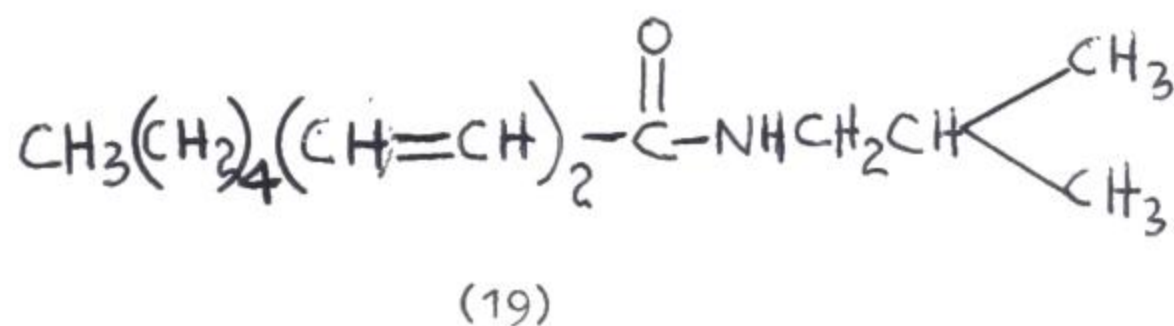
Piperlonguminine

Dasgupta et al also isolated and characterised piperlonguminine (18) from the roots of Piper attennatum



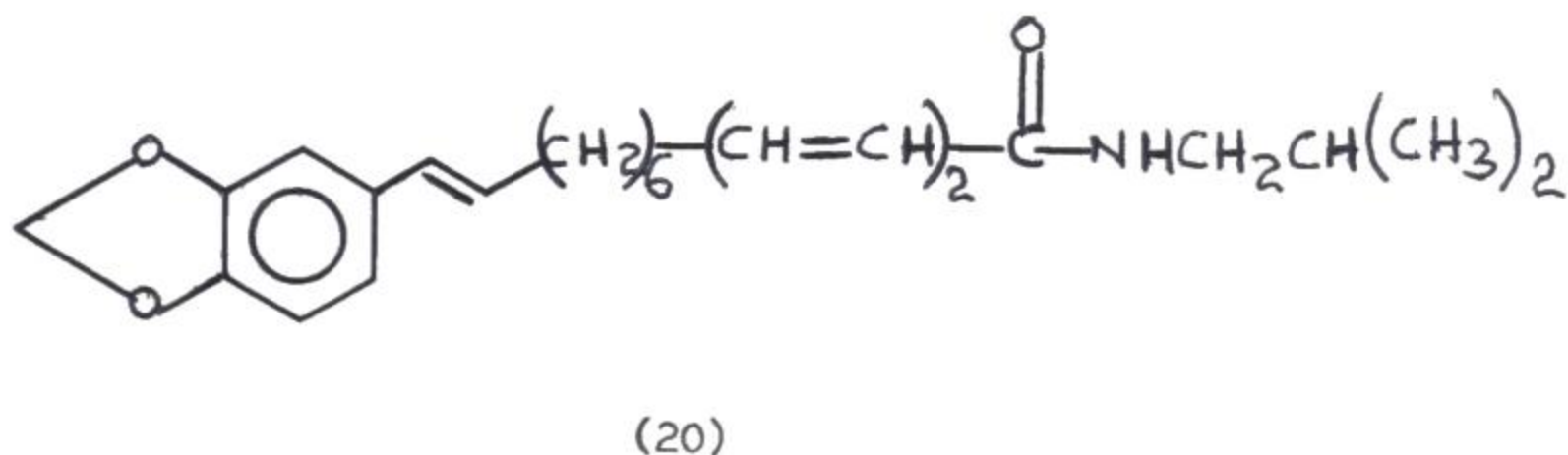
N-isobutyl-deca-trans-2 trans -4-dienamide

The above alkaloid was also isolated from the roots of Piper attennatum by Dasgupta and Ray⁽³⁵⁾.



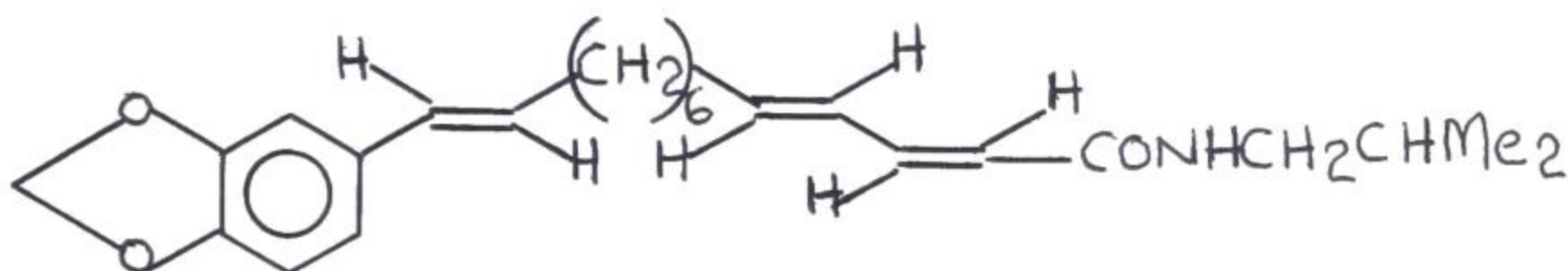
Guineensine

Guineensine (20) which was also reported to be present in the roots of Piper attennatum by the same workers above was found to have physical and spectral properties similar to sylvatine.



An Alkamide

Banerji et al⁽³⁷⁾ used chemical and spectroscopic studies to establish the structure and stereochemistry of an alkamide (21) isolated from Piper sylvaticum

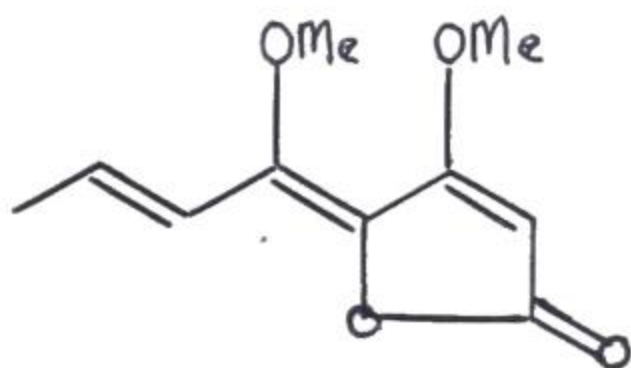


(21)

Non - Alkaloidal Compounds

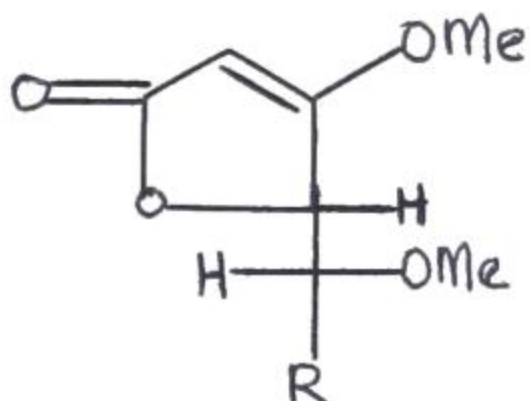
(5 E) - piperolide

Haensel, Schulz et al⁽³⁸⁾ using high performance liquid chromatography detected the presence of a piperolide-like substance from Piper sanctum plant. This substance was subsequently isolated by column-adsorption chromatography on silica gel and identified by spectroscopic techniques as (5E) - piperolide (22)



(22)

(22) was transformed first into a tetrahydro derivative and then into a threo-compound (23) which was correlated with a threo-compound (24), a derivative of known configuration.



They suggested that (22) might be an artifact produced from the Z isomer by the action of light. The two isomers when dissolved in benzene or chloroform and exposed to light produced an equilibrium mixture of 5Z - 5E isomers in the ratio 62:38. Upon dehydrogenation of either, the threo-or erythro- (24) with DDQ*, an equilibrium mixture of (5E) and (5Z) - piperolides was obtained.

*DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone.

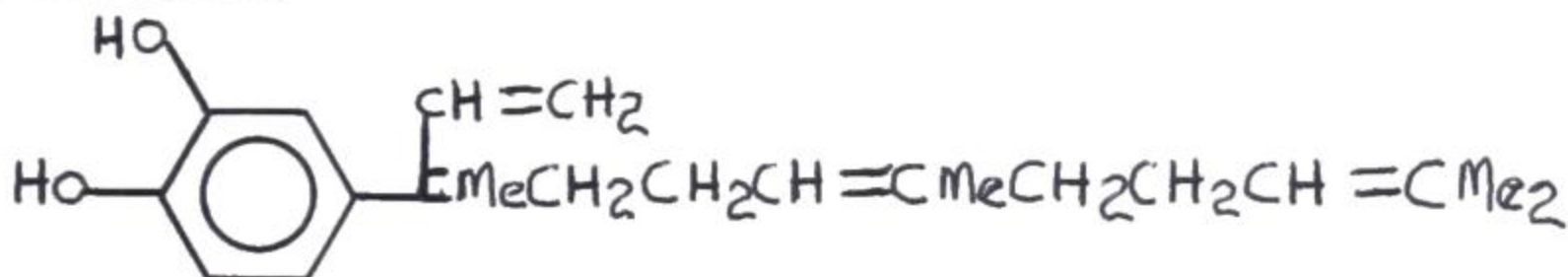
4-nerolidylcatechol

Kijjou et al⁽³⁹⁾ reported for the first time the isolation and characterisation of the compound 4-nerolidylcatechol (25) from the roots of

Piper unbellatum

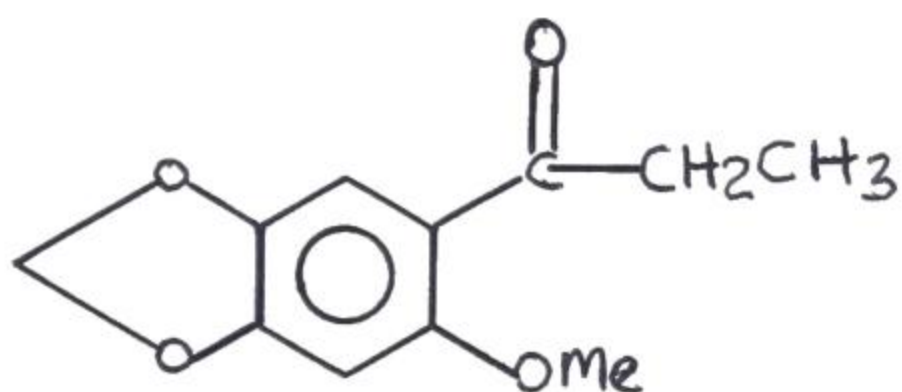
Specific rotation studies of the compound gave the following results,

$[\alpha]_D^{25} + 1^\circ$ (CH_2Cl_2). The same workers also isolated sitosterol from the roots.



Propiophenone

De Diaz et al⁽⁴⁰⁾ reported the isolation from the leaves of Piper marginatum some propiophenones. These compounds were isolated from the ethanol extract and characterised as 3,4-methylenedioxypropiophenone, 2-hydroxy-4,5-methylenedioxypropiophenone and 2-methoxy-4,5-methylene dioxypipropiophenone (26). The compounds were identified by spectral comparison with published data.



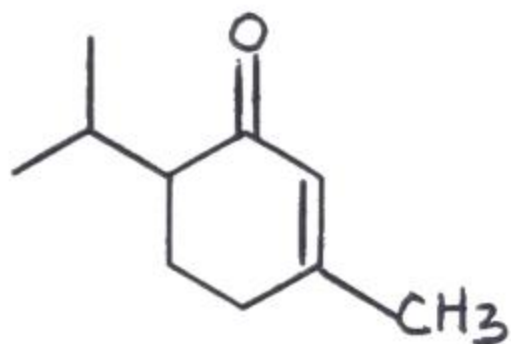
(26)

From the comparisons, (26) was found to be a novel natural compound. The structure was confirmed by synthesis through methylation of the 2-hydroxy derivative with dimethylsulphate. The leaves were also found to contain some terpenes, safrole, piperonal and stearic acid.

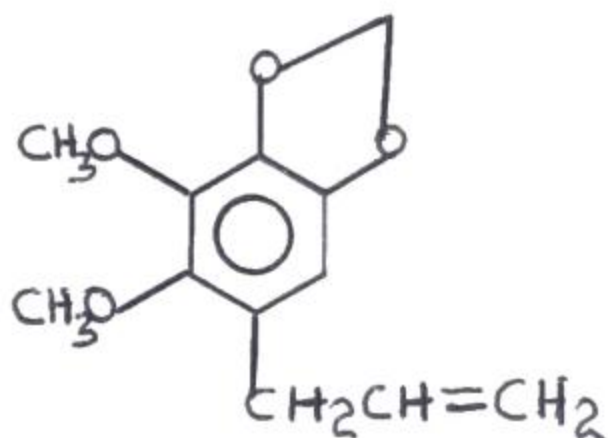
Essential Oil

Smith and Kassim⁽⁴¹⁾ reported the isolation of two essential oils, Dillapiole (27) and piperitone (28) from the leaf oil of Piper aduncum. The compounds were isolated by column and thin-layer chromatography. Dillapiole was obtained in 58% yield and piperitone 4%. The dried leaves were stored for sometime and the constituents investigated again.

They observed that some minor constituents which were present during the first investigation were absent. Secondly, the extract gave an oil which contained dillapoile in a high percentage, $> 90\%$.



Piperitone



Dillapiole

CHAPTER THREE

PREVIOUS WORK ON PIPER NIGRUM

In the introduction, Piperine, Piperetine, Piperoleine A and B were mentioned as the only alkaloids which had been isolated from the fruits of Piper nigrum and reported between 1908 - 1976. The other compounds known were terpenes and some hydrocarbons.

In this chapter, a summary of all the work done on the fruits and other parts of Piper nigrum after this period to 1980 is given. Like the approach in the previous chapter the method of isolation, characterisation and pharmacology of the constituents where available, are discussed.

FRUITS

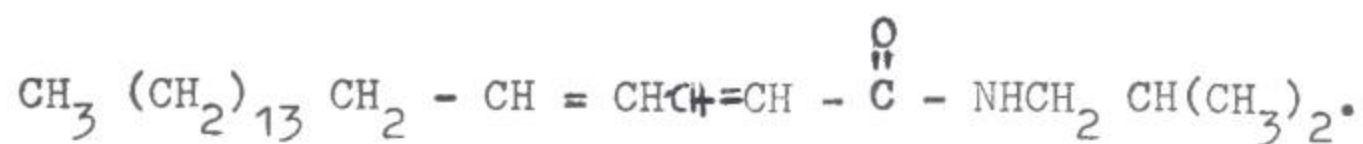
a) Isobutyleicosadienamide

Raina et al reported for the first time the isolation of N - isobutyleicosa - trans - 2 - trans - 4 - dienamide from the fruits of Piper nigrum⁽⁴²⁾.

From the petroleum-ether extract of the fruits they isolated a colourless crystalline compound $C_{24}H_{45}NO$ m.p. 85 - 86°C. The UV spectrum which showed a strong absorption, λ_{max} (MeOH) 260 nm, suggested the presence of a conjugated system which was found to be related to a known compound, sorbamide. The IR spectrum confirmed the **conjugation** involving an α , β , γ and δ conjugated carbonyl system at 1625 cm^{-1} . Absorptions at 3295 , 3075 cm^{-1} were assigned to an-NH group. The other peaks at 1655 and 1000 cm^{-1} were assigned to a trans-trans conjugated diene system.



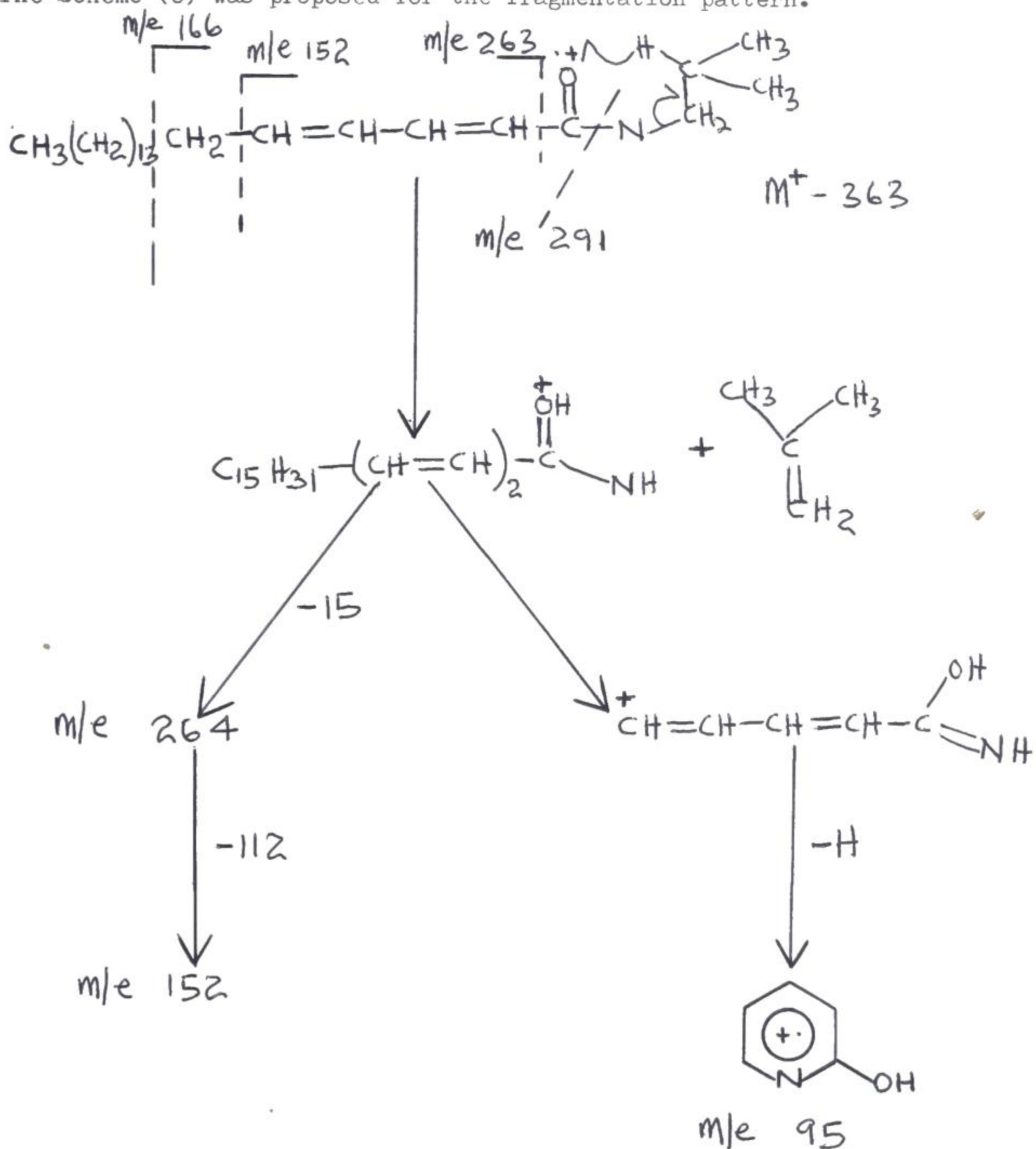
The PMR spectrum in deuterated chloroform indicated signals for the following features; a doublet at δ 0.95 ($J = 6$ Hz; 9H); a broad singlet at δ 1.25 (26H). The region between 1.8 - 2.25 contained a broad multiplet which was assigned to (3H). There was also a triplet at 3.19(2H, N-CH₂-C) which changed to a doublet on addition of D₂O. Other signals were a broad multiplet at 5.65(1H; -NH) and a doublet at 5.81(1H, $J = 15$ Hz) which was due to an olefinic proton adjacent to a carbonyl group. The multiplets between 6.0 - 6.4 (2H) were assigned to (γ and δ) olefinic protons. The structure (29) was proposed for the compound.



(29)

The mass spectrum of (29) showed a molecular ion at $M^+ - 363$ with other peaks at m/e 348, 291, 297, 264, 180, 166, 152, 96 and 95.

The Scheme (6) was proposed for the fragmentation pattern.



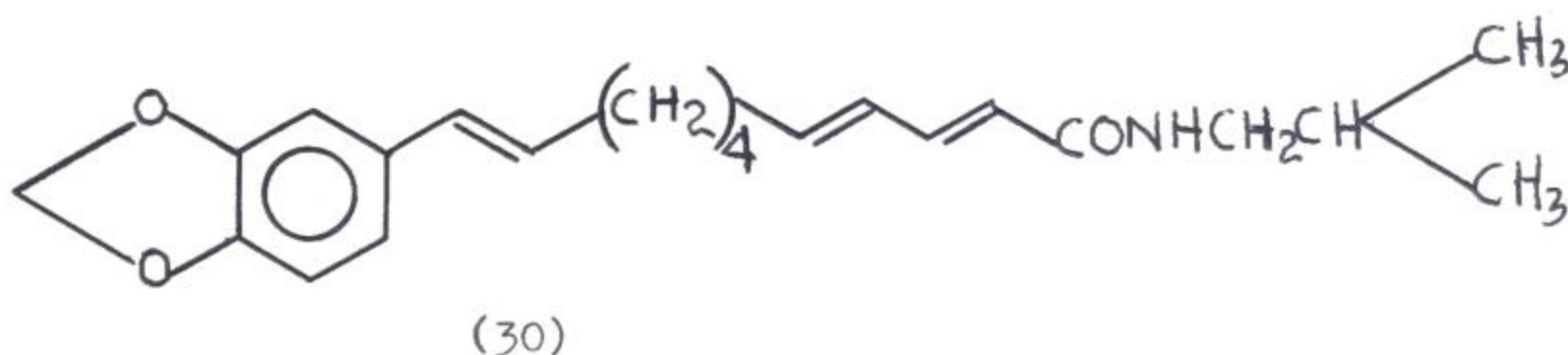
Scheme (6)

The compound on hydrogenation over Pd/C (10%) rapidly absorbed 2 moles of hydrogen to give a tetrahydro compound mp $72 - 74^{\circ}\text{C}$. The mass spectrum of the tetrahydro derivative indicated a molecular ion at $M^{+} - 367$. The hydrogenated product on acid and alkaline hydrolysis yielded (i) isobutylamine hydrochloride mp. $171 - 172^{\circ}\text{C}$, which was identified by mmp with an authentic sample, (ii) eicosanic acid, identified as its amide, mp $106 - 7^{\circ}$ (reported: $107 - 8^{\circ}$) and as a methyl ester, mp $44 - 6^{\circ}$ (reported $46 - 7^{\circ}$)^(42b), and by GLC. On the basis of these determinations, the compound was thus identified as N-isobutyleicosa-trans - 2 - trans - 4-dienamide.

Pipercide

Masakazu et al⁽⁴³⁾ isolated from the dichloromethane extract of the peeled black fruit powder an amide alkaloid which was identified as Pipercide (30). The peeled black fruits were crushed and the resulting powder (500 g) was extracted overnight with dichloromethane at room temperature.

The oil concentrate was subjected to silica gel chromatography with eluent, n - hexane-acetone. 3.50 mg of a compound was obtained which was identified by IR and NMR spectroscopy as Pipercide (30). This was a new amide alkaloid which was named as N-isobutyl-11- (3,4-methylenedioxyphenyl) - 2E; 4E, 10E - undecatrienoic amide. The structure below (30) was proposed for it.



The compound was found to show insecticidal activity against the adzuki bean weevil (Callosobrachus chinensis).

The same workers isolated from the fruits a mixture which was found to consist of pipericide, dihydropipericide and guineensine⁽⁴⁴⁾. A bioassay of this mixture was found to be toxic to the male adult adzuki bean weevil (Callosobrachus chinensis), whereas pure specimens of each component compound were found to be less toxic. They also observed that a simulated synthetic mixture of the three compounds in the ratio (75 : 5 : 2 wt/wt) was equally toxic as the crude natural mixture but the components in a 1 : 1 : 1 ratio were found to exhibit the highest synergistic action.

STEMS

Jagdev, Vasudevan et al⁽⁴⁵⁾ investigated the chemical constituents of the stems of Piper nigrum and isolated for the first time the following compounds from the petroleum-ether extract:

- a) Piperine
- b) 16 - hentriacontanone
- c) hentriacontane
- d) 16 - hentriacontanol
- e) β - sitosterol

Piperine

From the petroleum-ether extract of the air-dried coarsely powdered stems a greenish yellow residue deposited which was crystallized from petroleum ether - ethylacetate as yellow needles m.p. $128 - 129^{\circ}\text{C}$. The compound was identified as piperine by tlc mmp, UV, IR and NMR and mass spectral analysis with an authentic sample of piperine. It formed a bromoderivative, mp $115 - 116^{\circ}\text{C}$ and a waxy tetrahydro derivative on hydrogenation over Pd/C (5%). The derivatives were identified by UV, IR and NMR spectral analysis. When piperine was hydrolysed with alcoholic potassium hydroxide, piperic acid and piperidine were obtained and identified by mmp and Co-tlc with authentic samples of piperic acid and piperidine hydrochloride respectively.

16-hentriacontanone

After the removal of piperine, the extract deposited a whitish substance which was removed. Crystallization from alcohol gave a compound which was identified as 16-hentriacontanone by Co - t l c, mixed tlc, mmp and IR analysis with an authentic sample.

hentriacontane

The mother oil concentrate deposited a white compound from acetone, mp $67 - 69^{\circ}\text{C}$. They identified it as hentriacontane by mmp, Co - t l c, IR and NMR spectral analysis with an authentic sample.

16-hentriacontanol

They chromatographed the mother liquor over neutral alumina and eluted with petroleum ether-benzene (70 : 30) which afforded a white compound mp. 82 - 84°C. It was identified as 16-hentriacontanol by mmp, Co - t l c, mixed t l c, IR and comparison with the acetate derivative of an authentic sample of 16-hentriacontanol.

β -sitosterol

Elution with benzene-ethyl acetate (90 : 10) gave a white solid which was also identified as β -sitosterol by mmp, Co - tlc, IR, NMR and mass spectral analysis with an authentic sample.

CHAPTER FOUR

THE PRESENT INVESTIGATION



In the present investigation, two main alkaloids and steroids were isolated, purified and identified. The alkaloids were:

1. Piperine - isolated from both the fruits and roots of Piper nigrum and the Peperomia pellucida plant.
2. A compound with the characteristics of Piperettine - isolated from only the fruits of Piper nigrum. The steroids were:
3. Stigmasta - 4,22-dien-3-ol-isolated from the roots of Piper nigrum and Peperomia pellucida.
4. β -sitosterol - isolated from the fruits, roots and Peperomia pellucida.

Other amide alkaloids which were isolated from the fruits, roots and Peperomia pellucida as minor constituents could not be characterised fully. Similarly, compounds suspected to be lactones or aliphatic unsaturated esters were isolated from both plants.

The Peperomia plant also yielded a high molecular weight hydrocarbon and a long chain aliphatic ether which have not been fully characterised.

Upon comparing the amounts of alkaloids isolated from the petroleum ether extracts of the fruits and roots of Piper nigrum, it was observed that the fruits yielded piperine in an excess of 1.20% than the roots. Secondly, the petroleum ether extract of the fruits yielded 0.012% of Piperettine whereas the other amide alkaloids isolated from the same extract of the roots was just about $2 \times 10^{-4}\%$.

This suggested that the fruits were better synthesisers of petroleum ether soluble alkaloids since most of the fruit material used came from the same plant as the roots. It has been shown that in Piper guineense, the nature and distribution of alkaloids in the plant is dependent on seasonal variation and the geographical location of the plant.⁽⁴⁶⁾ Since the geographical location was the same for the fruits and roots, the results thus suggest that at the time of collection alkaloids were more abundant in the fruits than the roots. The roots were not extracted with chloroform as was done on the fruits, therefore a complete generalization could not be made on the alkaloid distribution. On the other hand, the petroleum ether extract of the roots yielded as major constituents, steroids which were characterised as β -sitosterol and stigmasta - 4, 22 - dien - 3 - ol.

These steroids were not obtained from the petroleum ether extract of the fruits but the chloroform extract yielded β - sitosterol. This observation also revealed that at the time of collection the roots were better synthesisers of these steroids than the fruits.

The results obtained for Peperomia pellucida indicated that it was better at synthesising steroids, hydrocarbons and lactone-like compounds than alkaloids.

The breakdown was steroids .015% , lactones .014% , hydrocarbons .022% and alkaloids $6.06 \times 10^{-4}\%$.

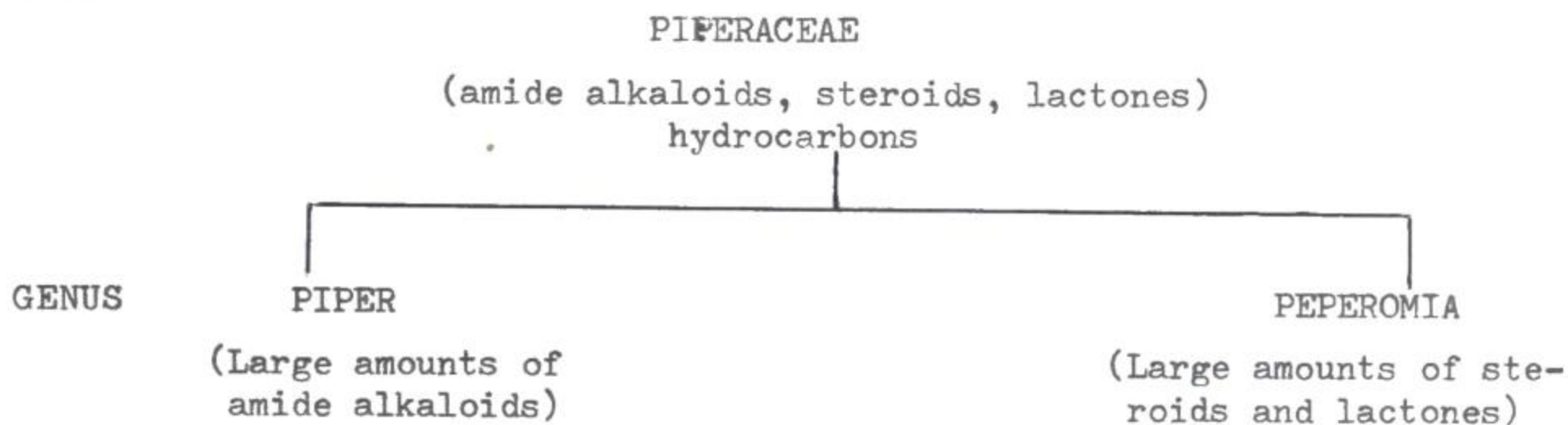
The investigations also showed that the constituents of Peperomia pellucida were also dependent on the geographical location of the plant.

A second collection of the plant material at a distance of about 400 m away from the first collection, but within the same time of collection, October - June, showed the complete absence of the two amide alkaloids isolated as minor constituents in the first investigation. The compound suspected to be a long chain aliphatic ether isolated from the first collection was also absent in the second collection.

These observations are not only significant from the distribution and geographical point of view, but also chemotaxonomically. Chemotaxonomically, because in the classificatory system of the family Piperaceae, as proposed purely on morphological grounds by Hutchinson et al⁽¹⁾, there are two main genera. The genera Piper (Linn) and Peperomia (Ruiz & Pav). Each genus contains several species which are widely distributed in the tropics. In the genus Peperomia, thirteen species are known to be distributed in West Africa, three of which have been identified in Ghana. They are P. pellucida, P. stahlmanii and P. rotundifolia. In the genus Piper, three wild growing species have been identified in West Africa. The genus Piper has been widely investigated and results reported have indicated that the various parts of the plant contain large amounts of amide alkaloids. The present investigation on the fruits and roots of Piper nigrum gave results which fairly agree with this observation. Thus these species are united as members of the same genus though the distribution of alkaloids varies from part to part. Piper species have also been shown to contain as minor constituents lactones, steroids and hydrocarbons (14, 38, 45, 47, 48) and the results so far obtained from Piper nigrum also agree with this observation.

On the other hand, the genus Peperomia has not been investigated ~~at~~ all. The results so far obtained upon investigating only one species, Peperomia pellucida, suggest that they may be capable of synthesising amide alkaloids containing the piperidine nucleus as has been shown to occur in Piper species. Generally, the isolation of two amide alkaloids from this species unite the two genera into the family Piperaceae as proposed by Hutchinson et al. This observation is also supported by the isolation from the two species of similar steroids and lactone-like compounds having the δ -ring size. As species belonging to different genera, the results lend support to Hutchinson classificatory system. It was observed that Piper nigrum excelled better in synthesising amide alkaloids whereas Peperomia pellucida showed an abundance of steroids, lactones and hydrocarbons. This generalization is not conclusive since the present investigation marks the beginning of future chemotaxonomic studies in the Piperaceae family. Scheme (7) below shows the chemical characters as applied to the classification.

FAMILY:



Scheme (7)

The results also supported the removal of Peperomia pellucida from the genus Piper as initially reported by Linnaeus who later on corrected his own classification. Reports on the nature of amide alkaloids present in the genus Piper indicate that the species are distinct from each other. This is exemplified by the fact that Piperettine is characteristic of Piper nigrum; wisanine and its cis isomer are characteristic of Piper guineense and Pipermethystine is also characteristic of Piper methysticum.

Isolation and Characterisation of Piperine

From the petroleum ether extracts of the fruits and roots of Piper nigrum and that of the whole plant of Peperomia pellucida was isolated piperine as yellow needles mp. 132 - 134°C (lit. 128 - 129°C)⁽⁴⁹⁾.

Inspection of the PMR, IR and UV spectra revealed that the compound was a substituted benzene derivative and that the substituents consisted of a long chain unsaturated amide group and a methylenedioxy group.

Thus the IR spectrum showed strong absorption at 1650 and 1625 cm^{-1} for an $\alpha\beta$ -unsaturated amide group and a conjugated double bond system at 1600 cm^{-1} . The extended conjugation in the molecule was confirmed by the UV absorption at a wavelength of 345 nm (ϵ -21000).

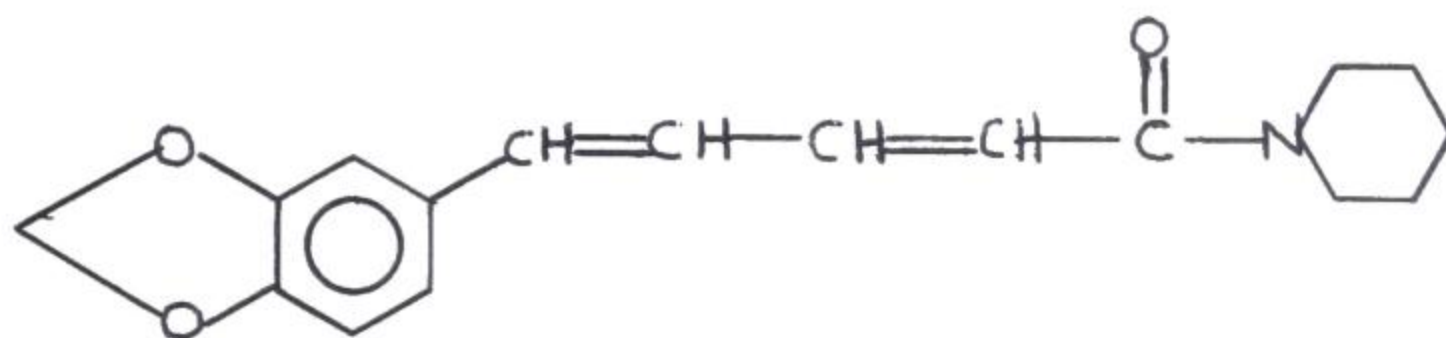
The PMR spectrum showed signals at δ 6.40 and 7.37 for two olefinic protons. The former signal, a doublet ($J = 14$ Hz), indicated a trans - $\text{CH} = \text{CH}-\text{CO}$ system which was further confirmed by the IR absorption at 925 cm^{-1} . This signal collapsed to a singlet when the olefinic proton at δ 7.37 was simultaneously irradiated.

A pair of split doublets at δ 7.37 ($J_{2,3} = 10.8$ Hz, $J_{2,4} = 2.9$ Hz) for the other : olefinic proton provided evidence of coupling ($J = 2.9$ Hz) between protons on C_2 and C_4 in the system, $\text{-CH} = \text{CH} - \text{CH} = \text{CH} - \text{CO}.$
 $\quad\quad\quad 4 \quad\quad 3 \quad\quad 2 \quad\quad 1$

A doublet at δ 6.82 ($J = 10.8$ Hz) was consistent with an ortho relationship of two aromatic protons, and a split doublet with further splitting at δ 6.91 ($J = 10.8$ Hz, 1.7 Hz) also indicated an ortho and meta relationship of aromatic protons.

The IR spectrum showed a $C = C$ stretching vibration for an aryl system at 1510 cm^{-1} and a PMR singlet at δ 5.9 was indicative of methylene protons of a methylenedioxyaryl group.

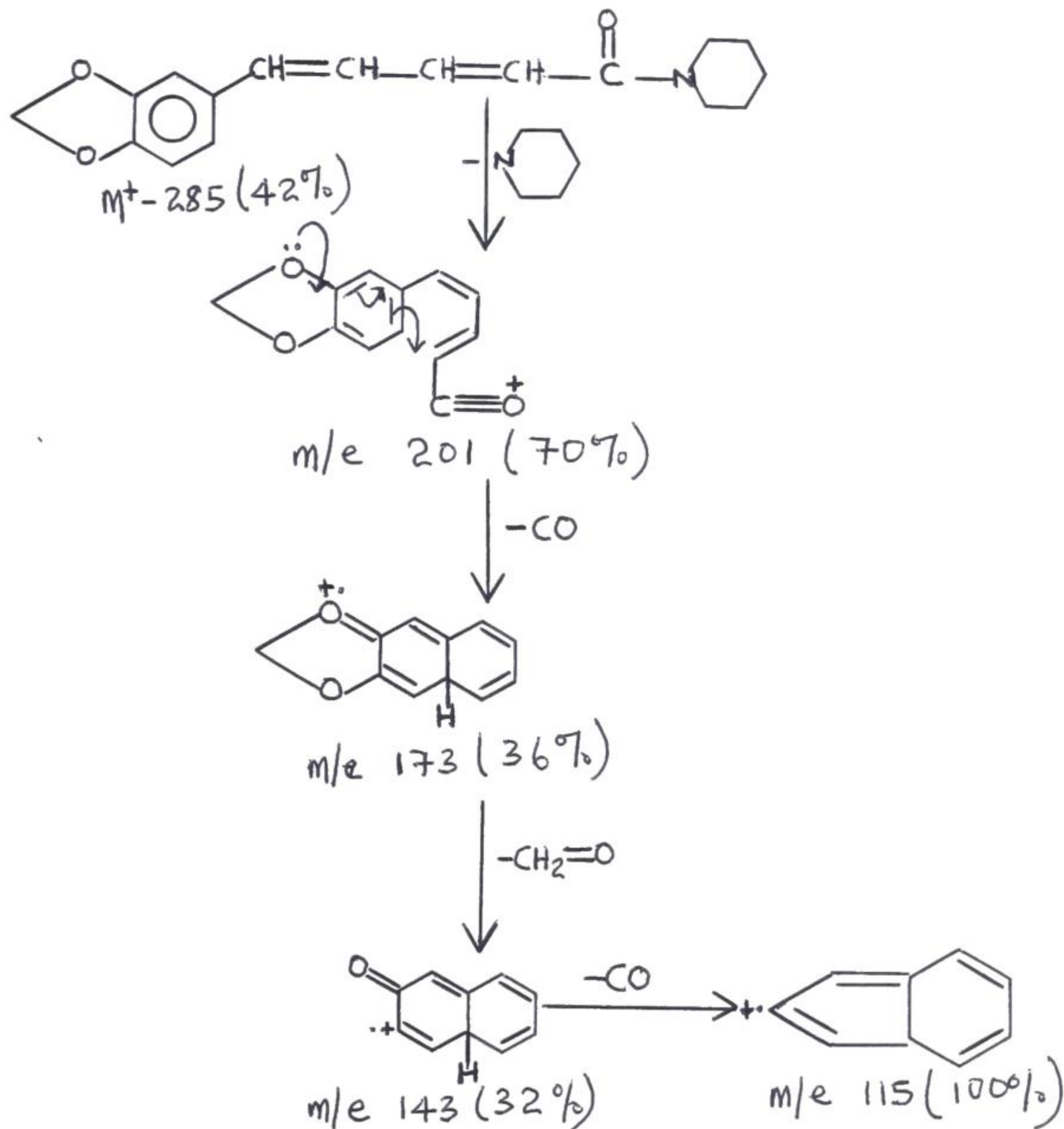
The lack of a signal at $3300 - 3550 \text{ cm}^{-1}$ indicated that the substituent consisted of a tertiary amide. The PMR signals of the tertiary amide were all upfield, at δ 3.5 and δ 1.5, relative to the unsaturated system. The δ 3.5 signal, a broad singlet was consistent with a $\text{CH}_2 - \text{N} - \text{CH}_2$ system and the δ 1.5 signal, a broad multiplet to $\text{CH}_2 - \text{CH}_2 - \text{CH}_2$. The spectra agreed with the structure (31) which is identical to piperine.



(31)

Mass spectral analysis established the molecular formula as $\text{C}_{17}\text{H}_{19}\text{O}_3\text{N}$. This formula requires ~~nine~~ sites of unsaturation. The spectrum showed an intense molecular ion peak at m/e 285 and a base peak at m/e 115.

Another intense peak at m/e 84 is a characteristic feature of compounds with the piperidine ring. Scheme (8) gives the fragmentation of the alkaloid.



Scheme (8)

In Peperomia pellucida, piperine was obtained from column chromatography of the extract and identified by mmp, mixed tlc Co - tlc, IR and UV spectra with an authentic sample.

Hydrolysis of piperine with 10% aqueous KOH gave a yellow solid, piperic acid, m.p. 218 - 220°C (lit. mp. 216 - 217)⁽⁴⁹⁾.

The IR (KBr) spectrum contained peaks at 3200 - 2800 cm^{-1} , which were indicative of a carboxyl - OH; 1715, 1700, 1625 cm^{-1} were suggestive of a conjugated carbonyl of a carboxyl group.

The peaks at 975, 940 cm^{-1} suggested the presence of a trans configuration of the olefinic group.

The UV spectrum showed absorption peaks at λ_{max} [EtOH] 330 nm and a shoulder at 310 nm. Piperidine, also obtained from the hydrolysis, was identified by its spectrum which was superimposable on that of an authentic sample.

ISOLATION AND CHARACTERISATION OF PIPERETTINE

The yellow solid separated mechanically from the solid mixture was purified by preparative tlc. The major fraction in diethyl ether deposited an alkaloid as yellow needles believed to be piperettine, mmp. 152-154°C (lit. mps., 146 - 149°C)⁽⁵⁰⁾.

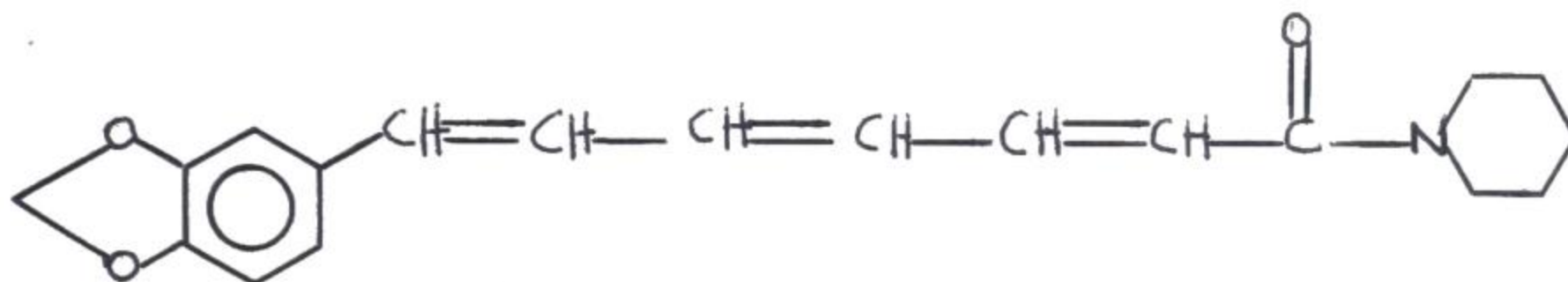
The mass spectrum gave the molecular formula of L 2A as $\text{C}_{19}\text{H}_{21}\text{NO}_3$. The formula indicates ten sites of unsaturation. The IR spectrum showed absorption at 1645, 1625 cm^{-1} for an $\alpha\beta$ -unsaturated amide system.

An absorption at 1600 cm^{-1} indicated the presence of a conjugated double bond system and a C = C stretching vibration for an aryl system occurred at 1580, 1510 and 1505 cm^{-1} .

The UV absorption at $\lambda_{\text{max}} [\text{EtOH}]$ 363, 207 nm (ϵ -40200, 18200) suggested the presence of an extended conjugated system.

The PMR spectrum showed signals for olefinic protons confirming the unsaturation in the compound. A doublet at δ 6.38, $J = 14\text{ Hz}$ was indicative of a trans - CH = CH - CO system and a quartet at δ 7.35 suggested a - CH - CH = CHCO system. The set of signals at δ 6.83, 6.9 and 6.99 indicated the resonance of aromatic protons. The methylene resonance signal of the methylenedioxyaryl group appeared as a singlet at δ 5.9.

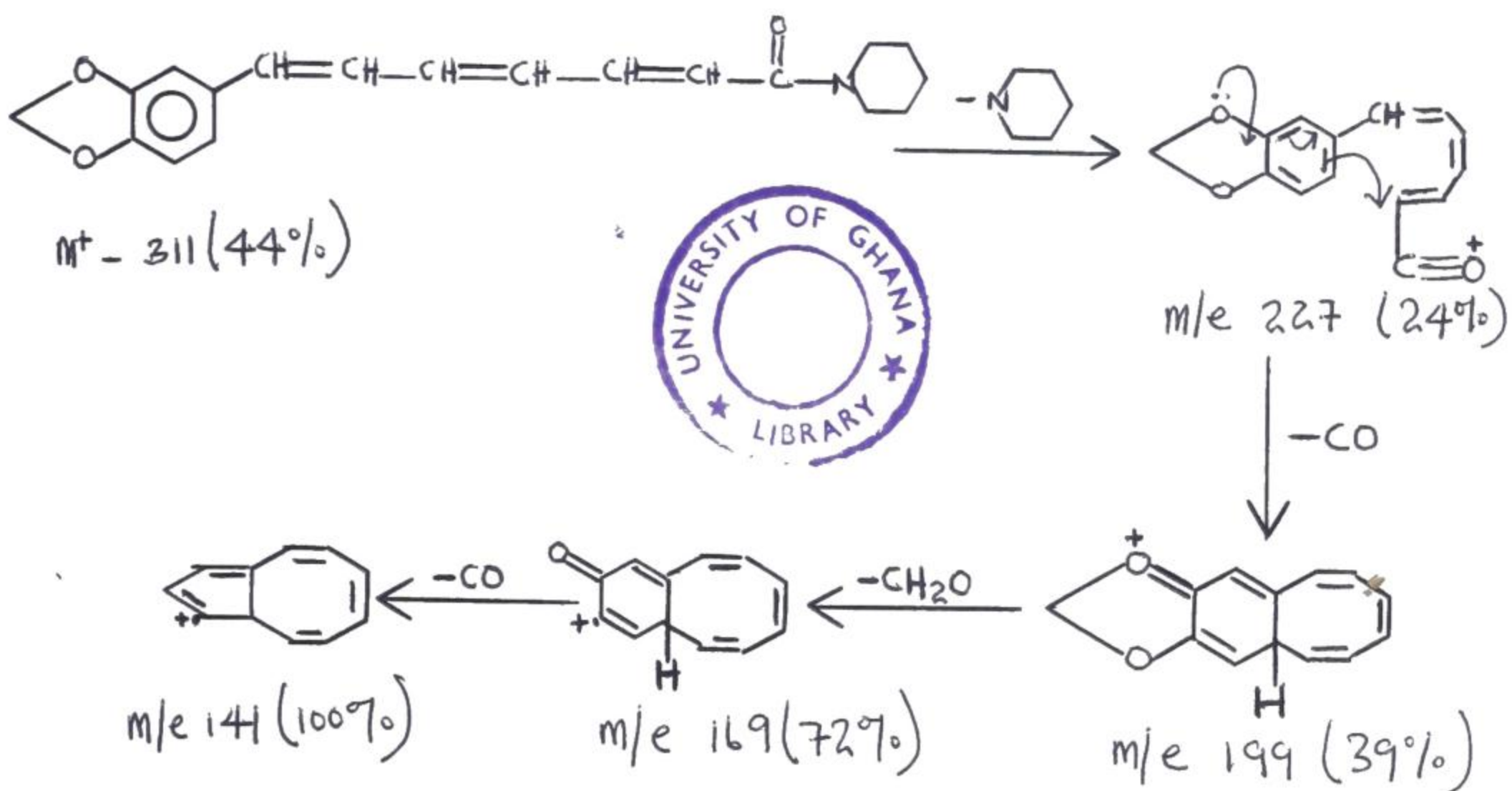
The lack of a signal at $3300 - 3550\text{ cm}^{-1}$ indicated the presence of a tertiary amide. The substitution of the amide group was identified upfield, in the PMR spectrum. A broad doublet at δ 3.5, $J = 25\text{ Hz}$ was assigned to $\text{CH}_2 - \text{N} - \text{CH}_2$ ⁽⁵¹⁾ and a broad multiplet centred at δ 1.5 assigned to $\text{CH}_2 - \text{CH}_2 - \text{CH}_2$. These findings were consistent with the structure (32) which is similar to piperettine.



(32)

The mass spectrum showed a molecular ion at M^+ 311.1526 (44%) (calc. 311.15214 for $\text{C}_{19}\text{H}_{21}\text{NO}_3$), and a base peak at m/e 141.

Scheme (9) below is in agreement with the fragmentation pattern.



Scheme (9)

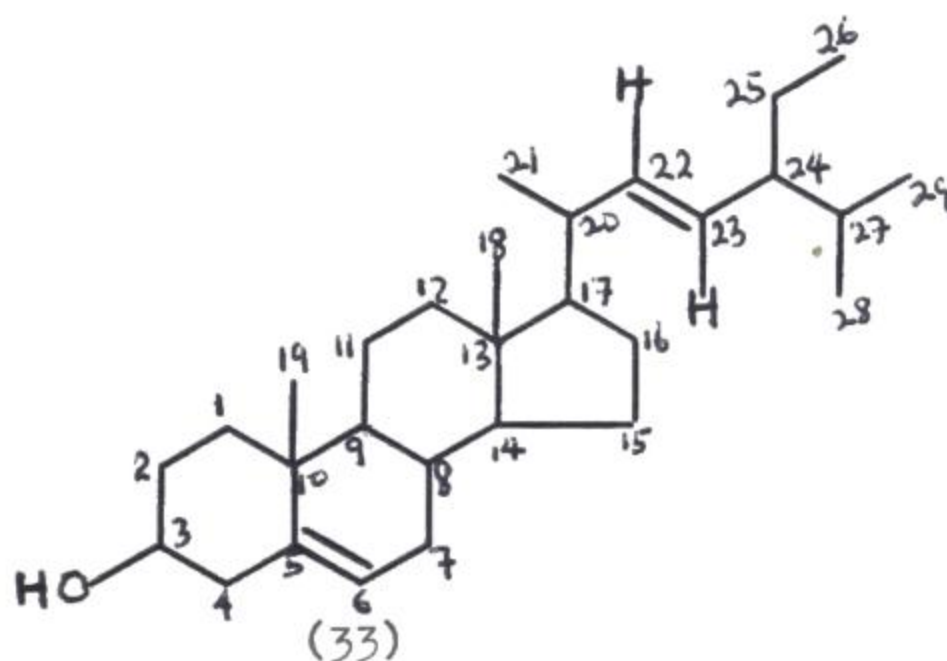
Hydrolysis of piperettine with 10% aqueous alcoholic KOH afforded yellow needles, piperetic acid, m.p. $226 - 228^\circ\text{C}$ (lit. mp., $223-224^\circ\text{C}$)⁽⁵⁰⁾. The IR (KBr) spectrum showed principal peaks at $3100 - 2900$ which suggested the presence of a carboxyl - OH, 1720 , 1638 for an $\alpha\beta$ - unsaturated acid and 1005 , 970 for a trans configuration of a double bond conjugated to a carbonyl group. The UV spectrum showed a maximum at $\lambda_{\text{max}} [\text{EtOH}] 353 \text{ nm}$ (lit. 360 nm)⁽⁴⁷⁾ which was similar to piperic acid $\lambda_{\text{max}} 335 \text{ nm}$. The difference of 18 nm between the two acids suggested that piperetic acid was a vinylogue of piperic acid.

ISOLATION AND CHARACTERISATION OF STIGMASTA - 4, 22-DIEN-3-OL
AND ITS DIHYDRODERIVATIVE

Fractionation of the petroleum ether extract of Peperomia pellucida by column chromatography on alumina gave nine fractions. Fraction seven, labelled D7, deposited white plates from acetone, identified as the steroid, stigmastera - 4,22 - dien - 3 - ol. The same steroid was also isolated from the roots of Piper nigrum after fractionating the petroleum-ether extract on an alumina column.

The determination of the structure of D7 was greatly aided by comparative studies involving the use of library research-computer mass spectral analysis of various sterols and D7. Results obtained from such studies showed that D7 belonged to the class of stigmasterols.

Stigmasterol (33) has a molecular mass of 412 but mass spectral analysis of D7 at different times of evaporation revealed that the compound was composed of a mixture made up of constituents having molecular masses 412 and 414.

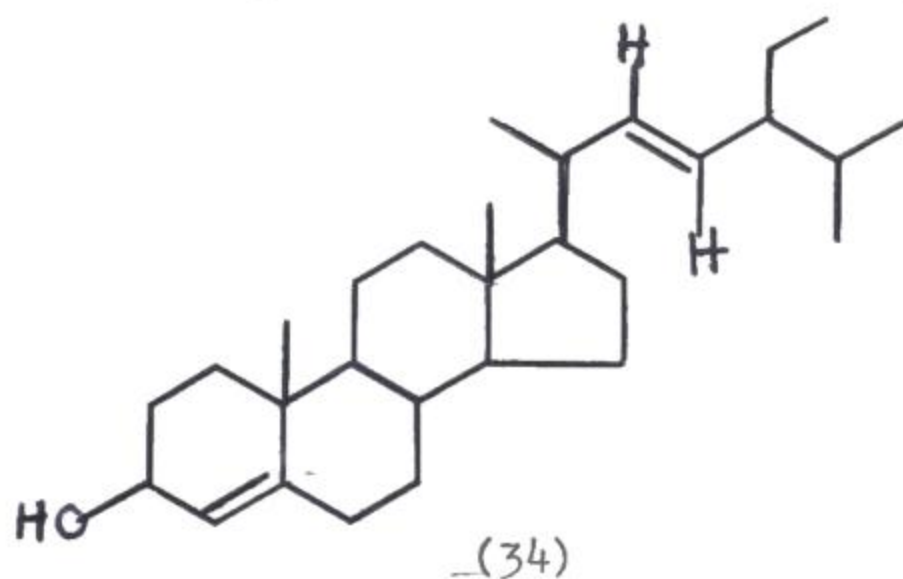


The IR spectrum of D7 showed strong-OH absorption at 3200 - 3600. The PMR spectrum indicated a singlet for the hydroxyl proton at δ 0.7 and the C - 3 proton a broad multiplet at δ 3.5.

The UV absorption at 207 nm (ϵ - 5670) indicated an unconjugated system which was confirmed by the lack of IR absorption at 1600 cm^{-1} . The PMR spectrum did not also show signals indicating that there was a conjugated double bond system. The IR spectrum showed a weak absorption at 985 and 995 cm^{-1} which suggested a trans - $\text{CH} = \text{CH}$ system. This was supported by the PMR spectrum which indicated an ABX quartet for each of the C-22 and 23 protons, centred at δ 5.0 and 5.13, $J_{AB} = 16\text{ Hz}$, $J_{AX} = 8.9\text{ Hz}$. H_X is either on C_{20} or C_{24} .

The most striking feature of the PMR spectrum of D7 was the presence of a vinylic proton, which resonated at a lower field at δ 5.35, appearing as a doublet, $J = 7.0\text{ Hz}$. This finding was suggestive of a double bond between C - 4 and C - 5. adjacent to the C-OH at C-3.

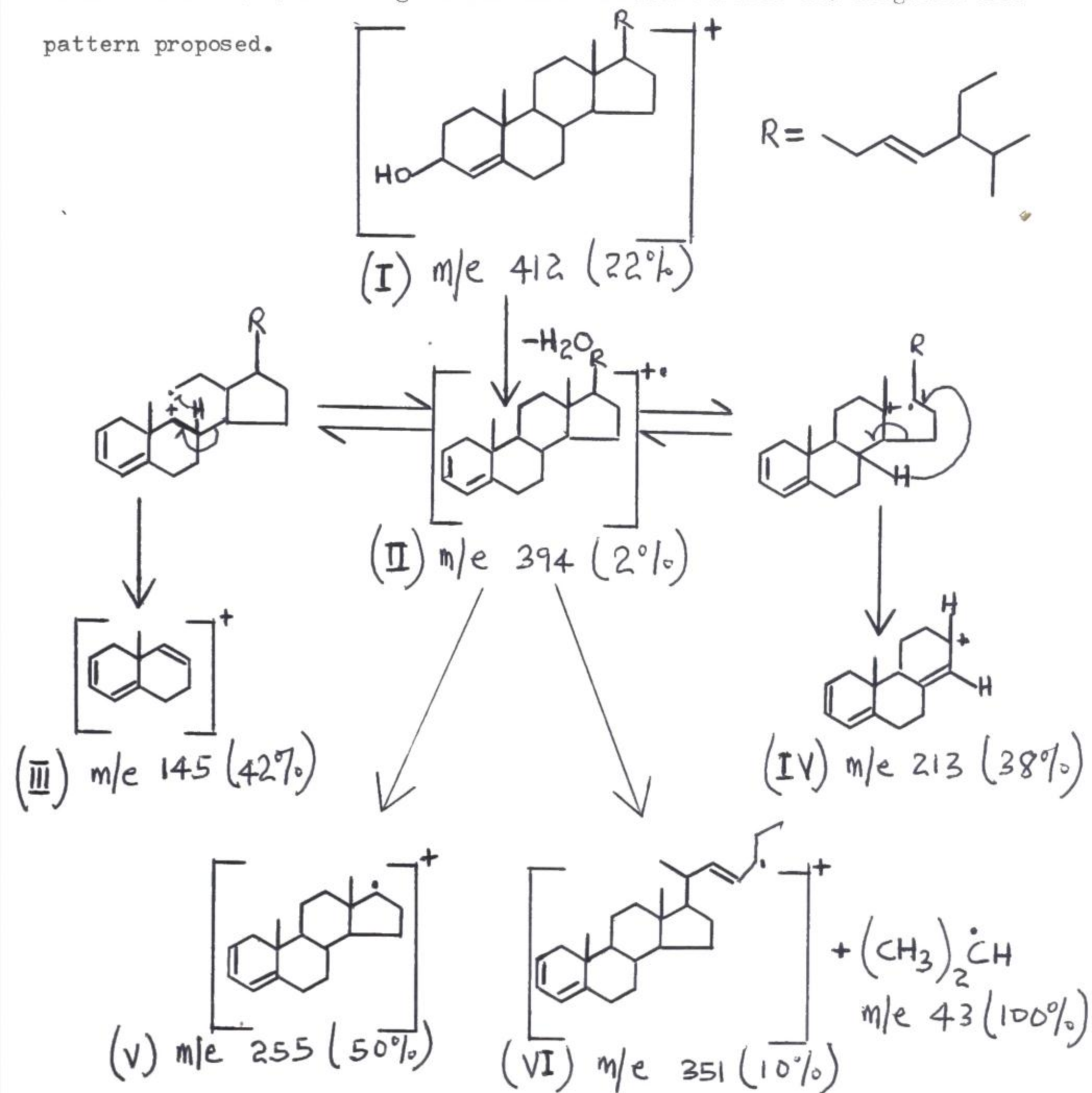
The PMR spectrum agreed with the structure (34).



It thus appears that the 412 mass unit constituent of D7 and stigmasterol are isomers and the 414 mass unit constituent a dihydro derivative of this isomer.

A comparison of the mass spectra of the two constituents gives an indication as to which $\text{C} = \text{C}$ is saturated.

The fragmentation pattern of the two compounds were similar, with prominent peaks at m/e 255, for the ion formed as a result of the loss of the side chain, (II) — (V), m/e 213 (IV) and m/e 145 (III) as in the Scheme. These findings indicated that in the dihydro derivative the Δ^4 double bond was intact and that the saturation involved the C - 22, C - 23 double bond. Scheme (10) is in agreement with and illustrates the fragmentation pattern proposed.



The acetate derivative of stigmasta - 4,22-dien-3-ol was prepared by condensing the compound with acetic anhydride. This afforded white plates mp 136 - 138°C. It gave a UV absorption at $\lambda_{\max}$ [EtOH] 208 nm. Its IR (KBr) spectrum gave peaks at 3000, 2905 (C - H), and a strong absorption at 1745 indicated the presence of a -CO of an ester supported also by a strong C - O - C peak at 1285 cm⁻¹.

The peaks at 990 and 980 confirmed the presence of the trans - CH = CH - system recorded in the original sterol.

ISOLATION AND CHARACTERISATION OF β -SITOSTEROL

β - sitosterol was isolated from the fruits and roots of Piper nigrum as well as Peperomia pellucida plant as white leaflets. It was obtained from the column chromatography of the diethyl ether soluble fraction of the chloroform extract of the fruits. It was obtained from the roots and Peperomia pellucida plant by a similar separating method applied to their petroleum-ether extracts.

Its IR (KBr) spectrum showed absorption at 3700 - 3200 for a hydrogen bonded-OH group and 3005 for C-H stretching vibrations of a =C - H system. The absence of two trans peaks at 995 and 985 or a cis peak between 950 - 965 cm⁻¹ indicated that the peak at 980 corresponded to a saturated system⁽⁵²⁾.

The UV absorption occurred at $\lambda_{\max}$ [EtOH] 207 nm. The spectra were identical in all respects with an authentic sample of β -sitosterol. The mobilities were also the same in the solvent systems employed.(p 79)

DISCUSSION OF PARTIALLY CHARACTERISED COMPOUNDS

The discussion under this section includes compounds which were isolated as minor constituents from the fruits and roots of Piper nigrum and the Peperomia pellucida plant. These compounds are divided into alkaloids and non-alkaloidal compounds.

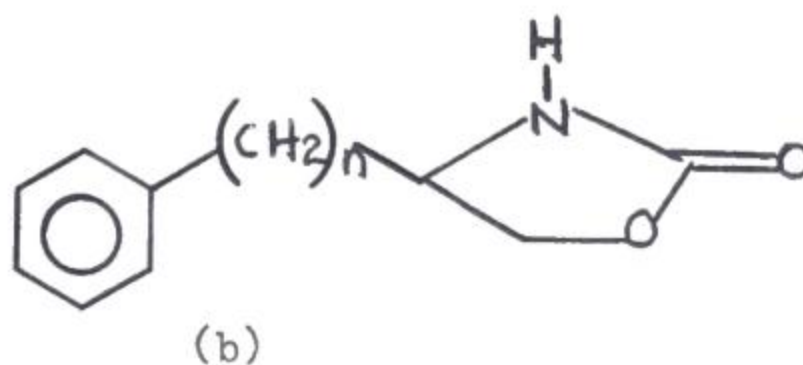
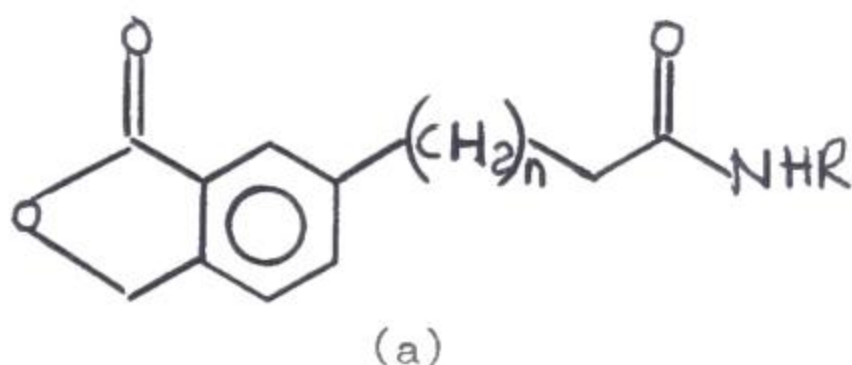
ALKALOIDS

C 11

The fruits of Piper nigrum were first extracted with chloroform then divided into a diethyl ether soluble extract and a chloroform mother liquor. Column chromatography of the diethyl ether extract on alumina afforded a greenish-yellow solid mp. $204 - 206^{\circ}\text{C}$, softened at 196°C . Its IR (KBr) spectrum contained weak peaks at 3460 and 3350 cm^{-1} which suggested the N-H stretching vibrations of a secondary amine. This suggestion was further supported by N-H peaks at 1570 and 1550 cm^{-1} . Weak peaks which appeared at 3100 and 3005 cm^{-1} were assigned to the C-H stretching vibrations of an Ar - H. The presence of an arene was supported by a weak peak at 1518 cm^{-1} and the conjugation in the benzene ring by another weak peak at 1600 cm^{-1} . A medium absorption at 1778 cm^{-1} suggested the presence of a five-ring lactone. The peaks at 1705 and 1700 cm^{-1} were assigned to -C=O group whilst a strong peak at 1680 cm^{-1} was indicative of an amide group of the form -CO-NH-R . There were weak absorptions at 3000 , 2995 and 1460 cm^{-1} for an alkyl group.

The UV spectrum showed an absorption at $\lambda_{\max}$ [EtOH] 208 nm and a shoulder at 232 nm. A minimum absorption was also observed at 345 nm. The UV spectrum indicated that the conjugation in the compound was not extensive.

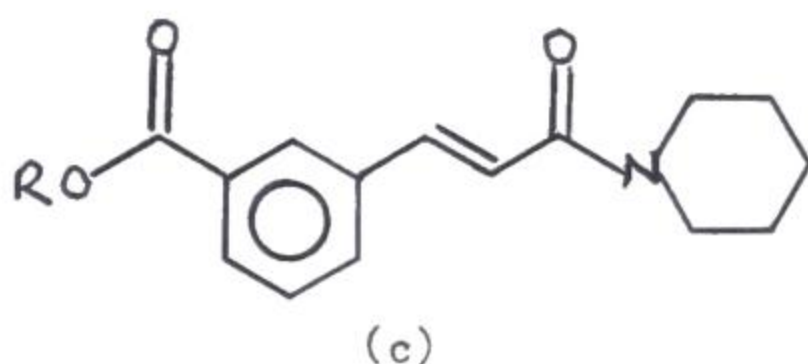
The structural features which best fit the IR and UV spectra of C 11 are



R 9

The compound was isolated from column chromatography of the petroleum ether extract of the roots as a pale yellow solid mp. $64 - 66^\circ\text{C}$. Its IR (KBr), spectrum showed an absorption at 1730 cm^{-1} which was suggestive of an α,β -unsaturated ester. A broad peak at 1300 cm^{-1} confirmed the $C=O$ stretching vibration of the ester.

The peaks at 1658 and 1538 cm^{-1} suggested the presence of a tertiary amide whilst the broad peak at 1620 cm^{-1} was indicative of C=C conjugated to an aromatic ring. The UV spectrum showed an absorption at λ_{max} [EtOH] 330, 207 nm and a minimum at 242 nm which suggested an extended conjugated system similar to piperine λ_{max} [EtOH] 345 nm, 207 nm. From the IR and UV spectra, R9 possibly contains the piperidine nucleus and shows the structural feature below.



D9

The compound was obtained from column chromatography of the petroleum ether extract of Peperomia pellucida as brown flocculent crystals mp. 173 - 176°C (<1 mg). The IR (KBr) spectrum of the crude material showed a peak at 3300 cm^{-1} which was suggestive of an N-H stretching vibration of a secondary amine. This was confirmed by a strong peak at 1640 cm^{-1} . A fairly strong peak at 1665 cm^{-1} was suggestive of an $\alpha\beta$ -unsaturated carbonyl system. Two medium peaks which appeared at 1560 and 1540 cm^{-1} were suggestive of an open chain amide. The UV spectrum was not determined due to paucity of material.

NON-ALKALOIDAL COMPOUNDS

C 5

The compound was obtained from the diethyl ether soluble extract as white needles from ethanol mp. $118 - 120^{\circ}\text{C}$. Its IR (KBr) spectrum showed a sharp peak at 3035 cm^{-1} which indicated the presence of a $\text{C}=\text{C}-\text{H}$ system. A sharp but medium peak at 1630 cm^{-1} suggested the presence of a non-conjugated $\text{C}=\text{C}$ and a strong peak at 1545 cm^{-1} was suggestive of a nitro group. The strong peaks between 1258 and 1200 cm^{-1} suggested the presence of a vinyl ether and those at 1058 and 1070 were suggestive of a $\text{C}-\text{O}-\text{C}$ system. The peaks between 818 and 905 were suggestive of a trisubstituted alkene of the form $\text{RCH}=\text{CR}_2$. The UV spectrum showed an absorption at λ_{max} [EtOH] 208 and a shoulder at 238 nm whilst a minimum was recorded at 296 nm . This spectra suggests that C 5 is a linear unsaturated nitro compound.

R 3

R 3 was isolated from column chromatography of the petroleum ether extract of the roots as white plates from acetone mp. $78 - 81^{\circ}\text{C}$.

Its IR (KBr) spectrum showed a strong absorption at 1759 cm^{-1} which was suggestive of a $\text{C}=\text{O}$ group of a vinyl ester. A weak but sharp peak at 1705 cm^{-1} confirmed the $\text{C}=\text{C}$ group in the vinyl ester. The strong peaks between 3000 and 2900 and between 1495 and 1400 suggested the presence of hydrogen atoms bound mainly to saturated centres.

A set of peaks of different intensities between 1290 and 1200 confirmed a C=C-O-C- system. Two weak peaks at 998 and 985 cm^{-1} were suggestive of a trans CH=CH.

The UV spectrum was not informative.

The PMR spectrum confirmed some of the structural features which were suggested from the IR analysis. A weak multiplet signal centred at δ 5.10, $J = 16$ Hz, 8.9 Hz confirmed the trans CH = CH in a system CH - CH = CH - CH. A 1H hump at δ 5.38 suggested the presence of a CH=C in a ring as has been suggested for stigmasta-4,22-dien-3-ol. A 4H quartet at δ 2.23, $J = 7.0$ Hz suggested that the structural feature $\text{CH}_3\text{CH}_2\text{-CO}$ occurred twice in the compound. A triplet at δ 0.9 supported the presence of $\text{CH}_3\text{-CH}_2$. The signals between 1.5 - 2.0 were suggestive of protons in a set of ring systems. An intense 32H singlet at δ 1.2 was suggestive of $\text{CH}_2\text{-(CH}_2)_n\text{-CH}_2$. The two other singlets upfield at δ 1.0 and 0.7 were suggestive of methyl groups in a $\text{CH}_3\text{-C-C=C}$ and $\text{CH}_3\text{-C}$ systems respectively.

The mass spectrum indicated that the compound had the stigmasterol/sitosterol nucleus and possibly an ester derivative of it with a long unsaturated side chain.

D1

The compound was isolated as white plates from the petroleum ether extract of Peperomia pellucida mp (63 - 64°C).



The IR, UV, PMR and **mass** spectra showed that it was a high molecular weight linear unsaturated hydrocarbon.

The IR (KBr) spectrum showed strong absorptions at 2960, 2900 and 1485 cm^{-1} which were suggestive of hydrogen atoms bound mainly to saturated centres. A sharp peak at 3005 cm^{-1} was suggestive of the C-H stretching vibrations of a C=C-H group. The presence of the double bond was confirmed by the UV absorption at 208 nm.

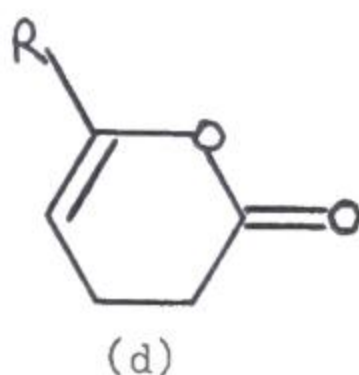
The PMR spectrum showed a 3H triplet at δ 0.9, $J = 7.0\text{ Hz}$ of a $\text{CH}_3\text{-CH}_2$ - system and an unresolved multiplet at δ 1.2 was consistent with the system $\text{CH}_2\text{-(CH}_2\text{)}_n\text{-CH}_2$.

D3

D3 was also obtained from column chromatography of the petroleum ether extract of Peperomia pellucida as white plates mp $63 - 64^\circ\text{C}$. Column chromatography of the petroleum ether extract of the roots of Piper nigrum also gave a similar compound R2 whose IR and UV spectra were superimposable on those of D3.

The IR (KBr) spectrum showed strong absorption at 1759 and 1705 cm^{-1} which were suggestive of the structural feature -CO-O-C=C . A medium absorption at 1200 cm^{-1} supported the presence of a C=C-O-C group. The strong absorptions at 2955, 2899 and 1485 cm^{-1} were suggestive of saturated alkyl centres. The UV spectrum which showed an absorption at λ_{max} [EtOH] 212 nm and a shoulder at 235 nm was suggestive of a non-conjugated system.

The spectra suggested the presence of a lactone having the δ -ring (d)



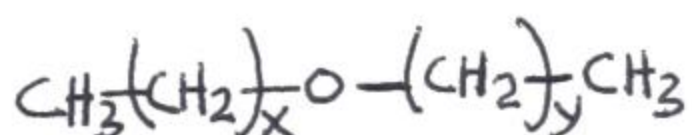
D6

Column chromatography of the petroleum ether extract of Peperomia pellucida afforded white plates identified as an ether from spectroscopic analysis mp. 82 - 84°C.

The IR (KBr) spectrum showed absorptions at 2960, 2900 and 1485 cm^{-1} which were all indicative of an alkyl system. A medium absorption at 1080 cm^{-1} supported the presence of a C-O-C system.

The PMR spectrum indicated a three proton triplet at δ 0.9, $J = 7.0$ Hz for the system CH_3CH_2 . An unresolved multiplet at δ 1.2 was assigned to $\text{CH}_2-(\text{CH}_2)_n$ and a triplet at 3.63, $J = 7.0$ Hz to the system $\text{CH}_2-\text{CH}_2-\text{O}$. A 4H hump at δ 1.53 was suggestive of a $\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{O}$.

The mass spectrum did not show a molecular ion but fragments around m/e 500 suggested that D6 was a high molecular weight ether having a structural feature of the form



(e)

where x and y are integers between 10 - 15.

CHAPTER FIVE

E X P E R I M E N T A L

Analytical thin layer chromatography was carried out on glass plates (75 x 25 mm) coated (~0.3 mm) with neutral alumina Type T 150 PF₂₅₄, Merck. Preparative thin layer chromatography was carried out on glass plates (20 x 20 cm) coated (0.5 mm) with the same adsorbent. Column chromatography was carried out using alumina Brockman II - III, Merck.

Mass spectra were recorded on a MAT 44S, SS 200. Data is reported as m/e VS relative intensity. The PMR spectra were measured on a Bruker WM 250 spectrophotometer with tetramethylsilane as an internal standard or with deuterium lock as internal reference. Both spectra were determined by Prof. Dr. Hans Achenbach. The IR and UV spectra were measured on a Perkin-Elmer spectrophotometer 720 and 700 respectively. All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. All compounds for which spectral data are reported showed single spots on tlc using at least three solvent systems. All solvent systems used are recorded as v.v.

Materials Collection

Piper nigrum

The fruits and roots of the plant were collected from the University Agricultural Research Station at Kade in the Eastern Region on 13th March 1981.

Peperomia pellucida

The plants were collected on the University Campus between October 1980 - June 1981. A second collection was made between 1981 - 1982 within the same period but at a distance of about 400 m away from the site of the first collection.

Identification of both plants was by Mr. O.B. Dokosi of the Botany Department, University of Ghana, Legon. Voucher samples have been deposited at the Herbarium of the Botany Department, University of Ghana, Legon.

The fruits and roots were thoroughly dried in the sun for about two weeks, after which they were finely ground. The Peperomia plants were dried for three weeks before grinding.

R E A G E N T S

Meyer's Reagent

1.36 g. of mercuric chloride was dissolved in 60 cm³. of water, 5 g. of potassium iodide dissolved in 10 cm³. of water were added and the solution diluted to 100 cm³ with water. Before use, the alkaloid solution in acetone was made distinctly acid with dilute hydrochloric acid and any precipitate that formed was redissolved by warming the solution. A few drops of the reagent were used for the tests. The reagent gives a yellowish precipitate with alkaloids.

Dragendorff's Reagent

8 g of hydrated bismuth nitrate were dissolved in 20 cm³ of conc. nitric acid and the solution was slowly added to 27.2 g of potassium iodide in 50 cm³ of water. The mixture was stirred well and allowed to stand for a time during which potassium nitrate crystallized out. The supernatant liquid was decanted and made up to 100 cm³ with distilled water.

Most alkaloids give a reddish brown precipitate with this reagent.

E X T R A C T I O N S

Approximately 0.8 kg of dried powdered fruits were extracted with petroleum ether (80 - 100°C), first by percolating for seventy-two hours, then extracting in a soxhlet apparatus for twenty-four hours. The extracts obtained by the two methods were worked on separately.

A) PERCOLATION

The supernatant liquid was decanted periodically and the combined extracts, approximately 2L was stored in the refrigerator for six days. An oily yellow and brown solid mass deposited. The mixture was removed by suction filtration and the mother liquor filtrate concentrated to dryness in vacuo to give a reddish-brown oily mass (25.4 g. P). The extract was stored in the refrigerator for several weeks.

The brown and yellow oily solid was washed thoroughly with methanol until all the yellow colour disappeared in the filtrate. The combined washings were evaporated to dryness in vacuo to give a dark brown oily mass L (12.5 g.).

Examination of L

The dark brown oily mass was triturated several times with diethyl ether and stored in the refrigerator for three days when a yellow and green solid mixture deposited. The mixture was removed and the solids separated mechanically. The yellow solid labelled L 1:1, mp. $130 - 132^{\circ}\text{C}$ weighed 0.18 g whilst the green solid L 1:2, mp. $141 - 150^{\circ}\text{C}$ weighed 0.11 g.

Tlc examination of L 1:1 on alumina in solvent system chloroform/heptane 3:1 showed two spots, which in UV light exhibited a light green fluorescence for the leading fraction and a blue for the trailing fraction. Purification by preparative tlc on alumina with eluent chloroform, followed by ethanol, gave a greenish-yellow solid from diethyl ether for the leading fraction. Crystallization from ethyl acetate-diethyl ether afforded yellow needles ~~0.091~~ 0.091 g. L 1:1:1) mp. $132 - 134^{\circ}\text{C}$, mmp with authentic sample of piperine $130 - 134^{\circ}\text{C}$. It was homogenous on tlc in solvent systems.

chloroform/heptane 3:1 (I), Rf 0.70, 1:1

benzene/chloroform 3:1 (II)

ethanol/petroleum ether 4:1 (IV).

Mixed tlc with an authentic sample of piperine in the same solvent systems gave a single spot which fluoresced blue in UV light. It gave a positive test for alkaloid with Meyer's and Dragendorff's reagents.

The IR(KBr) spectrum contained peaks at :

3060, 3000, 1680, 1660, 1650, 1630, 1605, 1525,
1510, 1485, 1470, 1385, 1270, 1219, 1159, 1138,
1058, 1018, 945, 865, 845, 825, 805, 738, and
720 cm^{-1} . The UV spectrum showed a maximum at λ_{max} (EtOH)

345 nm (ϵ - 34200) and a minimum at 205 nm (ϵ - 21000).

The PMR spectrum contained signals at δ (ppm) 1.5 (6H, hump),
3.5 (4H, hump) 5.9 (2H, s), 6.40 (1H, d, $J = 14$ Hz) 6.82 (1H, d,
 $J = 10.8$ Hz) 6.91 (1H, m, $J = 10.8$ Hz, $J = 1.7$ Hz), 7.37 (1H, m,
 $J = 10.8$ Hz, $J = 2.9$ Hz). The mass spectrum showed an M^+ peak at
 m/e 285 (42%) with the base peak at 115. Other significant peaks were
at 201 (74%), 173 (36%), 143 (32%), 84 (32%).

The second fraction labelled L 1:1:2 did not deposit any solid.
No further work was done on it.

The solid, L 1:2 was also examined on tlc in solvent system (I).
Three spots were observed, all of which gave different shades of yellow
fluorescence in UV light. Preparative tlc of L 1:2 on alumina gave,
for the major fraction, a yellow solid from diethyl ether. Crystallization
from minimum ethyl acetate-diethyl ether afforded greenish-yellow needles
mp 152 - 154°C (3.6 mg. L 1:2:1). It was homogenous on tlc in the
solvent systems (I), (II), (III), (IV) and benzene/ethyl acetate 1:1 (V).
It gave a positive identification for alkaloid with Meyer's and Dragendorff's
reagents. Rf. solvent system (I) 0.51.

The IR(KBr) spectrum contained peaks at :

3060, 3000, 2905, 1660, 1638, 1620, 1600, 1520, 1460, 1390, 1365, 1338, 1300, 1250, 1220, 1180, 1145, 1125, 1060, 1048, 950, 900, 885 and 830 cm^{-1} . The UV spectrum indicated a maximum at λ_{max} [EtOH] 364 nm (ϵ - 40200) and a minimum at 207 nm (ϵ - 18200).

The minor fractions did not deposit any solid. No further work was done on them.

FURTHER EXAMINATION OF L

The mother liquor on standing in the refrigerator for several days deposited a yellow crystalline solid which was removed, mp. 123 - 137°C (0.18 g. L 2). Tlc examination on alumina in solvent system (I) gave two spots, both of which fluoresced yellow in UV light. Preparative tlc on alumina gave a viscous yellow oil for the major fraction which was taken up in a minimum of diethyl ether. After storing in the refrigerator for two days a yellow solid deposited which crystallized from ethyl acetate-diethyl ether as greenish-yellow needles, mp. 152 - 154°C. (81 mg. L 2A), mmp with L 1:2:1 undepressed. It was homogenous on tlc in solvent systems (I), (II), (III), (IV) and (V), R_f 0.50 in solvent system (I). Mixed tlc and Co-tlc with L 1:2:1 gave the same R_f . It gave a positive test with Meyer's and Dragendorff's reagents for alkaloids.

The IR (KBr) spectrum showed peaks at :

3060, 3000, 2905, 1659, 1635, 1620, 1600, 1520, 1470, 1390, 1360, 1320, 1300, 1285, 1245, 1220, 1180, 1160, 1125, 1060, 1048, 950, 900, 885 and 830 cm^{-1} superimposable in all respects with L 1:2:1.

The UV spectrum was also superimposable on that of L 1:2:1. The PMR spectrum showed signals at δ (ppm) 1.5 (6H, hump), 3.5 (4H, d, broad, $J = 25$ Hz) 5.9 (2H, s), 6.38 (1H, d, $J = 14$ Hz), 6.83 (1H, d, $J = 8.9$ Hz), 6.9 (1H, m, $J = 8.9$ Hz, $J = 1.7$ Hz), 7.35 (2H, m, $J = 14$ Hz, $J = 10.8$ Hz). The mass spectrum showed a molecular ion at m/e 311 (44%) and a base peak at 141. Other significant peaks were m/e 227 (24%), 226 (38%), 199 (39%), 169 (72%), 112 (70%), 84 (33%).

The filtered mother liquor deposited more piperine (1.0 g, L 3).

Hydrolysis of Piperettine

10.3 mg. of piperettine was refluxed for eight hours with 10% aqueous alcoholic KOH (15 ml). The reaction mixture was allowed to cool to room temperature, then extracted three times with diethyl ether. The ether extract was evaporated to dryness to give an oil. The IR(film) was identical with that of piperidine.

The aqueous portion was treated with excess dilute hydrochloric acid. A deep yellow solid deposited which was removed by filtration, washed thoroughly with water and then crystallized from ethanol to give pale yellow needles, mp. $225 - 227^{\circ}\text{C}$ (lit. mp. $223 - 224^{\circ}\text{C}$ for piperettic acid). Its IR (KBr) spectrum contained peaks at 3100 - 2900, broad, 1720, 1638, 1538, 1485, 1460, 1378, 1358, 1320, 1300, 1240, 1185, 1140, 1080, 1045, 1005, 970, 905, 880 and 838 cm^{-1} . The UV spectrum showed an absorption at λ_{max} [EtOH] 353 nm (lit, 360 nm for piperettic acid).

Examination of P

The petroleum ether mother liquor was triturated with cold petroleum ether and then stored in the refrigerator for two weeks. A brown oily solid deposited; it was removed, dissolved in warm ethanol and then stored in the refrigerator again. After three days a pale brown solid deposited and was removed, mp. $72 - 77^{\circ}\text{C}$. (14 mg. 1A). Tlc examination on alumina in chloroform/heptane 5:1 showed one spot in UV light which did not fluoresce but appeared as a dark brown patch. Detection in iodine revealed two spots. Preparative tlc on alumina with eluent chloroform afforded pale yellow plates for the dark brown band in ethyl acetate. Crystallization from ethyl acetate afforded white plates, mp. $75 - 78^{\circ}\text{C}$ (5.8 mg. 1AA). It was homogenous on tlc but did not fluoresce in UV light. The spot was therefore detected in iodine vapour. **The following solvent systems were used**

chloroform/heptane 5:1 Rf. 0.85, 2:1

diethyl ether/cyclohexane 1:1 and

Pure cyclohexane.

The IR (KBr) spectrum showed peaks at 2970, 2900, 1760, 1485, 1190 and 740 cm^{-1} . Its UV spectrum was not clear since there was no correlation between the absorption at λ_{max} [EtOH] 240 nm with the PMR spectrum.

The PMR spectrum showed signals at δ (ppm) 0.9 (3H, t, $J = 7.0\text{ Hz}$), 1.25 (54H, s), 1.7 (5H, hump), 2.32 (1H, t, $J = 7.0\text{ Hz}$), 4.05 (1H, t, $J = 7.0\text{ Hz}$). The mass spectrum did not show a molecular ion peak neither was the fragmentation pattern clear. Fragments around m/e 760 indicated a high molecular weight compound.

The filtrate after removing the brown oily solid was concentrated to dryness in vacuo and examined on tlc in solvent system chloroform/hexane 1:1. Three spots were observed in UV light and appeared as a major dark brown fraction trailed by yellow and pink fractions. A fourth fraction was detected in iodine vapour. The oily mass, 22.0 g, was then subjected to column chromatography on alumina and eluted with hexane, hexane/chloroform 5:1, 3:1, 1:1 and then pure chloroform. Four fractions which were collected were labelled PA1 to PA4. PA2 and PA3 gave copious dark brown oils which were not analysed. PA1 which was eluted with hexane as a pale yellow oil was concentrated to dryness in vacuo and then taken up in a minimum of acetone. After storing in the refrigerator for one week, a yellow waxy mass deposited which was removed and dissolved in a minimum of warm ethanol. After storing in the refrigerator for two days, a pale yellow solid deposited, mp. $72 - 76^{\circ}\text{C}$ (10.2 mg. PA1). Tlc examination in chloroform/heptane 5:1 showed two spots in UV light; a leading dark brown fraction and a slower pale yellow fraction. Purification by preparative tlc on alumina afforded white plates for the leading fraction in ethanol. Crystallization from a minimum of ethanol gave white plates mp. $76 - 78^{\circ}\text{C}$. (6.8 mg. PA1:1). mmp with 1AA $75 - 78^{\circ}\text{C}$. Tlc examination on alumina showed a single spot in the solvent systems chloroform/heptane 5:1 Rf. 0.85, 2:1 diethyl ether/cyclohexane 1:1 and pure cyclohexane.

Mixed tlc with 1AA showed a single spot when the above solvent systems were used. The IR (KBr) spectrum was superimposable on that of 1AA.



WORK ON PA4

This fraction which was eluted as a pale green oil deposited specks of yellow solid in ethyl acetate. No further work was done on it due to paucity of material.

B) SOXHLET EXTRACTION

The extract obtained by this method was concentrated to about 3L with the same apparatus and the dark yellow liquid left to stand in the dark for two days. A brown oily mass and a greenish solid deposited. The supernatant liquid was carefully decanted and stored in the dark again (A). The oily solid mixture was removed from the flask by washing several times with warm methanol. The greenish solid dissolved and the supernatant liquid was decanted off. The washing was stopped when all the green colour disappeared in the supernatant liquid. A dirty brown solid residue was left in the flask. The supernatant liquid and the washings were combined, evaporated to dryness in vacuo to give a dark green oily mass. (10.6 g., B).

Examination of A

The supernatant liquid (A) which was kept in the dark deposited a greenish solid mp. $127 - 131^{\circ}\text{C}$ (5.18 g. A1). Tlc examination in solvent system (I) gave two spots in UV light. The major faster running fraction exhibited a blue fluorescence while the slower running fraction fluoresced purple. Purification by preparative tlc on alumina gave a viscous yellow oil for the major fraction which in diethyl ether gave a yellow solid.

The solid was crystallized from ethyl acetate-diethyl ether to give yellow needles. mp. $132 - 134^{\circ}\text{C}$ (2.14 g. A1:1). It was homogenous on tlc in solvent systems (I) Rf. 0.70, (II), (III), (IV) and (V), and was identified as piperine by mmp (undepressed), IR and UV spectra, which were identical in all respects to those of authentic piperine. It also gave a positive test for alkaloid with Meyer's and Dragendorff's reagents.

The mother liquor filtrate was concentrated to dryness in vacuo and then triturated again with diethyl ether. After storing in the refrigerator for one week a yellow solid deposited which was removed, purified and identified as piperine. (0.97 g. A2:2). The filtrate after removing piperine deposited a yellow solid mixture after twenty-four hours. The solids were separated mechanically on the basis of their colour and crystalline forms. They were labelled as A2:3, mp. $142 - 147^{\circ}\text{C}$, softening at 135°C . (0.97 g) and A2:4, mp. $128 - 132^{\circ}\text{C}$ (0.77 g). Purification of A2:4 by previous methods gave a yellow solid which was identified as piperine (0.24 g).



WORK ON A2:3

Examination on tlc in solvent system (I) gave three spots in UV light. The major spot exhibited a bright greenish-yellow fluorescence, the middle spot was pale yellow and the smallest fraction dark yellow. A2:3 was purified by preparative tlc on alumina and eluted with chloroform followed by ethanol to give different shades of brown oils labelled A2:3:1, A2:3:2 and A2:3:3. All the fractions were taken up in minimum amounts of diethyl ether and stored in the refrigerator.

After two days A2:3:1 deposited a yellow solid which was crystallized from ethyl acetate-diethyl ether to give yellow spheres, mp. 146 - 149°C which softened at 135°C (0.86g). It was homogenous on tlc in solvent systems (I), (II), (III), and (IV), and exhibited an unusual greenish-yellow fluorescence. It gave a positive test for alkaloid with Meyer's and Dragendorff's reagents.

Its IR (KBr) spectrum contained peaks at ;
3060, 3000, 2905, 1658, 1625, 1615, 1600, 1505, 1460, 1385,
1290, 1280, 1245, 1218, 1160, 1140, 1048, 1030, 945, 895,
, 880, and 825 cm⁻¹.

The PMR and mass spectra showed that A2:3:1 was a mixture of piperine and piperettine. Attempts made to separate them by tlc or fractional crystallization from ethanol were unsuccessful. No further work was done on it.

The mother liquor after removing A2:3 and A2:4 was concentrated to dryness in vacuo to give a reddish-brown oil (6.3 g) which was chromatographed on neutral alumina and eluted with hexane/chloroform 3:1, then pure chloroform. Forty fractions were collected. Fractions 10 - 28 upon concentrating and triturating with minimum diethyl ether gave a yellow solid which was purified to give yellow needles identified as piperine, mp. 132 - 134°C (2.27 g).

The other fractions did not give any significant solid. No further work was done on them.

Examination of B

The oily mass after repeated trituration with diethyl ether gave two main solids which were purified and identified as piperine (5.13 g) and piperettine (12 mg).

CHLOROFORM EXTRACT

In view of the low yield of alkaloid types isolated from the petroleum ether extract, the powdered fruits left over after the petroleum ether extraction (342 g) were extracted with 5L chloroform (60°C) in a soxhlet apparatus for 12 hours. The extract was concentrated to dryness to give a reddish-brown gum (29.0 g J.). It was divided into a diethyl ether soluble fraction and a chloroform soluble mother liquor.

WORK ON DIETHYL ETHER SOLUBLE FRACTION

The ether fraction was concentrated to dryness over a steam bath to give a dark brown oily mass (13 g. C). C was examined on tlc on alumina in solvent system hexane/chloroform 5:1. Four spots were observed in UV light; the major fastest running fraction exhibited a blue fluorescence, followed by a brown and two yellow spots. The extract was subjected to column chromatography on alumina and eluted with hexane, hexane/chloroform in increasing polarity, then pure chloroform. Several minor fractions were obtained from which four solids were obtained labelled C3, C5, C10 and C11. They were all isolated in smaller quantities.

WORK ON C3

C3 was obtained from a pale yellow oil as yellow needles in ethanol after storing the concentrated oil in the dark for two months. mp. 132-134°C (1.0 mg). No further work has been done on it except determining its UV spectrum which showed an absorption at $\lambda_{\max}$ [EtOH] 208 nm.

WORK ON C5

C5 was eluted as a dirty green oil with hexane/chloroform 5:1. The fraction was concentrated to dryness, taken up in minimum ethyl acetate, and stored in the refrigerator. After two weeks when no solid deposited, the solvent was removed and the oil was stored in the dark for two months. The oil was then swirled with ethanol during which fine white needles deposited and were filtered off. The solid was washed thoroughly with ethanol mp. 118 - 120°C (5.5 mg). It was homogenous on tlc in solvent systems (I), (II), and (III). Its IR (KBr) spectrum showed peaks at 3015, 2975, 2960, 2895, 2860, 1625, 1540, 1480, 1455, 1410, 1330, 1295, 1245, 1230, 1220, 1195, 1140, 1060, 1045, 900, 865, 840, 820 and 775 cm^{-1} . The UV spectrum showed an absorption at $\lambda_{\max}$ [EtOH] 208 nm, a shoulder at 238 nm and a minimum at 295 nm. Further work on the characterisation of C5 is required.

WORK ON C10

The oily concentrate obtained after evaporating the eluent was stored in the dark for one week and then swirled with ethyl acetate.

White plates deposited which were removed. Crystallization from ethanol afforded white plates mp. 136 - 138°C (4.0 mg). It was homogenous on tlc in the following solvent systems.

chloroform/petroleum ether 3:1

cyclohexane/acetone 5:1

chloroform/heptane 3:1



It did not fluoresce in UV light therefore was detected in iodine vapour. It was identified as β -sitosterol by mmp (undepressed), mixed tlc, Co-tlc with an authentic sample. The IR and UV spectra were identical in all respects with those of an authentic sample.

WORK ON C11

C11 was eluted with pure chloroform as a reddish-brown oil. The oily mass was kept in the refrigerator in ethyl acetate for two weeks after which it was removed and kept in the dark since no solid deposited. The ethyle acetate was removed to regenerate the oil. After two months, a minimum of ethyl acetate was again added and the mixture quickly swirled. Round greenish-yellow crystals immediately deposited and were removed. mp 204 - 206 softened at 196°C (2.7 mg). It was not homogenous on tlc, giving two spots in UV light; the faster running fraction which appeared as a tiny spot fluoresced greenish-yellow and the slower fraction which was the larger spot exhibited a similar fluorescence. Since the material obtained was very small, no attempt was made to further purify it.

The IR (KBr) spectrum of the crude material showed peaks at 3360, 3100, 3005, 2970, 1775, 1710, 1700, 1660, 1620, 1600, 1570, 1550, 1518, 1475, 1415, 1240 and 718 cm^{-1} . The UV spectrum showed an absorption at λ_{max} [EtOH] 208 nm, a shoulder at 232 and a minimum at 345 nm.

The chloroform mother liquor was not investigated due to lack of materials. Preliminary examination on tlc on alumina in solvent system hexane/chloroform 3:1 showed five spots in UV light.

EXTRACTION OF THE ROOTS

The dried pulverised roots (1.2 kg) were extracted with 5L petroleum ether (80 - 100°C) for forty-eight hours in a soxhlet apparatus. The extract was concentrated to about 800 cm^3 with the same apparatus and then transferred into a 2L flask. The extract was stored in the refrigerator for four days when a yellowish-brown solid deposited. The supernatant liquid was carefully decanted off, evaporated to dryness in vacuo to give a reddish-brown oily mass (28.0 g, R). The oily solid was washed thoroughly with methanol until all the yellow colour disappeared in the washings. Evaporation to dryness in vacuo gave a reddish-brown oily mass (10.2 g, M).

Examination of M

The oily mass was triturated several times with diethyl ether and stored in the refrigerator for three days. A yellow solid which deposited was removed. mp. 127 - 131°C (4.72 g. M1). Tlc examination on alumina in solvent system (I) showed two spots in UV light.

The major faster running fraction exhibited a blue fluorescence and the slower running fraction a purple fluorescence. Purification by preparative tlc on alumina gave a viscous yellow oil for the major fraction which was taken up in a minimum of diethyl ether. After storing in the refrigerator for twenty-four hours, a yellow solid deposited which crystallized from ethyl acetate-diethyl ether as yellow needles mp $132 - 134^{\circ}\text{C}$ (1.72 g. M1:1), mmp with authentic sample of piperine $132 - 133^{\circ}\text{C}$ (undepressed). It was homogenous on tlc in solvent systems (I), (II), (III), (IV) and (V). Rf in (I) 0.70. It was identified as piperine by its IR and UV spectra which were superimposable on those of an authentic sample. Since this investigation represented the first attempt at determining the alkaloid content of the roots the compound was further characterised.

Hydrolysis of Piperine

0.28 g of piperine was refluxed for twenty hours with 10% aqueous ethanolic KOH (30 cm^3). The reaction mixture was allowed to cool to room temperature and then extracted three times with diethyl ether. The ether fraction was evaporated to dryness to give a pale yellow oil whose IR (film) was identical with that of piperidine.

The aqueous portion was treated with excess dilute hydrochloric acid during which a pale yellow solid deposited. The solid was removed by suction filtration, washed thoroughly with water and crystallized from a minimum of ethanol to give pale yellow plates. mp. $218 - 220^{\circ}\text{C}$ (lit $216 - 217^{\circ}\text{C}$) (0.17 g). Its UV spectrum showed an absorption at λ_{max} [EtOH] 335 nm (lit. 340nm).

The IR (KBr) spectrum contained peaks at 3200 - 2800, 1715, 1700, 1625, 1525, 1518, 1478, 1435, 1395, 1338, 1310, 1280, 1220, 1180, 1138, 1060, 1025, 955, 875, 825 and 725 cm^{-1} .

Further Examination of M.

The mother liquor after removing piperine was further concentrated to dryness and triturated again with diethyl ether to give more piperine (0.11 g). The mother liquor filtrate was again concentrated to dryness in vacuo to give a dark brown oil (2.9 g) which was examined on tlc. Three spots were observed in UV light exhibiting a yellow, blue and purple fluorescence. Column chromatography on alumina with eluent hexane/chloroform 1:3 followed by pure chloroform gave four fractions. Each fraction was concentrated to dryness in vacuo, dissolved in a minimum of diethyl ether and then stored in the refrigerator. The second fraction (M4) deposited a yellow solid which crystallized from ethyl acetate-diethyl ether as yellow needles (0.091 g) identified to be piperine by IR spectrum superimposable on that of an authentic sample.

The first and fifth fractions (M3, M5) did not deposit any solid. No further work was done on them. M6 deposited brown specks of solid which was too small for any further analysis.

Examination of the Petroleum ether extract

Preliminary tlc of the extract on alumina using solvent system chloroform/heptane 1:2 did not show any fluorescence in UV light. Detection in iodine revealed seven spots.

The extract was then subjected to column chromatography on neutral alumina with eluents heptane, heptane/chloroform 10:1, 7:1, 4:1, 1:1 and then pure chloroform. Nine fractions were obtained R1 - R9. Each fraction was concentrated to dryness in vacuo to give an oil.

WORK ON R1.

R1 was triturated with acetone and stored in the refrigerator. After two days a white solid deposited but no further work was done on it due to paucity of material.

R4, R5 and R8 did not deposit any solid. No further work was done on them.

WORK ON R2

R2 gave a white waxy solid in acetone which crystallized from acetone as white plates mp. 60 - 62°C (6.3 mg). It was homogenous on tlc in the solvent systems

Pure cyclohexane Rf 0.46 (VI)

Cyclohexane/chloroform 3:1 (VII)

Cyclohexane/ethanol 2:1 (VIII)

Benzene/petroleum ether 1:1 (IX).

but did not fluoresce in UV light.

The IR (KBr) spectrum showed peaks at 2960, 2890, 1759, 1710, 1485, 1400, 1200, and 740 cm^{-1} .

The PMR spectrum showed signals at δ (ppm) 0.9 (6H, t, $J = 7.0$ Hz), 1.25 (6H, s), 1.65 (4H, hump), 2.25 (1H, m, $J = 7.0$ Hz), 4.05 (1H, t, $J = 7.0$ Hz), 6.43 (1H, t, $J = 7.0$ Hz). The mass spectrum did not show a molecular ion peak but indicated fragments around m/e 536 ($< 10\%$) and a base peak at m/e 57. Other significant peaks were at m/e 257 (22%), 97 (36%), 83 (38%), 72 (50%), 43 (78%).

WORK ON R3

R3 gave a white waxy solid in acetone after storing in the refrigerator for two days. Crystallization from acetone afforded white plates, mp. $78 - 81^{\circ}\text{C}$ (9.4 mg). The solid was homogenous on tlc in solvent systems (VI) (Rf. 0.48) (VII), (VIII) and (IX) but it did not fluoresce in UV light. The spot was detected in iodine vapour.

Its IR (KBr) spectrum showed peaks at 3000, 2960, 2900, 1759, 1490, 1405, 1290, 1265, 1245, 1220, 1200, 1038, 998, 985, 820 and 740 cm^{-1} . The UV spectrum was not clear.

The PMR spectrum showed signals at δ (ppm) 0.7 (34, s), 1.0 (34, s), 1.2 (32H, s) 1.66 (9H, unresolved multiplet) 2.00 - 1.85 (6H, hump,) 2.23 (4H, q, $J = 7.0$ Hz) 4.65 (1H, hump), 5.10 (1H, m), 5.38 (1H, d, 7.0 Hz). The mass spectrum indicated a molecular ion at M^+ 396 with the base peak at m/e 43. Other significant peaks were at m/e 382 (40%), 275 (14%), 255 (38%), 147 (60%), 145 (42%) 107 (30%), 83 (50%), 81 (66%), 57 (82%).

WORK ON R6

R6 was triturated with ethanol and then stored in the refrigerator. After five days a white solid deposited which crystallized from a minimum of ethanol as white plates mp. $147 - 149^{\circ}\text{C}$. Further crystallization from ethanol raised the melting point to $151 - 153^{\circ}\text{C}$. (65 mg). It was homogenous on tlc in the solvent systems

chloroform/petroleum ether 3:1 Rf 0.54

cyclohexane/acetone 5:1, 3:1

chloroform/heptane 3:1.

The IR and UV spectra were superimposable on that of a sample of stigmasterol - 4,22-dien-3-ol isolated from Peperomia pellucida. Mixed melting point with a sample of this compound was undepressed $151 - 153^{\circ}\text{C}$.

WORK ON R7

R7 was also dissolved in a minimum of ethanol. After one week during which no solid deposited, the precipitating solvent was removed and replaced with a more polar solvent, methanol. After two days standing in the refrigerator a mixture of white solids deposited, which were removed. Crystallization from methanol afforded white plates, mp $145 - 147^{\circ}\text{C}$, softened at 137°C (75 mg). Further crystallization from the same solvent did not alter the melting point. It was homogenous on tlc in solvent systems

cyclohexane/chloroform 1:1

hexane/chloroform 2:1

ethanol/petroleum ether 2:1 Rf 0.47

diethyl ether/cyclohexane 1:1

but did not fluoresce in UV light.

The IR and UV spectra were identical in all respects with R6 and stigmasta-4, 22-dien-3-ol. R7 could be a mixture of sterols.

The filtrate after removing R7 deposited some more white solid which crystallised from methanol as leaflets identified as β -sitosterol by mmp 137 - 139°C and IR spectrum which was superimposable on that of an authentic sample.

WORK ON R9

The oil in methanol deposited a yellow waxy solid after storing in the refrigerator. The oily solid (26.2 mg) was removed and examined on tlc on alumina in chloroform/hexane 2:1. Two spots were observed, both of which fluoresced blue in UV light. Purification by preparative tlc on alumina and eluent chloroform gave a pale yellow amorphous solid in methanol after storing in the refrigerator for twenty-four hours mp. 64 - 66°C (2.4 mg). It gave a positive test for alkaloid with Meyer's Reagent. The IR (KBr) spectrum showed peaks at 2960, 2905, 1730, 1658, 1620, 1538, 1490, 1285, 1058, 1000, 975, 940 and 745 cm^{-1} . Its UV spectrum showed an absorption at λ_{max} [EtOH] 330 nm, 208, shoulders at 220 and 305 nm and a minimum at 242 nm.

PEPEROMIA PELUCIDA

Preliminary Investigation

A total of 329.7 g of dried pulverised plant material collected on the University campus between October - June 1981 were boiled in three batches with petroleum ether (80-100°C) in a 0.5L beaker. Each batch was boiled for an hour after which the supernatant liquid was decanted.



The supernatant liquids were combined, concentrated to about 0.3L and then stored in the refrigerator for several weeks during which no solid deposited. The extract was therefore concentrated to dryness in vacuo to give a dark green oily mass. (10 g. D).

Examination of D

Tlc examination on alumina in solvent system, chloroform/petroleum ether 3:1 gave several spots, two of which were detected in UV light as a blue and brown fluorescence. The extract was subjected to column chromatography on alumina and eluted with petroleum ether, petroleum ether/chloroform in varying proportions and then pure chloroform. Nine fractions were obtained D1 to D9. Each fraction was concentrated to dryness to give an oil. Fractions D5 and D8 did not deposit any solid. No further work was done on them.

WORK ON D1

D1 which was eluted as a colourless oil with pure petroleum ether gave a white waxy solid from acetone. Crystallization from acetone afforded white plates mp $63 - 64^{\circ}\text{C}$. (73 mg). It was homogenous on tlc in solvent systems

Petroleum ether (X) Rf . 0.90

Petroleum ether/acetone 2:1 (XI)

Cyclohexane/chloroform 3:1 (XII)

and Pure chloroform (XIII).

The compound did not fluoresce in UV light.

Its IR (KBr) spectrum contained peaks at 3005, 2960, 2900, 1485, 1405, 750 cm^{-1} . The UV absorption occurred at λ_{max} [Cyclohexane] 208 nm. The PMR spectrum showed signals at δ (ppm) 0.9 (3H, t, $J = 7.0$ Hz), 1.23 (approx. 60H, unresolved multiplet).

The mass spectrum did not show a molecular ion peak but indicated a peak at m/e 464 ($< 10\%$). Other peaks were at m/e 408 (20%) followed by fragments which differed from the next by 14 mass units. The base peak occurred at m/e 43, 57, 71 and 85.

WORK ON D2

D2 was eluted as a thin yellow band on the column with petroleum ether/chloroform 7:1. Evaporation to dryness in vacuo gave a very small yellow oil which was kept in the refrigerator for one week. After this period the oil was swirled with minimum diethyl ether during which yellow needles deposited and were removed, washed thoroughly with diethyl ether until all the yellow colour disappeared from the filtrate.

The compound melted at $131 - 133^{\circ}\text{C}$ (2.0 mg) and was identified as piperine by mmp with an authentic sample of piperine $131 - 134^{\circ}\text{C}$ undepressed. It was homogenous on tlc and gave one spot on mixed tlc with authentic piperine in solvent systems (I) Rf. 0.70, (II), (III), (IV), and (V). The spot fluoresced blue in UV light. Its IR and UV spectra were identical in all respects with those of authentic piperine.



WORK ON D3

D3 was also eluted with petroleum ether 7:1 as a yellow oil which gave an oily yellow solid in acetone. The solid was removed and washed with acetone until all the yellow colour disappeared from the filtrate. Crystallization from methanol afforded white plates mp $63 - 64^{\circ}\text{C}$ (11.4 mg). It was homogenous on tlc in the solvent systems (X) (Rf. 0.66), (XI), (XII) and (XIII) but the spot did not fluoresce in UV light.

The IR (KBr) spectrum contained peaks at 3005, 2990, 2905, 1760, 1719, 1665, 1485, 1405, 1200 and 745 cm^{-1} . The UV spectrum showed an absorption at λ_{max} [EtOH] 212 nm and a shoulder at 235 nm.

WORK ON D4

D4 was also eluted with petroleum ether/chloroform 7:1 as a deep yellow oil which in acetone gave an oily pale yellow solid after storing the mixture in the refrigerator for twenty-four hours. The solid was removed and washed thoroughly with acetone. Crystallization from methanol afforded white plates mp $63 - 64^{\circ}\text{C}$ (35 mg). It was homogenous on tlc and identified as D3 by mixed tlc and mp. Its IR and UV spectra were superimposable on those of D3.

WORK ON D6

D6 was eluted as a yellow oil with petroleum ether/chloroform 5:1. The oil was triturated with acetone and was stored in the refrigerator. After twenty-four hours a pale yellow solid deposited which was removed and crystallized from methanol to give white plates mp. $82 - 84^{\circ}\text{C}$ (24.2 mg).

It was homogenous on tlc on alumina in solvent systems chloroform/petroleum ether 3:1 (Rf 0.64), (XI) and (XII). The spot did not fluoresce in UV light therefore it was detected in iodine vapour. Its IR (KBr) spectrum showed peaks at 2955, 2905, 1485, 1080, 750 and 740 cm^{-1} .

The PMR spectrum showed signals at (ppm) 0.9 (3H, t, $J = 7.0$ Hz), 1.3 (54H, unresolved multiplet), 1.53 (4H, hump) and 3.63 (2H, t, $J = 7.0$ Hz). The mass spectrum did not show a molecular ion peak but intense peaks occurred at m/e 125 (28%), 102 (50%), 97 (76%), 83 (72%), 70 (58%), 57 (100%), 43 (68%). Peaks of m/e less than 10% appeared between 168 and 504.

WORK ON D7

D7 was eluted with petroleum ether/chloroform 1:1 as a dirty green oil which was **taken** up in a minimum of acetone and stored in the refrigerator. After twenty-four hours a dirty white shiny solid deposited which crystallized from ethanol as white plates mp. 151 - 153°C (50 mg). It was homogenous on tlc on alumina in solvent systems

chloroform/petroleum ether 3:1 Rf. 0.53

cyclohexane/acetone 5:1, 3:1

chloroform/cyclohexane 2:1

and chloroform/hexane 3:1

The compound did not fluoresce in UV light. Its IR (KBr) spectrum indicated peaks at 3600 - 3200(broad), 3005, 1485, 1405, 1080, 1045, 995, 985 and 830 cm^{-1}

The UV spectrum showed an absorption at $\lambda_{\max}$ [EtOH] 207 nm.

The PMR spectrum showed signals at δ (ppm) 0.7 (1H, s), 0.9 (3H, t, $J = 7.0$ Hz) 1.0 (3H, s), 1.5 (3H, s), 1.9 (4H, hump), 2.0 (4H, hump), 2.3 (2H, hump), 3.5 (1H, m) 5.0 - 5.13 (2H, m. $J = 16$ Hz, $J = 8.9$ Hz), 5.35 (1H, d, $J = 7.0$ Hz).

The mass spectrum indicated a molecular ion at 412 (414) (22% and a base peak at m/e 43. Other peaks were 394 (4%), 351 (10%), 300 (20%), 255 (50%), 213 (38%), 145 (44%), 107 (38%). 81 (52%).

Acetate Derivative

31.3 mg. of the sterol was mixed with fused powdered sodium acetate (0.15 mg) and acetic anhydride (3 cm^3) and the mixture heated on a steam bath, with occasional shaking for two hours. At the end of this time, the warm solution was poured, with vigorous stirring, into iced-water (50 cm^3). The mixture was allowed to stand with occasional stirring, until the excess acetic anhydride was hydrolysed during which white plates deposited. The solid was removed, washed thoroughly with water and recrystallized from ethanol. mp. $136 - 138^\circ\text{C}$ (27.7 mg). Its IR (KBr) spectrum showed peaks at 3000, 2905, 1745, 1485, 1405, 1390, 1285, 1179, 1060, 990, 980, 925 and 820 cm^{-1} . The UV spectrum showed an absorption at $\lambda_{\max}$ [EtOH] 208 nm.

WORK ON D9

D9 was eluted with petroleum ether/chloroform 1:1 as a dark brown oil which in diethyl ether deposited three large specks of brown flocculent crystals. The crystals were removed and washed with cold diethyl ether, mp 173 - 176°C (< 1 mg).

The IR (KBr) spectrum indicated peaks at 3300, 2950, 2895, 1665, 1645, 1560 and 1540 cm^{-1} . There were weak peaks at 1460, 1398, 1340 - 1300, 1100 - 1040 and 710 cm^{-1} .

It gave a positive identification for alkaloid with Meyer's reagent.

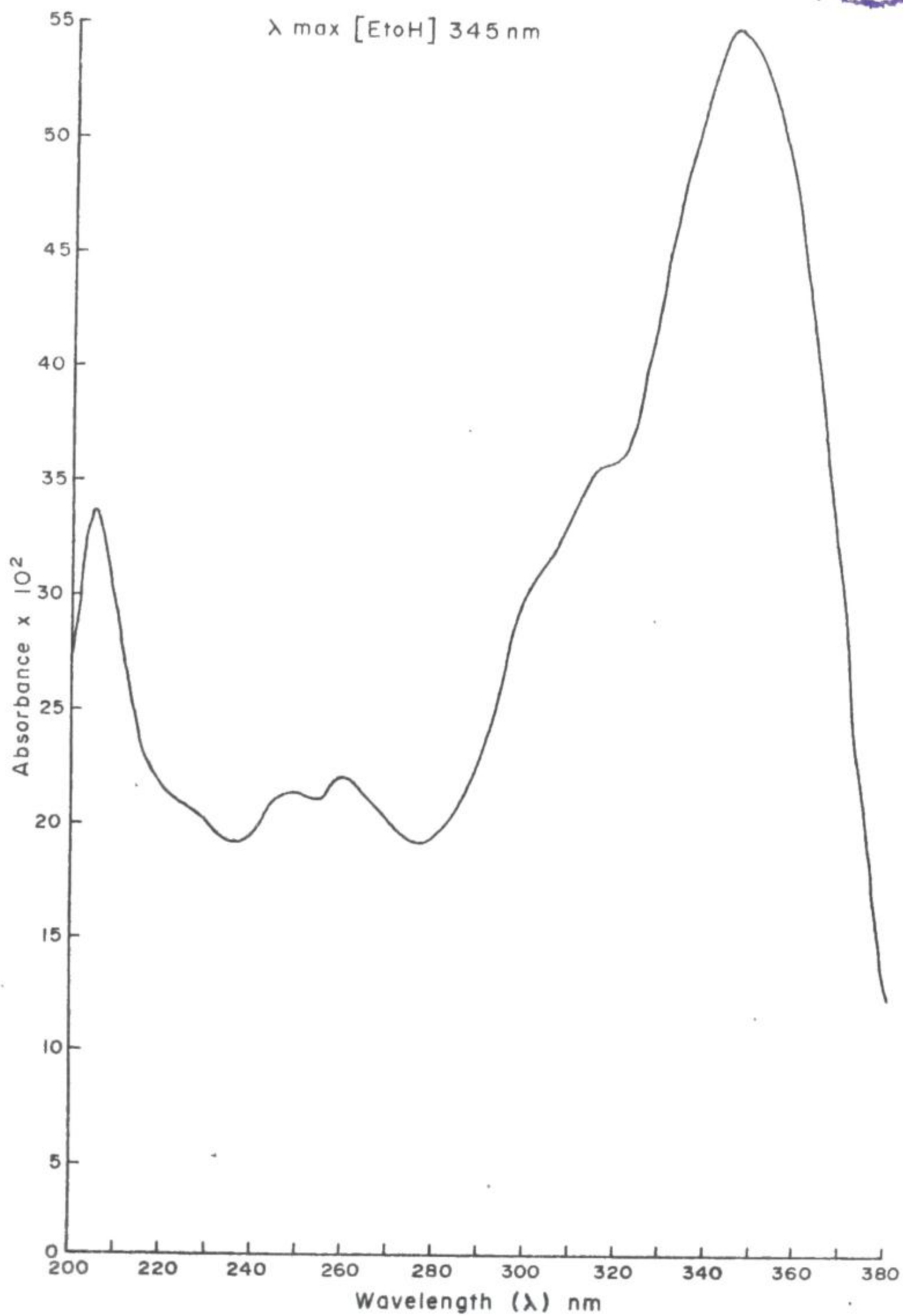
APPENDIX I

UV Spectra of Compounds

- FIG. IA UV Spectrum of Piperine
- FIG. IB UV spectrum of Piperic acid
- FIG. IIA UV spectrum of Piperettine
- FIG. IIB UV spectrum of Piperettic acid
- FIG. IIIA UV spectrum of stigmasta-4,22-dien
- 3-ol
- FIG. IIB UV spectrum of stigmasta-4,22-dien -3- acetate.
- FIG. IV UV spectrum of β - sitosterol
- FIG. V UV spectrum of C5
- FIG. VI UV spectrum of ALKALOID C11
- FIG. VII UV spectrum of ALKALOID R9
- FIG. VIII UV spectrum of LACTONE D3



UV SPECTRUM OF PIPERINE

 λ_{max} [EtOH] 345 nm

UV SPECTRUM OF PIPERIC ACID

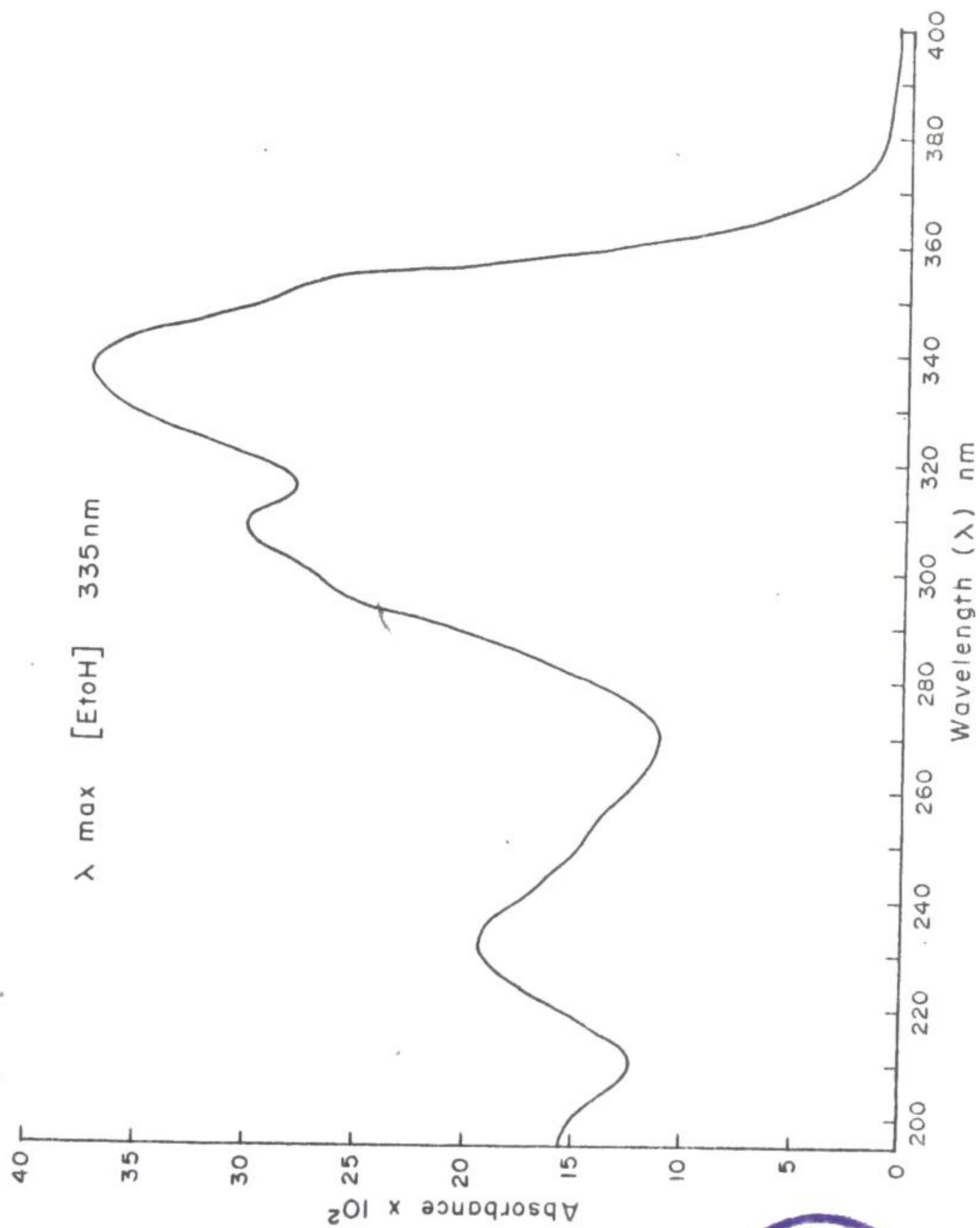


Fig. 1B



UV SPECTRUM OF PIPERETTINE

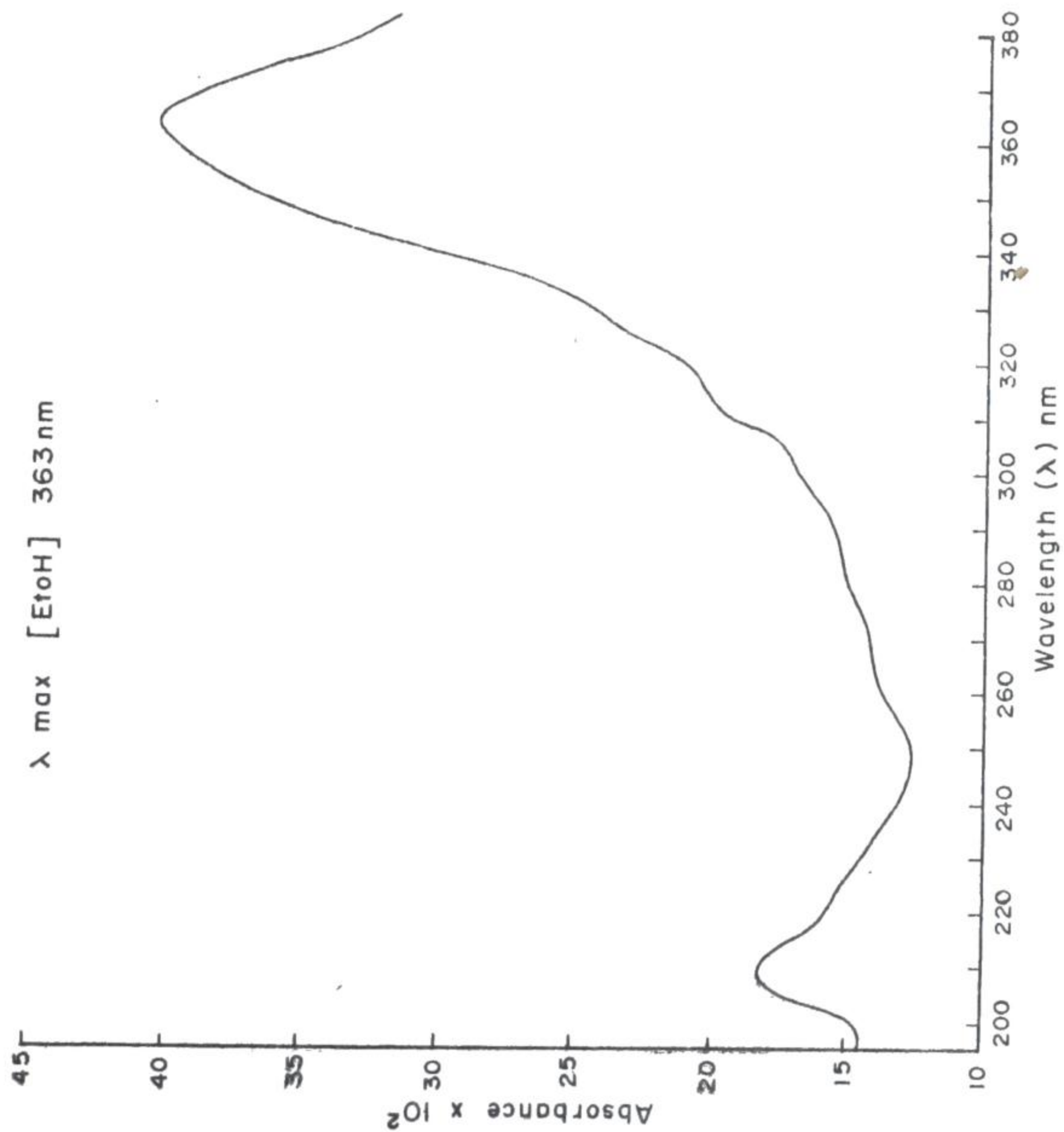


Fig. II A.



UV SPECTRUM OF PIPERETTIC ACID

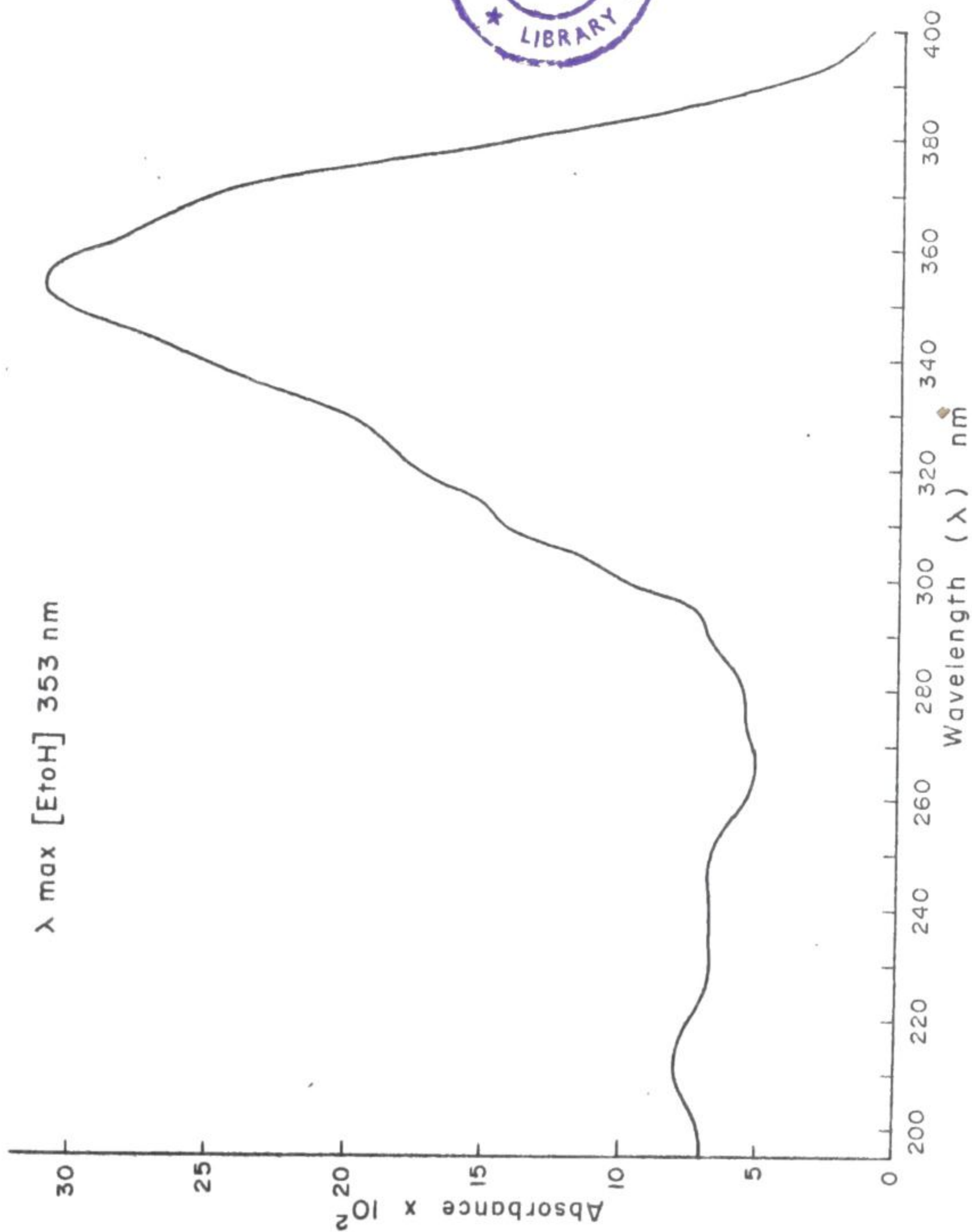


FIG. II B.

UV SPECTRUM OF STIGMASTA -4, 22-DIEN-3-OL

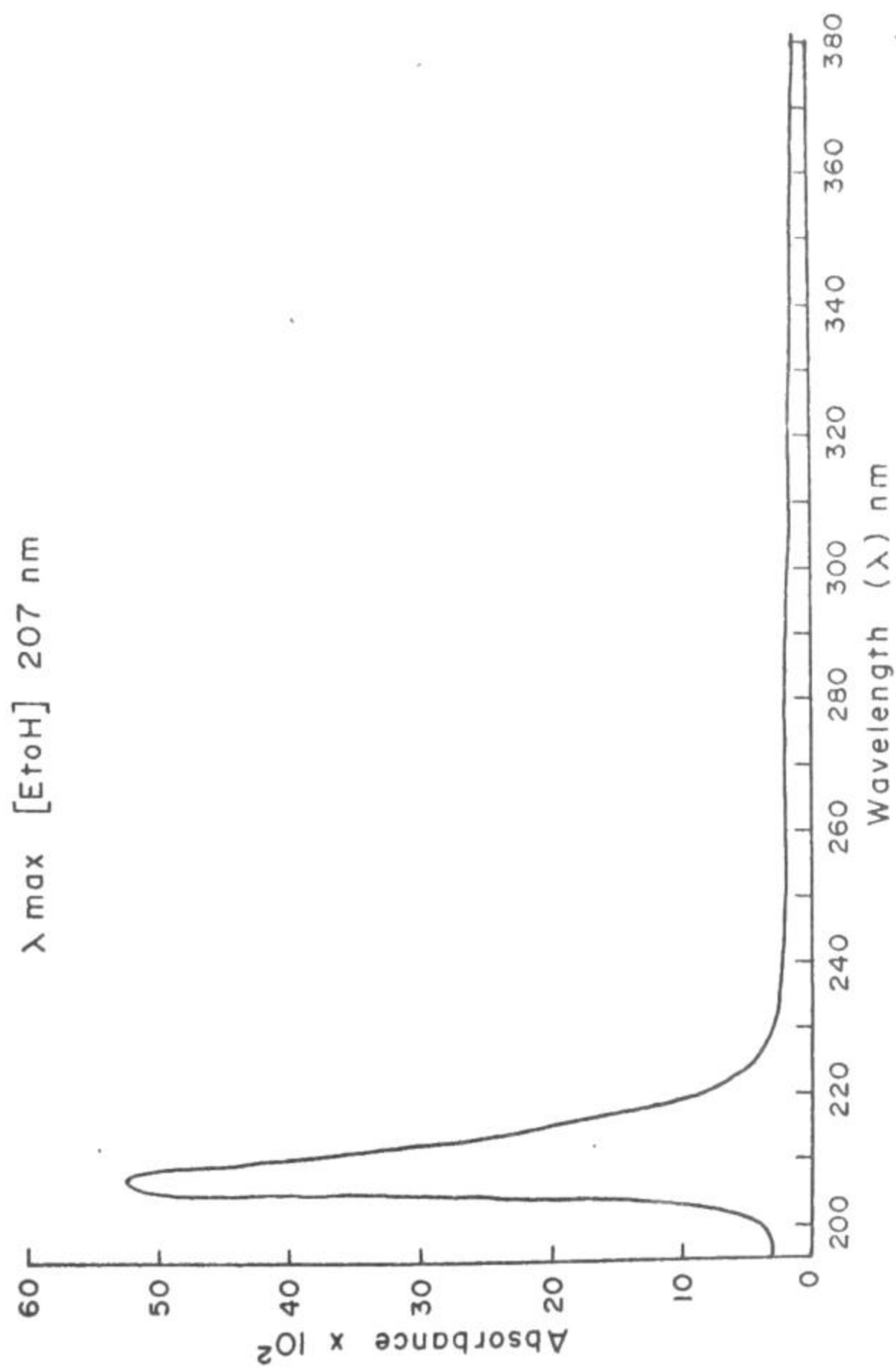


Fig. III A.

UV SPECTRUM OF THE ACETATE DERIVATIVE

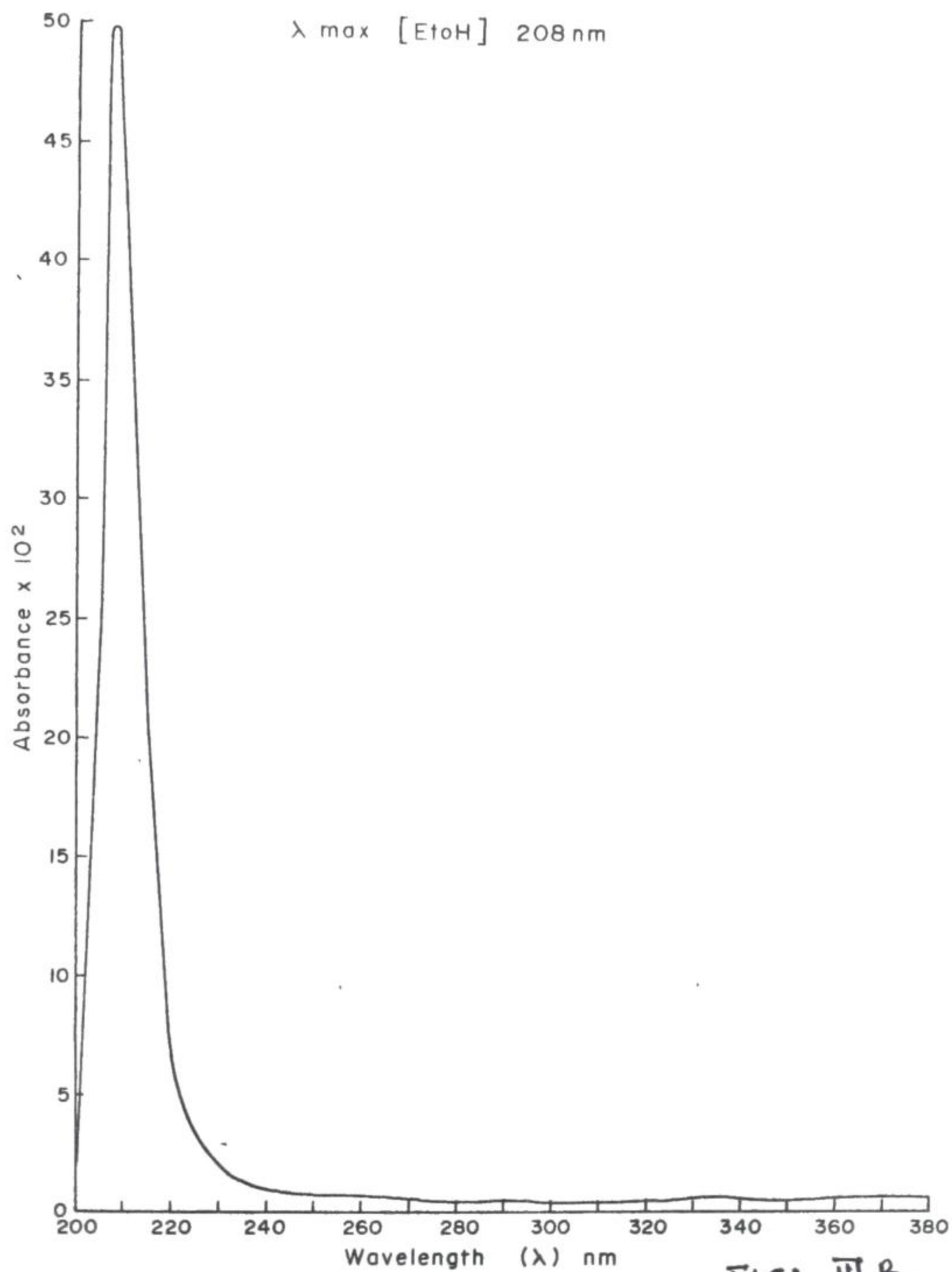


FIG. III B.

UV SPECTRUM OF β - SITOSTEROL

λ_{max} [EtOH] 207 nm

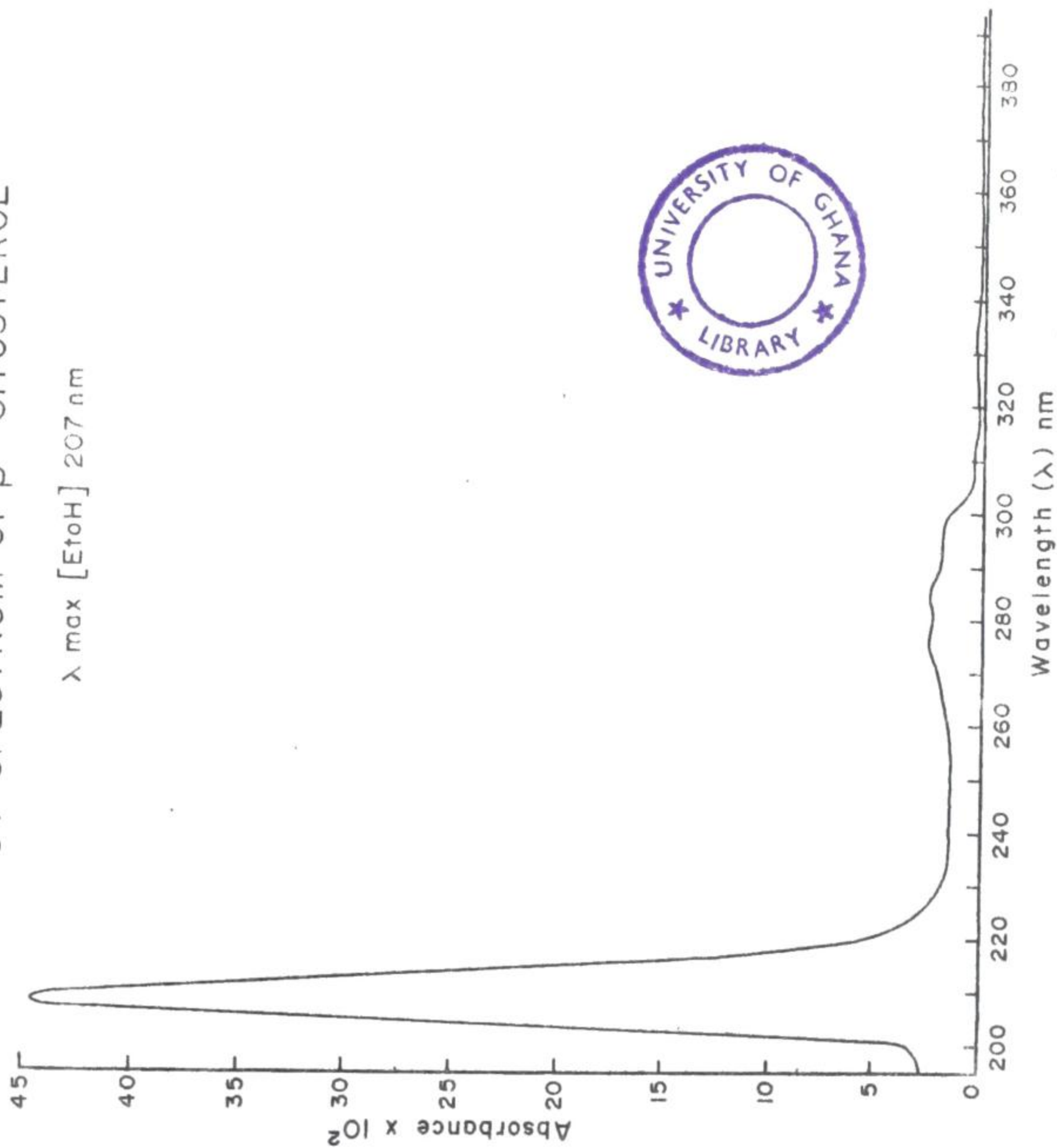
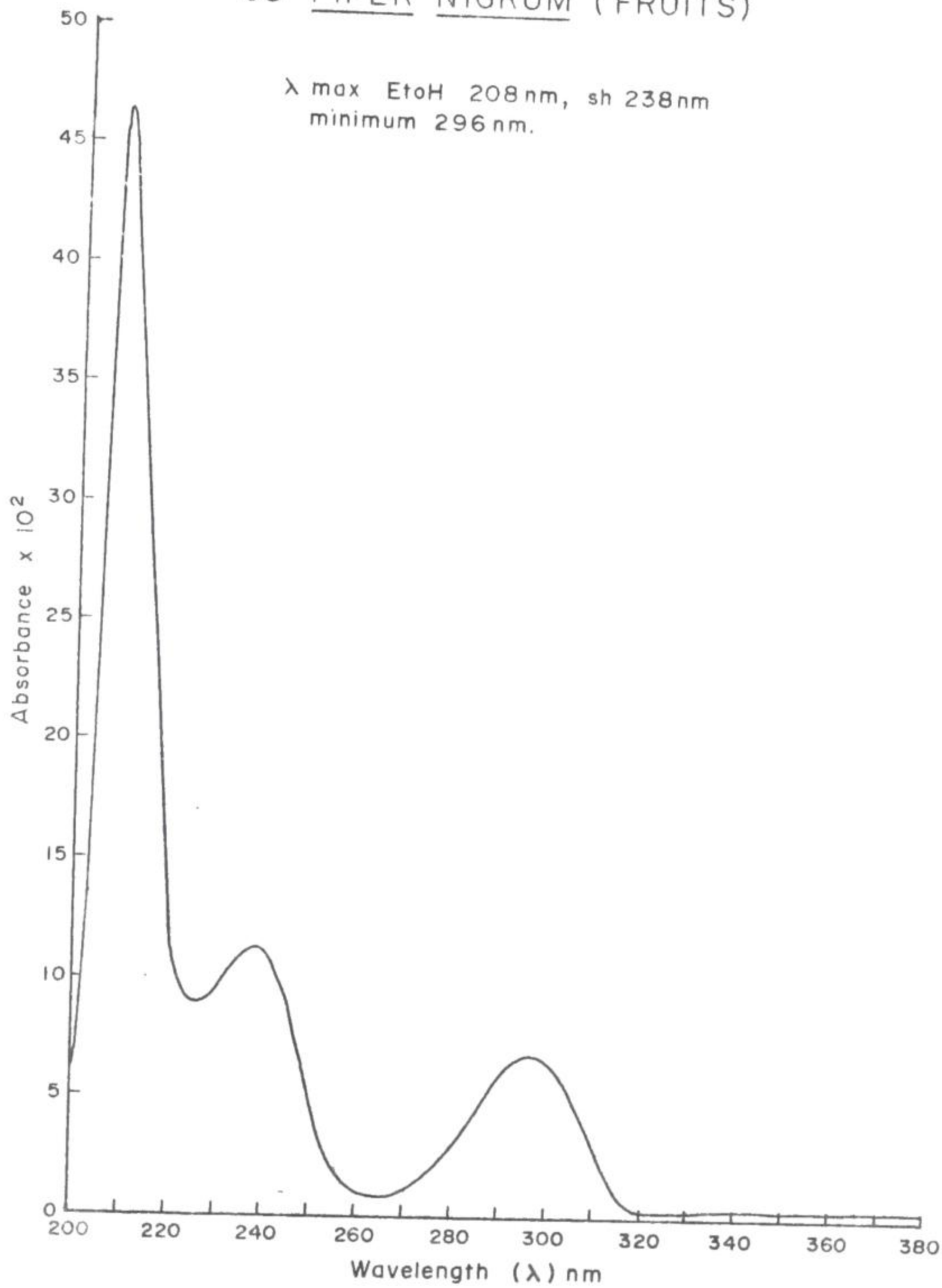


FIG. IV.

C5 PIPER NIGRUM (FRUITS)

λ max EtoH 208 nm, sh 238 nm
minimum 296 nm.

FIG. V.

UV SPECTRUM OF CII PIPER NIGRUM (FRUITS)

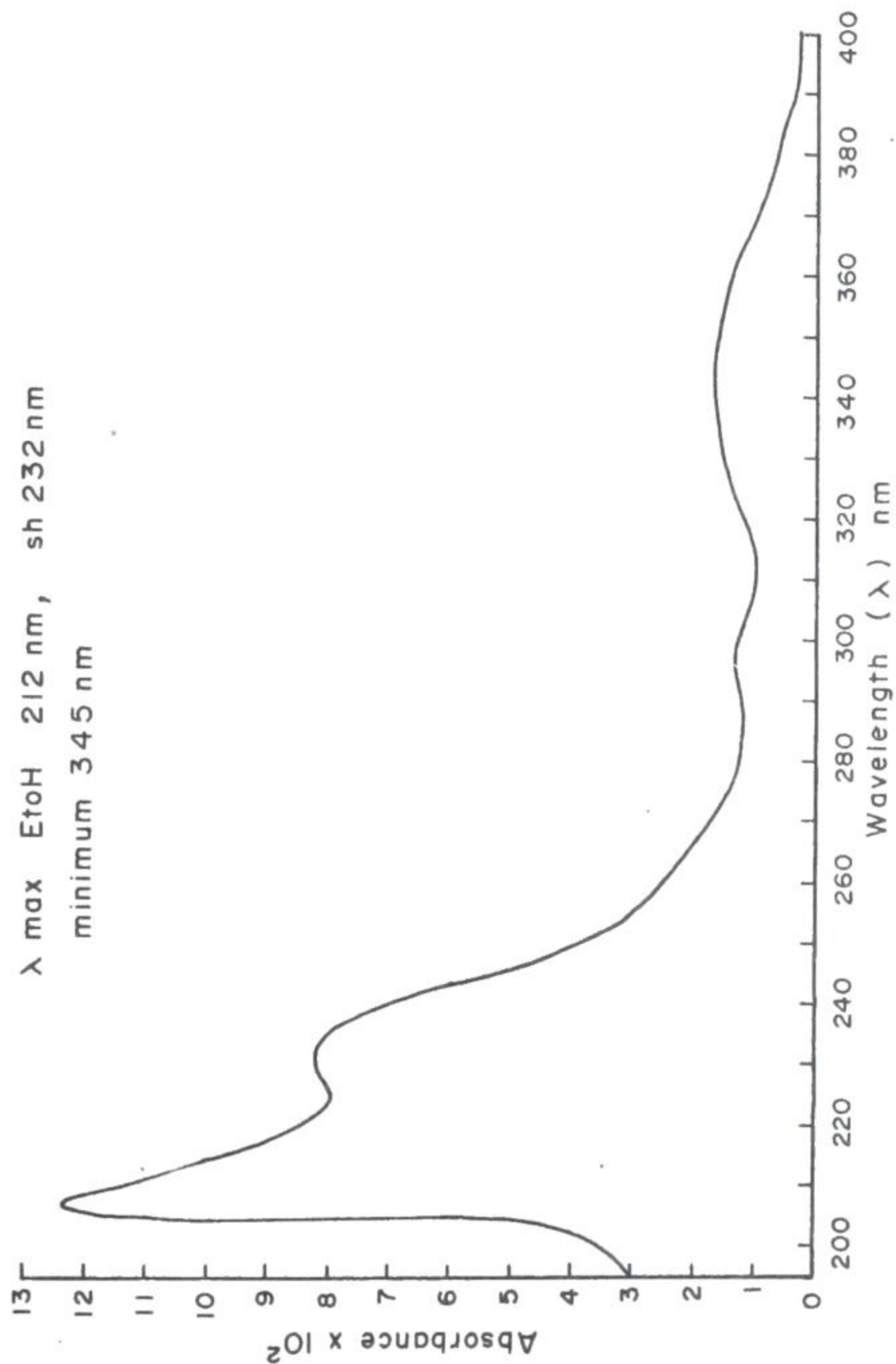


Fig. VI.



PIPER NIGRUM (ROOTS)
UV SPECTRUM OF R9
 λ_{max} [EtOH] 330 nm

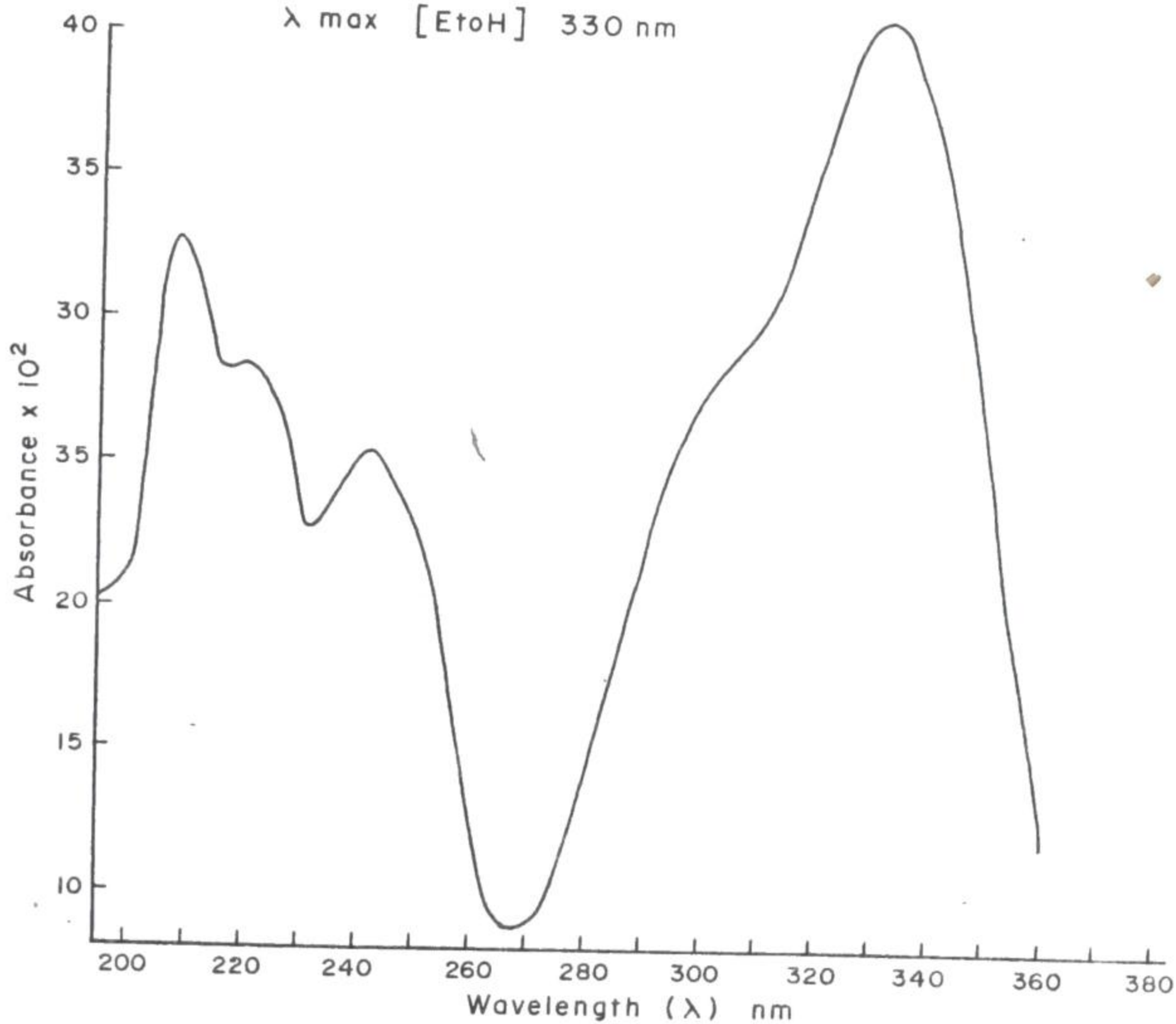


FIG. VII

UV SPECTRUM OF D3 PEPEROMIA PELLUCIDA

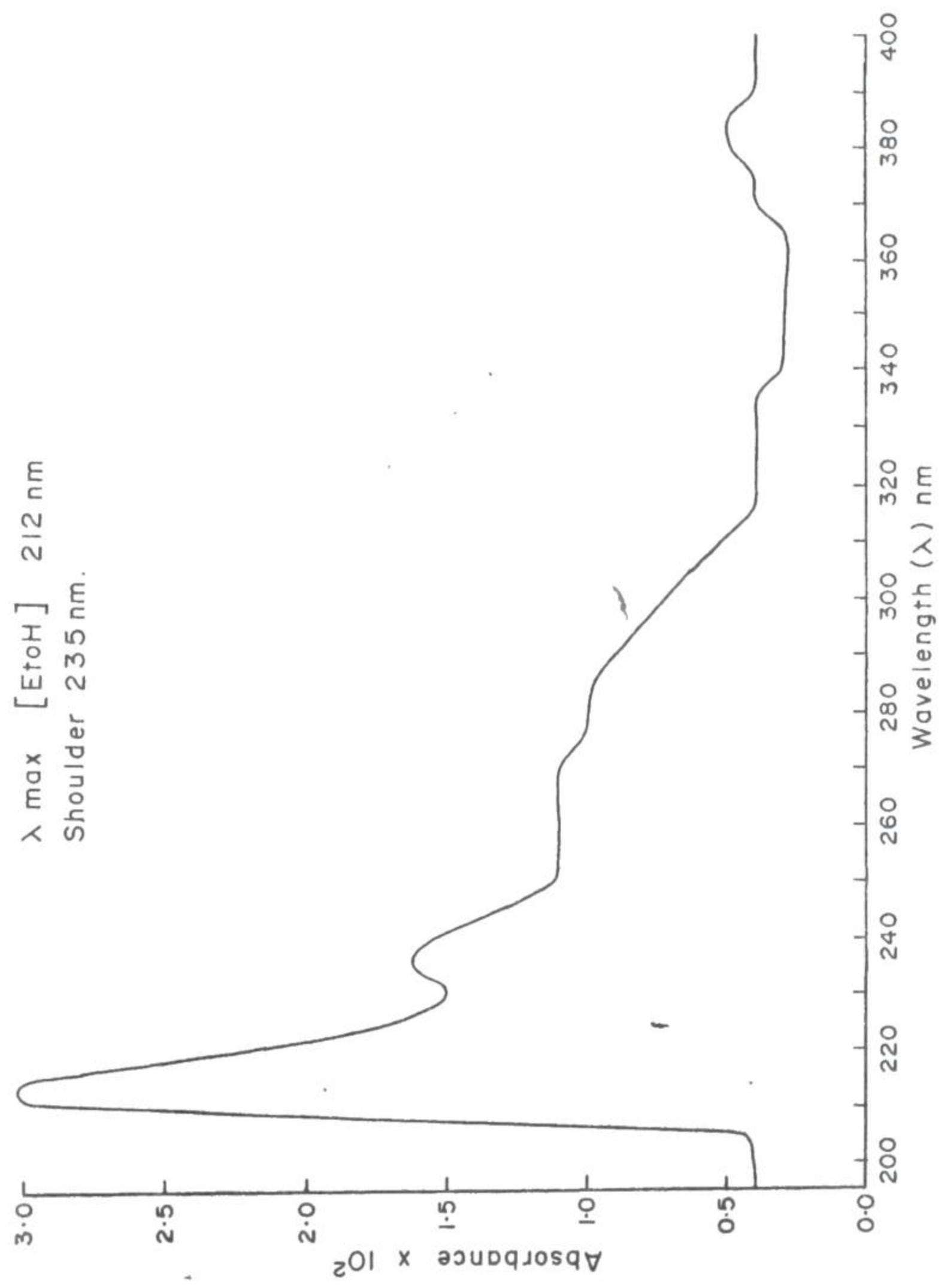


Fig. VII.

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APPENDIX II

IR Spectra of Compounds

- FIG. IXA IR spectrum of Piperine
- FIG. IXB IR spectrum of Piperic acid
- FIG. XA IR spectrum of Piperettine
- FIG. XB IR spectrum of Piperettic acid
- FIG. XIA IR spectrum of Stigmasta-4,22-dien-3-ol
- FIG. XIB IR spectrum of Stigmasta-4,22-dien-3-acetate.
- FIG. XII IR spectrum of β -sitosterol
- FIG. XIII IR spectrum of C5
- FIG. XIV IR spectrum of ALKALOID C11
- FIG. XV IR spectrum of ALKALOID R9
- FIG. XVI IR spectrum of LACTONE D3.



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PERKIN-ELMER

ORIGIN

SPECTRUM NO.

SAMPLE NO.

IR SPECTRUM OF

IR SPECTRUM OF PIPERINE

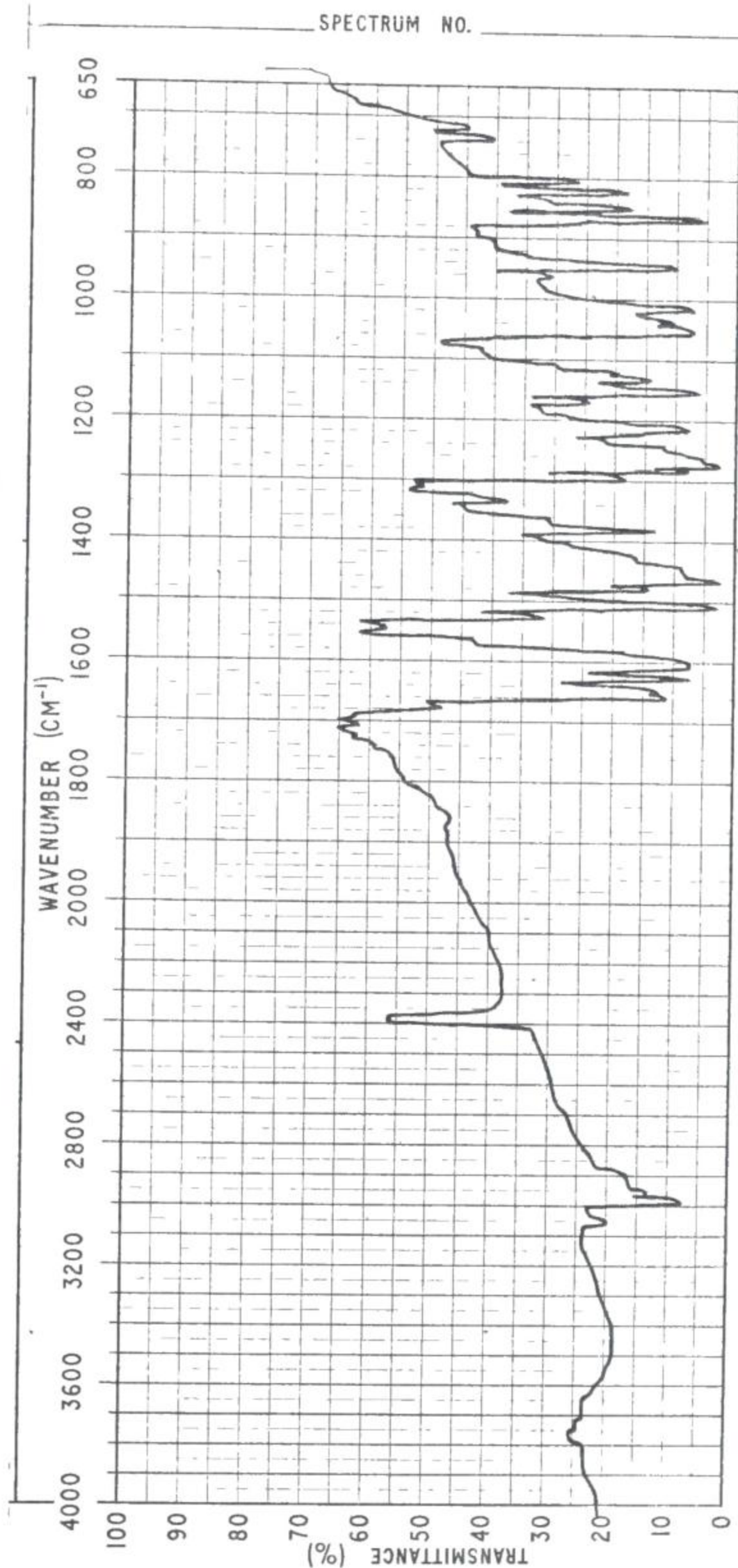


FIG. IXA.

REMARKS	PERKIN-ELMER	
	MODEL 700	
ORIGIN	SPECTRUM NO.	
PURITY	SAMPLE I	

IR SPECTRUM OF PIPERIC ACID

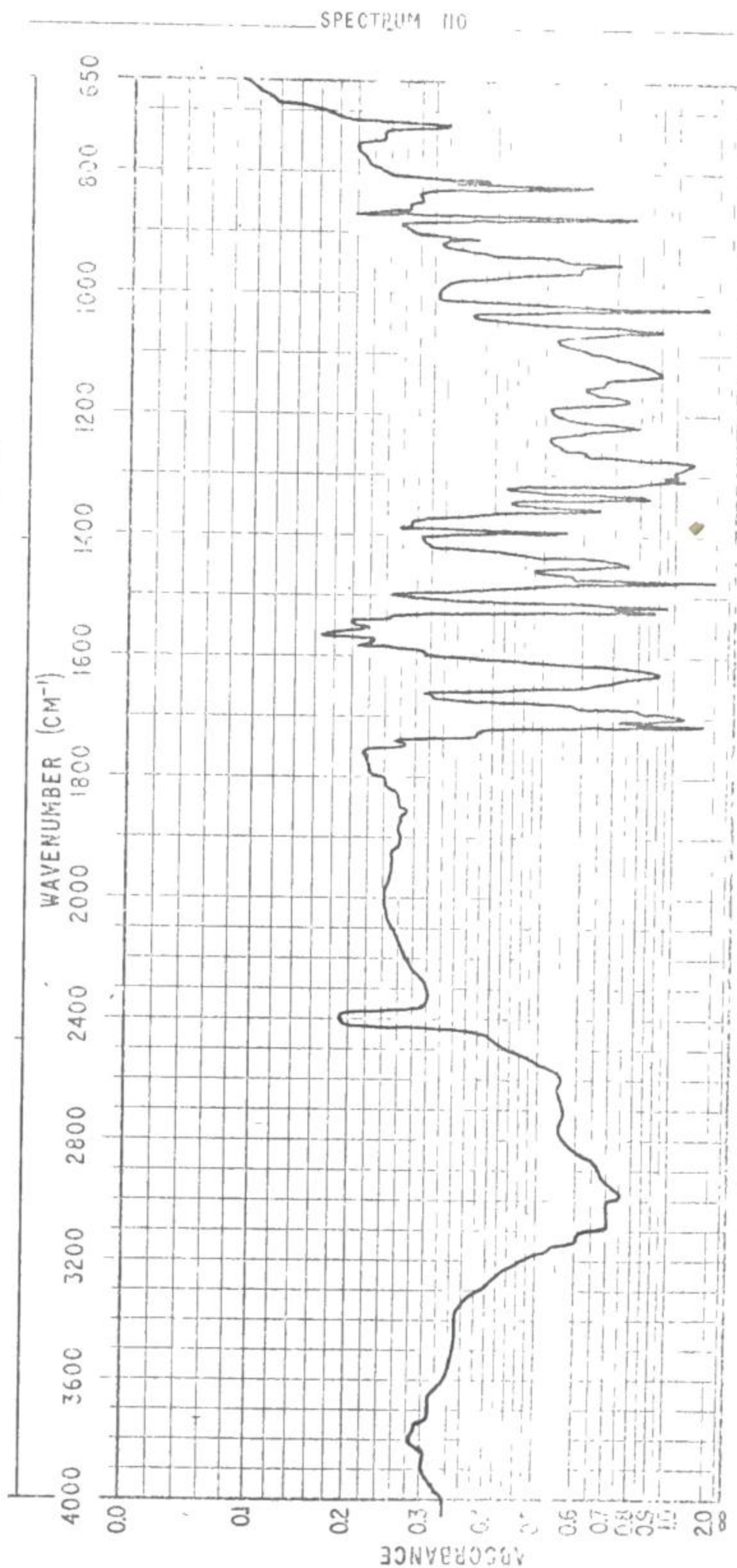


FIG. IXB.

NO. 5-100-0309

REMARKS

ORIGIN

PERKIN-ELMER

SPECTRUM NO.

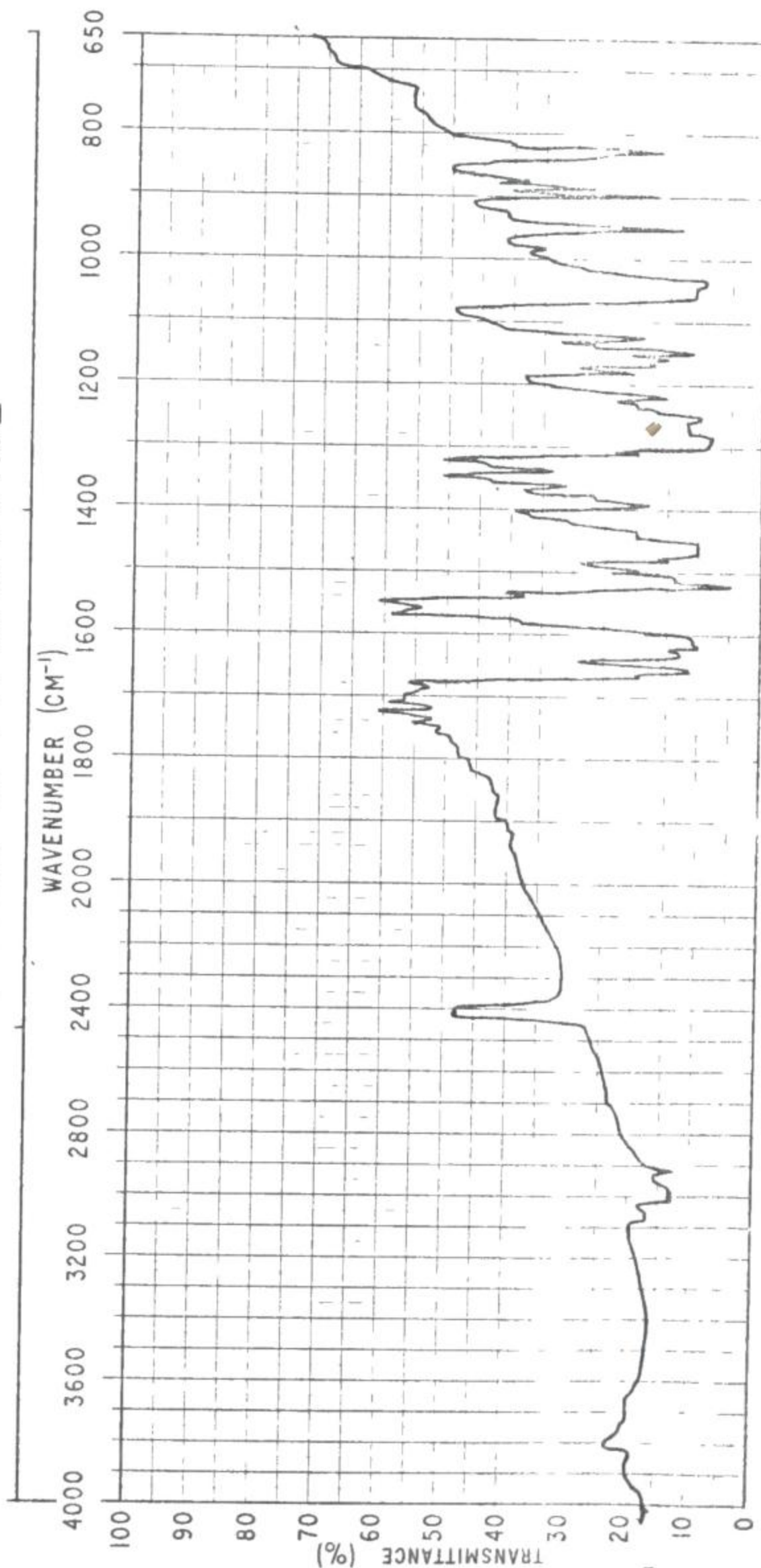
PURITY Very Good.

SAMPLE N₃ LA

159-1540

SAMPLE

IR SPECTRUM OF PIPERETTINE



108



FIG. XA.

NO. 5-000-0310

611
9

REMARKS

Piperetic acid from me
hydrolysis of piperettin

ORIGIN

PERKIN-ELMER
MODEL 700

SPECTRUM NO.

SAMPLE

SPECTRUM NO

IR SPECTRUM OF PIPERETTIC ACID

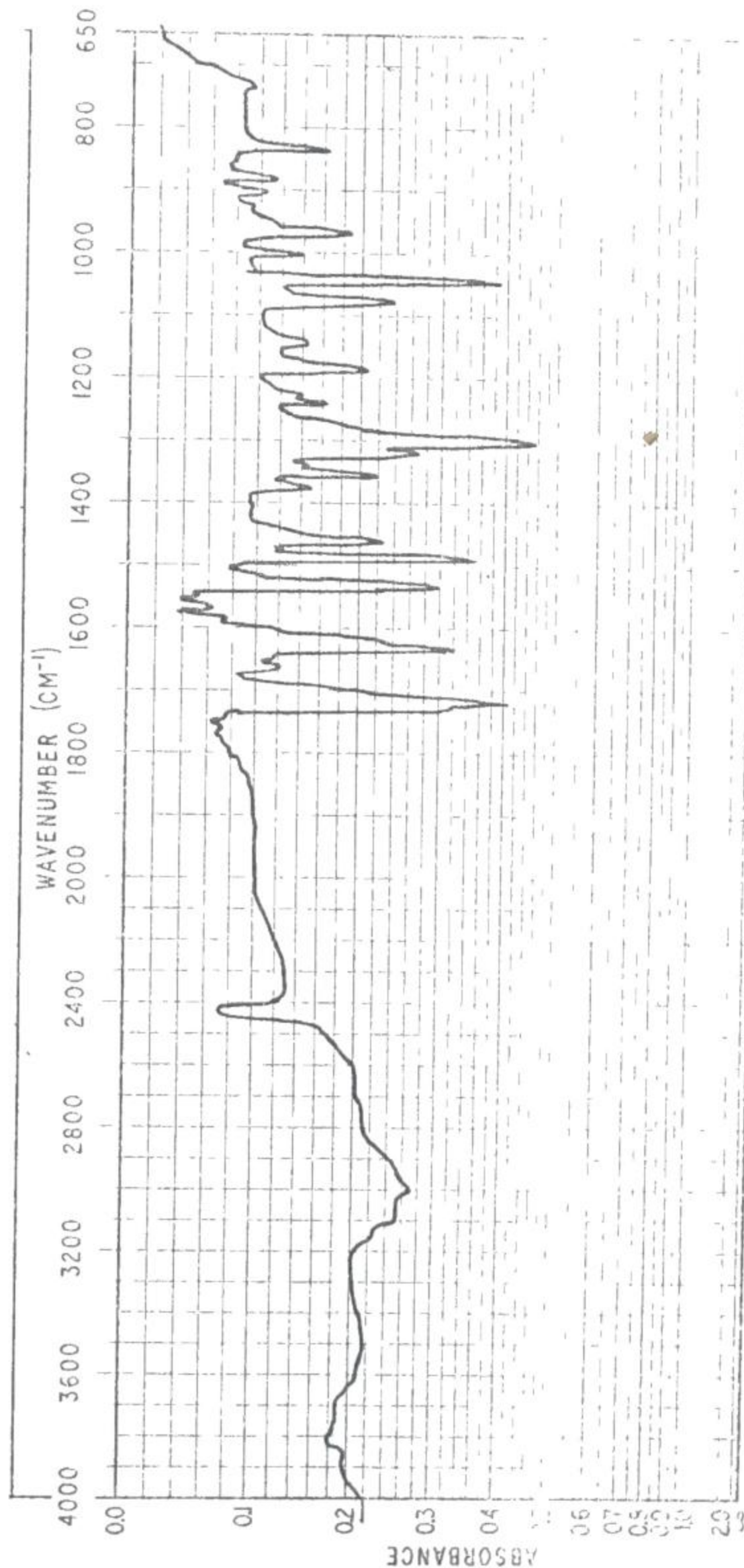


FIG. X8

REMARKS

ORIGIN

PERKIN-ELMER

PURITY

SPECTRUM NO.

PHASE

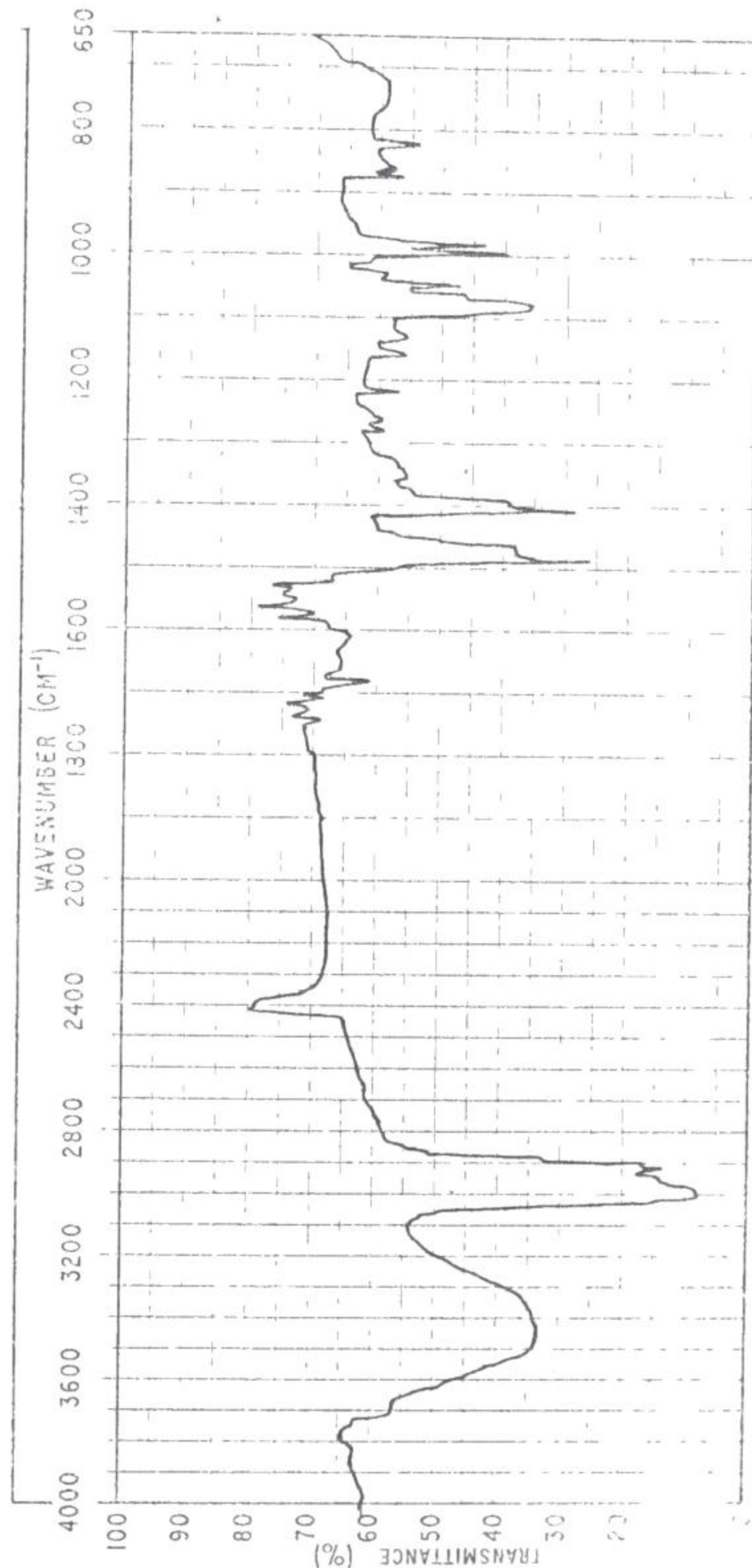
SAMPLE NO.

CONCENTRATION

IR SPECTRUM OF

SAMPLE

IR SPECTRUM OF STIGMASTA-4,22-DIEN-3-OL



SPECTRUM NO.

110



FIG. XIA.

REMARKS	OPICIN	PERKIN-ELMER
		MODEL 700
		SPECTRUM NO.
	PURITY	

CAMPIE

IR SPECTRUM OF THE ACETATE DERIVATIVE

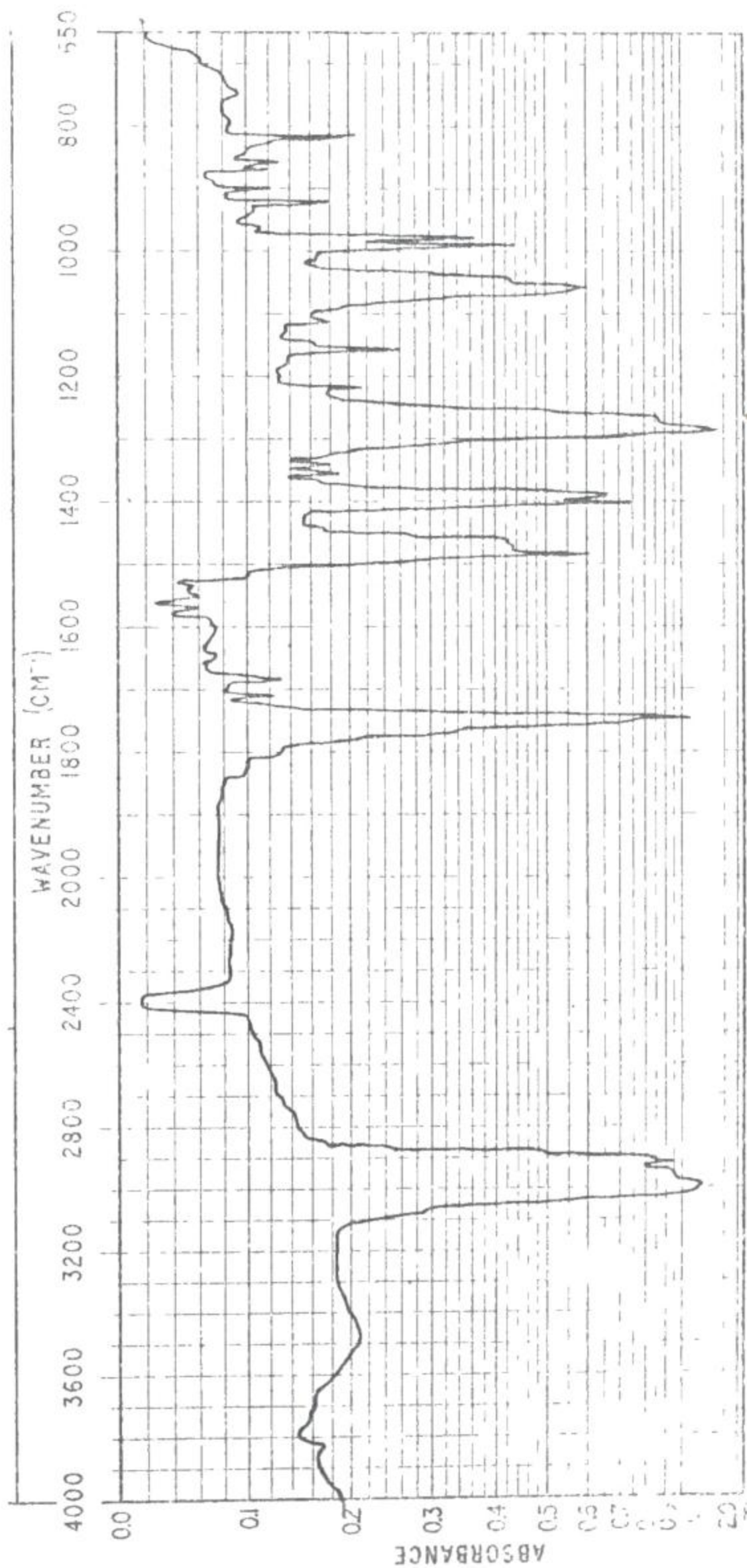


FIG. XIB.



SAMPLE _____

SPECTRUM NO. _____

REMARKS <u>IR SPECTRUM OF</u> <u>β-SITOSTEROL</u>	ORIGIN _____	PERKIN-ELMER
	PURITY _____	SPECTRUM NO. _____
	PHASE <u>solid</u>	SAMPLE I _____

IR SPECTRUM OF β -SITOSTEROL

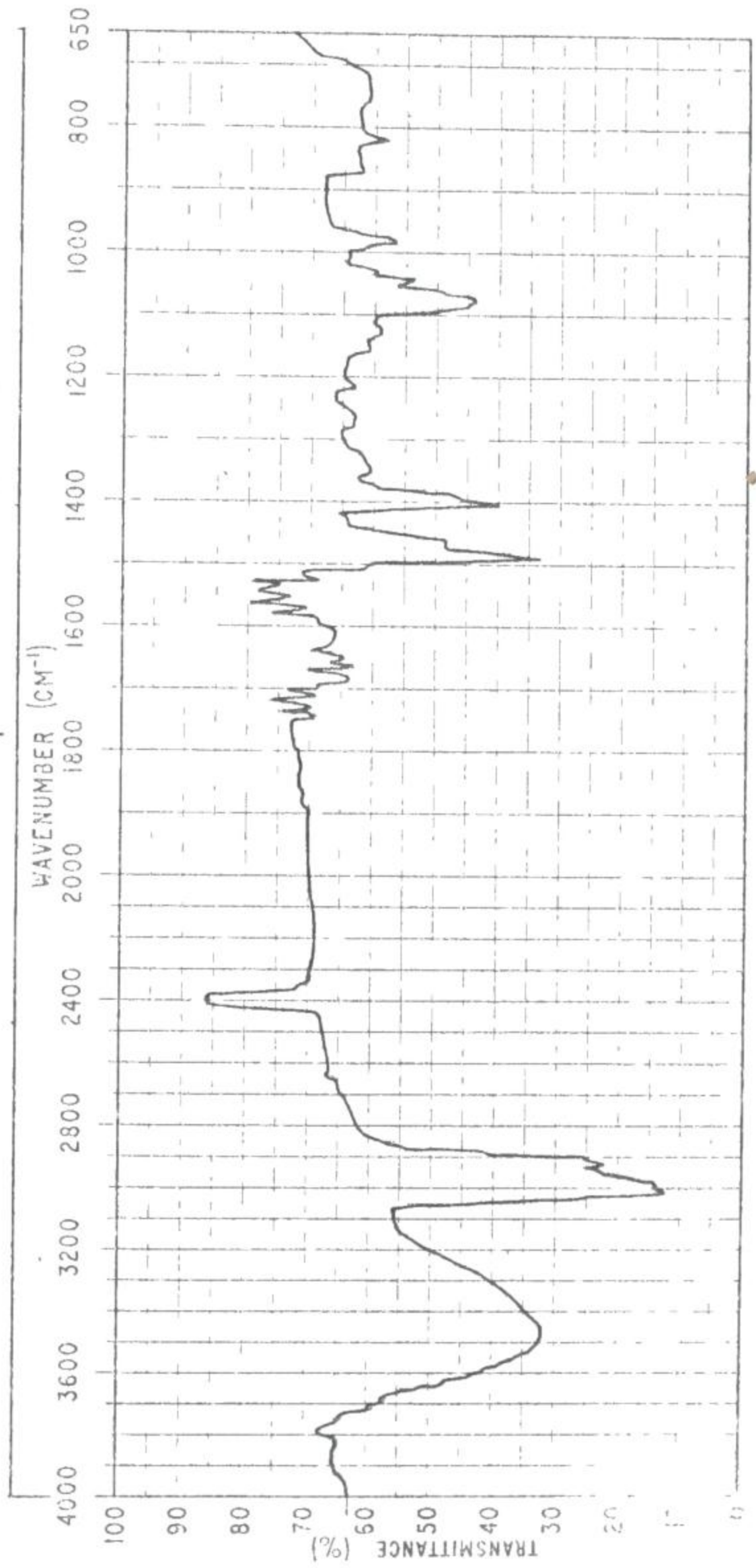


FIG. XII.



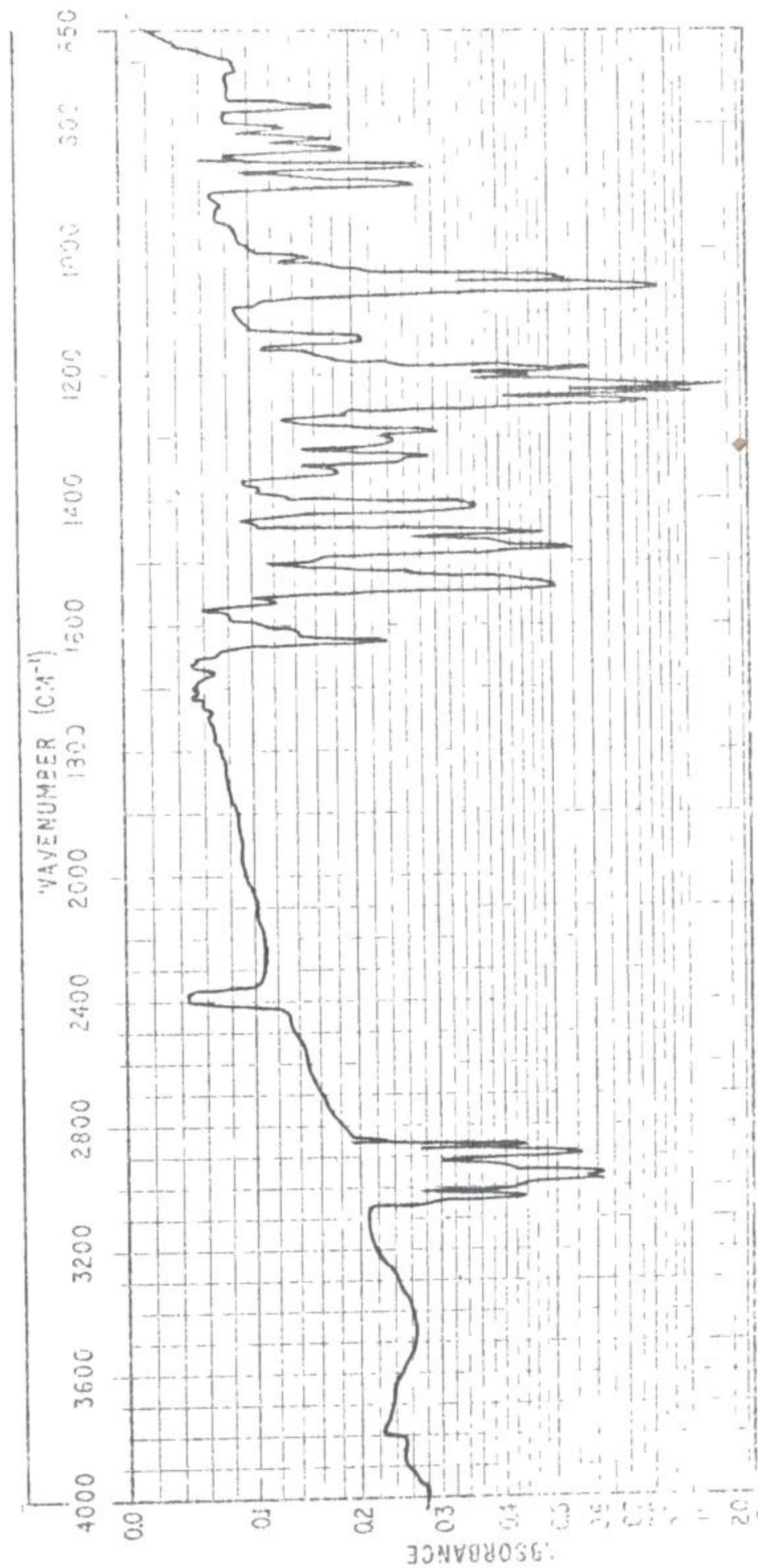
CRMDIT

SPECTRUM NO.

REMARKS	PERKIN-ELMER MODEL 700	
	ORIGIN	
	PURITY	
	SAMPLE	1

IR SPECTRUM OF

IR SPECTRUM OF C5

FIG. X^{IV}.

REMARKS	PERKIN-ELMER	
	ORIGIN	SPECTRUM NO.
IR SPECTRUM OF ALKALOID (CII)	PURITY	SAMPLE

SAMPLE _____ SPECTRUM NO. _____

IR SPECTRUM OF ALKALOID (CII)

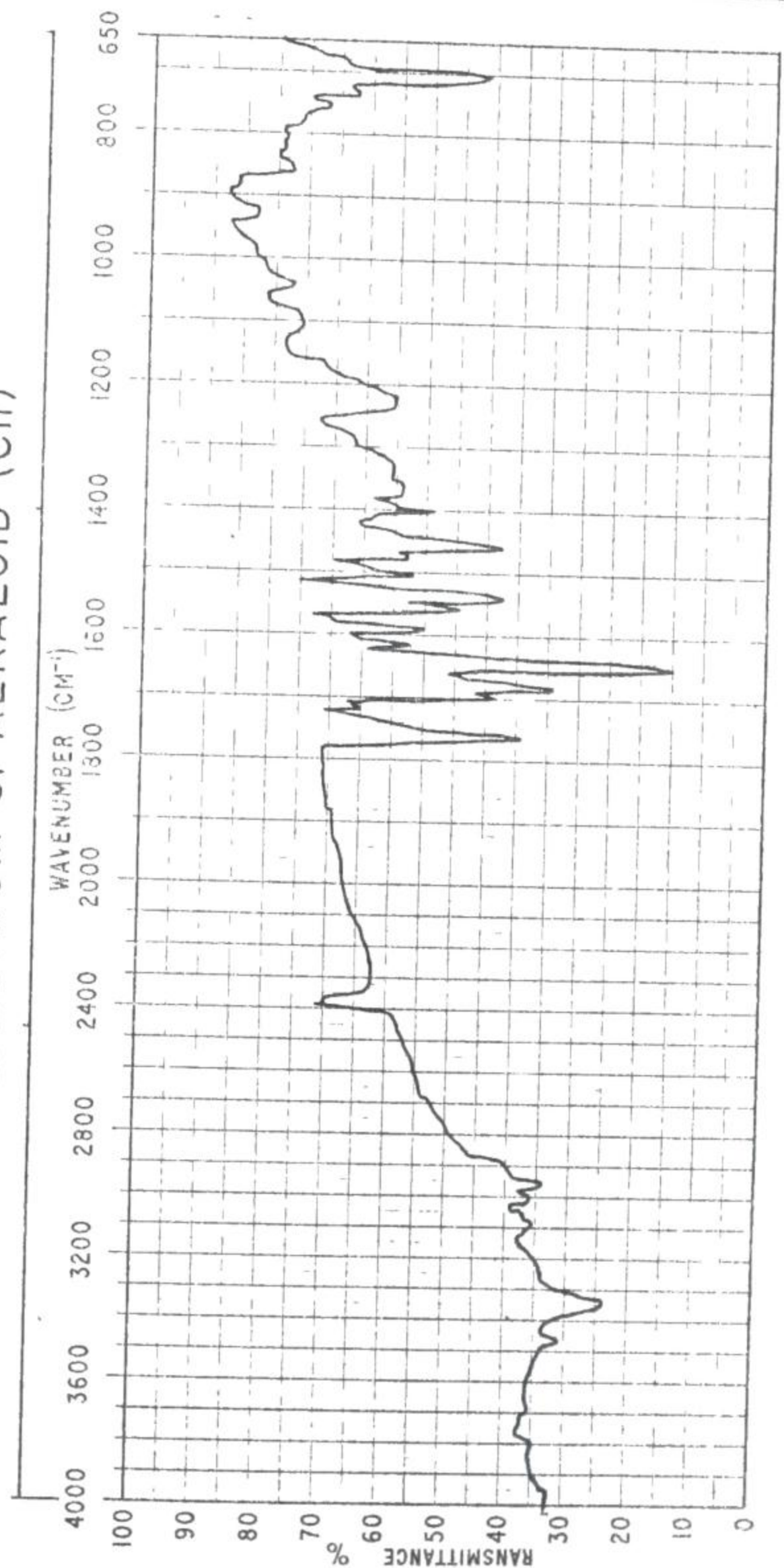


FIG. X IV.



SAMPLE _____

SPECTRUM NO. _____

PERKIN-ELMER

ORIGIN _____

SPECTRUM NO. _____

PURITY _____

SAMPLE N^o R9:2

IR SPECTRUM OF ALKALOID (R9)

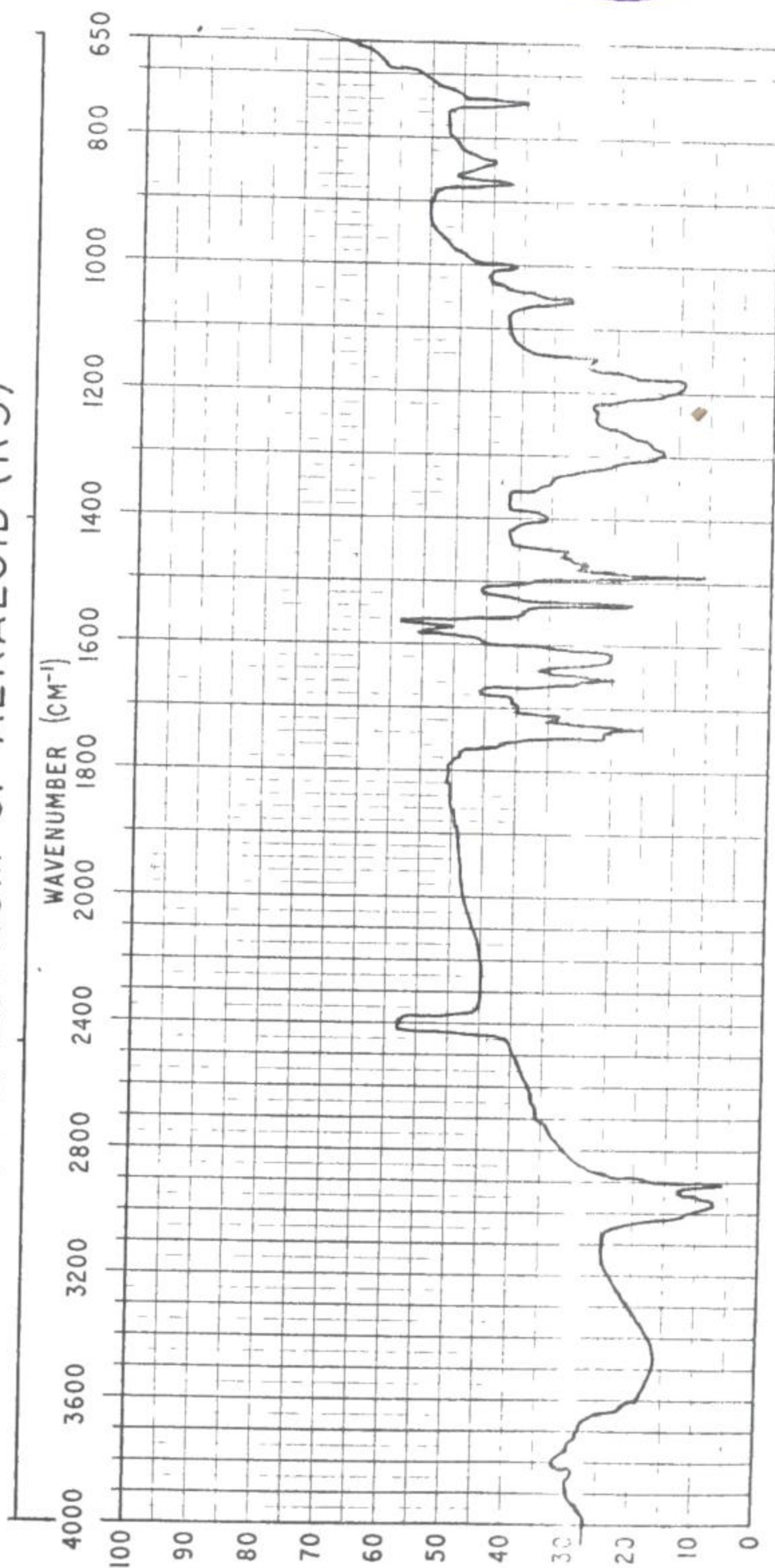


FIG. XV.

REMARKS

ORIGIN

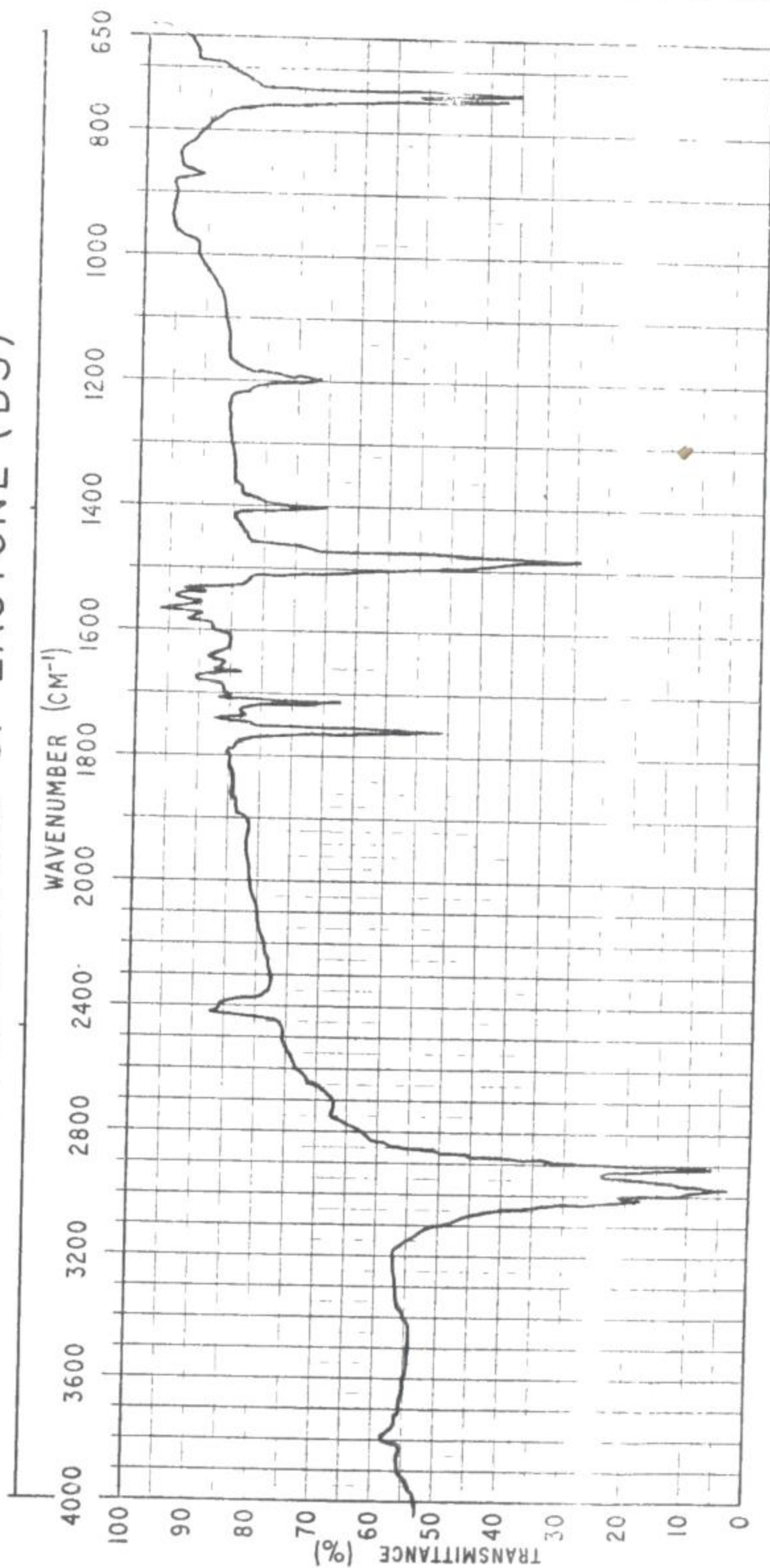
PERKIN-ELMER

PURITY

SPECTRUM NO.

SAMPLE 1

IR SPECTRUM OF LACTONE (D3)



SPECTRUM NO.

FIG. XVI.

APPENDIX III

PMR spectra of Compounds.

FIG. XVII PMR spectrum of Piperine

FIG. XVIII PMR spectrum of Piperettine

FIG. XIX PMR spectrum of Stigmasta-4,22-dien -3 -ol.



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