EXTRACTION OF WATER SOLUBLE ALKALOIDS FROM HUNTERIA UMBELLATA

BY

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M.Sc.. Chem. (Lagos)

A DISSERTATION IN THE DEPARTMENT OF CHEMISTRY

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF PHILOSOPHY OF SCIENCE OF THE UNIVERSITY OF LAGOS

SCHOOL OF POSTGRADUATE STUDIES
UNIVERSITY OF LAGOS

CERTIFICATION

THIS IS TO CERTIFY THAT THE THESIS -

EXTRACTION OF WATER SOLUBLE ALKALOIDS FROM HUNTERIA UMBELLATA

SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES

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PHILOSOPHY OF SCIENCE

IS A RECORD OF ORIGINAL RESEARCH CARRIED OUT BY

MRS FELICIA OLATUBOKUN OGUNSULIRE

IN THE DEPARTMENT OF

CHEMISTRY

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ABSTRACT

This thesis describes the extraction, isolation, purification of water soluble alkaloids from the seed, leaf and heart bark of the plant Hunteria Umbellata. Characterization of the alkaloids was achieved via spectroscopic methods. A total of ten carbazole alkaloids were isolated, two from the seeds and four each from the leaves and bark. Those from the seed and one from the leaves are inferred from the data available to be dimeric. Structures are proposed for most of them, but the lack of some essential facilities do not allow for an unambiquous structural assignment.

ACKNOWLEDGEMENTS

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Finally, I thank God Almighty for providing me with good health and courage throughout my stay in the Chemistry Department, University of Lagos.

CERTIFICATION

I certify that this work was carried out by Mrs. Felicia Olatubokun Ogunsulire in the Department of Chemistry, University of Lagos, Lagos, Nigeria.

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DEDICATION

TO GOD WITH GRATEFUL

THANKS.

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CHAPTER 1

1.1 HISTORY OF ALKALOIDS:

The use of alkaloids is almost as old as civilization. Mankind has used drugs containing alkaloids in potions, medicines, teas, poultices and poisons for over four thousand years, yet no attempts have been made to isolate any of the therapeutically active ingredients from the crude drugs until the early nineteenth century.

The first crude drug to be investigated chemically was opium, the dried latex of the poppy Papaver somniferum. Opium had been used for centuries in popular medicine, and both its analgesic and narcotic properties were well known. In 1903, Derosane isolated a semi pure alkaloid from opium and named it narcotine[1]. Serturner in 1805 isolated morphine from the same plant and he was the first to identify its basic character.

Between 1817 and 1820 Pelletier and Caventon isolated nine different alkaloids. These are strychnine, emetine, brucine, piperine, caffeine quinine, cinchonine and colechicine. These alkaloids with a wide spectrum of biological activities are the cornerstone of all that has transpired in alkaloid chemistry in the past one hundred and sixty years.

In 1826, Pelletier and Caventon also obtained coniine an alkaloid of considerable historical significance . Not only is the alkaloid responsible for the death of Socrates

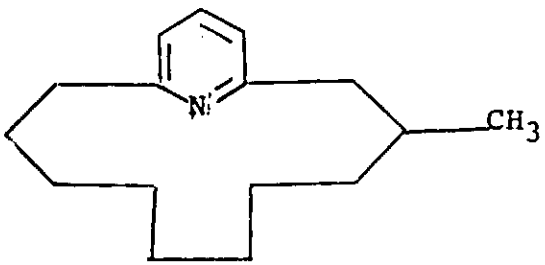
from a draught of poison hemlock, it also has simple molecular structure. It was the first alkaloid to be fully characterized (1870) and the first to be synthesized (1886).

The molecular complexity of the majority of these alkaloids precluded an early structure elucidation. For example, strychnine was first obtained in 1819 by Pelletier and Caventon but it took nearly one hundred and forty years of extremely arduous, very frustrating chemical investigation before the structure was finally determined in 1946 by Robinson and co-workers.

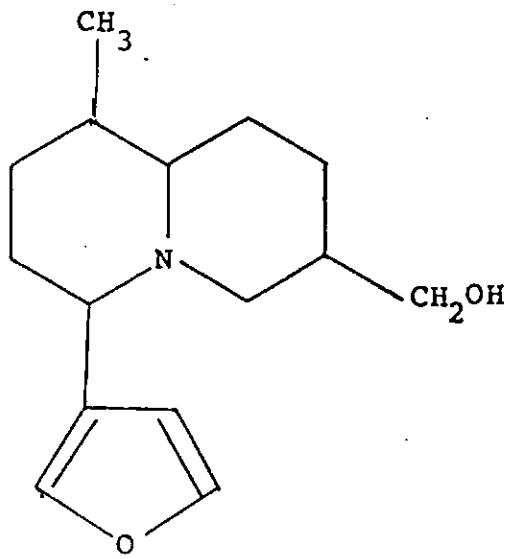
By 1939 nearly three hundred alkaloids had been isolated of which two hundred had well defined structures. A review to the middle of 1973 counted 4959 alkaloids of which 3293 had known structures. By late 1978, the number stood nearly at 4000 . i.e. those having defined structures.

1.2 NATURAL OCCURRENCE OF ALKALOIDS

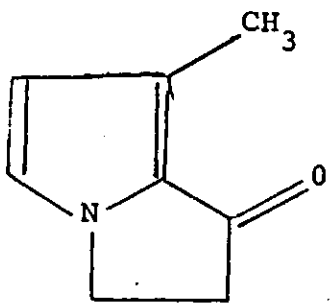
The major source of alkaloids in the past has been the flowering plants, the angiosperms. In recent years, there have been increasingly numerous examples of alkaloids occurring in animals, insects, marine organisms, micro-organisms, and the lower plants. Some examples of these are muscopyridine (1) from the musk deer; castoramine (2) from the Canadian beaver; the pyrrole derivative (3), a sex pheromone of several insects, saxitoxin (4) the neurotoxic constituent of the red tide Gonyaulax catenella, pyocyanine (5) from the bacterium Pseudomonas aeruginosa; Chanoclavine -1 (6) from the ergot fungus, Claviceps Purpurea; and lycopodine (7) from the genus of club mosses, Lycopodium[2].



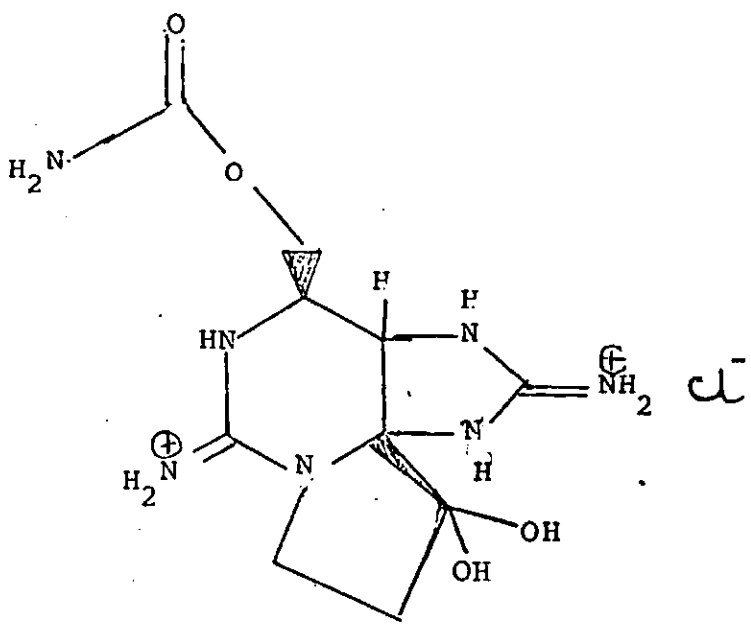
Muscopyridine
(1)



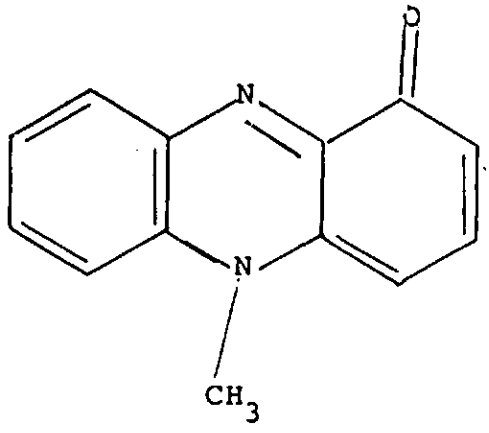
Castoramine
(2)



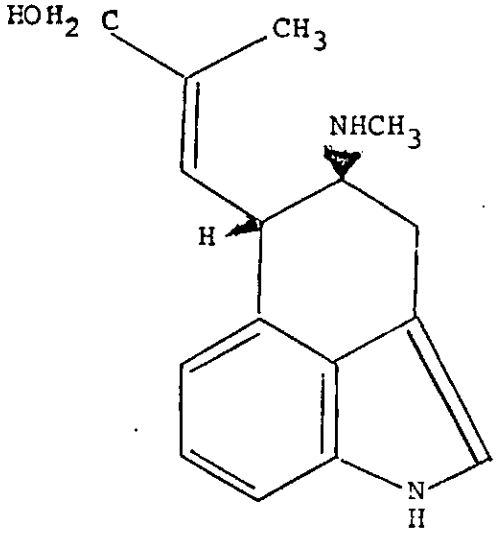
Pyrrole derivative
(3)



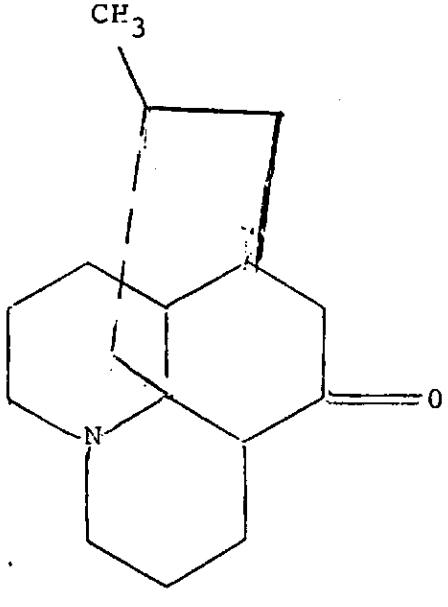
Saxitoxin cation
(4)



Pyocyanine
(5)



Chanoclavine - 1
(6)



Lycopodine
(7)

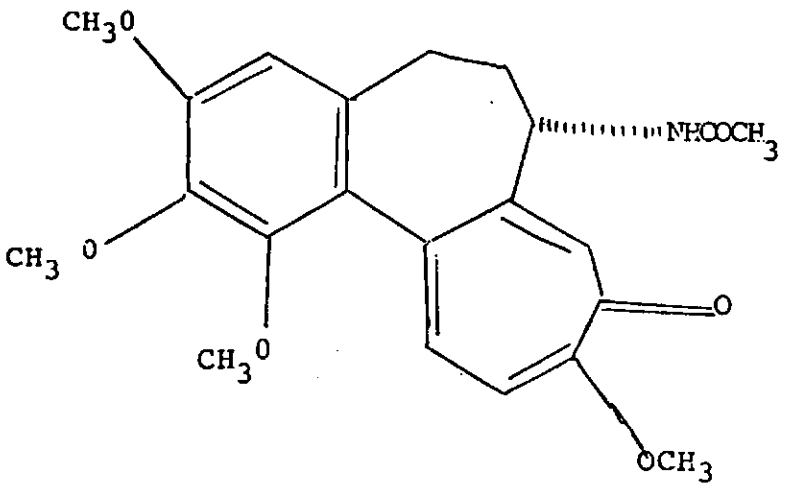
Within a given alkaloid - containing plant, the alkaloids may be highly localized (concentrated) in a particular plant part. For example morphine, occurs in the latex of Papaver somniferum. This does not necessarily mean that the alkaloids are formed in that part of the plant. For example alkaloids in Datura and Nicotiana species are produced in the roots but are translocated rapidly to the leaves. In addition plants in the same genius may even produce the same alkaloid in different plant parts.

1.3 CLASSIFICATION OF ALKALOIDS

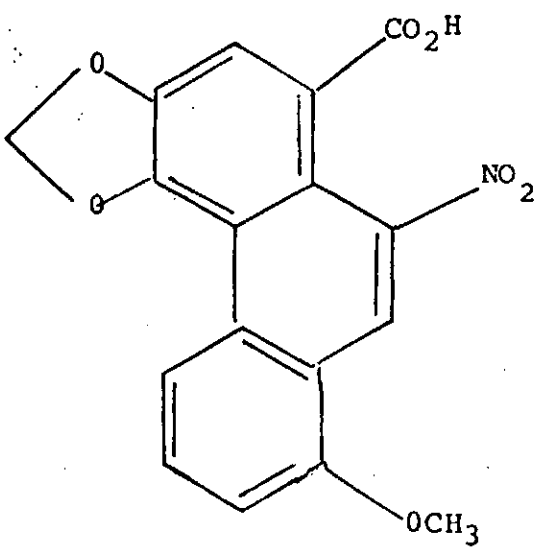
Alkaloids are grouped into (a) true alkaloids, (b) protoalkaloids, and (c) pseudoalkaloids.

True Alkaloids are often basic and are organic compounds with a wide range of physiological activity. They normally contain nitrogen in a heterocyclic ring. They are derived from amino acids of limited taxonomic distribution, and they normally occur in the plant as the salt of an organic acid. Some exceptions to these are colchicine (8) and aristolochic acid (9). They are not basic and have no heterocyclic ring. Quarternary alkaloids are acidic rather than basic.

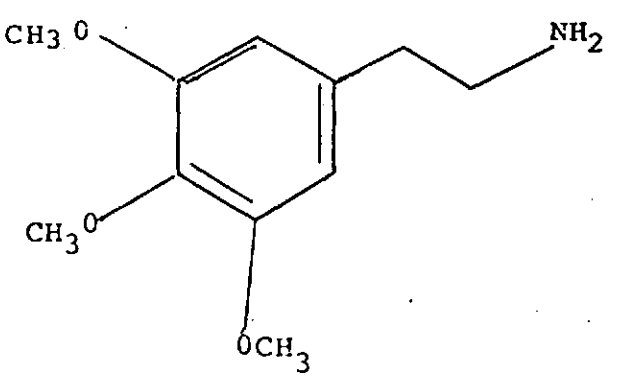
The Protoalkaloids: The protoalkaloids are simple amines in which the amino acid nitrogen is not in a heterocyclic ring. They are biosynthesized from amino acids and are basic. Often, the term "biological amines" is used for this group of compounds, e.g.mescaline (10),ephedrine (11), and N, N-dimethyl tryptamine (12),



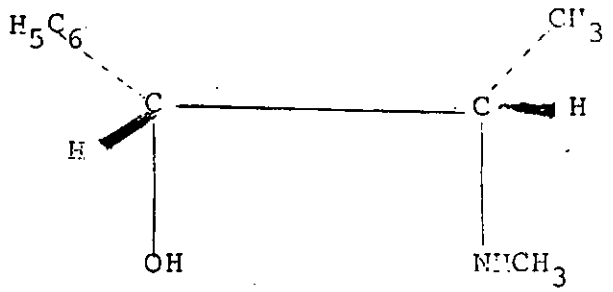
colchicine
(8)



Aristolochic acid
(9)

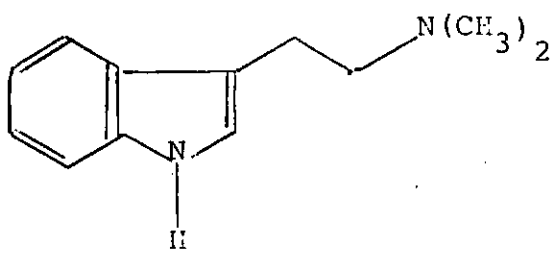


Mescaline
(10)



Ephedrine

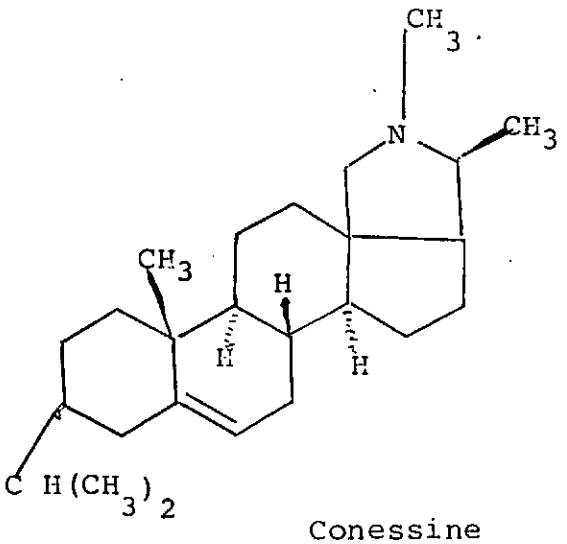
(11)



N,N-Dimethyltryptamine

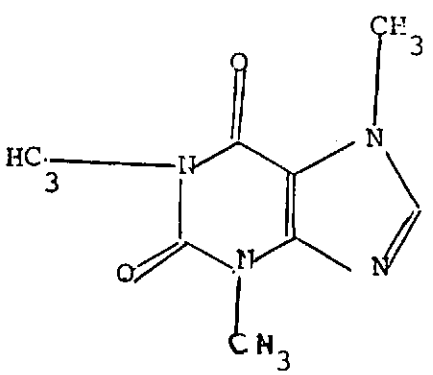
(12)

The Pseudoalkaloids: The pseudoalkaloids are not derived from an amino acid precursor. They are usually basic and only two series are important e.g. Conessine (13) and the purines e.g. Caffeine (14).



Conessine

(13)



Caffeine
(14)

1.4 NOMENCLATURE OF ALKALOIDS

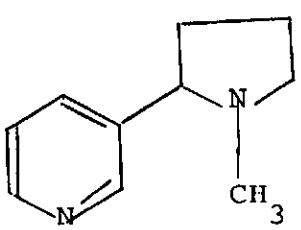
The only common characteristic of alkaloid nomenclatures is the suffix "ine". Like other natural products, they are also given "trivial" (i.e. nonsystematic) names.

They may derive from genus names (e.g. atropine from Atropa belladonna); from the species name (e.g.) cocaine from Erythroxylon coca; from a common name for the drug e.g. ergotamine from ergot; from the physiological action of the compound e.g. emetine, an emetic or from the name of a famous alkaloid chemist e.g. pelletierine.

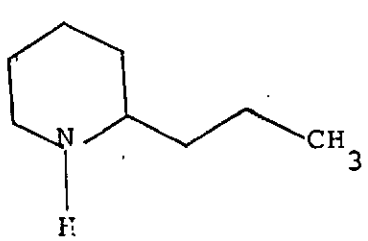
1.5 PHYSICAL PROPERTIES OF ALKALOIDS

Most isolated alkaloids are crystalline solids with a defined melting point or decomposition range. A few are amorphorus gums, and some, such as nicotine (15) and coniine (16) are liquids.

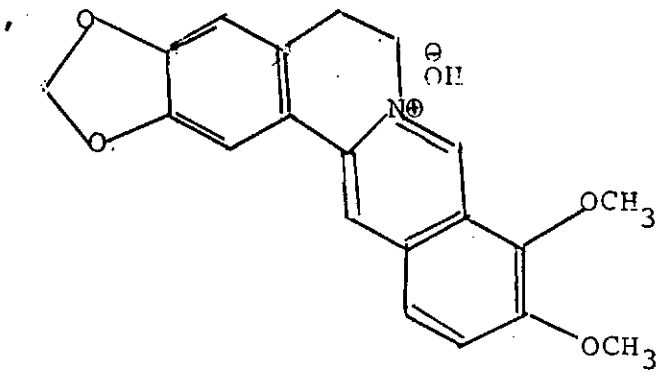
Most alkaloids are colourless, but some of the complex, highly aromatic species are coloured e.g. berberine (17) is yellow and betanin (18) is red.



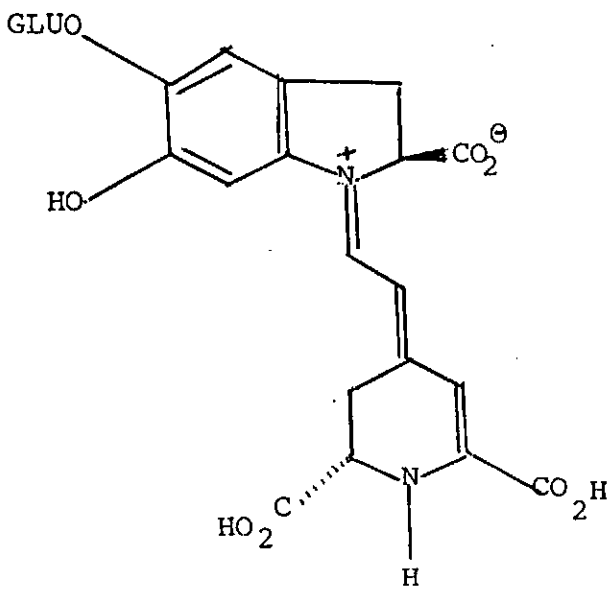
Nicotine
(15)



Coniine
(16)



Berberine
(17)



Betanin
(18)

The solubility of the alkaloids and their units is of considerable significance in the pharmaceutical industry, both in the extraction of the alkaloid from the plant, or

fungus, and in the formulation of the final pharmaceutical preparation. In general the free base of the alkaloid is soluble only in an organic solvent, although some of the pseudo and proto-alkaloids are substantially soluble in water. The salts of alkaloids and the quarternary alkaloids are normally highly water soluble.

1.6 Detection of Alkaloids in Plant Material

The most distinct chemical property of most alkaloids is that they are basic. Two methods are probably the most reliable for the screening of potential alkaloid, containing plants. The wall procedure involves the extraction of dried plant material with refluxing 80% ethanol. After cooling and filtering the residue is washed with 80% ethanol and the combined filterates evaporated. This residue is taken up in water, filtered, acidified with 10% hydrochloric acid, and the alkaloid precipitated either with Mayer's reagent or with silicotungstic acid. If either test is positive, a confirmatory test is made in which the acid solution is basified. The alkaloids are extracted into organic solvent. Then the alkaloids are back extracted into aqueous acid. If this acid solution gives a precipitate with either reagent, the plant contains alkaloids. The basified aqueous phase should also be examined for the presence of quarternary alkaloids.

The Kiang-Douglas procedure is somewhat different in that the alkaloidal salts present in the plant (normally citrates, tartarates, or lactates) are first converted to free bases by moistering the dried plant material with dilute aqueous ammonia. The alkaloids are then extracted with chloroform. The extract is concentrated, and the alkaloids are removed as their hydrochlorides by the addition of 2N hydrochloric acid. The filtered aqueous solution is then screened for alkaloids by the addition of Mayer's, Dragendorff's or Bouchardat's reagent.

The methods described above have their limitations. One disadvantage of the second procedure is that the quarternary ammonium compounds, which are not converted to their free bases by the addition of ammonia, remain in the plant material and are not detected. Similarly, in the standard Wall procedure, quarternary alkaloids appear as "false-positive" since they are not extracted into organic solvent in the acid-base partition.

The reagents used to precipite these alkaloids are based on the property of most alkaloids to combine with high atomic weight metal salts such as those of mercury, bismuth or tungsten, or with complex iodides. Mayer's reagent, contains potassium iodide and mercuric chloride. Dragendorff's reagent contains bismuth nitrate and potassium iodide in aqueous nitric acid. Bouchardat's reagent is similar to Wagner's reagent and contains potassium iodide and iodine and reacts by halogenation.

The silicotungstic acid reagent contains a complex of silicon dioxide and tungsten trioxide.

Chromatography on a suitable absorbent is the normal method for separation and the isolation of pure alkaloids from the crude mixtures. Like other natural products, the column fractions are conveniently monitored by thin - layer chromatography (TLC).

Detection Chromatographically:

One general reagents used for the detection of alkaloids chromatographically is Dragendorff's reagent, which in the form of a spray produces orange - coloured spots for alkaloidal materials. Other reagents used are phosphomolybdic acid, iodoplatinate, iodine, vapour and antimony (III) chloride.

Detection by U. V. Lamp:

Many chemicals may be excited to fluoresce when irradiated by light with a wavelength of between 250 and 360 nm. Once located a permanent record can be made photographically or by simply outlining the detected zones with a pencil line.

Alternatively, fluorescence quenching may be employed. The silica adsorbent used incorporates a fluorescent indicator which absorbs light at 254 nm and re-emits light in the green end of the spectrum. Any compound that absorbs in the 254 nm region will quench the fluorescence and show

up as a dark spot against a green background.

This alternative method is the method used in the present work. Each impure compound was spotted on a thin layer sheet which is 0.25mm silica gel containing fluorescent indicator UV_{254}. Two UV lamps of wave length 360 nm and 254 nm were used. The former excited the fluorescent indicator and the latter induced fluorescence in some of the alkaloids being isolated.

Ehrlich's reagent which is acidified p-dimethyl - amino-benzaldehyde gives a quite characteristic blue or gray-green colour with the ergot alkaloids. Acidified (surphuric or phosphoric acid) ceric ammonium sulfate (CAS) reagent yields different, quite distintive colours with many indole alkaloids. The colours are dependent on the ultraviolet chromophore of the alkaloid and are therefore of quite considerable structural significance.

CHAPTER 2

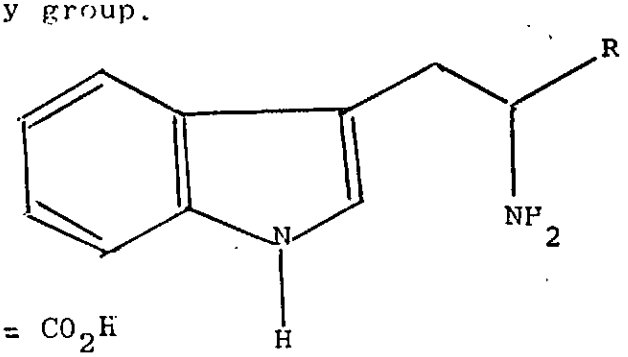
2.1 BIOSYNTHESIS OF MONOTERPENOID INDOLE ALKALOIDS

The plant Humteria umbellata (K. Schum) is a member of the Apocynaceae family. This family of plant contains an indole or related fragment and a rearranged monoterpene unit. Apocynaceae is also grouped with the Loganiaceae and Rubiaceae families on the basis of the isolation of structurally similar alkaloids.

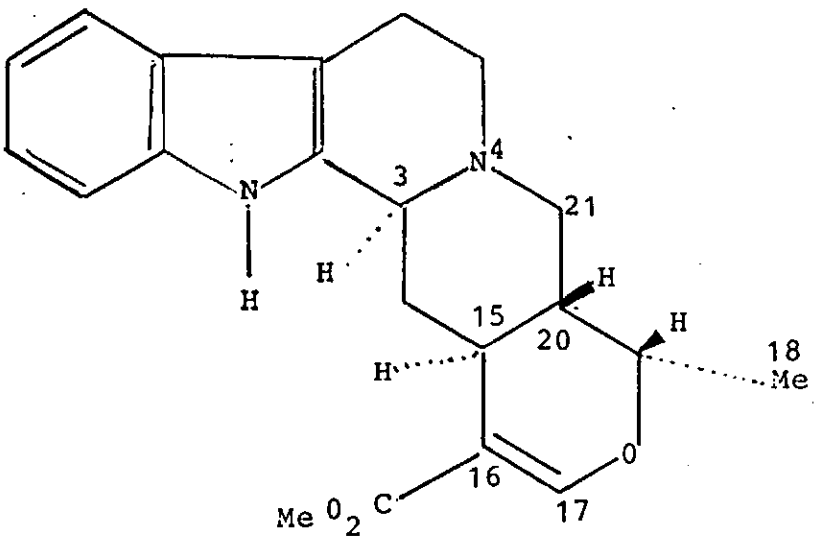
Common to all the monoterpenoid indole bases is a tryptamine unit which has its genesis in tryptophan[3-5] (19) via tryptamine[6,7] (20). The enormous variation in terpenoid indole structure is associated not with the tryptamine unit but with the remaining monoterpene unit and its derivative.

The terpenoid units reveals broadly three major types, first that of the corynanthe-strychnos group, for example, adjalicine (21) and akuamicine (22) where the unit is simplified as in (28) second, that of the Aspidosperma group, e.g. vindoline (23) with the C_9/C_{10} fragnenthas been established as being monoterpenoid unit represented by (29a) and (29b); and the third Iboga group, e.g. Catharanthine (24).

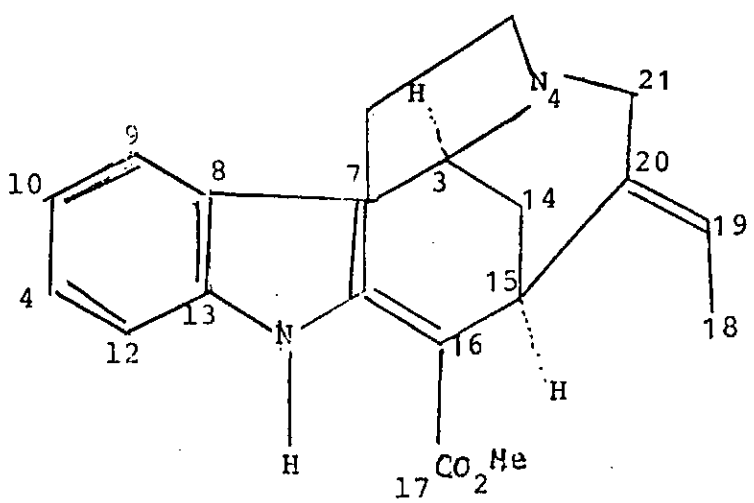
The relationship of these skeletal types to a common cyclopentane monoterpene skeleton is illustrated in scheme 1, in which the tenth atom (C-22), which is sometimes missing, is depicted as a carbomethoxy group.



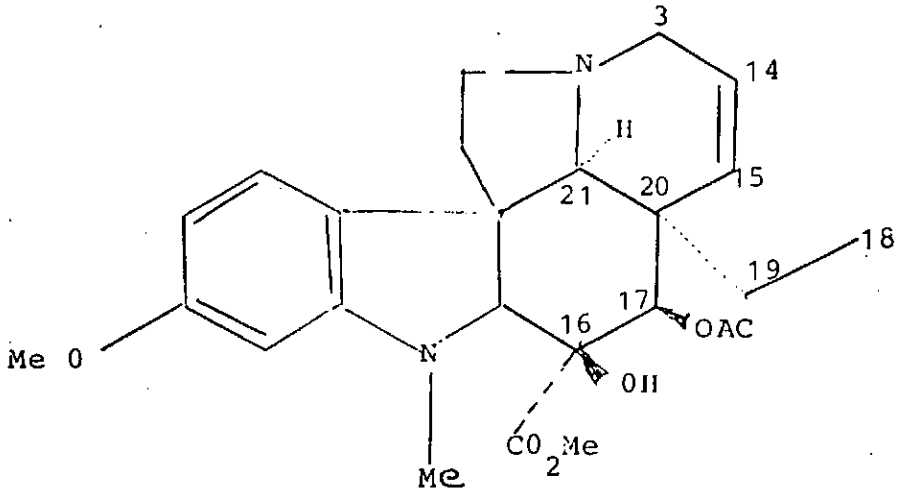
(19) R = CO_2H
(20) R = H.



Ajmalicine
(21)

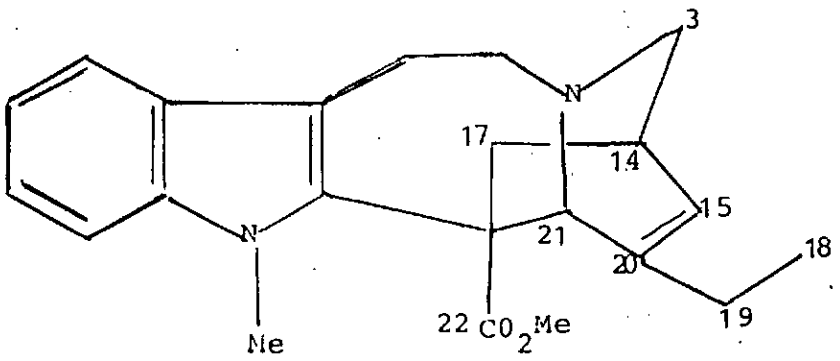


Akuammicine
(22)



Vindoline

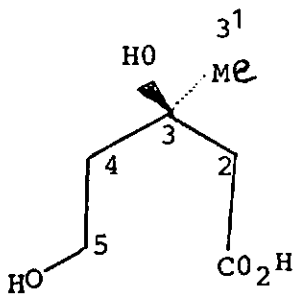
(23)



Catharanthine

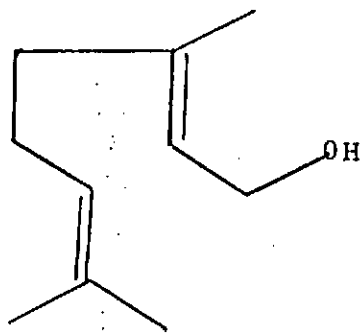
(24)

Scheme 1



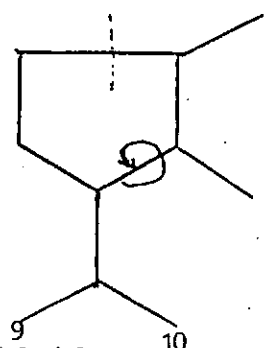
Mevalonic Acid

(25)



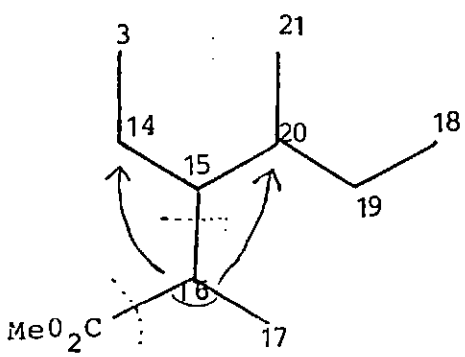
Geraniol

(26)



Iridoid Skeleton monoterpene

(27)

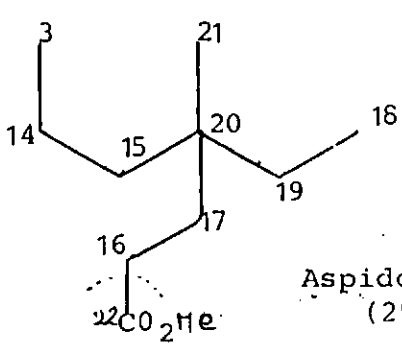


Corynanth-estrychnos

(35)

(35)

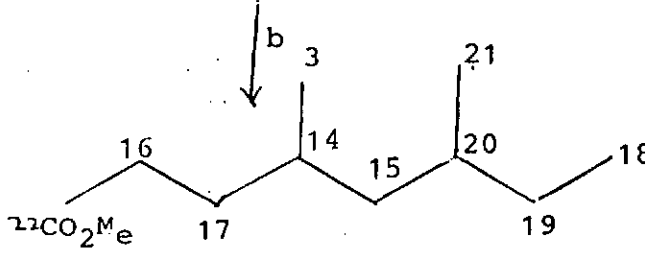
a



Aspidosperma

(29a)

b



Iboga

(29b)

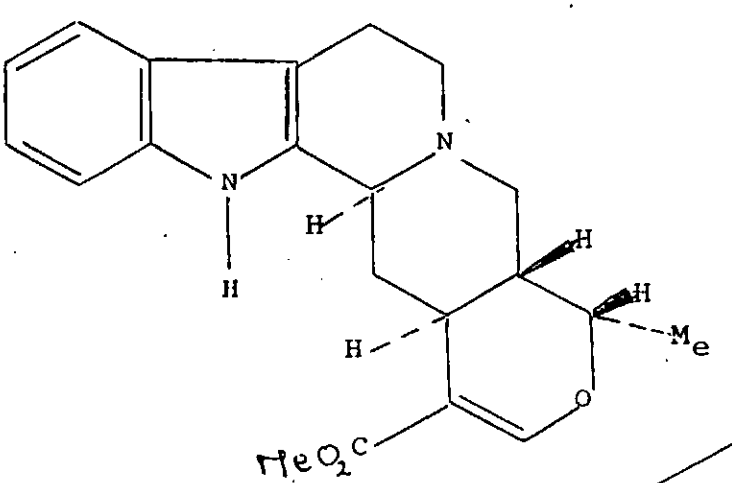
Essential proofs for the C_9/C_{10} units are terpenoid in origin and related in the way shown has come from extensive and rigorous experimentation. It was established that the C_9/C_{10} units of the three groups of alkaloids are each derived from two molecules of mevalonic acid[5,8-11] linked initially in the normal head to tail fashion, elaborated along a path-way which includes geraniol (26)/nerol (38)[9-12] and the cyclopentane monoterpene, loganin (32a)[12,13].

Not only was loganin (30), in contrast to three other cyclopentanoid monoterpenes, a specific precursor, but also its biosynthesis from geraniol and its presence in Catharanthus roseus G. Don, the plant used for most of the experiments (Scheme 2; (31) has like (21), the skeleton of type (28). These results secure loganin as an intermediate in monoter-penoid indole alkaloid biosynthesis. It stands as a key compound along the biosynthetic pathway.

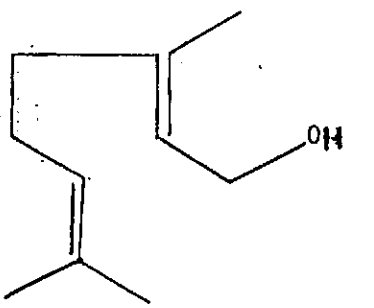
Similar to other systems, the biosynthesis of the alkaloid monoterpene unit is from (3R) - mevalonic acid not the (3S) isomer[11]. The transformation of C-2 or C-3[1] of mevalonate through C-9 and C-10 of the intermediate (27) into alkaloids was observed to occur with loss of identity between these termini, as observed in the biosynthesis of cyclopentanoid monoterpenes.[14]

It has also been established[15] experimentally that deoxyloganin (33) should be sited as an intermediate in the biosynthesis before loganin (30) and that the hydroxy deriva-tives (39a) and (39b) of geraniol (26) and nerol (34) be included in the path-way[14,15] scheme 3.

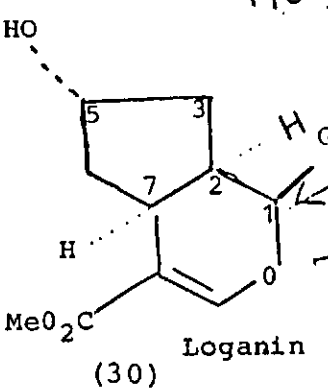
Scheme 2



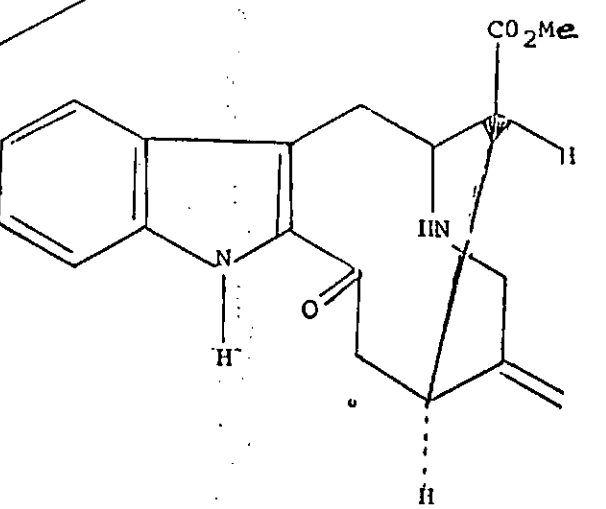
Ajmalicine
(21)



Geraniol
(26)

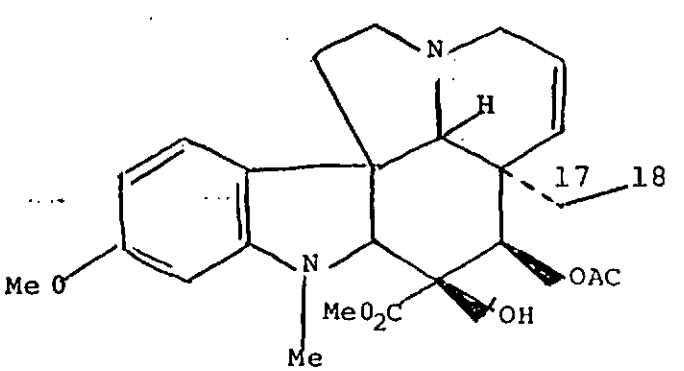


Loganin
(30)

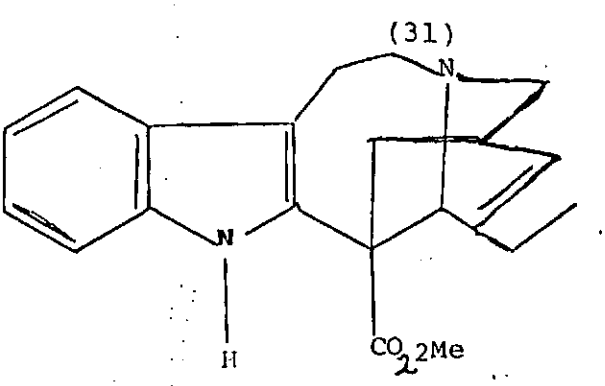


Perivine

(31)



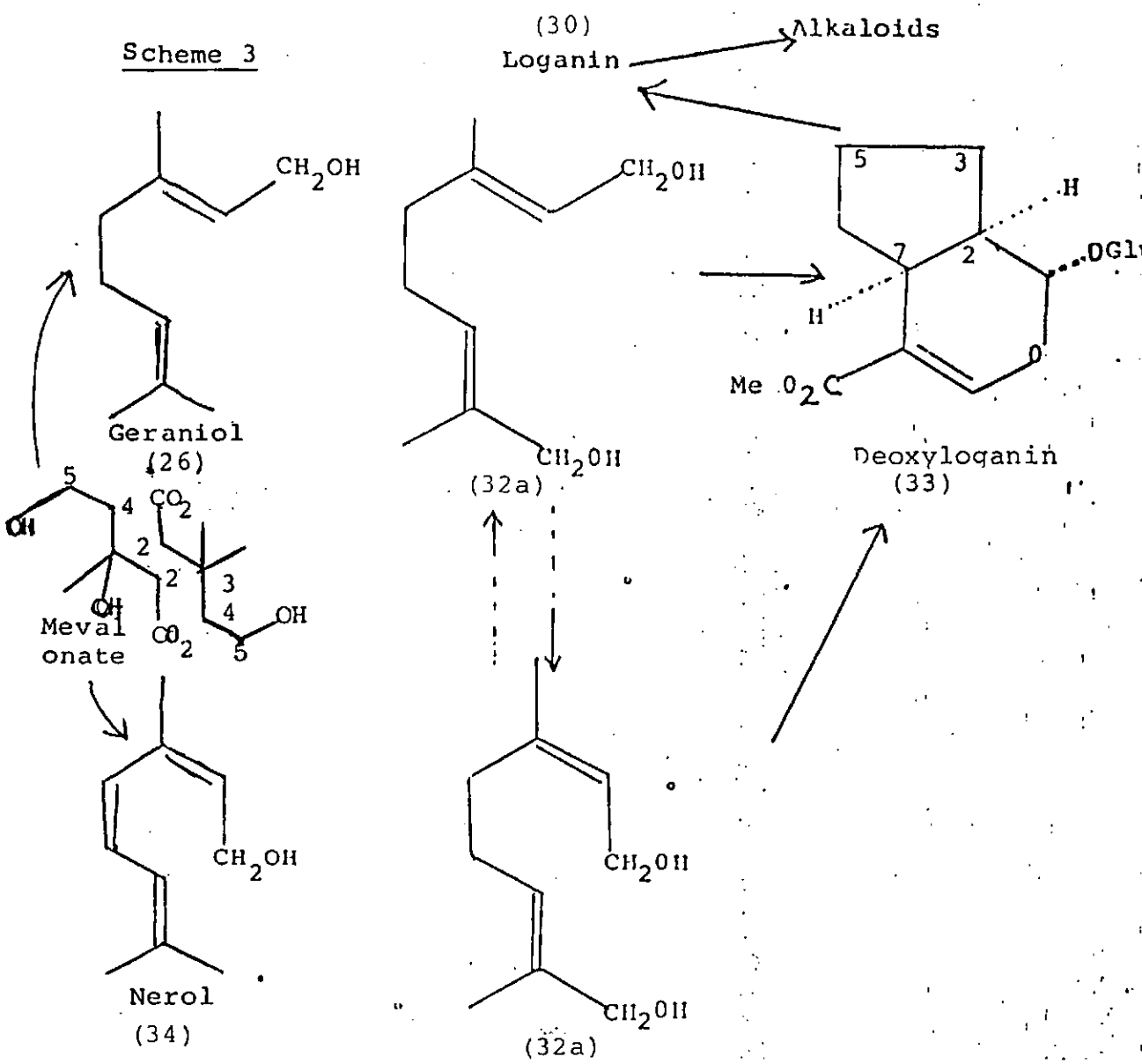
Vindoline
(23)



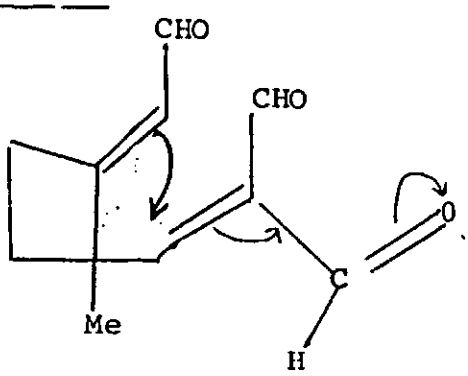
Catharanthine
(24)

The failure of various other derivatives of geraniol and nerol to act as precursors[15,16] restricted the range of possible intermediates beyond (32a) and (32b) and this led to plausible mechanism for cyclization via trialdelyde (Scheme 4) which accounted for the observation that label passing from the mevalonate through C-9 and C-10 of the a cyclic terpenes was equally distributed between the corresponding positions in loganin and the alkaloids[15,17].

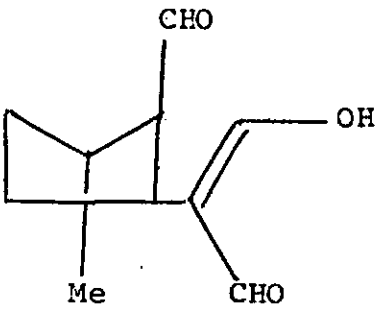
Scheme 3



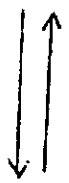
Scheme 4

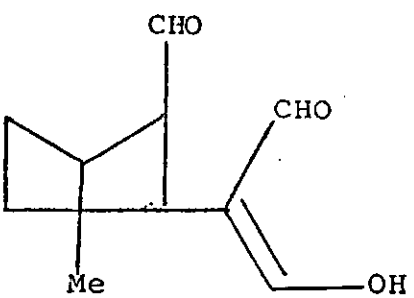


(35)

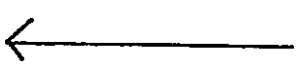


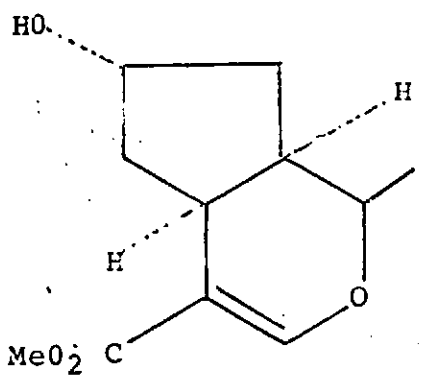
(36)





(37)





Loganin

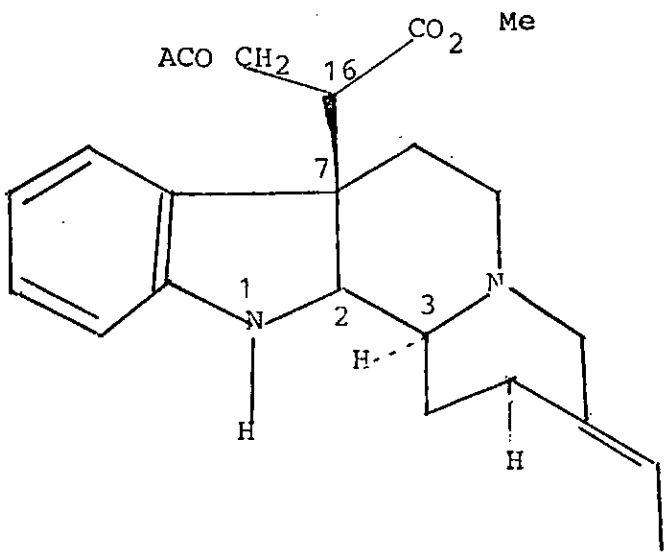
(30)

2.2 SUMMARY OF RECENT WORK ON AKUAMMILINE GROUP OF MONOTERPENOID INDOLE ALKALOIDS

The Akuammiline Group:

Alkaloids with a 7, 16 bond

Most of the alkaloids known to be present in Hunteria umbellata are of the structural type havig C7 - C16 bond; it is relevant therefore to review this group of indole alkaloids. There are between 40 and 50 indole bases having a common carbon skeleton formally derivable from the corynanthine type by the introduction of an extra carbon-carbon bond between C-7 and C-16. Akuammiline (38)[18] represents the prototype in its skeletally most straight forward form.

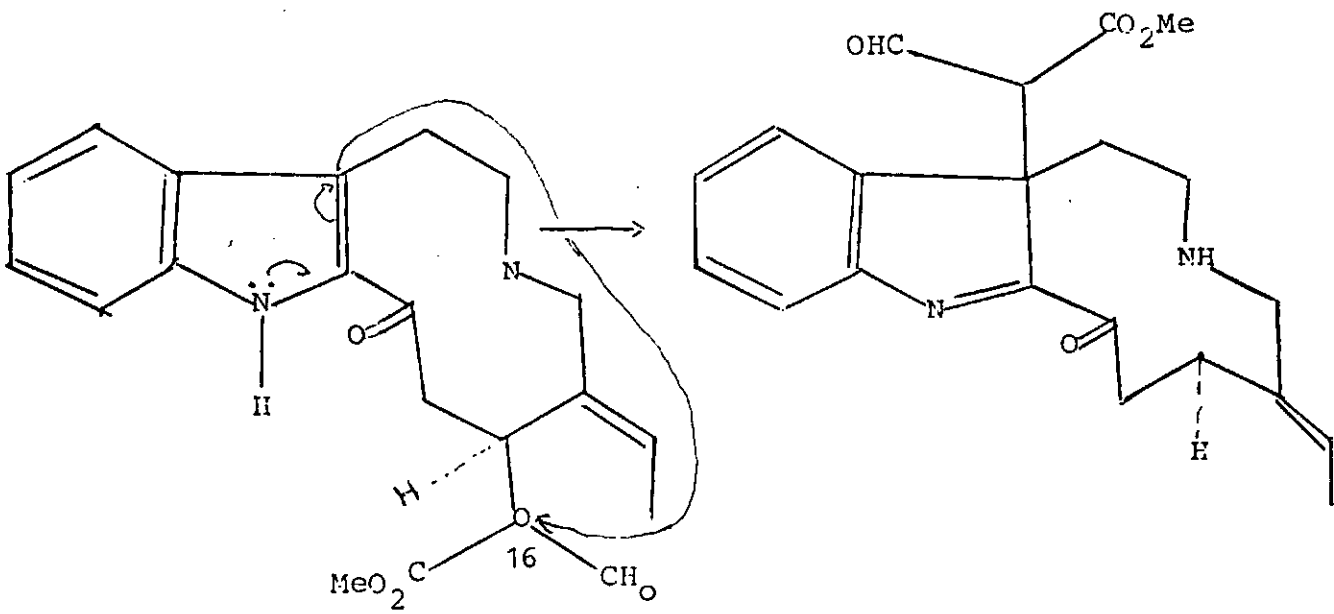


Akuammiline

(38)

The mechanism of the biosynthetic formation of the 7, 16 bond poses an intriguing question, for in a corynanthine type precursor, C-7 as an indole β position and C-16 as a

potential enol/enolate site are nucleophilic. 90% of the known bases in this group have a C-3 oxygen substituent. These alkaloids may be produced by a nucleophilic attack as shown in the equation below:

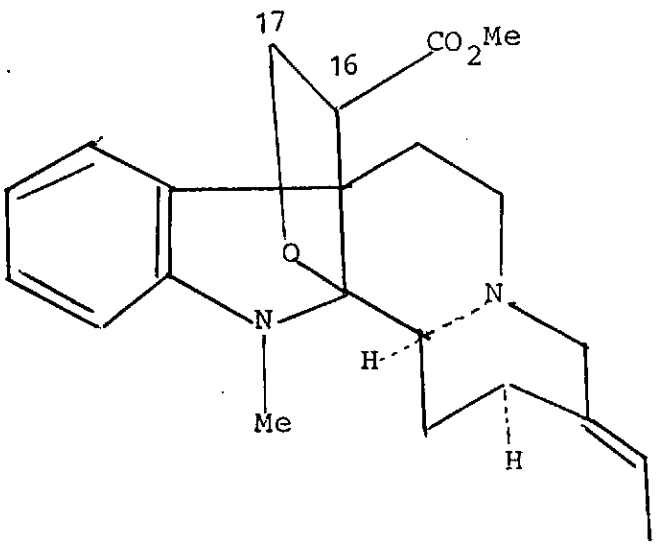


(39) (40)

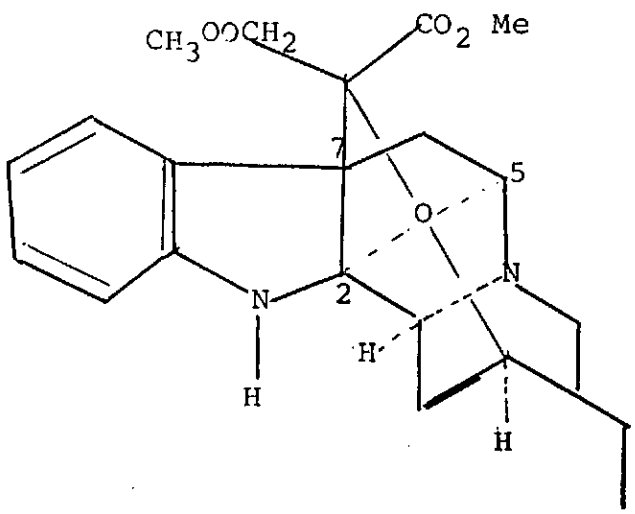
Besides the akuammiline type there are nine other variations in which, although the carbon skeleton remains unchanged (leaving aside the occasional absence of C-17 or C-22), additional ether links are present and/or bonds to N_a or N_b have been broken and/or made.

Pseudoakuammigine (41)[19], picraline (42)[20] and quaternoline (43)[21] (from Alstonia quaternata v. Henrcket Muell, Arg.) with structures established by x-ray crystallography differs from akuammiline only in having an extra oxygen - containing ring, between C-2 and C-17, C-2 and C-5, and C-20 and C-22 respectively.

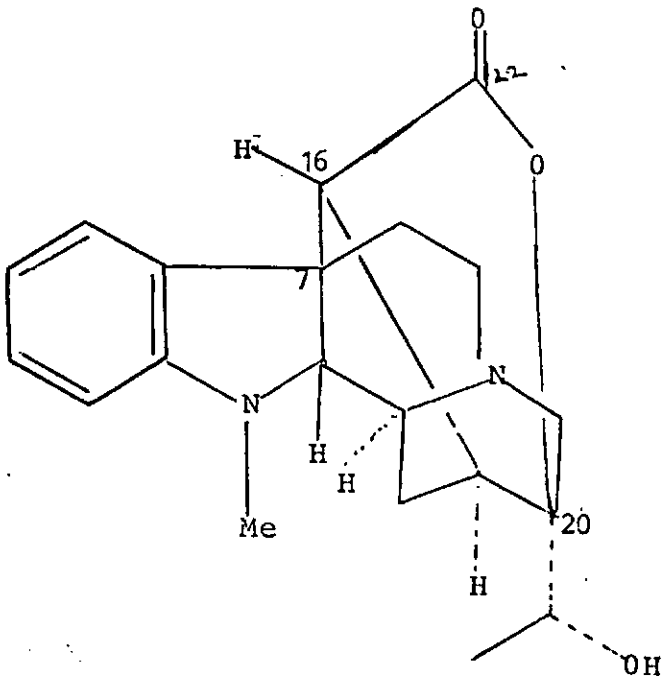
In echitamine (44)[21] a 2, N_b bond replaces the 3, N_b; the corymine (45a)[23] type is derivable from this by the formal introduction of a cyclic hemiacetal, formed from a hydroxyl group at C-3 and a C-17 aldehyde group. The absence of a 21, N_b bond characterizes the 7th and 8th variations, exemplified in such a way as to emphasize their structural relationship to the other alkaloids in this group. Both alkaloids also have additional oxygen - containing rings, in which C-3 is attached via oxygen to C-22.



Pseudoakuammigine
(41)

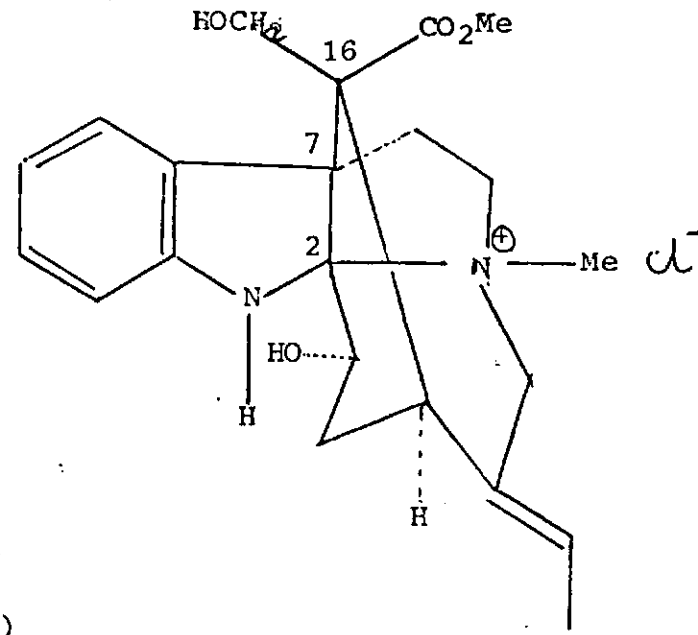


Picraline
(42)

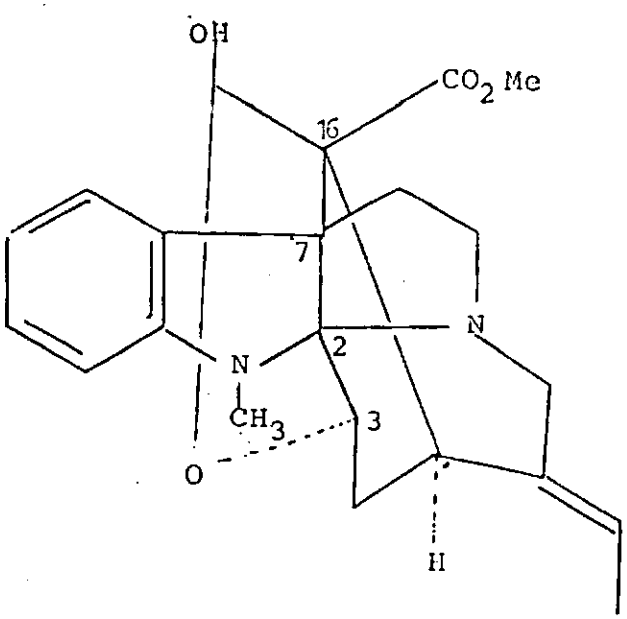


Quaternoline (= Rancubaine)

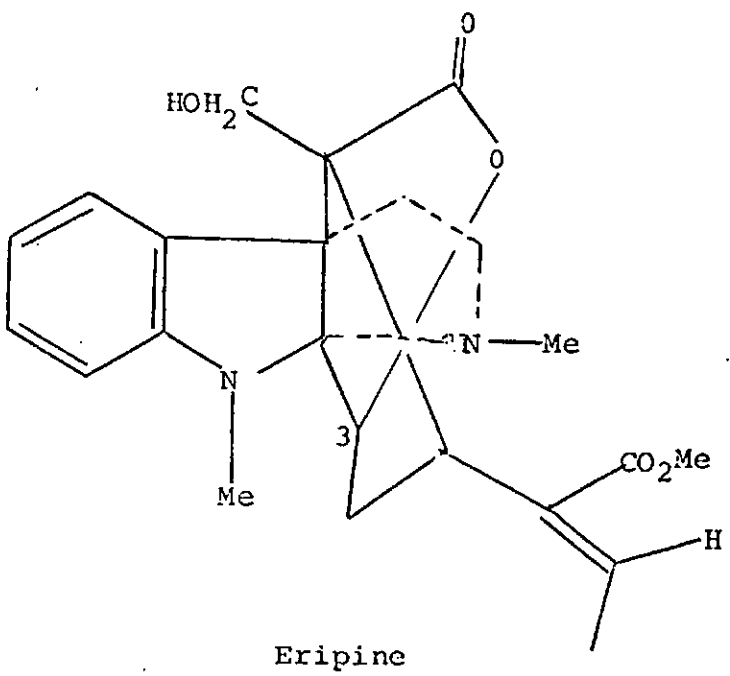
(43)



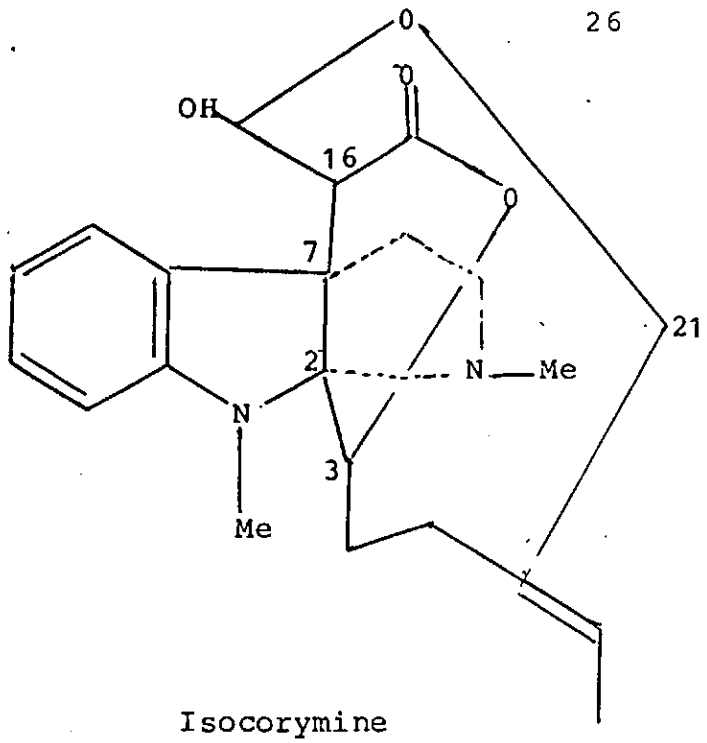
Echitamine Chloride
(44)



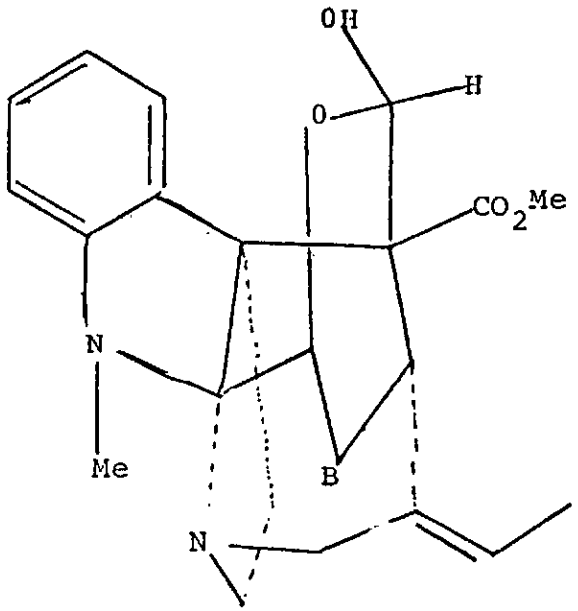
Corymine
(45a)



Eripine
(46)

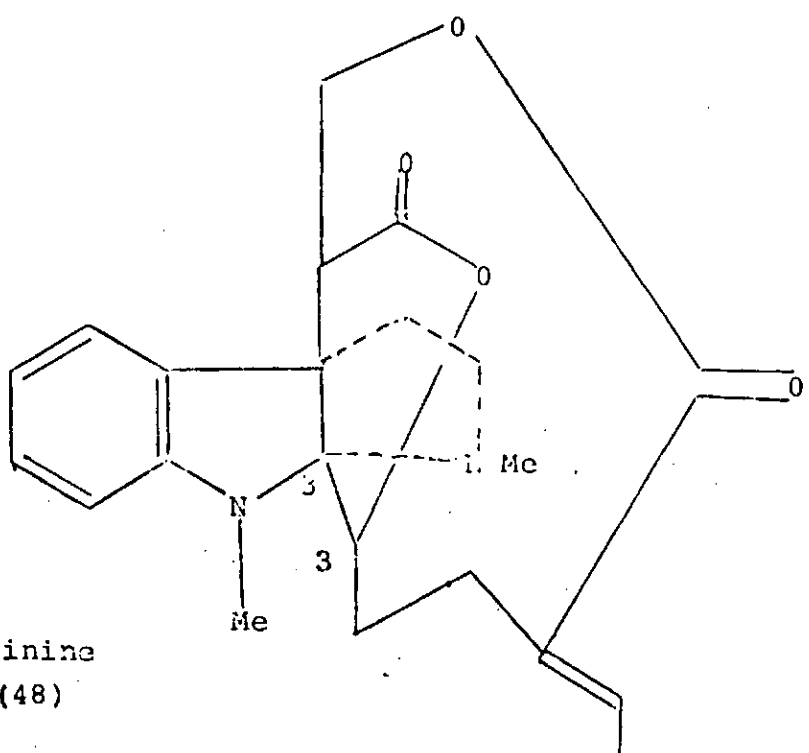


Isocorymine
(47)



Corymine

(45b)

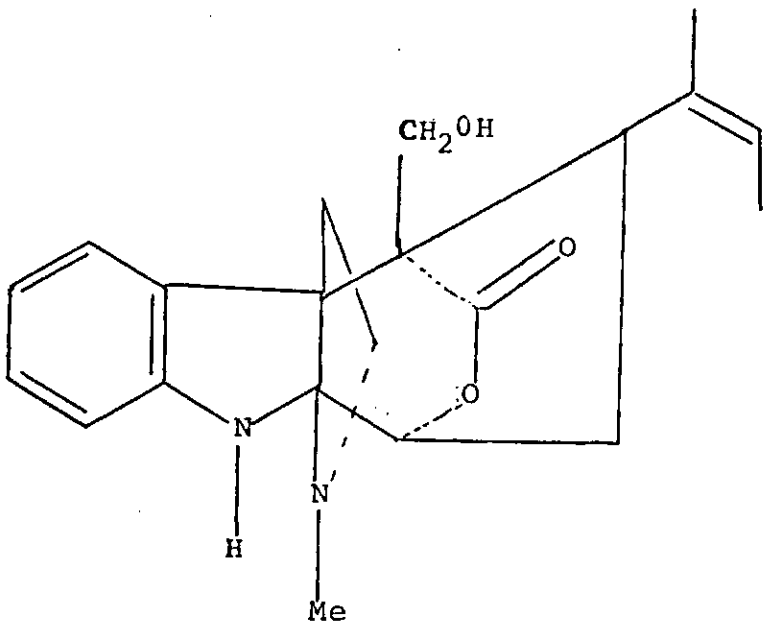


Erinine
(48)

Structural studies by ^{1}Hnmr and mass spectrometric analysis showed that corymine and 0-acetyl corymine from Hunteria umbellata (K. Schum) Hall. f. contain N_a.C. N_b. system. The former was shown by X-ray crystallographic analysis to have the structure (45a - 45b) closely related to that of echitamine, but one which also incorporates a cyclic hemiacetal, formed from the C-3 hydroxyl group and an aldehyde group attached to C-16.

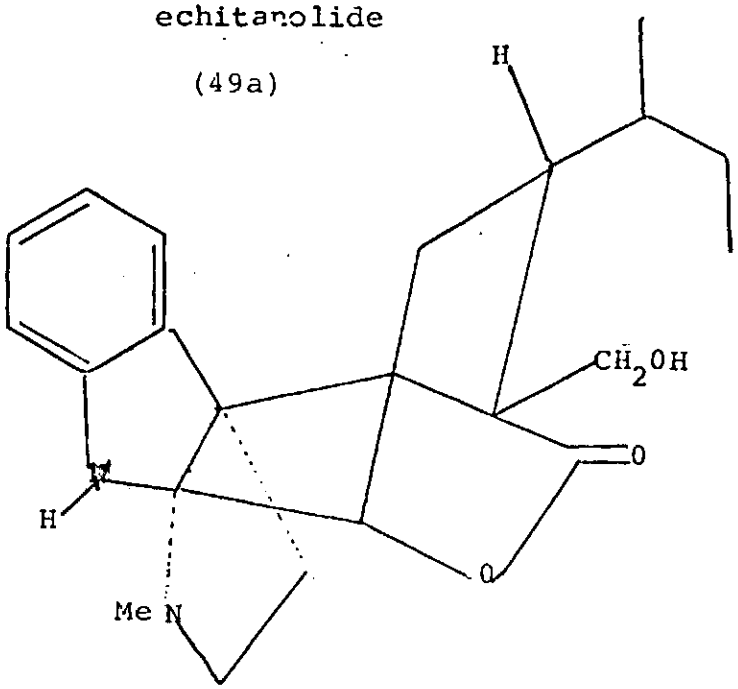
Erinine (48) and erinicine (57) (19, 20-dihydro erinine) are two other alkaloids of Hunteria umbellata which also incorporate N_a. C. N_b units. Their structures were demonstrated[26] mainly by mass spectra comparisons with echitanolide ( ) and derivatives. Eripine (29)[25], another base from this plant, could be thermally cyclized to a mixture of erinine and an unnatural 19, 20 double bond isomer. Isocorymine (from Hunteria umbellata) has a related structure (47)[27].

While retaining the ubiquitous configuration at C-15, (as in structure on page 32) the complex ring system of erinine, erinicine, and isocorymine can be constructed in two ways, both stereoisomers differ in the configurations at the pair of centres, C3 and C16. A priori, all three alkaloids could have either of the stereo - chemistry, especially since in this sub group, alkaloids epimeric at C-16 have been isolated. The conformation depicted in (47), (48) and (49b) shows one of the two C-3/C-16 possibilities.



echitanolide

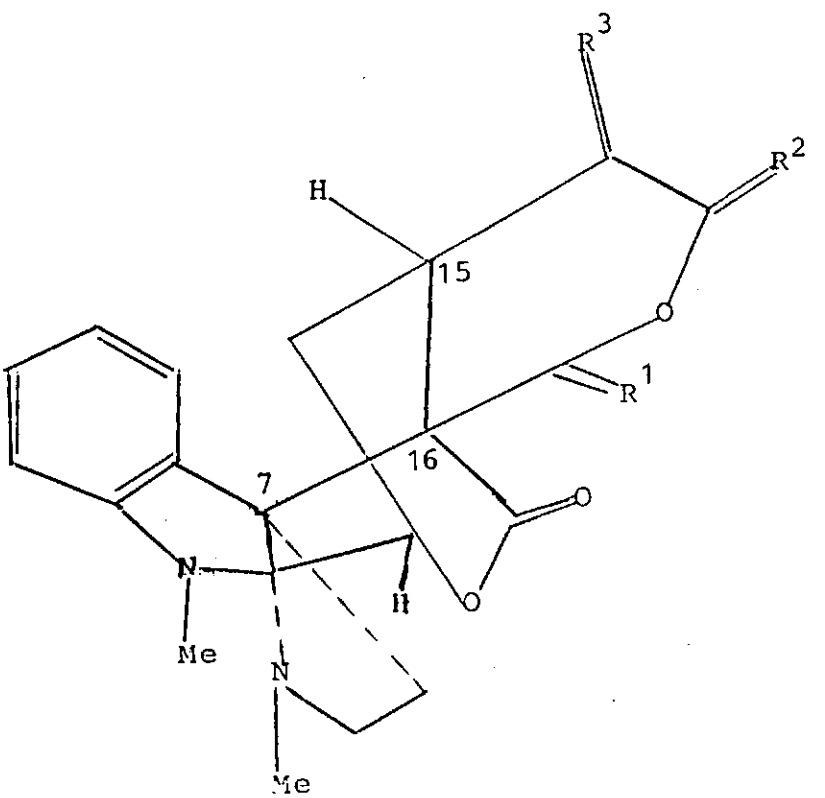
(49a)



echitanolide

(49b)

Conformations (47), (48), (50) show configuration at C-3 and C-16, whereas (47) emphasizes the configuration at C-17 and the geometry about the 18, 19 double bond in isocorymine (erinnine has the same configuration about the double bond); and (50) illustrates the configuration at C-20 in erinicine. This study shows that three - dimensional detail, approaching that available from x-ray crystallographic studies in the solid state, can be achieved in suitable cases by nmr measurements in solutions.



		R^1	R^2	R^3
Isocorymine	(47)	H, OH	H_2	CHMe
Erinine	(48)	H_2	0	CHMe
Erinicine	(50)	H_2	0	H, Et.

CHAPTER 3

3.0 BIOLOGICAL ACTIVITY

The most characteristic biological effect of these drugs is the arrest of cell division at the metaphase, in a manner resembling the effect of colchicine. There is an attack on the spindle itself which then undergoes attrition and finally disappearance. These changes may be reversible, chromosomes may adopt unusual metaphase configurations[26-29]

Cytitoxicity was first recognized in the P1534 leukaemia system[30], but a wide range of experimental animal tumors respond to drugs of this class.[31] The most sensitive tumors are P388 and P1534 leukaemia Ehrlich, Freund, Sarcoma 180 and Walker 256 ascites tumors, Ridgeway osteogenic sarcoma, and B82A leukaemia; vinblastine and vincristine are generally the most active alkaloids against these tumors.

The drugs block progression of cells through the cell cycle during mitosis, but appear to exert greatest cytotoxicity on cells in the DNA synthetic S phase[32].

Antiviral activity has been demonstrated for vinblastine, vincristine, leurosivine, leurosidine, and desacetyl vinblastine, but not for leurosine and lochnerinine. Interestingly, the monomeric alkaloid apparicine, which is not cytotoxic, does have antiviral activity.

Neuromuscular activities are common among this group of alkaloids, and in the case of vincristine are the dose

limiting side effects. Vindesine is also markedly neurotoxic. Depression of deep tendon reflexes, paresthesias of the extremities, cranial and sensory nerve involvement constipation, paralytic ileus, muscle weakness [34], and reduced nerve conduction velocities [35] have all been reported.

In rat, skeletal muscle vincristine causes a fall in calcium uptake and alterations in the phospholipid composition of the microsomes [36]. The uptake of norepinephrine by brain synaptosomes is inhibited by vinblastine, and there is evidence for other autonomic effects that may alter cardiovascular function.

In terms of the effects on the behaviour of mice, vincristine, desacetyl vinblastine, and leurosidine are central nervous system depressants, whereas leurosine behaves as an adrenergic blocking agent.

Among other actions of these alkaloids, tetratogenesis has been well established in animals, although there is no evidence of mutagenicity in the Ames test or of direct damage to chromosomes [37]. Vincristine may produce inappropriate secretion of antidiuretic hormone, reduced secretion of thyroid hormone [38], insulin, [39] renin [40], and plasma lipoproteins [41]. Vinblastine is known to bind to, and cause aggregation of, ribosomes [42,43] Of these cytotoxic alkaloids, only leurosine causes a delay on depression of blood sugar levels, the activity for which the plant extracts were originally assayed, this alkaloid also produces transient hypotension probably by α - adrenergic blockade [44].

3.1 Biological Activities of Indole Alkaloids

Like any other alkaloid, the indole alkaloids process biological activities. It is relevant to this thesis to review the biological activities of some of the more important known alkaloid salts. Also since some of the Hunteria alkaloids under study were probably dimeric, this chapter also briefly reviews the most important biologically active known dimeric indole alkaloids. In most cases the activity depends on the structure of the alkaloid. Interesting biochemical reactions are shown by these group of alkaloids resulting from usually complicated metabolism.

3.2.1 STRUCTURE - ACTIVITY RELATIONSHIP

Bisindole skeletons appear essential for cytotoxic potency in alkaloids. Only two of the known monomeric alkaloids, lochnericine and lochnerinine are the ones found to have even a very modest activity[45]. Apropriate stereochemical configuration about the Catharanthine - vindoline linkage is very critical, and failure to achieve this has been the major obstacle to the synthesis of active bisindoles. This has been over come[46,47]. Among other features that have been identified as being important for cytotoxic potentiality are the need for a basic nitrogen in the catharanthine nucleus, the requirement for at least one free hydroxyl group, and partial loss of activity after reduction of the 14, 15 double bond or dehydration at the 16, 17 position. Changes of 16 and 17 substituents only modify the spectrum of activity.

3.2.2 Biochemical Actions

The many biological effects produced by these alkaloids suggest the existence of a number of different biochemical interactions. Two of the latter, interaction with microtubule system and inhibition of biosynthetic pathways, are particularly important, and may explain most of the biological effects.

The microtubule system consists of tubular elements of diameter about 250°A in eukaryote cells, often singly, but usually in groups near cell membranes or intracellular orga-

nelles, or as components of structures such as flagella or the mitotic spindle[48-50]

Tubules apparently containing tubulin, the basic component of eukaryote microtubules, have also been found in certain spirochetes which are prokaryotes[51]. It would appear that the microtubule system is primarily involved in maintaining rigidity and some forms of motion.

Microtubules in neurons, termed neurotubules, are associated with the movement of mitochondria and vesicles[52] whereas in the mitotic spindle a combination of poleward sling and opposite end assembly and disassembly may be responsible for mitotic movements[53].

Drugs sc such as the Vinca alkaloids resemble colchicine in being able to bind tightly to tubulin, although at different sites, and interfere with the functioning of the microtubule system. For the Vinca alkaloids, it appears that the configuration at C-14' and C-16' as well as the presence of methoxy-carbonyl at C-16' play essential roles in the microtubular interaction . Since the polymerized tubulin in the form of microtubules and formed tubular structures is in equilibrium with the tubulin pool diminution of the latter by complex formation with drugs soon depletes the whole system at rates dependent on the kinetics of the various equilibria, this interaction, which involves both high affinity and low affinity sites, has been reviewed[55,56].

The low affinity sites relate to microtubule crystal formation. Recent findings indicate that the alkaloids actually inhibit the polymerization of tubulin[57]. In addition, the site for binding of vinblastine has cysteine residues in its immediate vicinity[58]. Most striking has been the identification of an endogenous tubulin-binding protein in rat brain that inhibits polymerization of the protein to microtubules, and completes with colchicine for binding[59]. This may be a natural regulator for the microtubule system much like the endorphanoplate receptor complex. It is quite logical to ascribe many of the common biological effects of the Vinca alkaloids - mitotic arrest, interference with phagocytosis and secretion, and the neurologic toxicity to interaction with microtubules that are major elements in these processes.

Interference with biosynthetic pathways may be a contributing factor to the cytotoxicity of the Vinca alkaloids. Published reports show that DNA, RNA, and protein, synthesis may all be inhibited to various degrees in different systems[60]. In general, much higher levels of drugs are needed than those that block mitosis or lead to significant binding to tubulin when in vitro systems are studied, but these biochemical effects have been observed in animals treated with therapeutic doses. The underlying mechanisms are not clear, but reduced amino acid transport, inhibition of respiration[61], and inhibition of RNA polymerase[62,63] seem to be among the processes that could be involved.

Miscellaneous biochemical effects of the Vinca alkaloids include a fall in liver coenzyme A level[64], inhibition of histamine release from most cells[65], and an increase in cyclic AMP concentrations in lymphoma cells[66].

3.2.3. Metabolism:

Of the three major Vinca alkaloids (vinblastine, vincristine, and vindesine), vincrstine has the largest plasma half-life especially in the tertiary phase of the triphasic clearance curve. About 75% of the alkaloid content of the plasma is bound to protein, especially α- and β-globulin[67]. In addition to this bound component, very high levels of these alkaloids enter the platelets which may carry more total drug than is present in the plasma, thereby acting as a reservoir[68]. It appears that the platelets retain vincristine more tenaciously than vinblastine, although the latter drug enters these structures faster than vincristine[69]. There is also some concentration of drug by leukocytes, and the evidence obtained with cells in vitro, as well as the extremely rapid initial distributive phase in the plasma, suggest that the Vinca alkaloids are avidly endowed with many tissues.

The major route for excretion of these drugs is biliary, with urinary excretion in any species examined reaching no more than 23%. Most of the urinary excretion products consists of unchanged alkaloids, and this true of the bile also, but degradation by intestinal flora is virtually complete so that little or none appears in the

feaces. Vincristine appears to undergo less metabolism than vinblastine[70]. The combination of more prolonged retention by platelets, slower elimination, and less breakdown underlies the greater potency of vincristine.

3.2.4. Clinical Antitumor Activity

Vinblastine and Vincristine have been employed successfully as important components of complex regiments of combination chemotherapy, rather than alone. Vincristine is most effective in the therapy of acute lymphoblastic leukemia, Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and Burkitt's lymphoma and is valuable in the treatment of Wilm's tumor, rhabdomyosarcoma, testicular tumors, carcinomas of the breast and bronchus, Ewing's sarcoma, and the nonmalignant letter Siwe disease.

Vinblastine is of somewhat more limited use, particularly because it is not effective against - acute lymphoblastic leukemia. Its major clinical applications have been for Hodgkin's disease, other lymphomas, choriocarcinoma, testicular cancers, and carcinoma of the ovary. The fact that its limiting toxicity is marrow depression, the most frequently encountered toxicity among cancer chemotherapy agents, rather than the unusual neurological toxicity of vincristine, makes it a less versatile component of combination regimens. Leurosine resembles viblastine in its spectrum of activity and toxicity, but may produce in addition a shock like syndrome when injected

rapidly; this may result from the transient hypotensive and marked hypoglycemic activities of this alkaloids.

Vindesine resembles vincristine in its antitumor activity; but exhibits the toxicities of both vinblastine and vincristine. Although platelets accumulate high levels of the Vinca alkaloids, thrombocytopenia has not been a prominent side effect of these agents, infact, thrombocytosis elevation of platelet levels has been more commonly observed[71,72] However, in the treatment of idiopathic thrombocytopenia purpura, where platelets levels are high, Vinca alkaloids may be useful in those patient refractory to the usual therapy such as prednisone and azathioprine[74] .

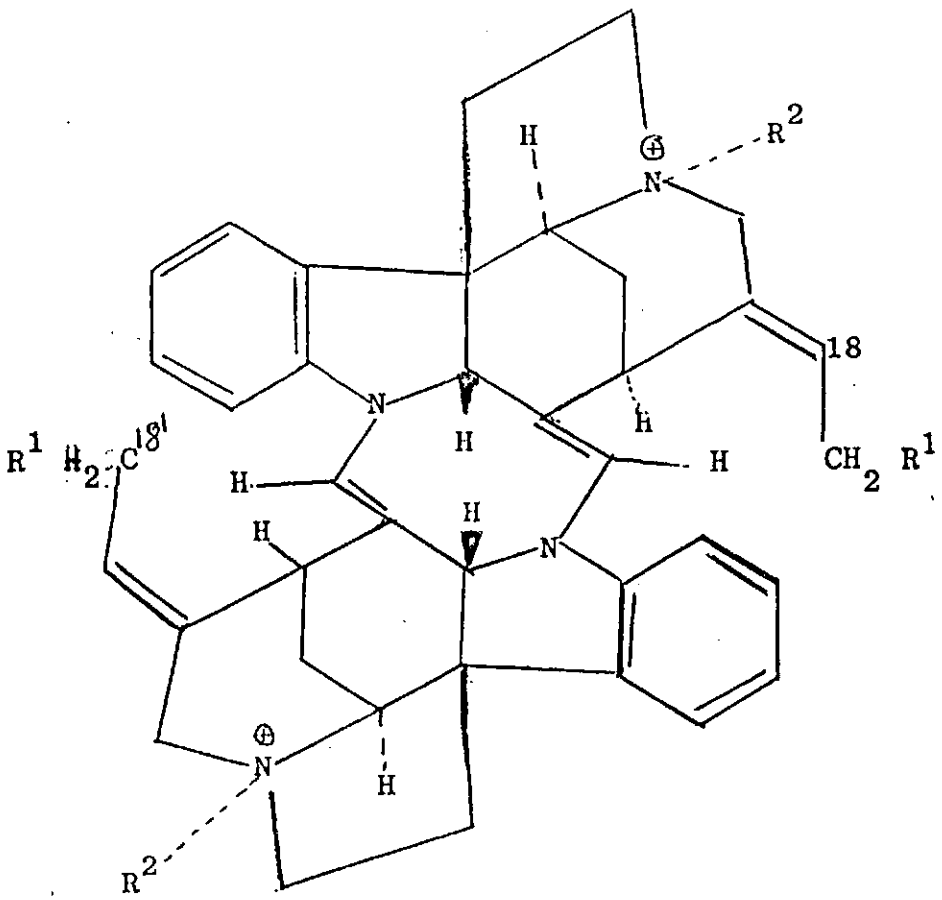
Vinblastine has been found to relieve the pain of acute gouty episodes in the same manner as does colchicine[74] . probably through reduction of metabolic activity and phagocytosis in the leukocytes in the joints, but no further clinical use has been made of this observation.

3.2.5 Indole Alkaloids As Neuromuscular Blocking Agents - Curare Alkaloids

Neuromuscular blocking agents are found in Calabash curare used for preparing poisoned arrowheads. These agents are derived from many different plants, including Strychnos toxifera, Chondrodendron tomentosum, and other species of the Loganiaceae[75]. Although d-tubocurarine is the component that has been studied most extensively and is in widespread clinical use as neuromuscular blocking agent during surgery; there are at least forty other alkaloids that contribute to

the overall toxicity of curare; most of these are indole derivatives[76].

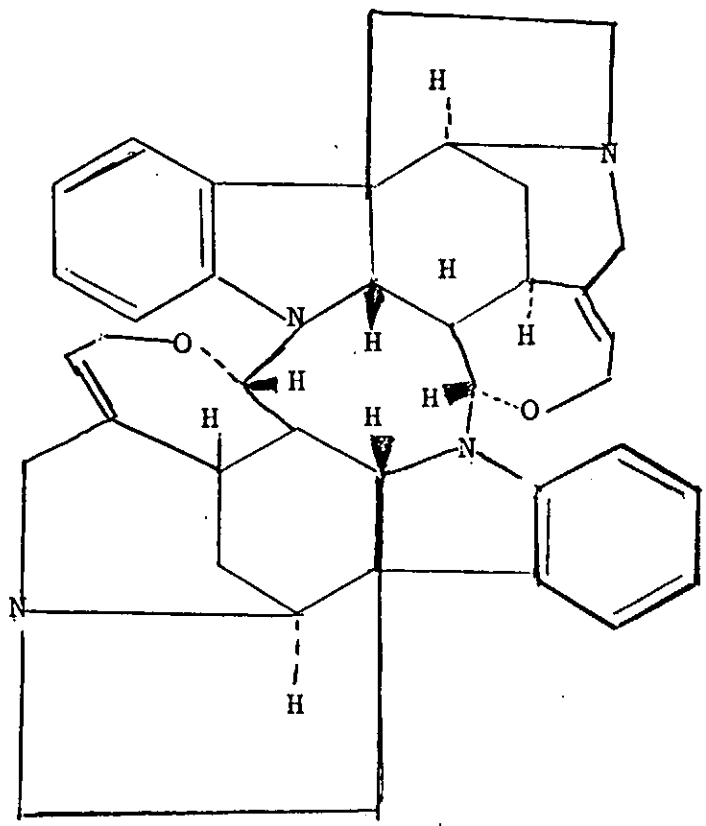
The most active compounds in curare are the toxiferines dihydrotoxiferine I (51) and toxiferine (52)[77]. Although these are not presently used, the diallybis derivative of nortoxiferine known as alcuronium (53) is used clinically in Europe. Among other weak muscle relaxing alkaloids are caracurine V (54) and its N - oxides.



51 Dihydrotoxiferine I R^1 = H, R^2 = Me

52 Toxiferine R^1 = OH, R^2 = Me

53 Alcuronium R^1 = OH, R^2 = CH_2 CH = CH_2.



Caracurine V (54)

There is no central action or direct effect of curare on the muscles by itself, but high doses block autonomic ganglia[77]. The end plate receptor, a lipoprotein subunit with a molecular weight of 50,000, seems to be associated with, or actually to be, the ionophore for sodium transport[78]. Although several neuromuscular blocking agents are extensively used for muscle relaxation during surgery, thus permitting the use of lighter and safer planes of anesthesia, most of the indole derivatives mentioned above have only limited clinical use, e.g. toxiferine where long-term paralysis is needed, and alcuronium for short operations[78].

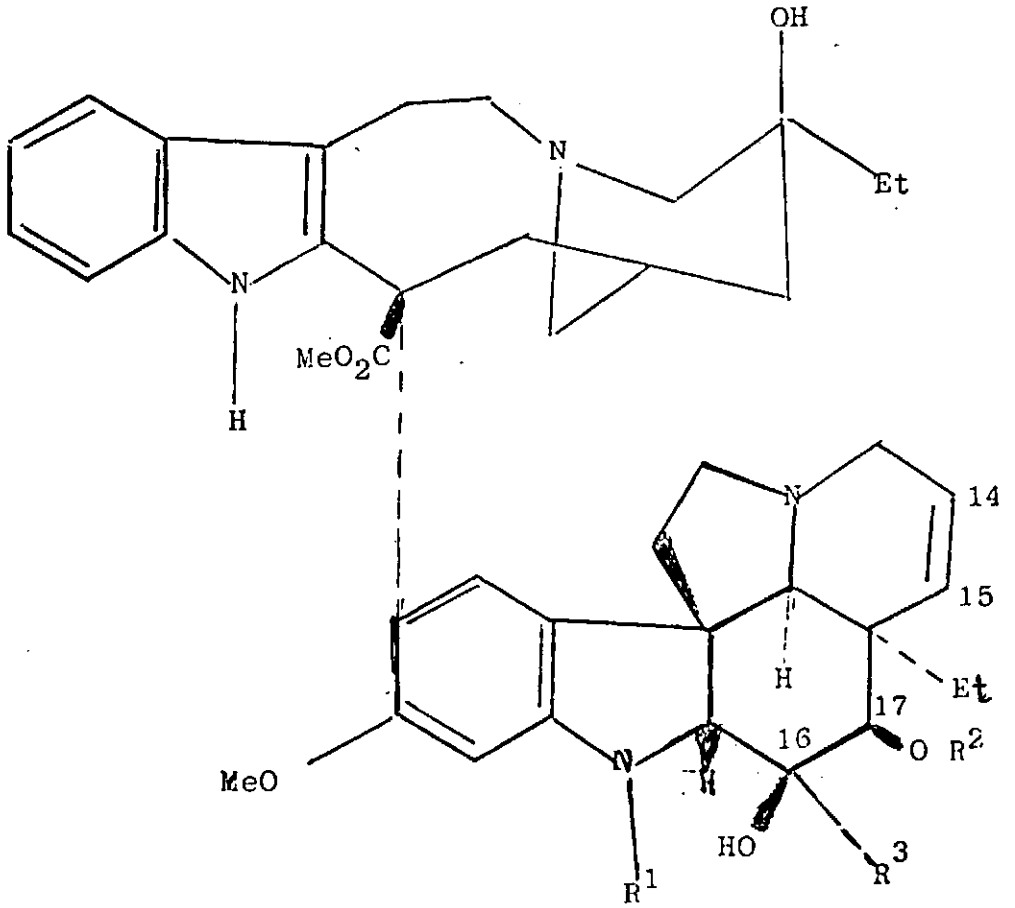
Tubocurarine has a half-life of between 0.25 and 3 hours, it undergoes 40 per cent binding to plasma protein, and is excreted to an extent of about 43 per cent of administrered dose in humans [78]

3.2.6 Biological Activity of Indoles - Vinca Alkaloids

These bisindole derivatives are isolated in yields of only a few milligrams per kilogram from the leaves of the Madagascan periwinkle, Vinca rosea L., correctly referred to as Catharanthus roseus G. Don (Apocynacea), a plant with a long- standing reputation for its medicinal value [79]. It was an investigation of the reputed usefulness of the plant for treating diabetes that led to the isolation of the cytotoxic alkaloids [80]. Of more than 70 alkaloids that have been identified in this plant, eight are cytotoxic bisindoles composed of a vindoline and a Catharanthine moiety.

The active alkaloids are vinblastine (vincaleukoblastine; velban or velbe) (55), Vincristine (leurocristine, Oncovin) (56), vinleurosine (leurosine) (59) vinrosidine (leurosidine) (60), leurosivine 4 - desacetyl vinblastine, rovidine, and leurocolombine.

There are, in addition, several semisynthetic derivatives including 14, 15-dihydrovinblastine, vinglycinate (17-N, N-dimethyl aminoacetyl - 17-desacetylvinblastine) (57), vindesine (17-desacetylvinblastine amide) (58), and several 15', 20'-dehydro compounds [31].

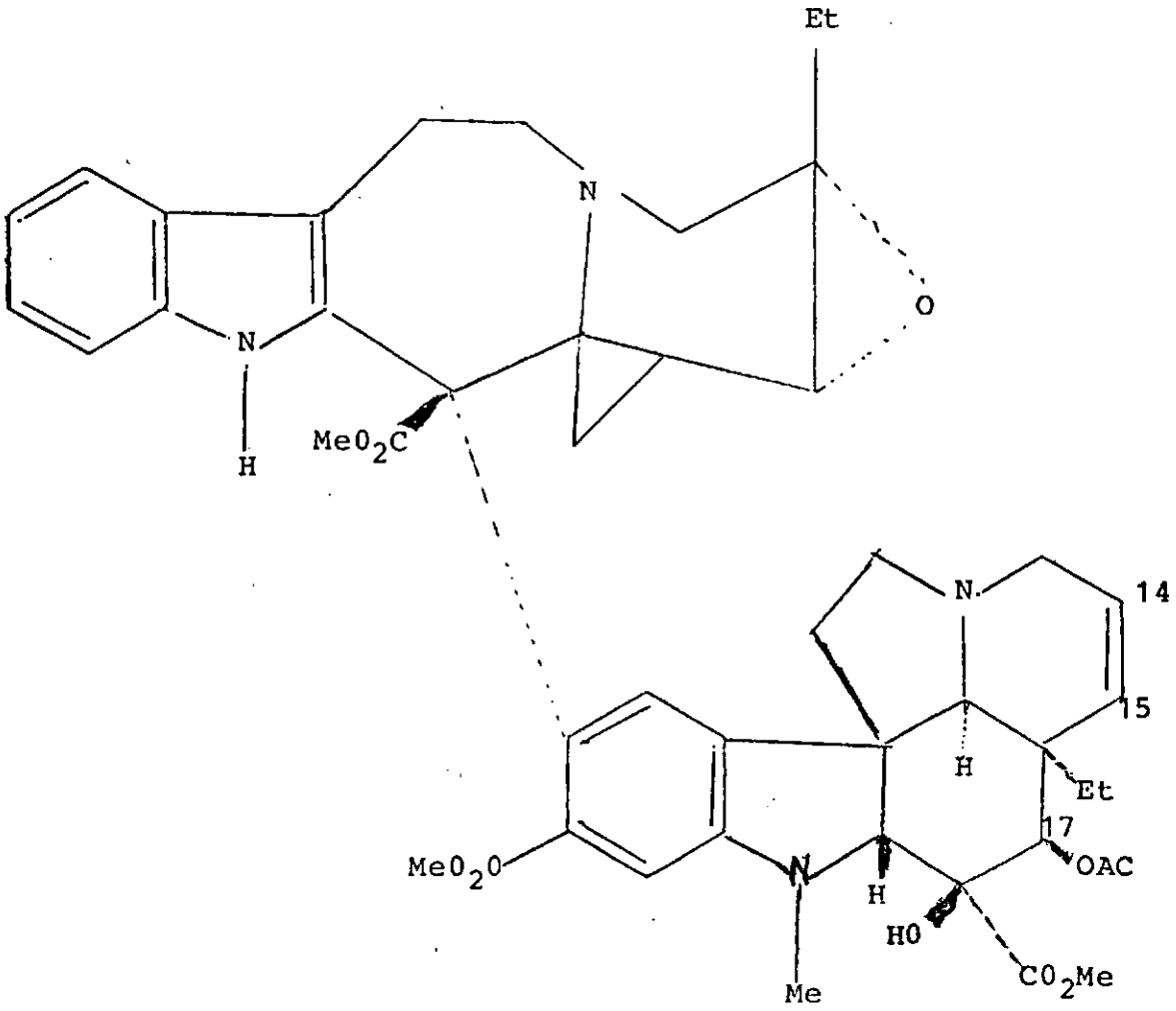


Vinblastine (55): R^1 = Me, R^2 = CO Me, R^3 = CO_2Me

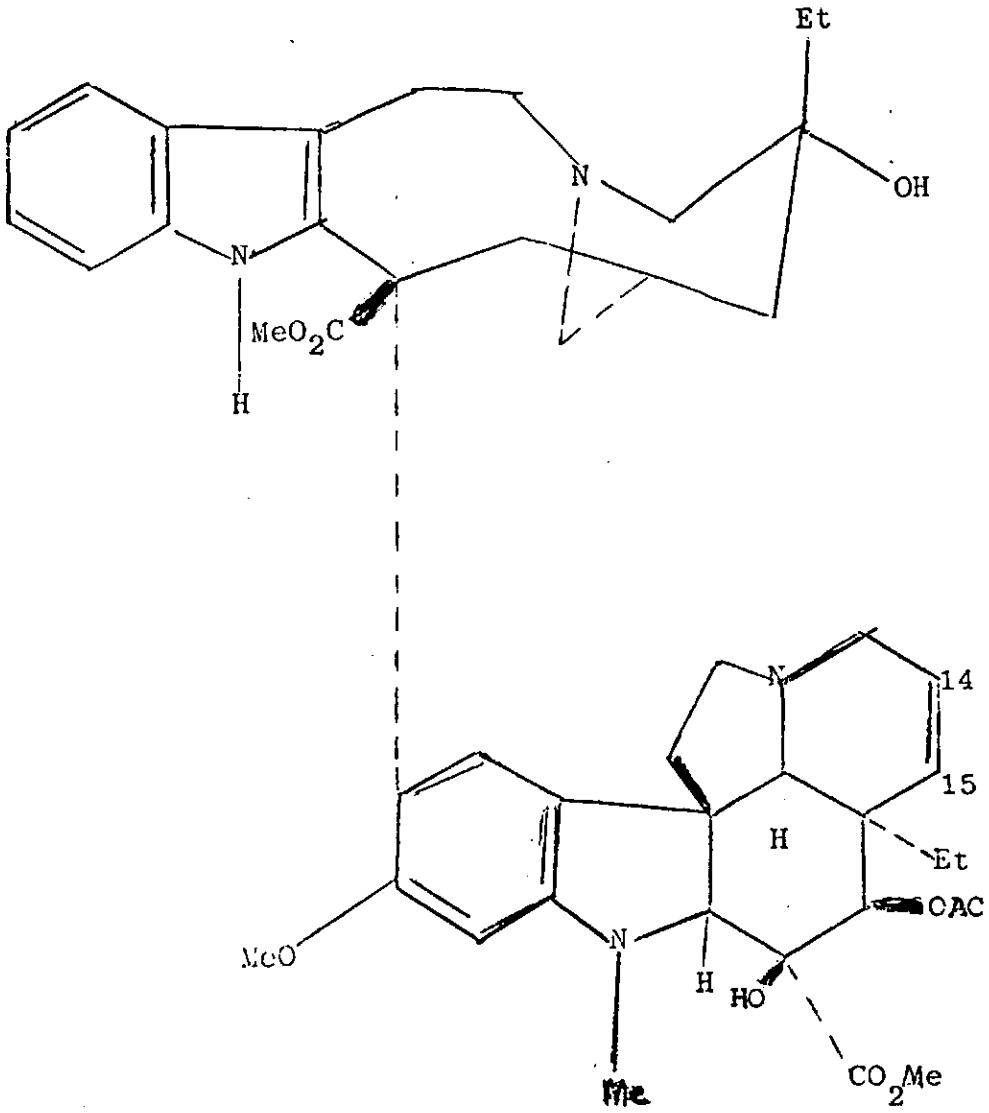
Vincristine (56): R^1 = CHO, R^2 = CO Me, R^3 = CO_2Me

Vinglycinate (57): R^1 = Me, R^2 = $COCH_2NMe_2$, R^3 = CO_2Me

Vindesine (58): R^1 = Me, R^2 = H, R^3 = $CONH_2$



Leurosine (59)



Leurosidine (60)

CHAPTER 4

PREVIOUS WORK ON THE ALKALOIDS OF HUNTERIA UMBELLATA

4.1 Table of Alkaloids* Isolated from Hunteria Umbellata

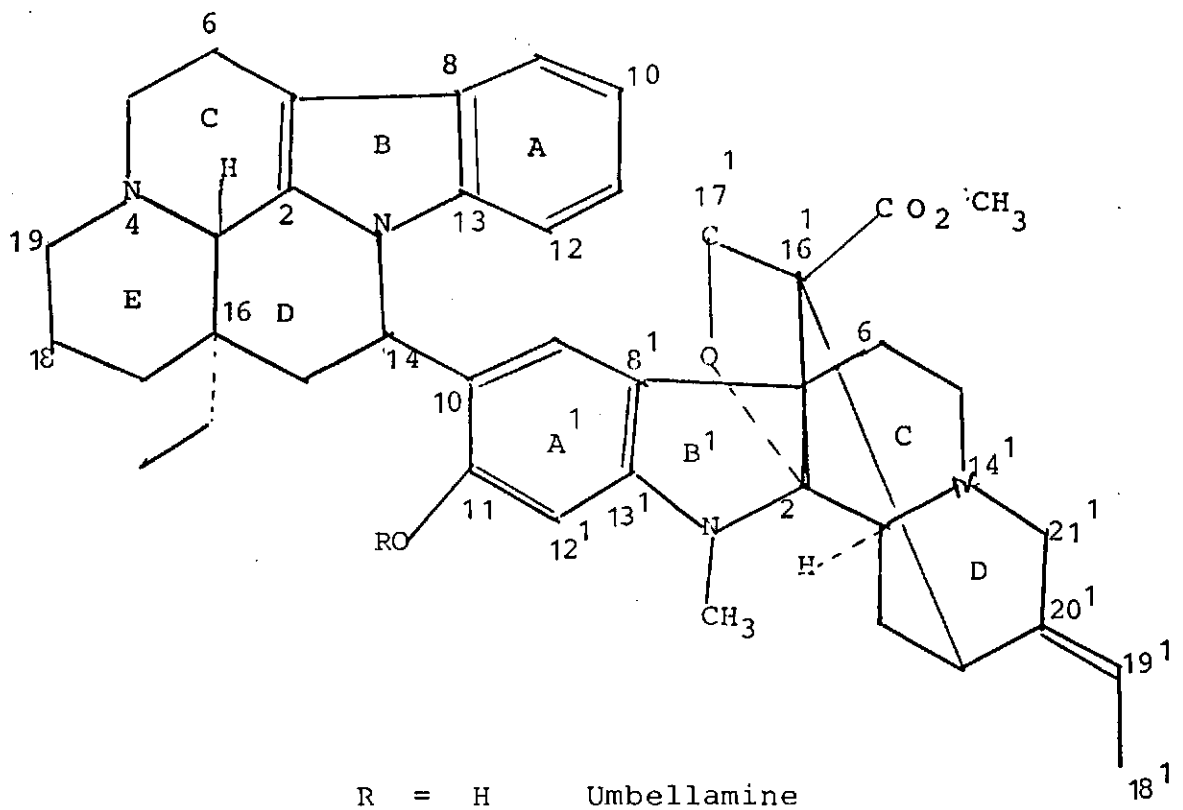
Seeds	Leaves	Root Bark
Water Insoluble Alkaloids	Water Insol. Alkaloids	Water Insol. Alkaloids
Corymine		
Corymine acetate	Erinine	Umbellamine
Isocorymine	Corymine	
HU5, HU6, HU8, HU9 and	PU.6-Picra-	
HU11, HU 12, lanceomigine	line	
	Umbellata,	
N-oxide, Picraline,	Eripine	
Akuamimidine,		
17-methoxy pseudoakuammigine		
Water Soluble Alkaloids		
Four Isomeric		
14-Isopropyldihydroxy-		
deoxisocorymines		

* References to the above compounds are given later on in the text.

4.2 Hunteria Umbellata (K. Schum) a member of the Apocyanaceae family was previously classified as Picralima umbellata. It is a smooth skinned tree about 16 metres in height, found growing abundantly in Lagos, Oyo, and Ogun and

Bendel States of Nigeria[81]. All parts of the plant are used in local medicine as a bitter tonic. Its wood is yellow, very hard and is used for making native combs and tool handles as it is considered very durable and immune to termites.

The plant has been extensively investigated, and all its parts have been shown to contain alkaloids[82]. As already shown in the Table (4.1) above, the one reported so far from the root bark is Umbellamine



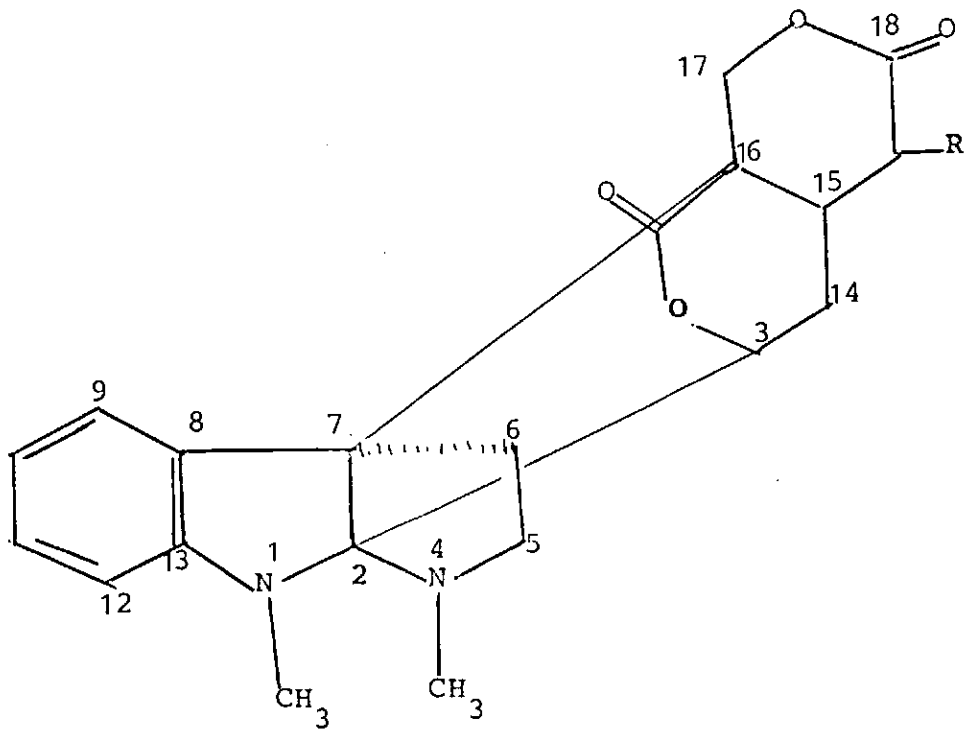
R = H Umbellamine

(61)

The alkaloid, of molecular formula $C_{41}\ H_{48}\ N_4\ O_4$ was isolated by Morita, et al in 1969[83]. It is a dimeric

indole-indoline alkaloid, probably identical with the alkaloid from Hunteria unbellata. The two monomeric units have been identified as (+) -eburnamenine and a phenolic hydroxy pseudoakuammigine moiety. The proposed structure (61) was based on spectroscopic data obtained from umbellamine and its derivatives.

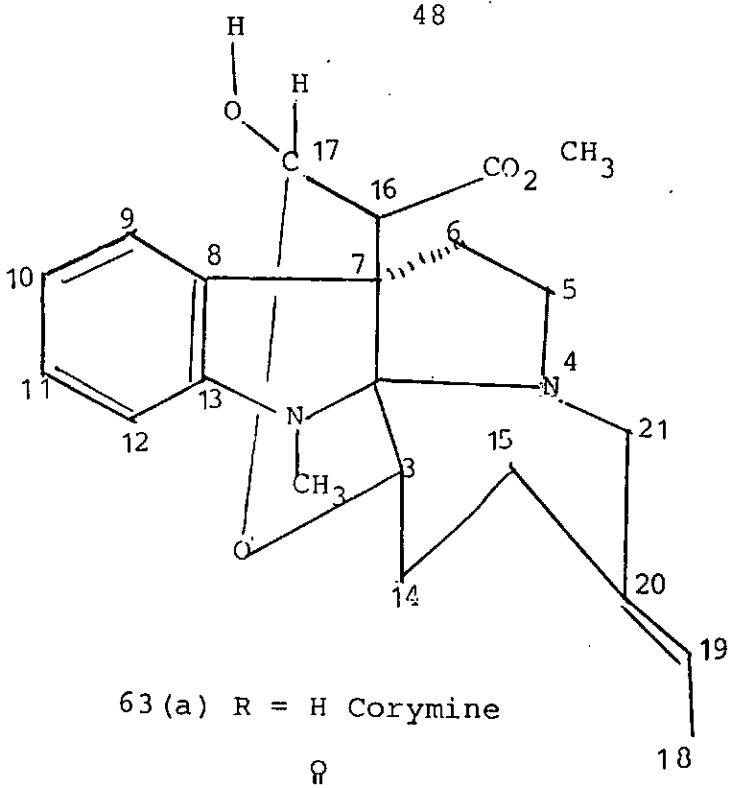
The alkaloids reported from[25,84] the leaves are erinine (62a), corymine (63a) eripine (64) and 'pu6'. 'pu6' has not yet been assigned a structure. These water insoluble alkaloids were isolated by Bycroft in 1965. Since then, no other alkaloid has been reported from the leaves.



62(a) R = CH-CH_3 ERININE

(b) R = H, CH_2-CH_3 Erinicine

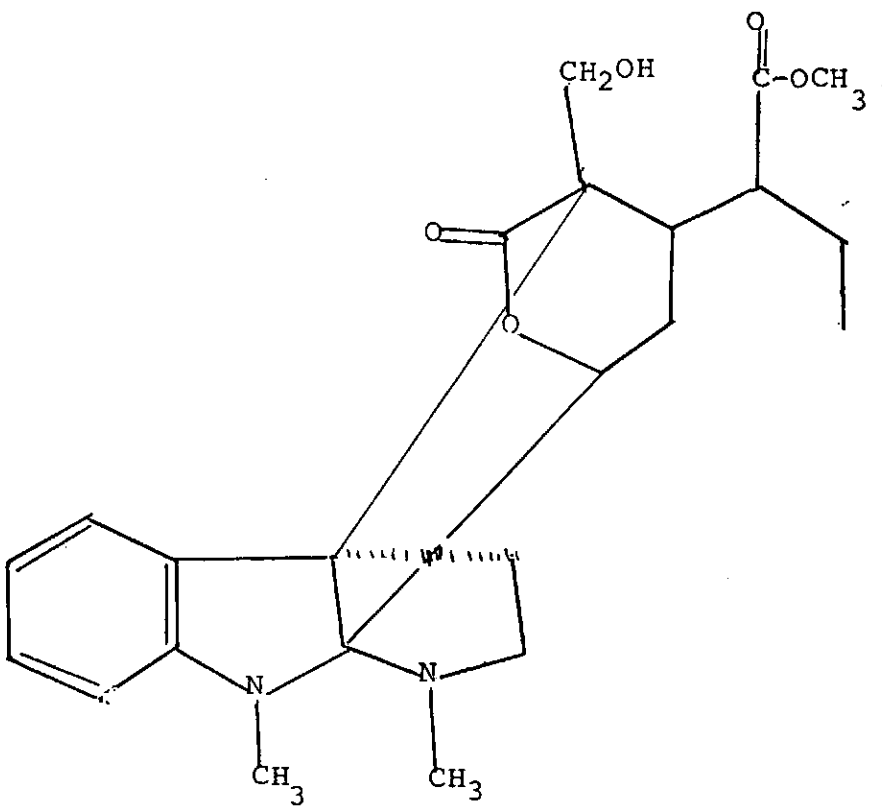
erinicine



63(a) R = H Corymine

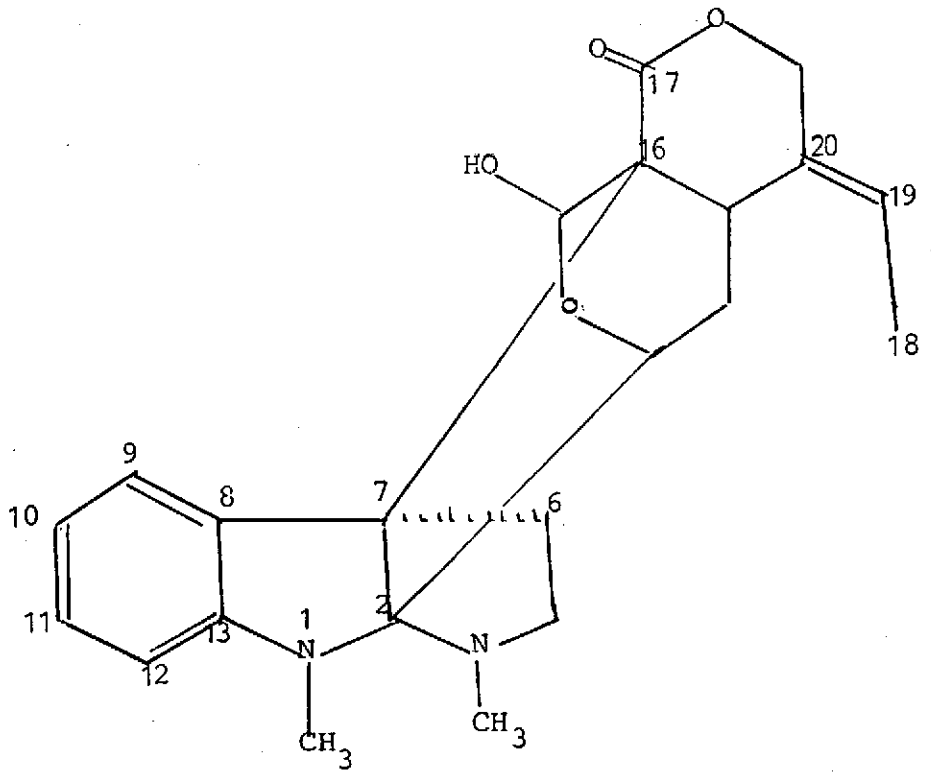
(b) R = $\overset{O}{\overset{\|}{C}}-CH_3$

Corymine Acetate

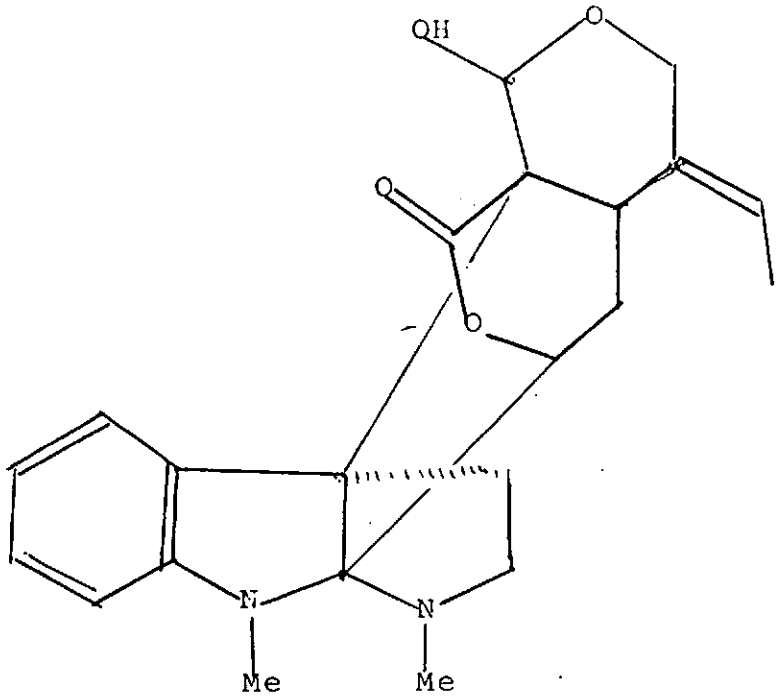


(64) Eripine

Bevan and his group in 1967 isolated corymine (63a) corymine acetate (63b) and isocorymine 27b (66), which was originally thought to have structure (65) but later assigned structure (66) mainly on mass spectral evidence[23] .

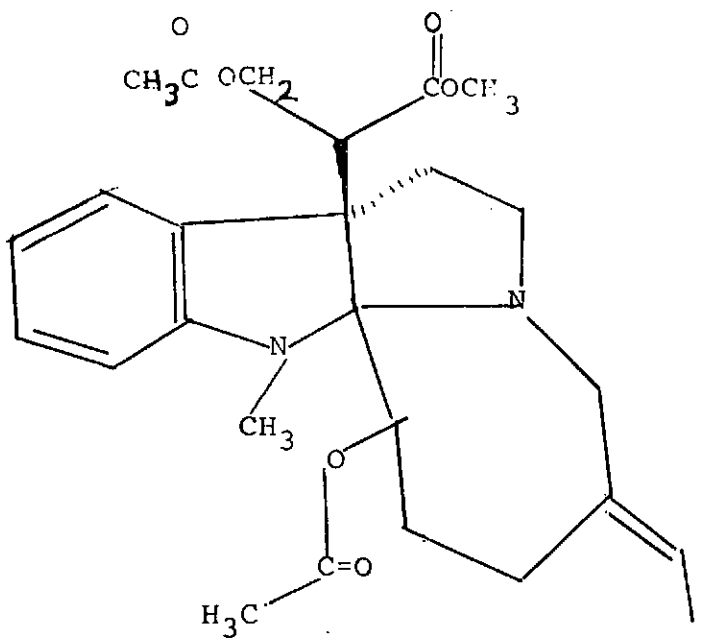


(65) Old structure of Isocorymine.

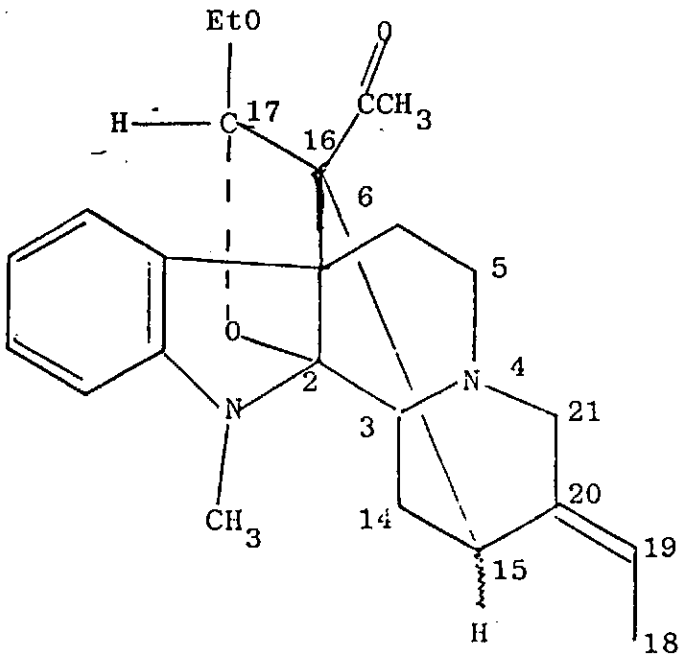


(66) True Structure of Isocorymine.

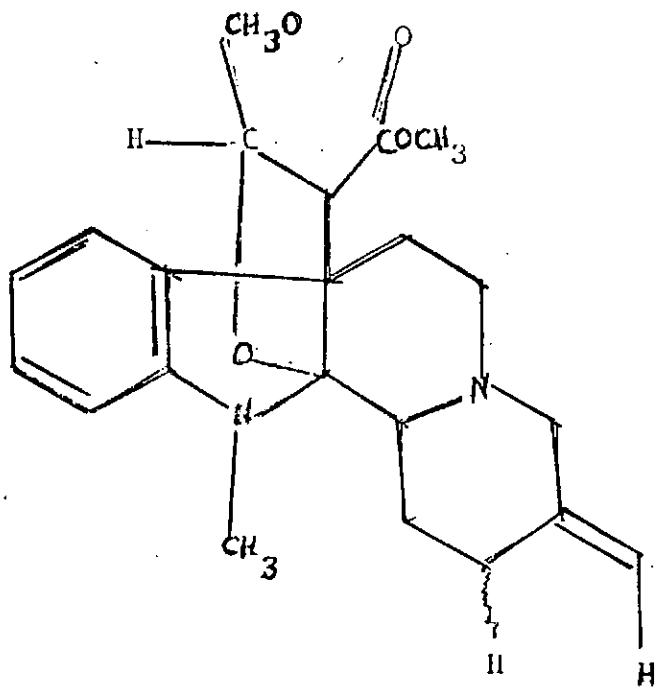
Jackson[84] had isolated five other new alkaloids from the seeds denoted HU5, HU6, HU8, HU9, and HU11 and these have been assigned the structures (67), (68), (69), (70) and (71) respectively on spectral data and chemical transformation evidence



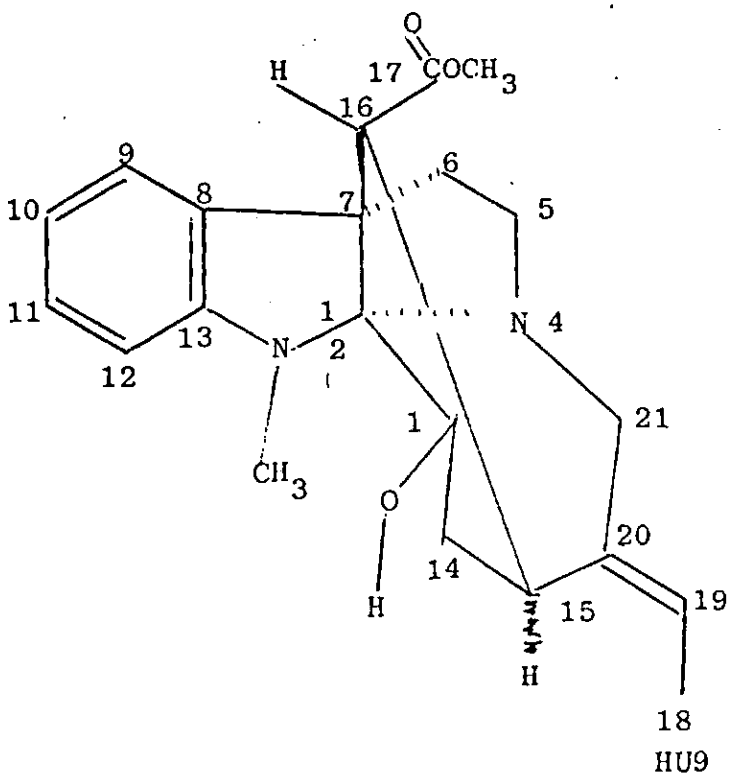
HU5 (67)


EtO
O
H
C
17
CCH3
16
6
5
O
2
N
4
N
21
3
20
CH3
14
19
15
H
18

HU6
(68)

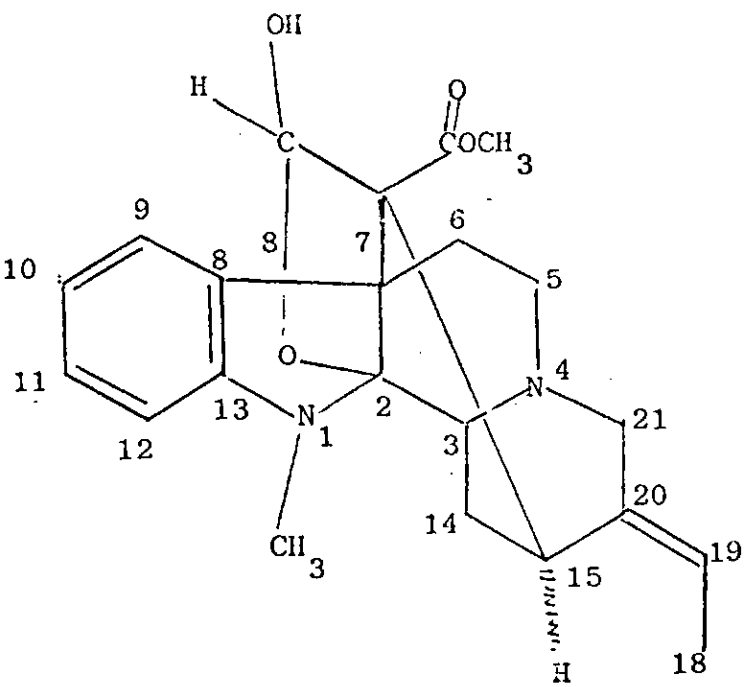

CH3O
O
H
C
COCH3
O
N
CH3
H
H

HU8
(69)



HU9

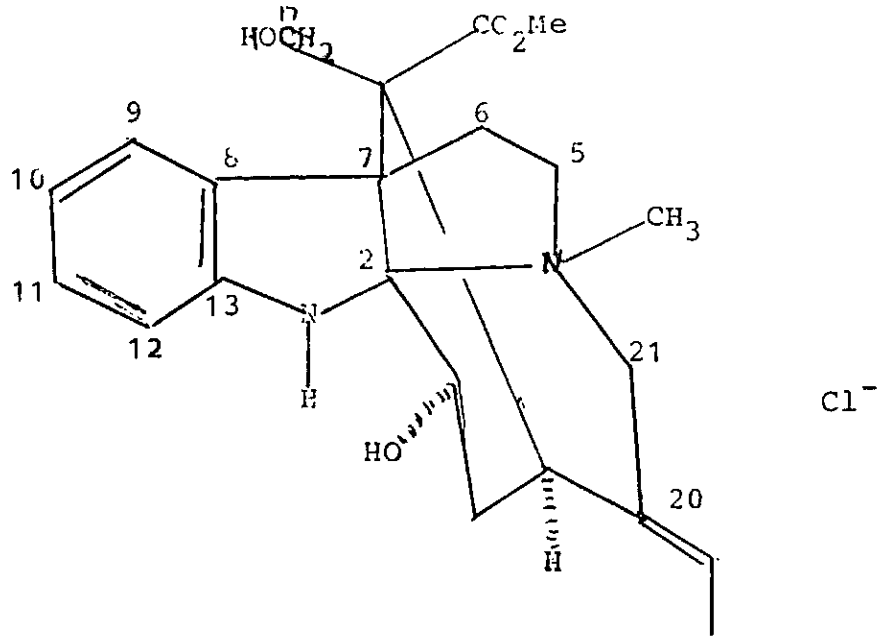
(70)



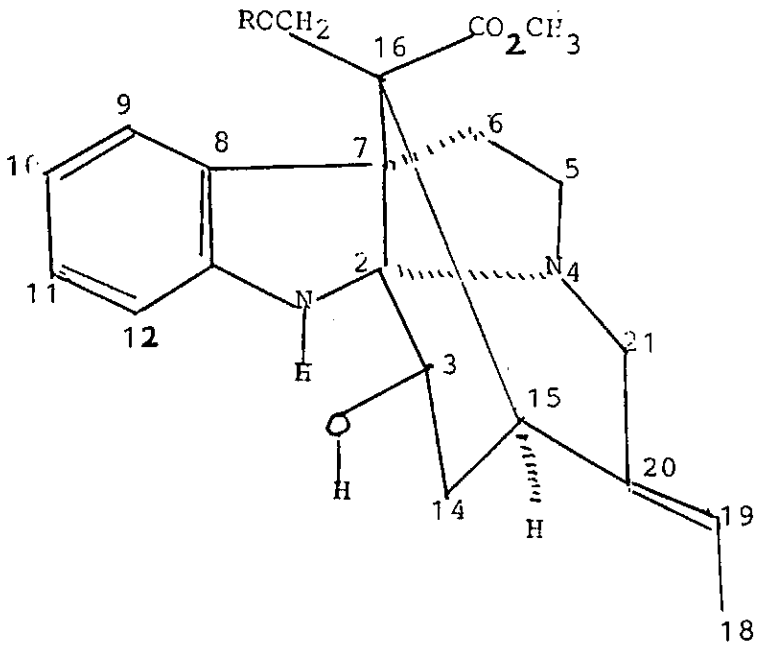
HU 11

(71)

Corymine was first isolated from Hunteria corymbosa[85] and was shown to be closely related to echitamine (72). The presence of a hemiacetal linkage was demonstrated by both the nmr data and treatment with sodium borohydride to give the dihydro-compound (73a). On treatment with hot 1% aqueous potassium hydroxide, corymine methiodide gave methylalloechitamine (73b) and the structure was therefore formulated as (63a).



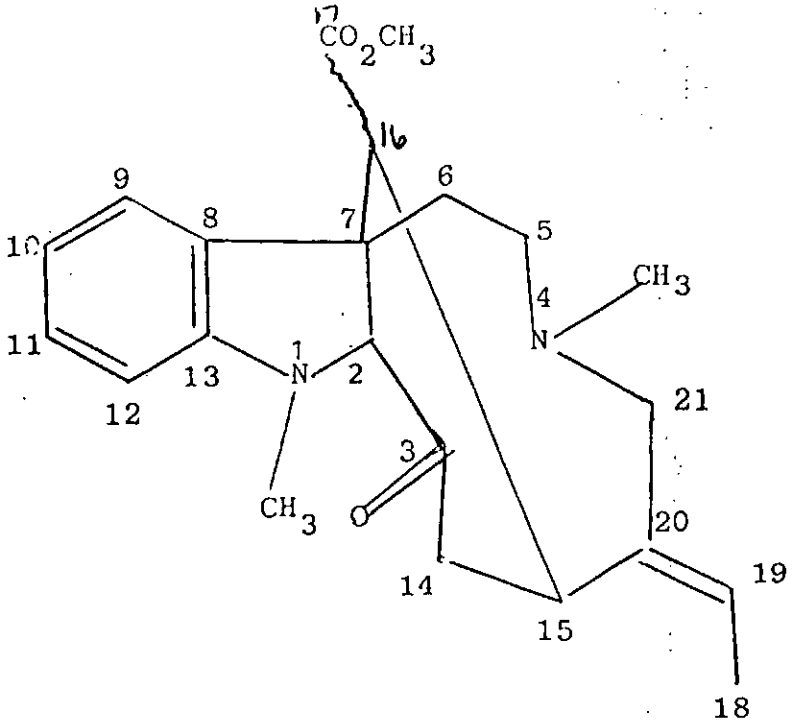
Echitamine
(72)



(a) R = H Dihydrocorymine

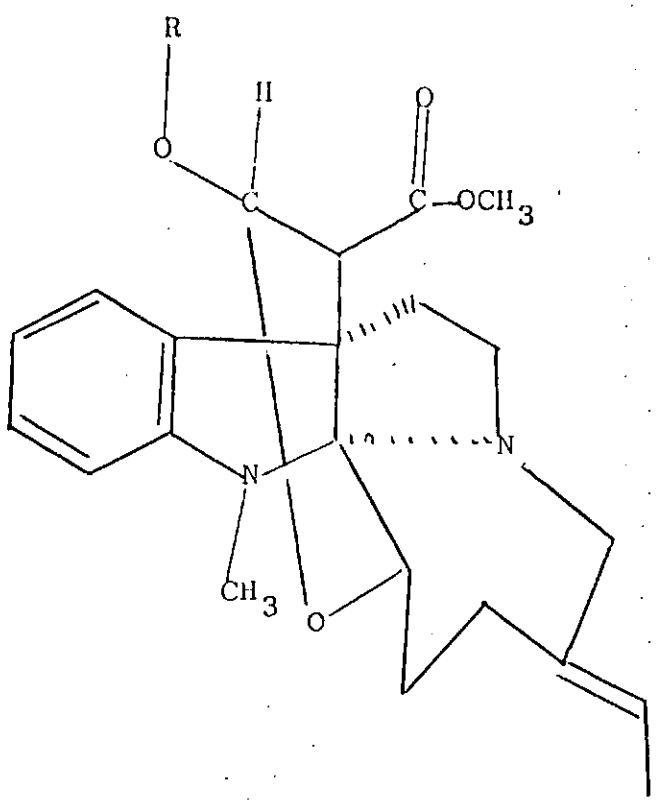
(b) R = CH_3

(73)



N_a-methylalloechi-tatime

(81)



R = H Corymine

(70a)

Later when corymine was isolated from Hunteria umbellata its structure was obtained using X-ray crystallography.[25] The corymine structure was further confirmed by Acetylation.

Four alkaloids from Hunteria Umbellata isocorymine[23a], erinine, erinicine and eripine have been assigned related structures (66), (62a), (62b) and (64) respectively. These are placed in that sub-group of indole alkaloids derived from tryptamine and an unrearranged secologanin unit but lacking the usual 21, N_b - bond and having additionally a 7, 16-bond.

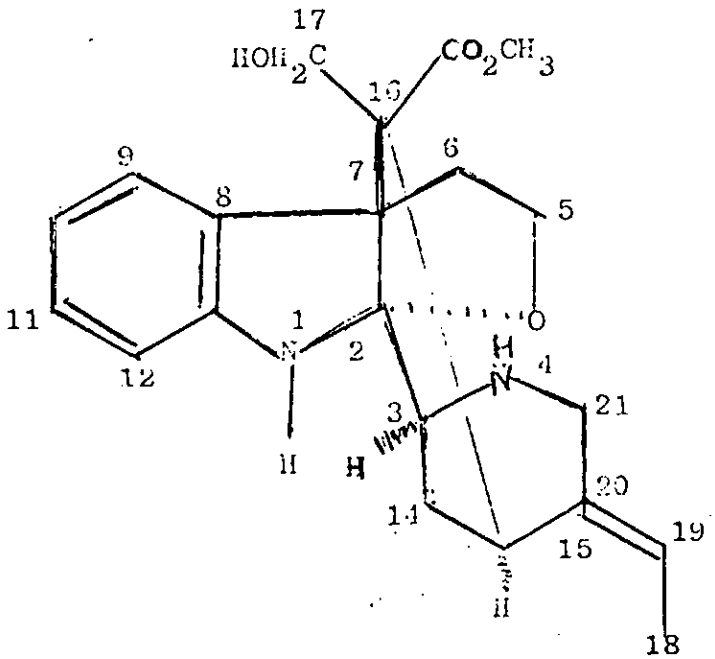
Erinine, erinicine and eripine were chemically interrelated in that eripine (64) was lactionised by heating to give erinine (62a) together with the 18, 19-double bond isomer of erinine; the same mixture of double bond isomers was obtained by similarly heating either erinine or its double bond isomer and erinine was clearly catalytically reduced to give erinicine (62b).

4.3 Absolute Configuration of Erinine, Erinicine, Eripine and Isocorymine

The absolute configurations at C-2 and C-7 in eripine and therefore by correlation in erinine and erinicine were established by optical rotary dispersion comparisons technique. The linkage of C-16 at C-7 is the irrevocable consequence of the ubiquitous 15-α - H configuration typical of most indole alkaloids. An assumption of this absolute configuration for isocorymine would therefore imply a C-2/C-7 cis or trans stereo-chemistry.

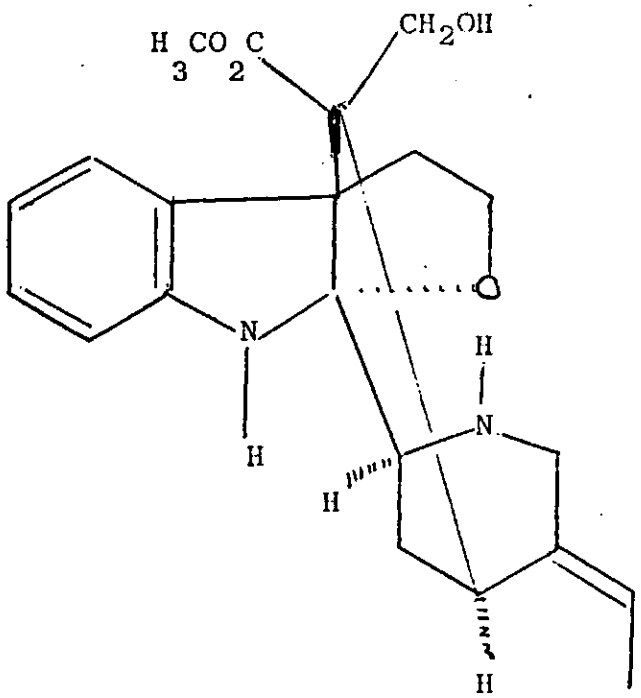
4.4 Unresolved Stereochemical Features in Isocorymine Erinine, Erinicine and Eripine: Several factors of stereochemical uncertainty remained to be established for these bases. Firstly, the geometry of the 18, 19-double bond in isocorymine (66), erinine (62a) and thus in eripine (64) has not been elucidated; the E configuration would be expected by comparison with nearly all established instances, which possess unsaturation at the 19, 20-position.

Secondly, the stereochemistry of the fusion of the eserine unit to the lactone or lactol ring, i.e. the stereochemistry of C-3 and C-16 relative to C-15 was not considered for isocorymine and only tentatively assigned[28a] for erinine and by deduction for erinicine and eripine by noting the similarity in the infrared spectra of an eripine degradation product and that of a compound derived from the alkaloid echitamine, the stereochemistry of which had been established by X-ray crystallography. The stereochemistry of C-16 and implicitly that of C-3 has acquired more significance in the wake of the more recent isolation[86] of Aspidoasycarpine (75) and ionicerine[87] (83), both indole alkaloids of possible C-16 epimeric types.



Aspidoasycarpine

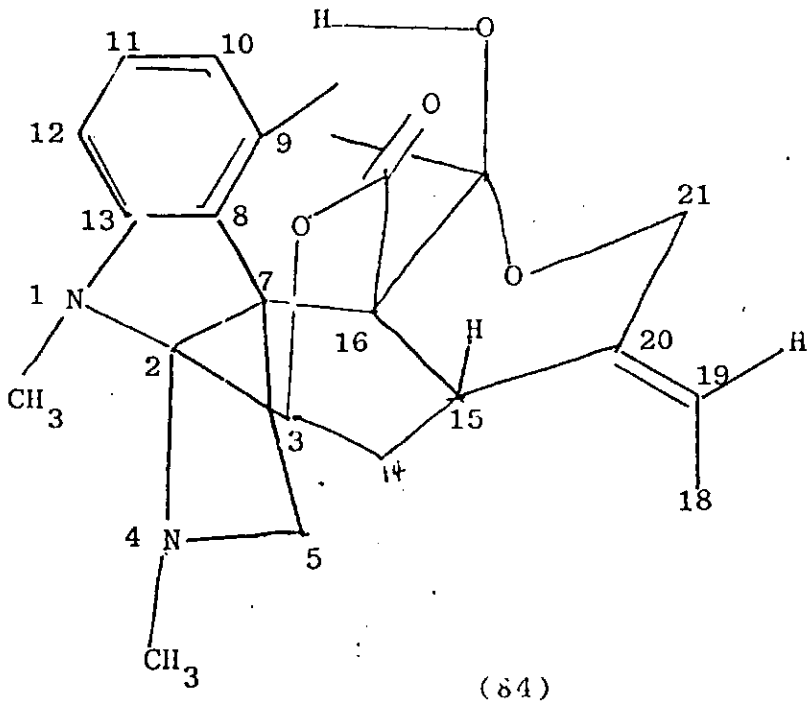
(82)



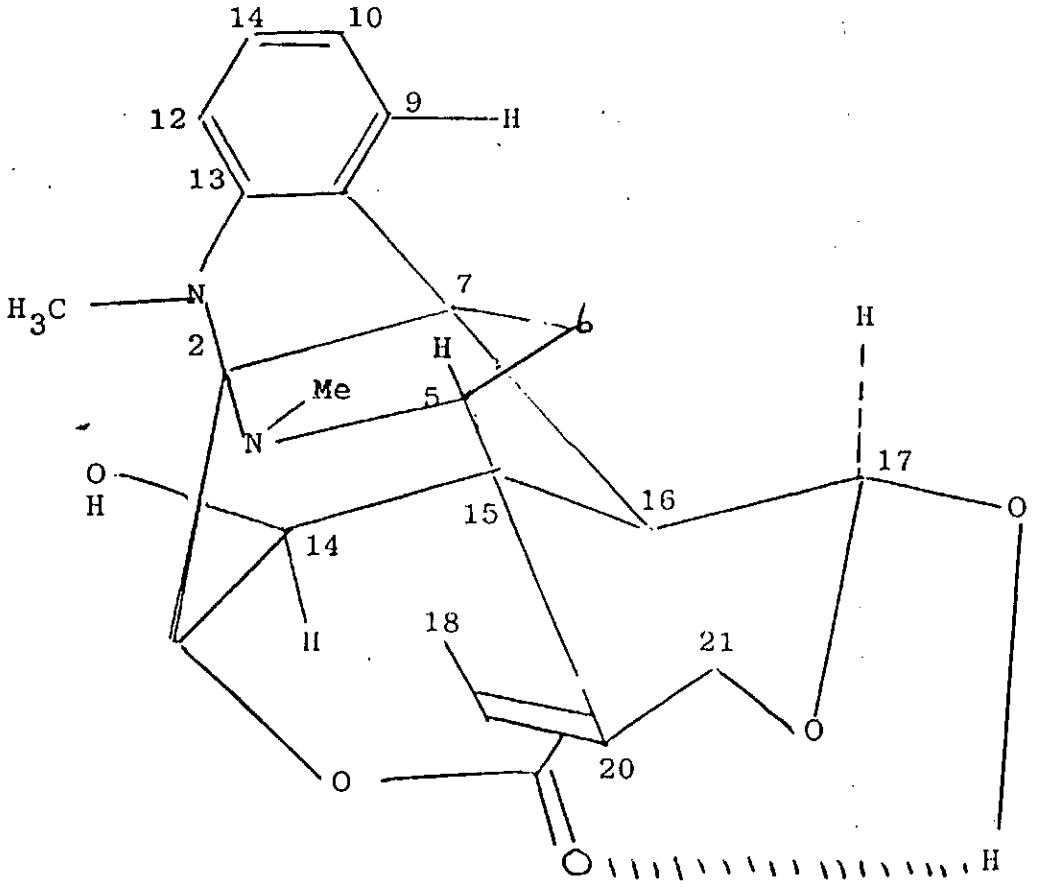
Ionicerine

(85)

Thus a priori considering the pentacyclic bases (66) (62a) and (62b) they could have stereochemical structures represented by either (77) or (78), whilst in each case retaining the same configuration at C-15, shown in these diagrams on the assumption of the ubiquitous α-H-15 configuration.



(84)



(85)

Finally, neither the configuration at C-17, the hemi-acetal carbon in isocorymine (69) nor the stereochemistry of reduction of erinine, i.e. the configuration at C-20 in erinicine 69b had been elucidated.

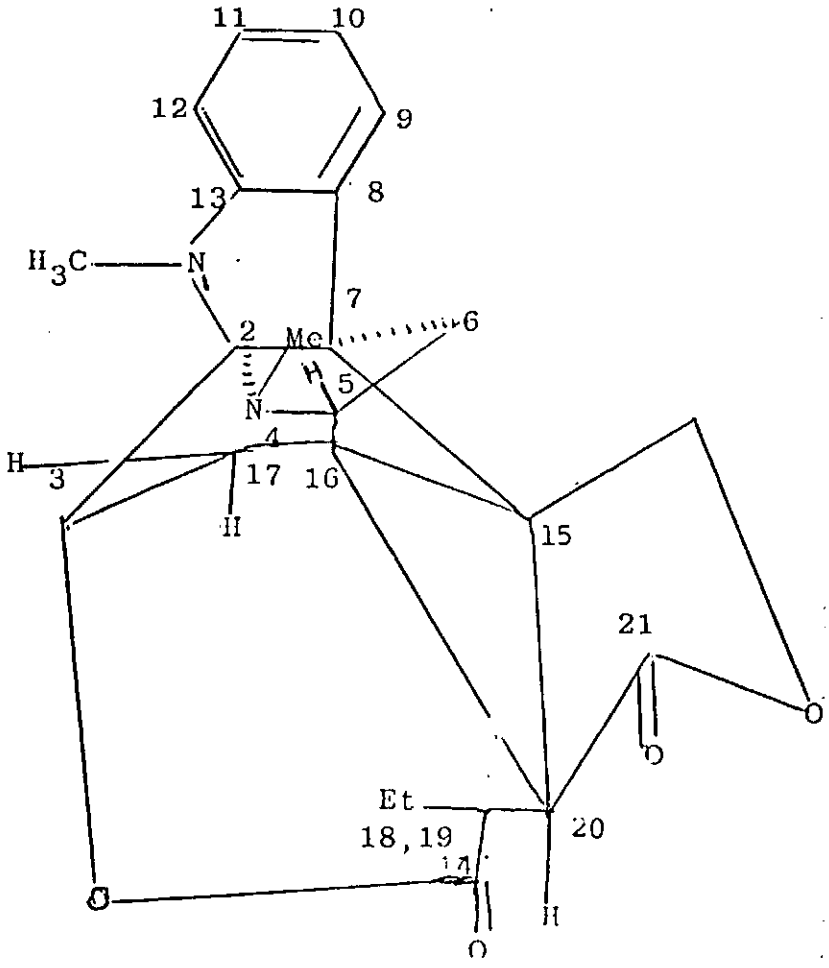
4.5 A Proton Magnetic Resonance Study of Isocorymine, Erinine, and Erinicine Determination of the Stereochemistry at C-16 and the Geometry of the Ethylidene System

It was considered that p.m.r spectroscopy would likely be the most convenient method of determining some of the unknown stereochemical features. In particular the application of transient nuclear Overhauser enhancement (NOE) of a signal following selective inversion of another signal seemed appropriate. This inversion was achieved by the application of a frequency selective pulse[88], followed, after a variable relaxation interval, by a non-selective high power pulse which monitored the partially relaxed intensity of all peaks. This technique had been particularly useful in the recently reported determination of the conformation of apparicine. Similar experiments were carried out on isocorymine, erinine, and erinicine.

A study of models seemed to indicate that the C-14 protons would be in close priximity to the N_a-methyl group if the structure (78) was correct and that these same protons would be in the vicinity of the N_b-methyl if the alternative (97) configuration for C-16 was correct. Therefore having fully assigned the spectrum of isocorymine, which confirmed the structure, the selective inversion of the N_a-methyl signal was carried out by Frank Healthey and John A. Joule on this compound .

A transient NOE. for proton C-14(H_o) was observed, indicating that the configuration at C-16 was present in structure (78). A similar result was also obtained for erinine by this group of co-workers. Thus the configuration at C-16 for erinine and by implication erinicine was established.

The geometry of the ethylidene system was also ascertained for the two bases, erinine and isocorymine to be of the E type system. A small but significant n.o.e. was observed for the C-14 - proton signal on selective inversion of the C-18 methyl signal. The configuration at C-20 in erinicine was determined by the observation of a coupling constant with a value of 12Hz, between H-15 and H-20 such a value indicated that the protons were trans to one another, thus fixing the configuration at C-20 in structure (79).



(86)

4.6 Determination of Configuration at C-17 for Isocorymine

A large transient nuclear Overhauser enhancement n.o.e. was observed for C-9-H on inversion of C-17-H indicating that they are neighbours in space, as depicted in (78). Thus the hydroxyl group as shown is α-to the lactol ring. Confirmation was derived from analysis of the p.m.r. spectrum. The chemical shift position of the exchangeable proton, i.e. C-17-OH appeared towards the low field limit of the range for aliphatic hydroxyl groups and was insensitive to temperature, indicating a fair degree of intramolecular hydrogen bonding.

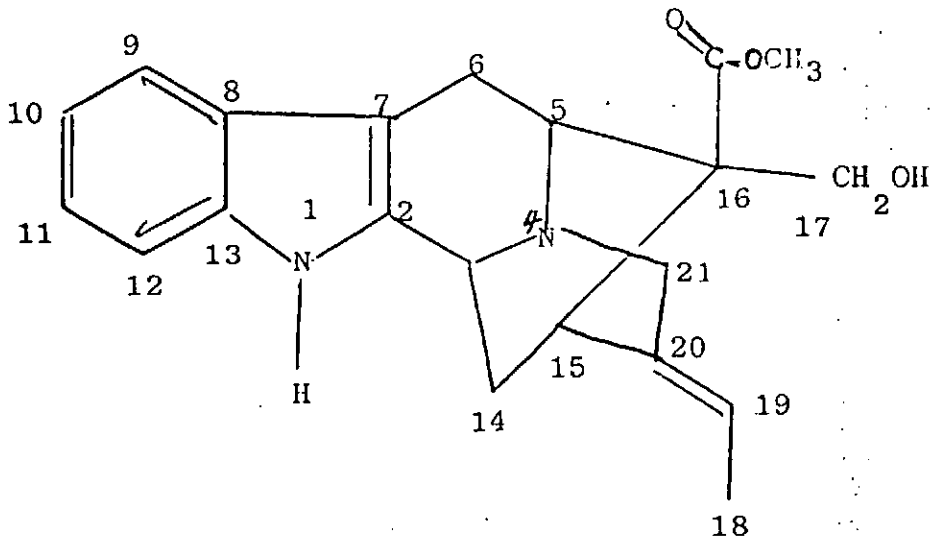
In addition the coupling constant $J_{H,COH}$ is large, about 12Hz, thus the protons are in a trans arrangement. These ctriteria are only accommodated by the configuration at C-16 as in (78) and an α-OH group with intramolecular hydrogen bonding to the carbonyl oxygen.

4.7 Isolation of Picraline, Akuammidine, 17-methoxy Pseudoakuammigine and Hu12.

David I. Bishop isolated Picraline, Akuammidine, 17-methoxy Pseudoakuammigine and Hu12 from the seed of Hunteria umbellata. Akuammidine (80) and 17-methoxy pseudoakuammigine were obtained by chromatographic separation on silica of the mother liquors of the fractions from which erinine, and isocorymine had been co-crystallized.

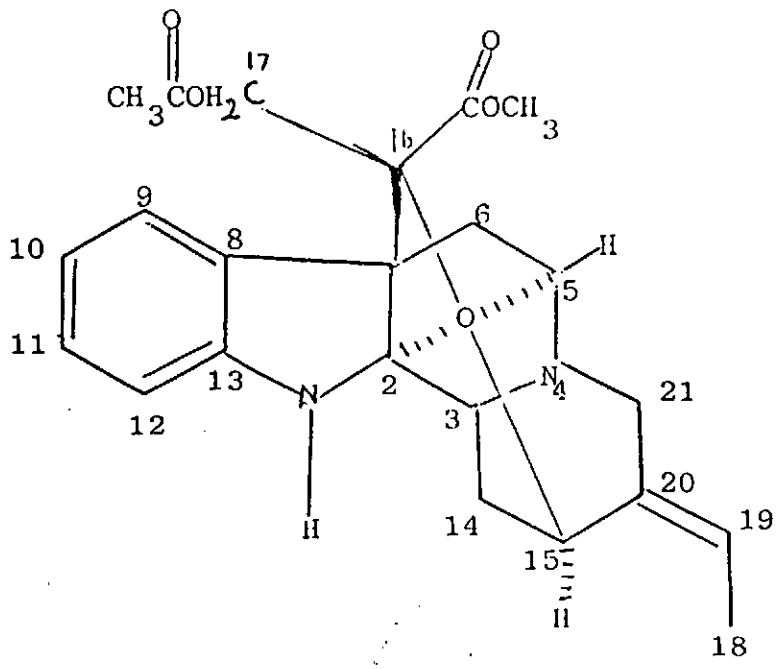
Crude chromatographic separation on deactivated alumina of the more polar material gave many fractions, two of which by t.l.c. were shown to contain alkaloids, some novel to this species. The less polar fraction was re-chromatographied on

silica to give picraline (81) along with the ubiquitous corymine. The more polar fraction was flash chromatographed on silica to give an amorphous alkaloid Hu12.



Akuammidine

(80)



Picraline

(81)

4.8 Picraline and Akuammidine, Alkaloids novel to Hunteria Umbellata.

Both akuammidine[89] and picraline[20a], although known alkaloids are unreported for Hunteria umbellata. The identity of picraline was proved by observation of an indoline type chromophore in the UV spectrum, a molecular ion at m/z 410 with ion fragments represented by m/z 337 $(M-CH_2OAC)^+$ and m/z 239 which correlated with data reported and finally by t.l.c. analysis involving a comparison on silica plate with an authentic sample.

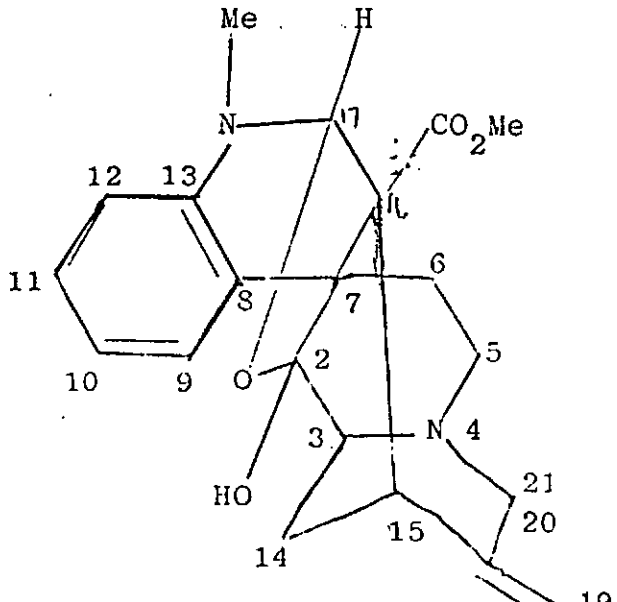
Akuammidine was similarly characterized. Again the UV spectrum obtained showed the molecule to prossess a known chromophore, in this case that of indole. The mass spectrum gave a molecular ion at 352 with ions at m/z 321, 249 and 169 in accord with published data[90]. Confirmation was obtained by a similarity in melting point 238 - 242°C (249°C) and by a p.m.r. spectrum indicating an ethylidene group, a quartet at δ 5.47 coupled to a doublet at δ 1.74 and a methoxy group with a three proton singlet at δ 3.8.

4.9 Hu12

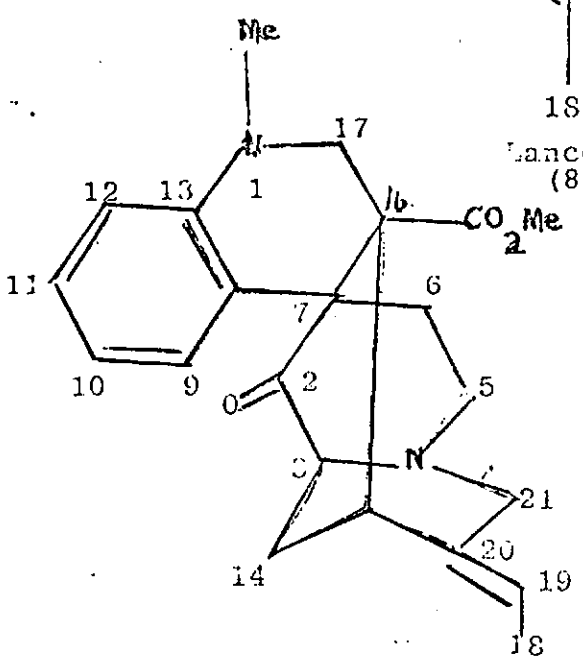
The alkaloid originally designated as Hu12, has been isolated from the seed and the structure shown to be identical with lanceomigine - N-oxide from Alstonia lanceolata, H. congolana and H. zeglancia. This is an amorphous base, the structure of which proved difficult to elucidate by conventional chemical degradative methods.

The polarity of the new alkaloid and presence of a substantial N-16 peak in its mass spectrum strongly suggested the presence of an N-oxide grouping though the compound was unaffected by treatment with triphenyl phosphine.

Lanceomigine (89) from Alstonia lanceolata[91], Hunteria congolana[92] and Hunteria cylancia[93], also an amorphous base, was reduced[44] with a combination of triethylsilane and trifluoroacetic acid to the crystalline deoxy-derivative (83) the structure of which was established[95] by X-ray crystallography.



Lanceomigine (82)



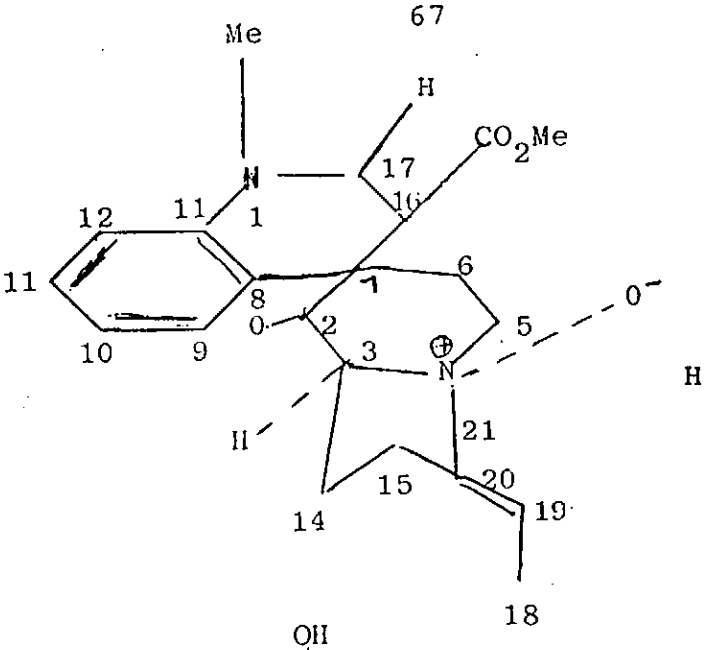
deoxy- derivative of Lanceomigine.

(83)

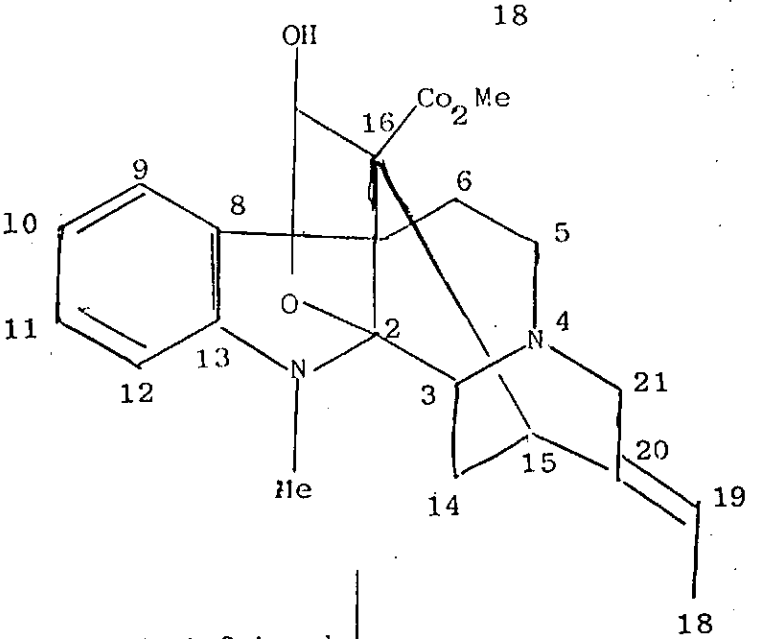
The location of the hydroxy-group at C-17 in the alkaloid itself was deduced from the presence of a sharp singlet signal for H-17. That lanceomigine exists in a pentacyclic from with a C-17-O-C-2 hemiacetal link followed from the absence of a ^{13}C signal for C-2 in the carbonyl carbon region.

Lanceomigine is one of the only two indole alkaloids so far reported , the other being lanceomigine N-oxide (87) which have an N-1'-C-17 bond instead of the usual N-1-C-2 bond present originally in biogenetic precursor tryptophan - tryptamine It was demonstrated[94] that although 17-hydroxy-Ψ-akuammigine (85) which occurs with lanceomigine in Hunteria-congolana could be transformed smoothly into lanceomigine with mineral acid at room temperature, nevertheless the latter is not an artefact of the isolation procedure.

Lanceomigine N-oxide, isolated as an amorphous compound from Alstonia lanceolata, was reduced[91] to the same ketone (83) obtained from lanceomigine and could be formed[91] from the latter by N-oxidation using P-nitroperbenzoic acid. A comparison of the published data[91] for lanceomigine N-oxide with those for the amorphous Hunteria umbellata alkaloid, suggested their identity and this was confirmed by direct comparison with spectrum of sample provided.

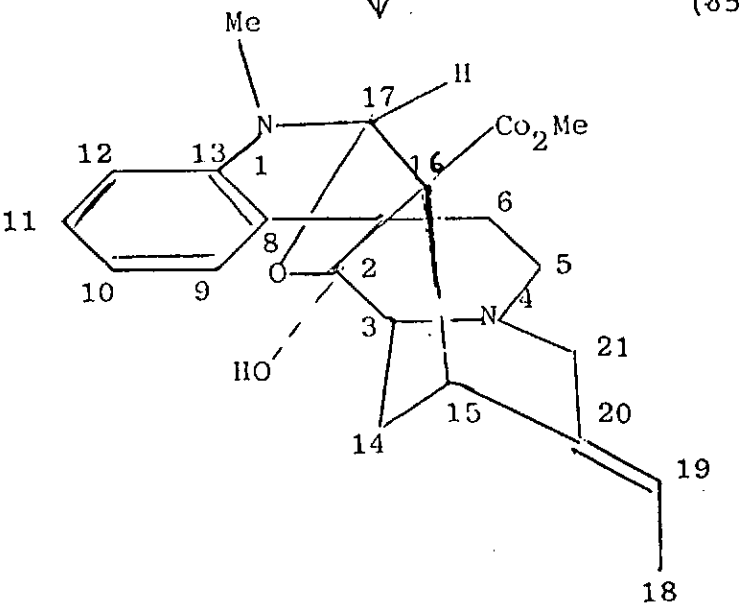


Hu12 Lanceomigine N - Oxide (84)



break 1-2 bond.

17-Hydroxy-Ψ-akuamigine (85)

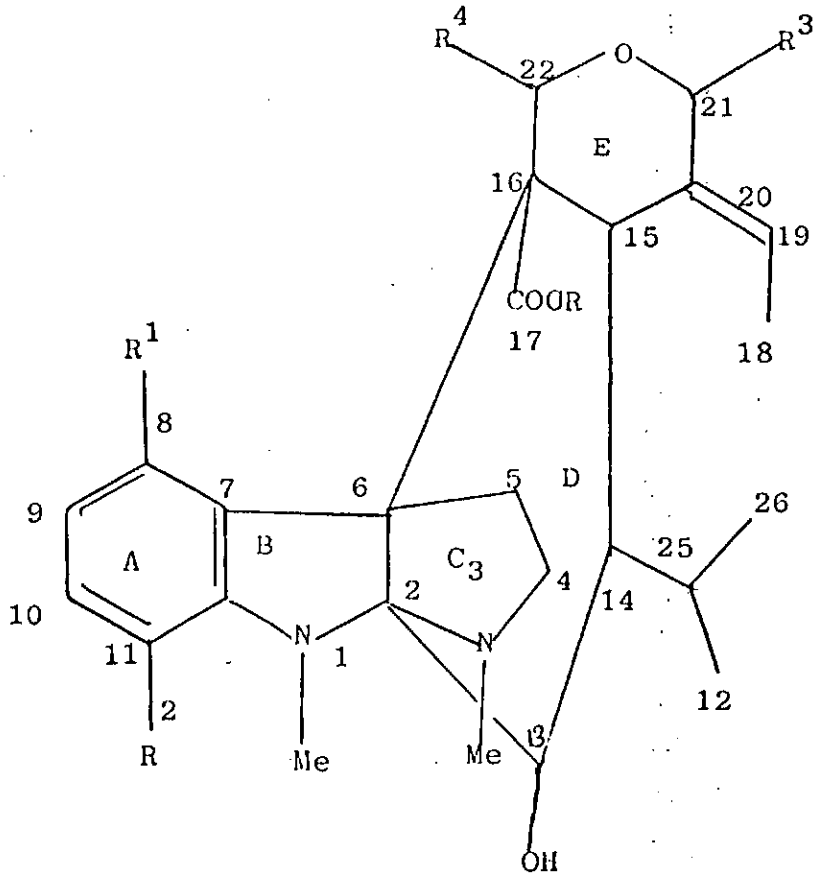


Lanceomigine (86)

4.10 Four Isomeric Water - Soluble Alkaloids from Hunteria umbellata.

Four isomeric 14-isopropyl-dihydroxydeoxyisocorymines with the lactone bridge opened were extracted from the seed of Hunteria umbellata.[95]

Spectral data showed that compounds (87-90) were isomeric, having a relative molar mass of 458 and a molecular formula of $C_{25}H_{34}N_2O_6$.



(87)

87 $R^1 = R^2 = OH$; $R^3 = R^4 = H_2$

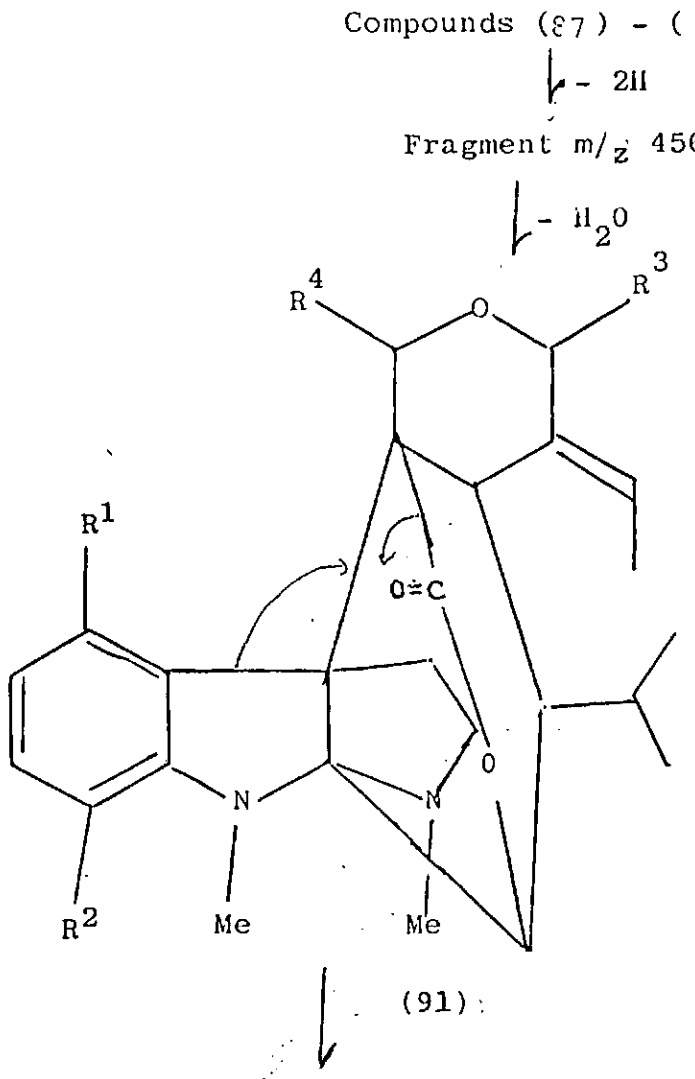
88 $R^1 = H$; $R^2 = R^4 = OH$, $R^3 = H_2$

89 $R^1 = R^2 = H$; $R^3 = R^4 = OH$

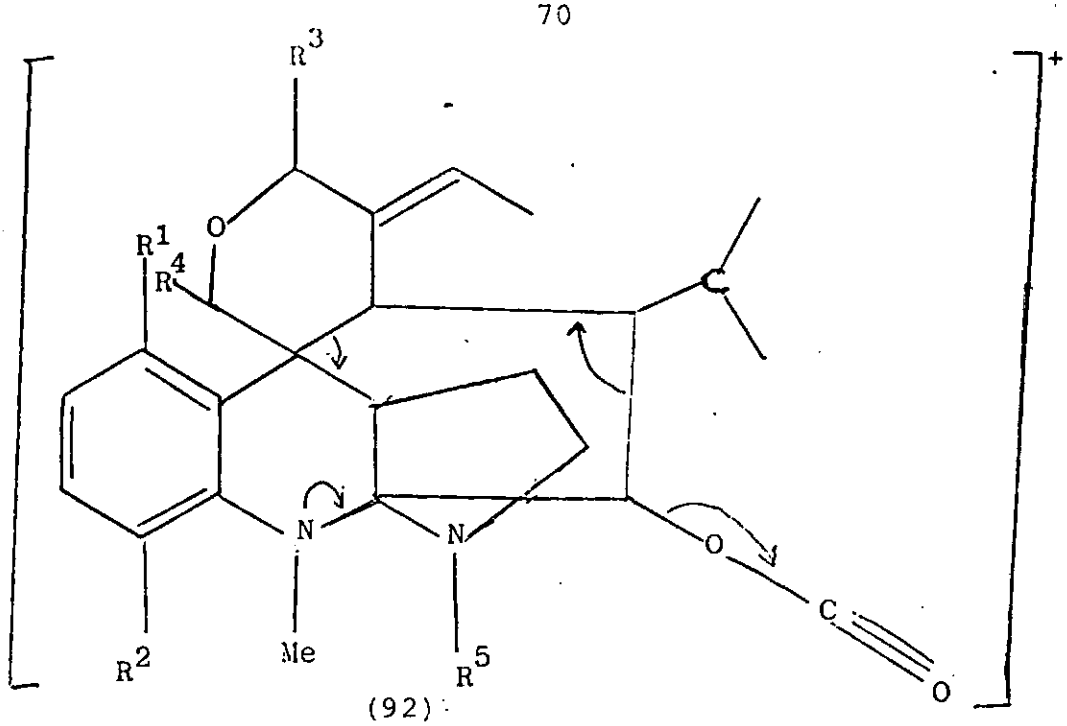
90 $R^1 = H$, $R^2 = R^3 = OH$; $R^4 = H_2$.

The four indole alkaloids were precipitated from aqueous acid solution by means of Mayer's reagent and the dry complex decomposed by prolonged standing in 95% ethanol. Removal of solvent gave a mixture which was initially separated by flash chromatography on deactivated alumina followed finally by purification on silica gel preparative TLC. The four isomers were distinguished by their R_f values of 0.1, 0.3, 0.6 and 0.9 respectively.

The mass spectrometric fragmentation pattern of each of the isomers 1-4 was paralleled by that proposed for isocorymine. The following ions present in the four isomers are also characteristic of isocorymine.



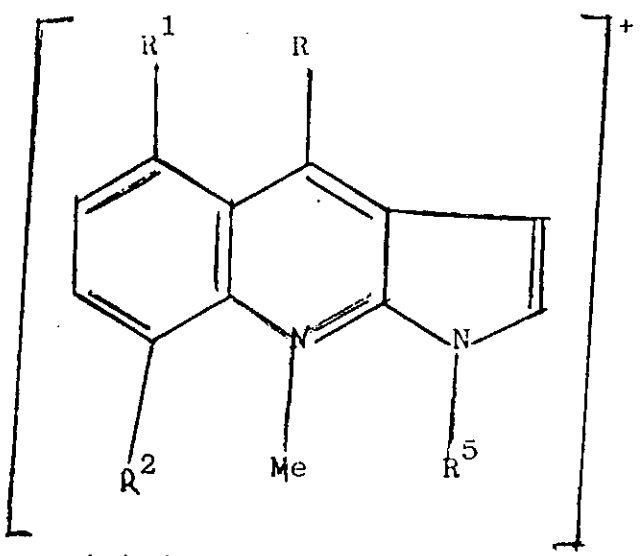
(91)



(92)

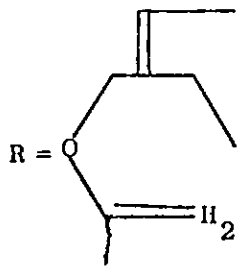
m/z 209

$C_{12}H_{17}O_3$



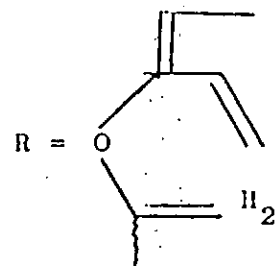
(a) R = H

(93)



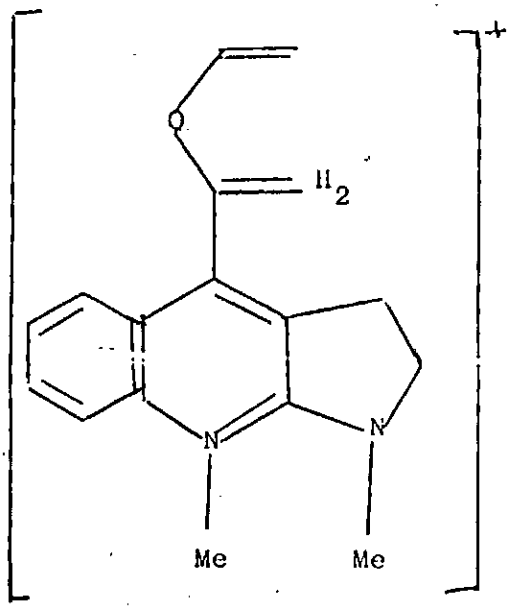
(94a)

$R^1 = R^2 = OH$, m/z 325



(94b)

$R^1 = R^2 = OH$, m/z 327



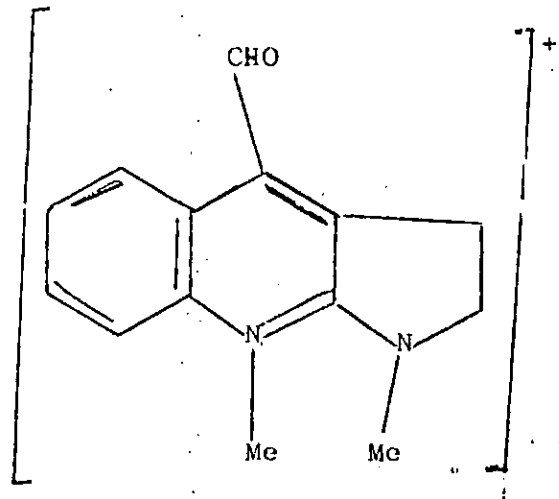
m/z 267 $C_{17}H_{19}N_2$

(95)

loss of

C_3H_4

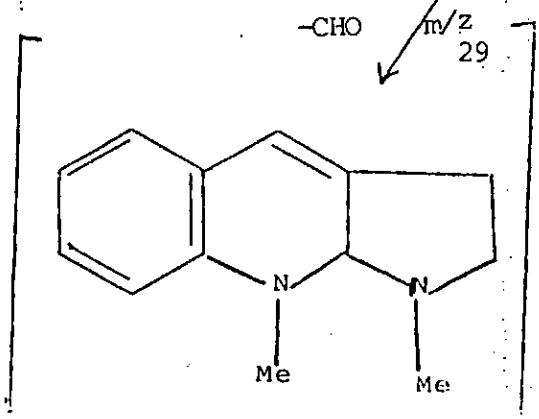
m/z 40



m/z 227

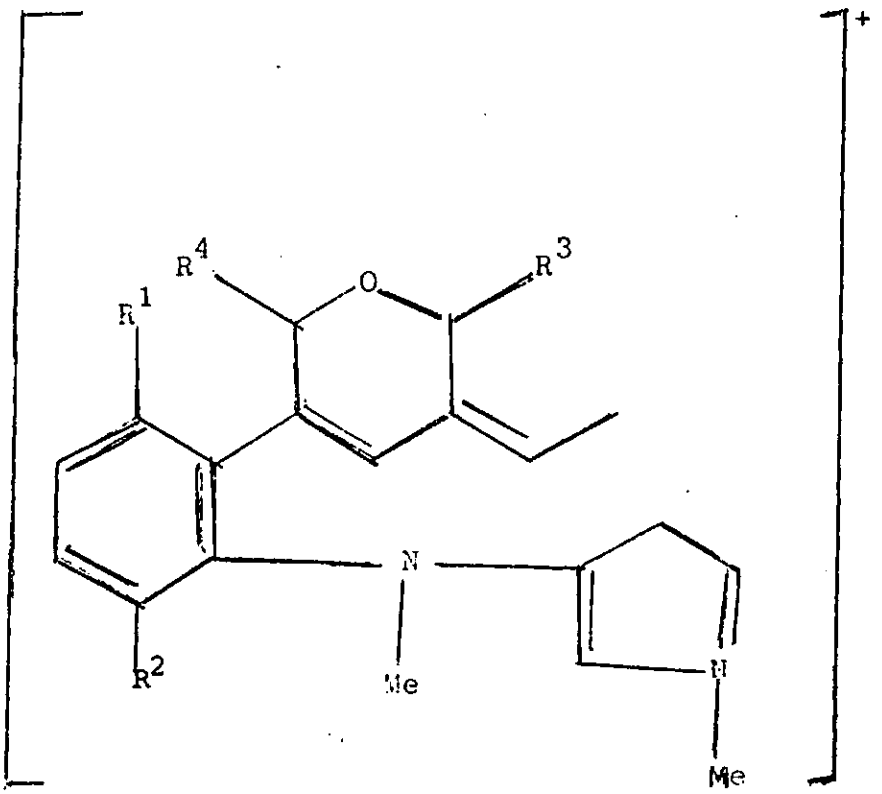
(96)

-CHO m/z 29



(97)

The prominence of fragment m/z 327 in the four probe spectra was significant. This was 16mu greater than the corresponding iso-corymine ion and indicated the presence of an additional hydroxyl group in 1,2,3 and 4, consistent with the existence of rings A,B (ruptured) C and the tetrahydropyran ring E.



(98)

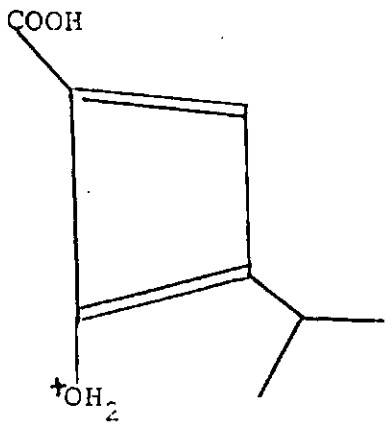
(a) $R^1 = R^2 = OH$; $R^3 = R^4 = H_2$; m/z 327.

(b) $R^1 = H$; $R^2 = R^4 = OH$; $R^3 = H_2$; m/z 327

(c) $R^1 = R^2 = H$; $R^3 = R^4 = OH$; m/z 327

(d) $R^1 = H$; $R^2 = R^3 = OH$; $R^4 = H_2$; m/z 327.

The presence of fragment m/z 155, in all the spectra could possibly arise as a result of bond ruptures between C-2 and C-13, C-15 and C-20, C-6 and C-16 and C-16 and C-22. The fragment contained an isopropyl substituent as reflected by the presence of the ion m/z 155 in all the spectra examined.



(99)

m/z 155

The present work is aimed at isolating more water soluble alkaloids from the same source as encouraged by recent findings of Adegoke et al[95] that the water soluble alkaloid mixture possesses hypoglycemic properties and also serves as antiharmoroid agents. Other ethnomedical beliefs of the natives that the plant is a potential breast anticancer agent also prompted the intensive search for the active alkaloid reported here. In all ten new water soluble alkaloids were isolated and using spectra analysis acceptable structures were proposed. All these are reported.

CHAPTER 5

RESULTS AND DISCUSSION

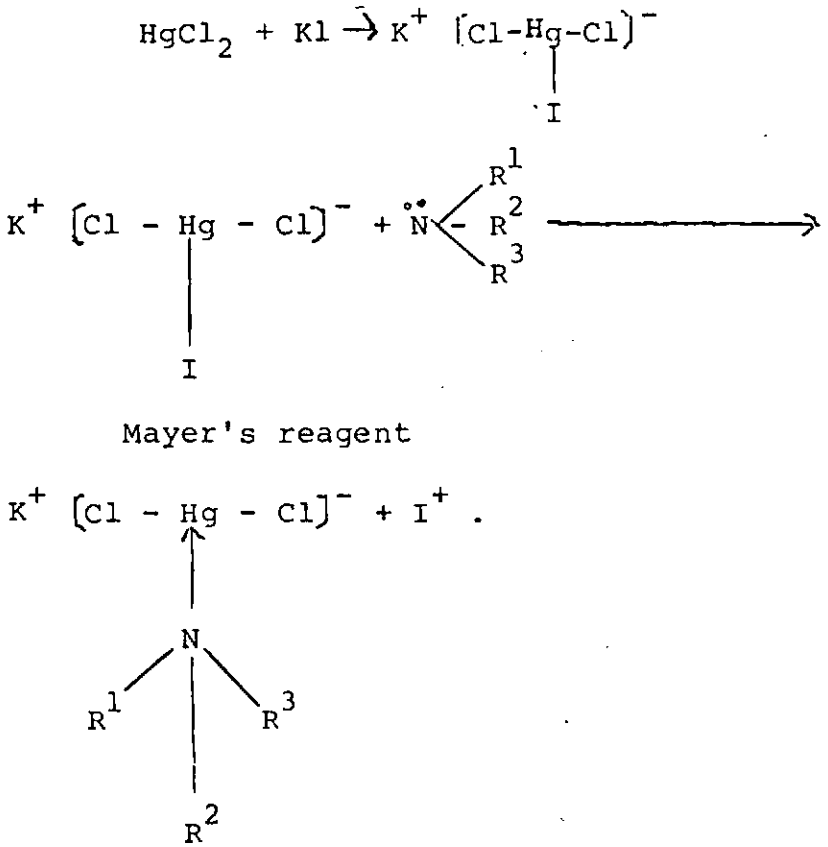
5.1 Extracting

The seeds, leaves and bark Hunteria umbellata were collected and subjected to continuous extraction. Before extraction, the plant was identified by a botanist and confirmed by comparison with authentic samples in the Forest Research Institute, Reference No. FH122857, Photographs on pages 266 - 271.

The plant material, particularly the seeds and leaves often contain quite substantial quantities of non-polar fats and waxes. These were removed by percolation of the dried pulverised material (leaves, seeds, or bark) with petroleum ether. Further extraction was achieved by refluxing with ethanol for forty eight hours.

The extract was treated with aqueous mineral acid and the solution filtered basified, and extracted with chloroform to yield a mixture of crude alkaloids. The basic aqueous layer contained the water soluble alkaloids. This solution was acidified and the alkaloid was obtained as a complex of Mayer's reagent. The Mayer's reagent was prepared from potassium iodide and mercuric chloride. Mercury has a vacant f orbital and is therefore capable of forming stable organic complexes by dative bonding. The lone pairs of electron present in the nitrogen atoms of the alkaloids are possible donated to the 5f orbitals to give the insoluble complexes.

A possible chemical reaction for the formation of these complexes is shown below:



Mayer's reagent

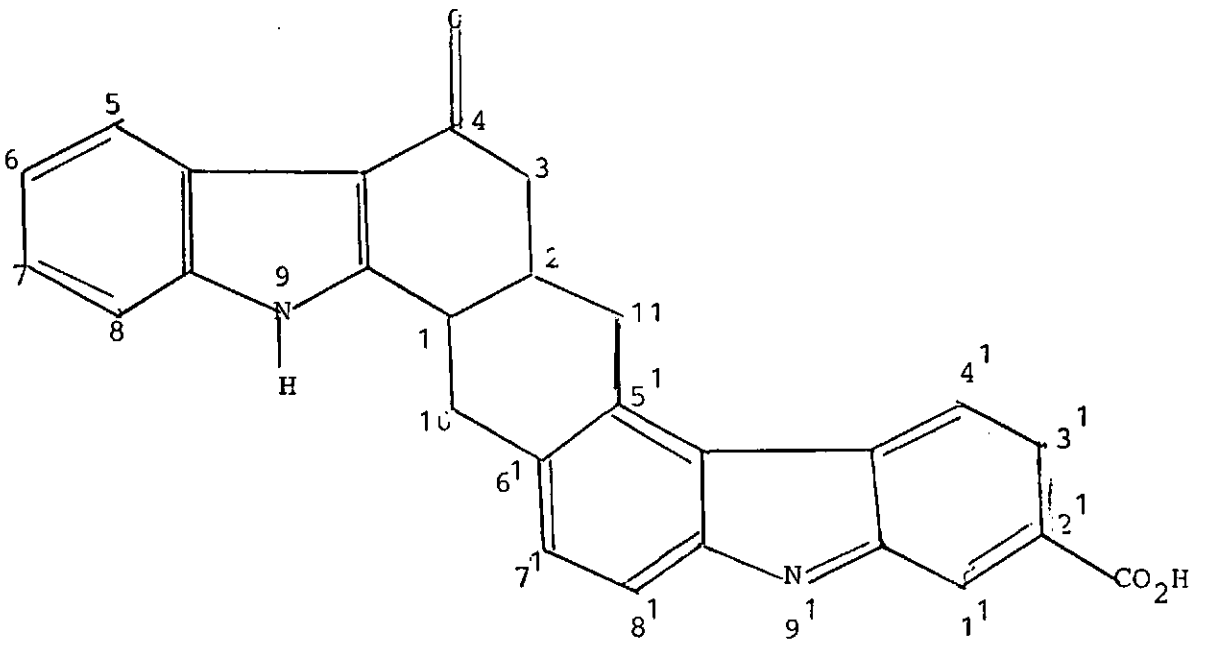
Complex

The complex, precipitate, after drying was suspended in ethanol, for six weeks and was allowed to decompose, at room temperature. The ethanol layer was decanted from the remaining undecomposed complex. Excess mercury was removed from the decantant by passing hydrogen sulphide into the filtrate and then through amberlite resin. Removal of the ethanol gave a crude mixture of the water soluble alkaloids in each of the seeds, leaves nd and back reported.

From 2,400 gms of seed, 14.6gm of mixture of crude water soluble alkaloid were obtained (0.61%). From 784 gms of the bark, 6.17gm were obtained (0.79%) and from 400 gms of the leaves 5.6gms were obtained (1.4%)

5.2 Isolation Procedure for the Seeds

The mixture of water soluble alkaloids of the seed in ethanol was initially separated by flash chromatography on deactivated alumina followed finally by the use of silica gel preparative plates. Three different alkaloids named SSHU1, SSHU2 and SSHU4 were isolated from the seeds. Here, only two were obtained in workable quantities.



SSHU 1 (100)

The compound SSHU1 is a light brown, amorphous glass, R_f 0.56 (silica gel, n-butanol: NH_3: H_2O, 15:1:05). The UV spectrum in ethanol absorbed at λ_{max} of 220nm, 264nm, 271nm and 284nm. In dilute ethanolic hydrochloric acid the maxima absorbed at 264nm, 271 nm, and 289nm, showing a

small bathochromic shift. The UV data are identical with those obtained for a carbazole.

The I.R. spectrum in chloroform showed band at 3064 cm^{-1}, 1728 cm^{-1} aryl and αβ unsaturated carbonyl (CCO), 1677 cm^{-1} (-COOH) and 1596cm^{-1} (benzene).

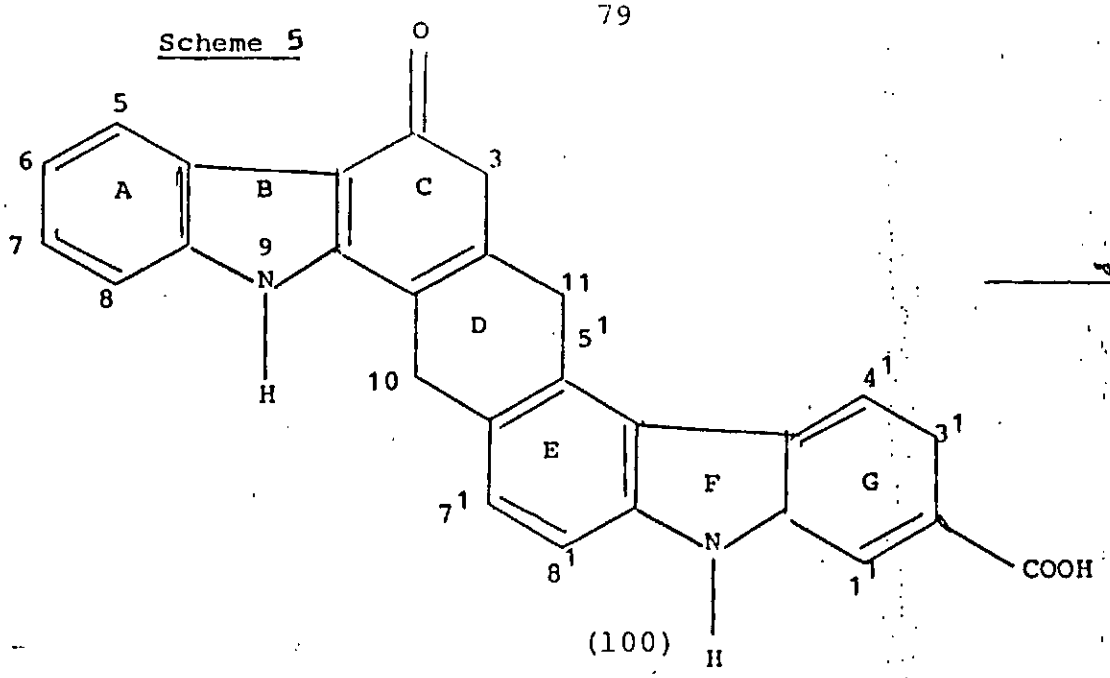
The 'H-nmr spectrum showed six aromatic protons, two protons as a doublet at δ7.8, three protons as a quartest at δ7.6, and one proton each as a single at δ7.35, and δ7.1 respectively. The methylene protons each absorbing at δ3.9, δ3.6, and δ3.1, and methine protons at δ1.6 and δ1.4 as a triplet.

The mass spectrum showed a molecular ion of 418. This plus other spectral data, inclusive of rules such as the Nitrogen Rule gave a molecular formular $C_{27}H_{18}N_2O_3$. The double bond equivalent is therefore 2 0. From the spectra data available structure 100 was proposed for SSHU1.

The aromatic protons were distributed in the following manner: the protons H-8 and H-8^1 absorbed at δ7.8. The nitrogen deshields proton H-8 and H-8^1 because N is orthoto to them. They however appear as doublet. Protons H-5, H-6 and H-7 absorbed at δ7.6 each and H-7^1 absorbed at δ7.35. The carbazole N-9 hydroqen absorbed at δ7.1. The methylene protons however absorbed at δ3.9 for H-11 at δ3.6 were for H-10 and at δ3.3 for H-3^1. The methine protons at δ1.6 was H-1^1, δ1.4 was H-4^1 and H-3 at δ1.7.

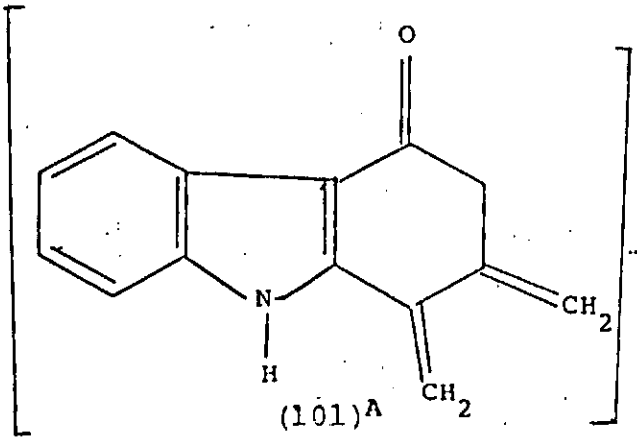
The compound is essentially a carbazole derivative linked via C-10 and C-11 methylenes to another carbazole derivative. Fragmentation pattern of the molecule confirmed the proposed structure.

Scheme 5



(100)

A + B

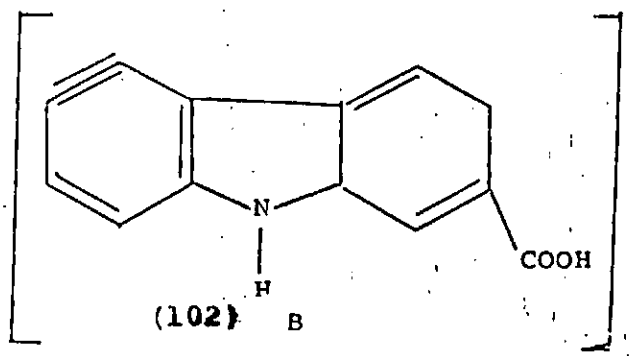


(101)A

m|z 209

$C_{14}H_{11}NO$

+



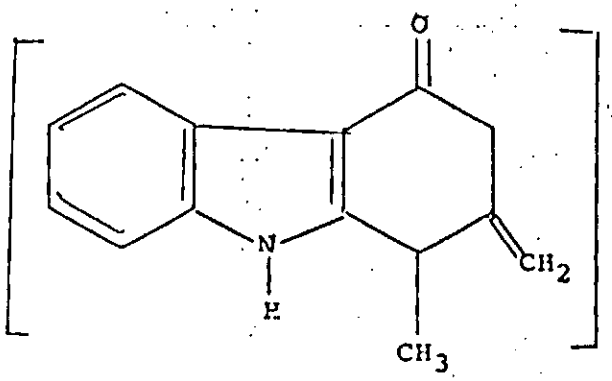
(102) B

m|z 209 (88.5%)

$C_{13}H_7NO_2$

rearranges as follows:

101 + H →



m|z 210, (88.6%)

(103)

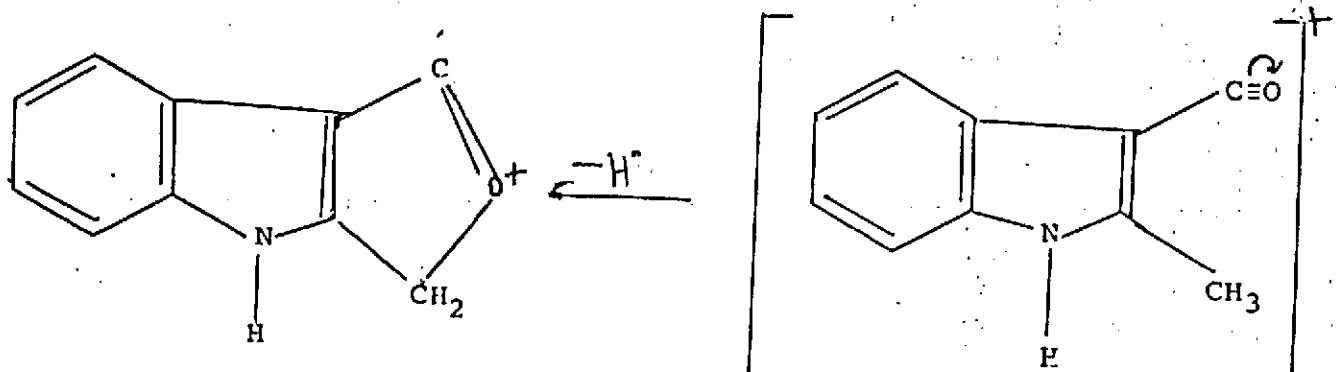
(103)

$m|_z$ 210, (88.6%)

$C_{14}H_{12}NO$

$- CH = C = CH_2$

$- CH_2$

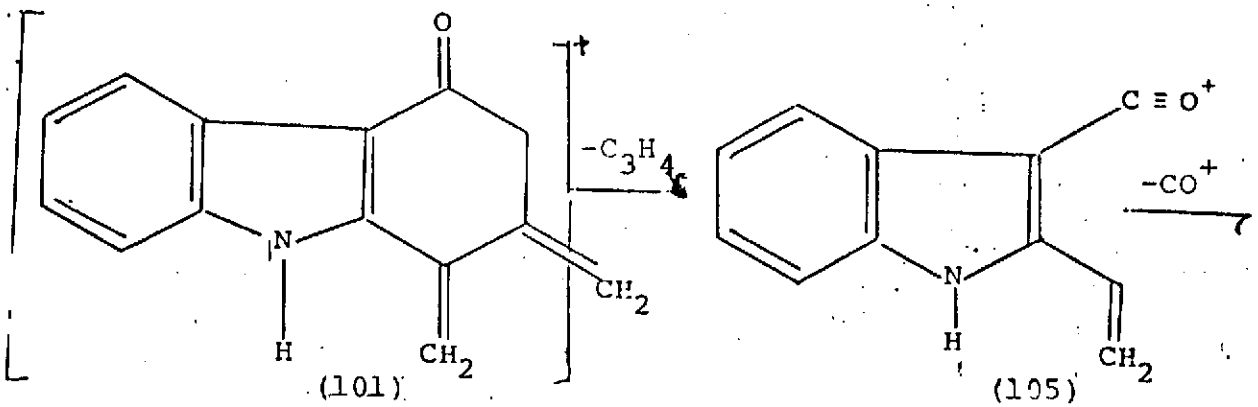


(105) $m|_z$ 158, (104)

$m|_z$ 157 (8.4)

$C_{10}H_7NO$

The presence of the minor ion $m|_z$ 157, 105 confirmed the position of the oxo group in ring c.

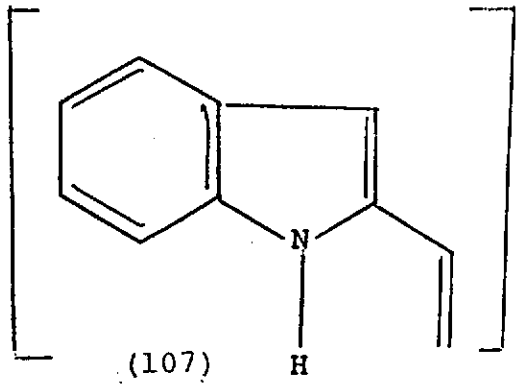


(101) (105)

$m|_z$ 170

(24%)

$C_{11}H_8NO$



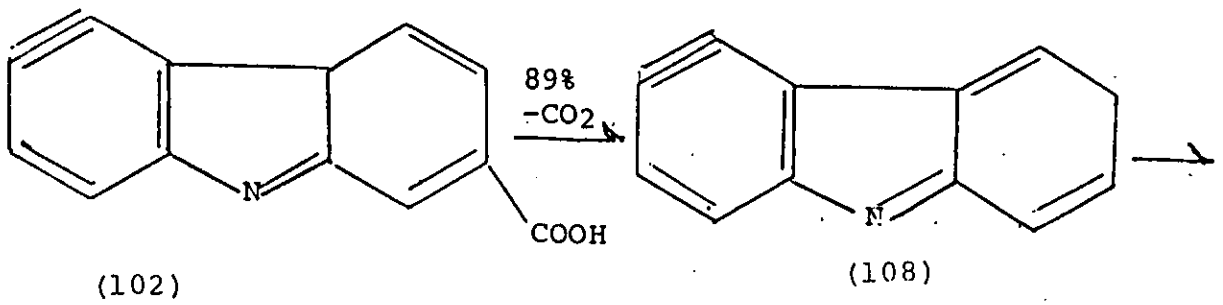
(107)

$m|_z$ (142) (21.9%)

$C_{10}H_6N$

An alternative route showed that 101 fragmented to 127, $m|_z$ 170, which by loss of carbon monoxide followed by loss of two protons gave the acetylenium ion 128 $m|_z$ 140, thus providing concrete proof for the siting of the oxo group in ring C.

The monomer 102 gave the following ions:

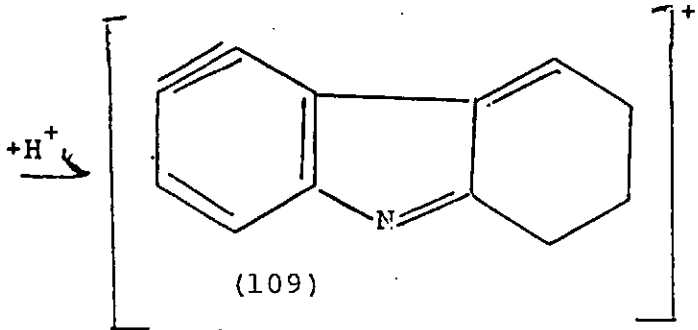


(102)

(108)

$m|_z$ 165 (4.6%)

$C_{12}H_7N$

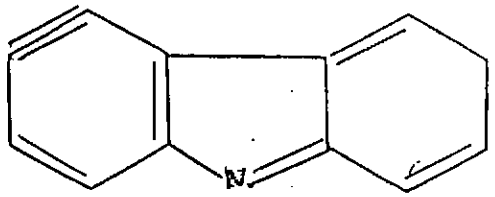


(109)

$m|_z$ 166 (3.6%).

$C_{12}H_8N$

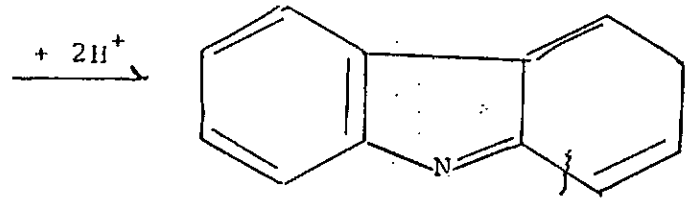
Ion 165 108 was produced from the monomer B 102 by loss of carbon dioxide. This established the position of the side chain carboxylic acid.



(108)

$m|_z$ 165

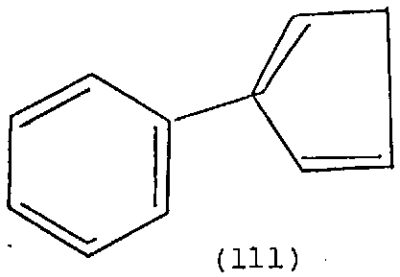
$C_{12}H_7N$



(110)

$m|_z$ 167

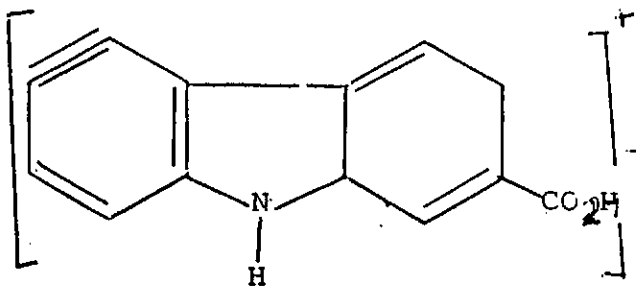
$C_{12}H_9N$



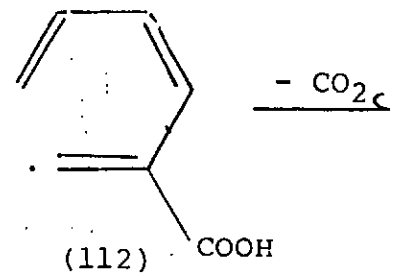
(111)

$m|_z$ 140 (44.1%)

$C_{11}H_8$



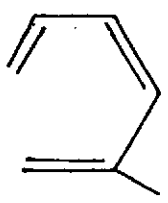
(102)



(112)

$m|_z$ 109 (11.5%)

$C_6H_5O_2$



(113)

$m|_z$ 65 (20.7%)

C_5H_5

$CH \equiv C - COOH$

(114)

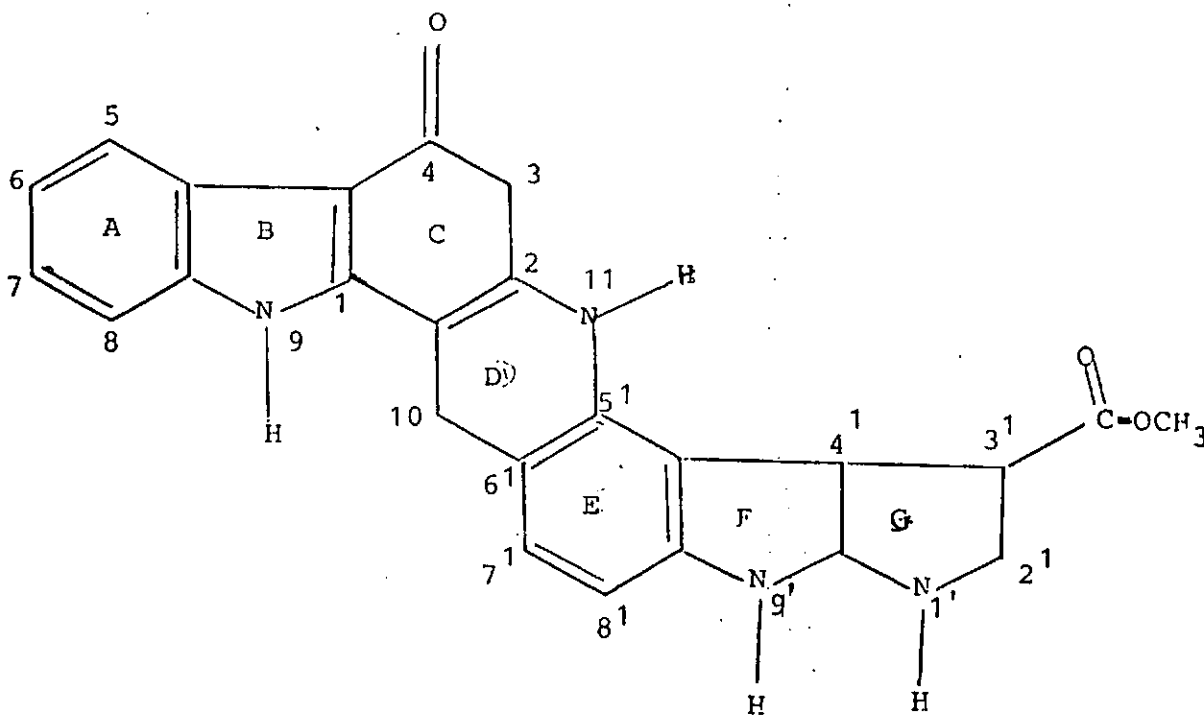
(62.1%)

$m|_z$ 70 $C_3H_2O_2$

Fragmentation of 102, produced the acetylenic - vinylic ion (112), $m|_z$ 109, which lost carbon dioxide to give 114 $m|_z$ 65. The ion 112 can also fragment to give the acetylenic acid (114). The production of the three entities established the positions of the $C1^1$ - $C2^1$, $C4^1$ - $C4a^1$, and $C9^1$ - $C9a^1$ double bonds and the $C2^1$ - position of the carboxyl group.

The mass spectral evidence thus made possible the structure proposed for SSHU1.

SSHU4



(115)

SSHU4

Compound SSHU4 is a light yellow glassy solid. It has a UV maximum absorption at λ_{max} 386nm and a shoulder at 260nm. In acid it exhibited λ_{max} 261nm and 436nm. For the

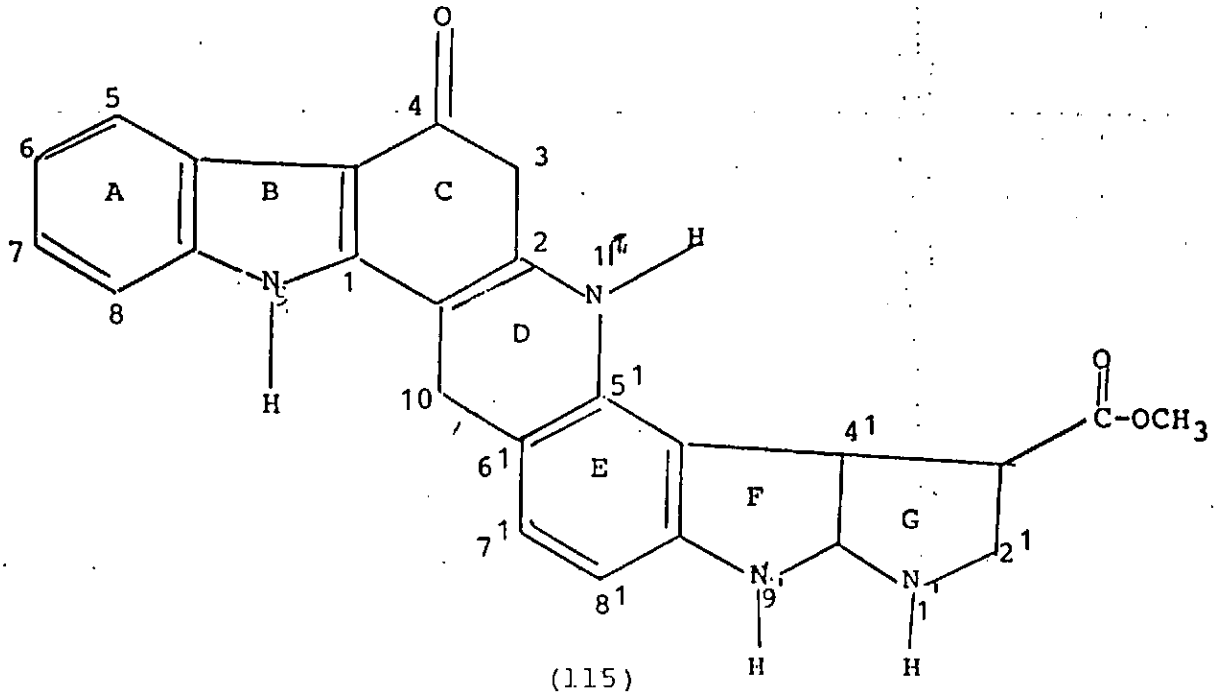
former this was a large bathochromic shift of 50nm. SSHU4 was therefore different from SSHU1. The chromophore responsible for this absorption appeared to be different from any one previously known in indole alkaloids. From the UV absorptions, the systems Ar-N-C-and Ar-N-C-N) were present. The compound is therefore a dimer of two different units. UV absorptions also indicated an extensive conjugation.

The IR spectrum showed absorptions at 3377 (N-H), 1744 (- CO of an ester), 1660 (= C - C = 0) and at 1548 benzene) cm^{-1}.

The NMR spectrum ran in CD_3OD showed six aromatic protons, one proton each as singlet at δ7.96 and δ7.4, J = $2H_z$ as doublet two protons as a doublet at δ7.38, J = $2H_z$ one proton as a triplet at δ7.1 J = $2H_z$ and a proton as a doublet at δ6.8. There were other absorptions at δ5.6 (1H,br, N-H), δ4.0 (1H, br, N-H), δ3.7 (2H, S, 2-N-H), δ3.62 (1H, S, CH) δ2.85 (2H, d, $-CH_2$), δ2.5 (2H, br, CH_2), and δ1.6 (1H, d, CH). These absorptions are summarized in table 2.

The mass spectrum gave a molecular ion of 426 and this, in addition other spectra data, the corresponding molecular formular, $C_{25}H_{22}N_4O_3$. The double bond equivalent for this molecule is therefore $\overline{17}$.

From the above spectra data structure (115) was proposed for SSHU4.



(115)

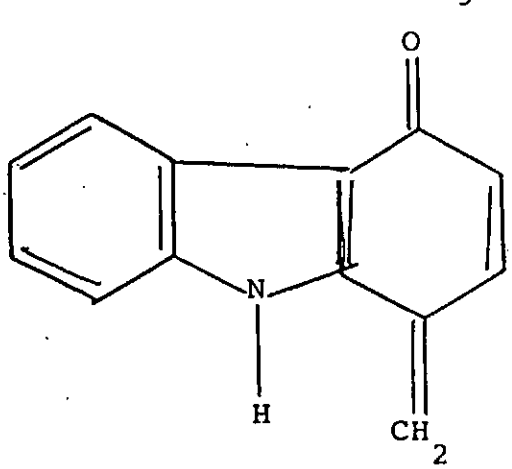
The aromatic protons were assigned as follows: the proton H-8 absorbed at δ7.9, H-8^1 at δ7.4, H-7 and H-5 each at δ7.3, H-6 at δ7.1, H-7 at δ6.9, H-9 at δ5.6, H-9^1 at δ4.0, H-11 at δ3.7, H-1^1 at δ3.7, H-3^1 at δ3.62 H-10 at δ2.90, H-3 at δ2.5, and H-4^1 at δ1.6.

The presence of the oxo group and the ester carbonyl group was confirmed by the IR absorptions at 1660cm^{-1} and 1744cm^{-1} respectively. The proposed structure was confirmed by fragmentation pattern as indicated by the mass spectrum.

Scheme 2a

(115) ⟶ A + B

There were three possible cleavages of the compound 136 into different ions which were prominent in the mass spectrum. The first involved a cleavage between bonds C2-N and C10-C6 to give ions A and B where A and B have the structure given below.

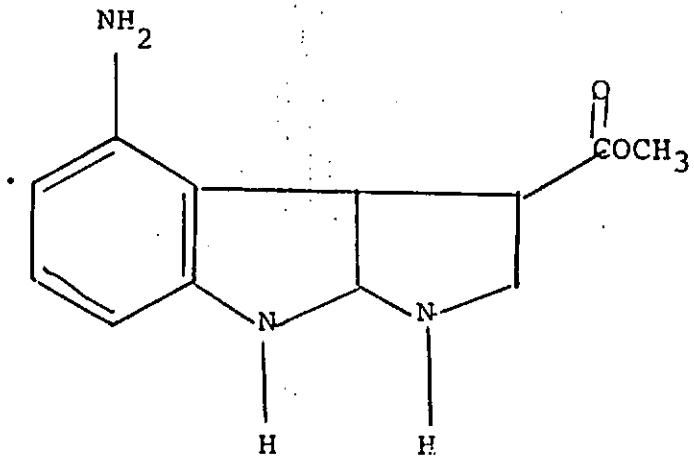


A

(116)

Ion $m|_z$ 195 (44.70%)

$C_{13}H_9NO$

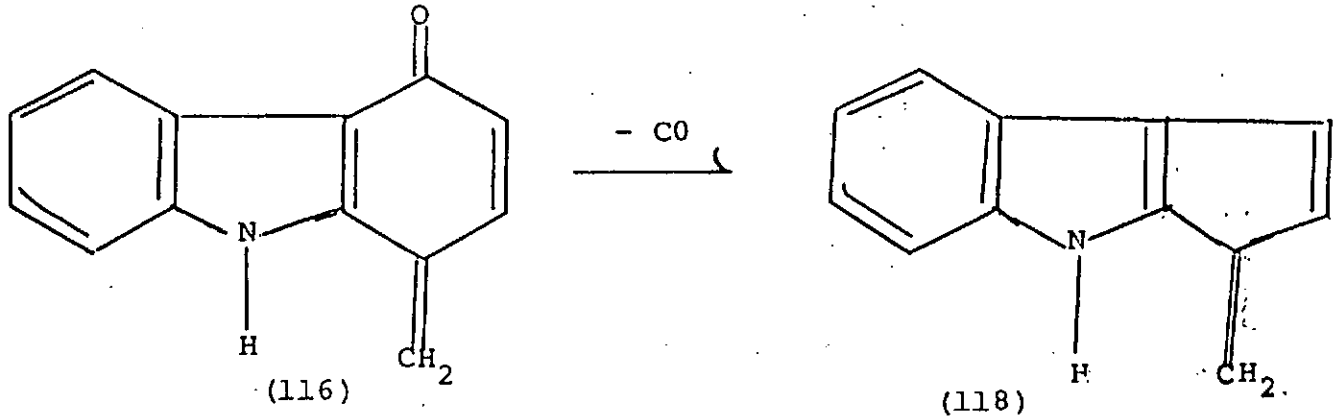


B (117)

Ion $m|_z$ 231

$C_{12}H_{13}N_3O_2$.

Ion B, structure 117 is not stable but fragmented immediately to minor ions. Ion A, 116 is quite stable as indicated by a moderately high intensity.



(116)

(118)

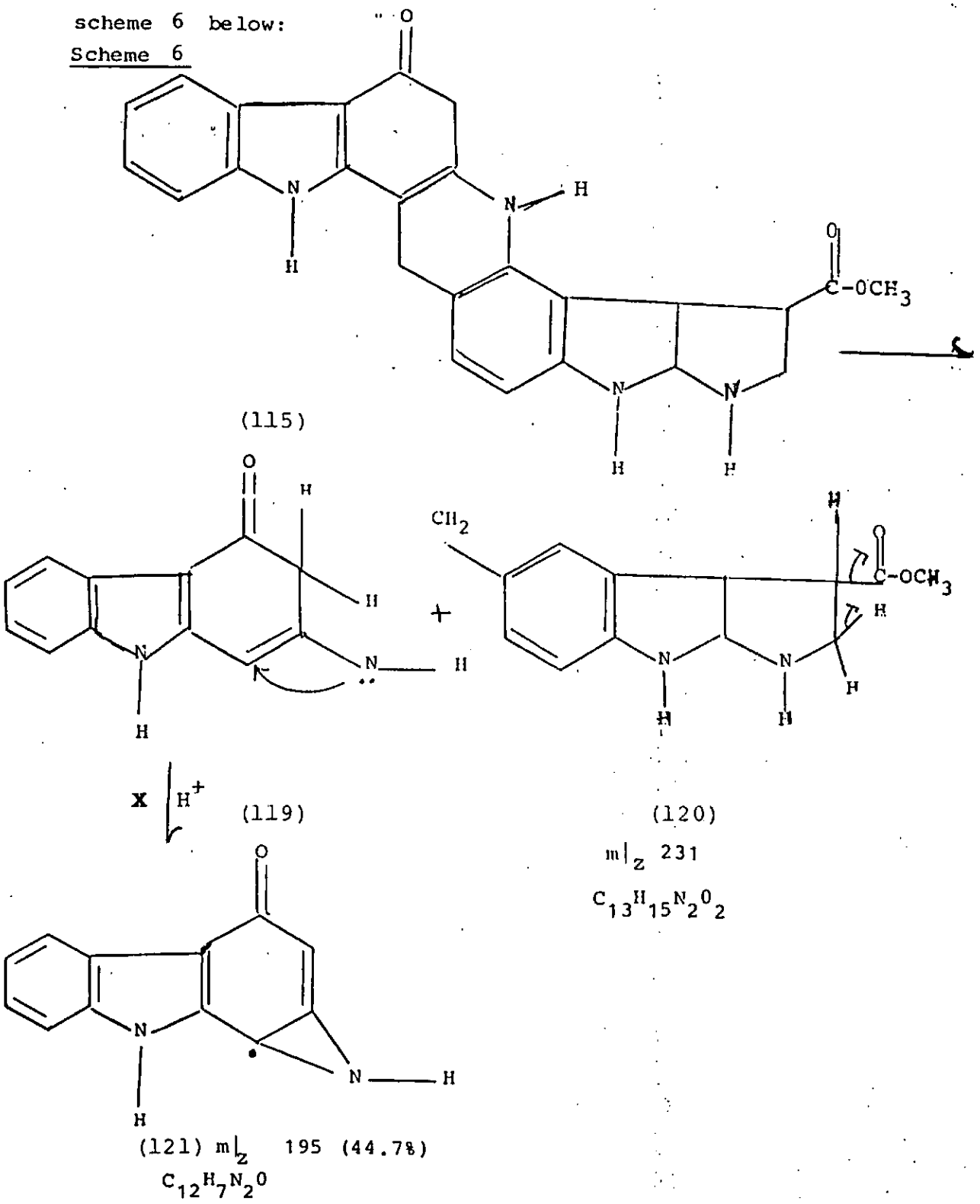
$m|_z$ 167 (34.3%)

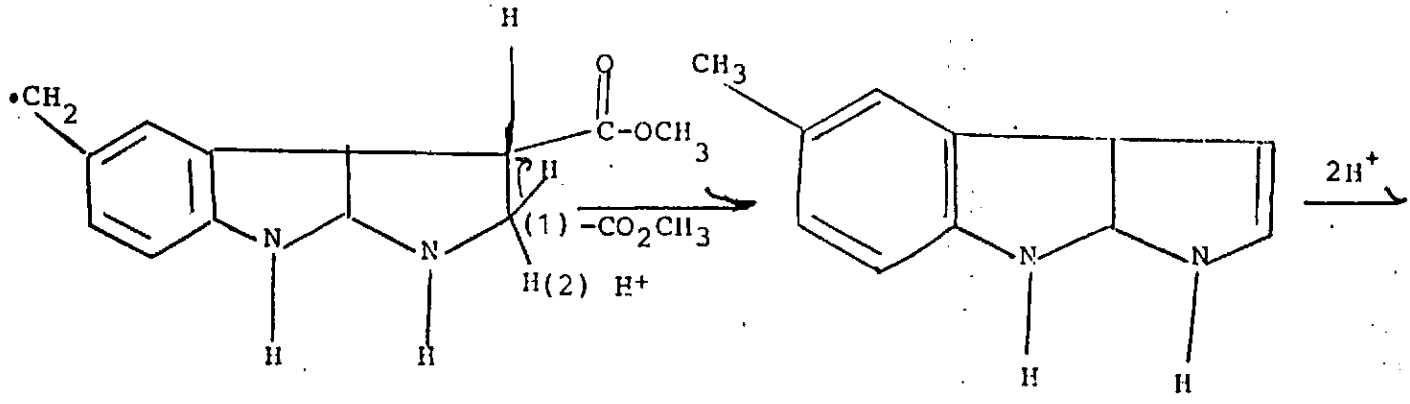
$C_{12}H_9N$

Ion 118 was formed by the loss of CO $m|_z$ 28, (32.5%) from ion 116.

The second route of fragmentation of 115 is shown in scheme 6 below:

Scheme 6



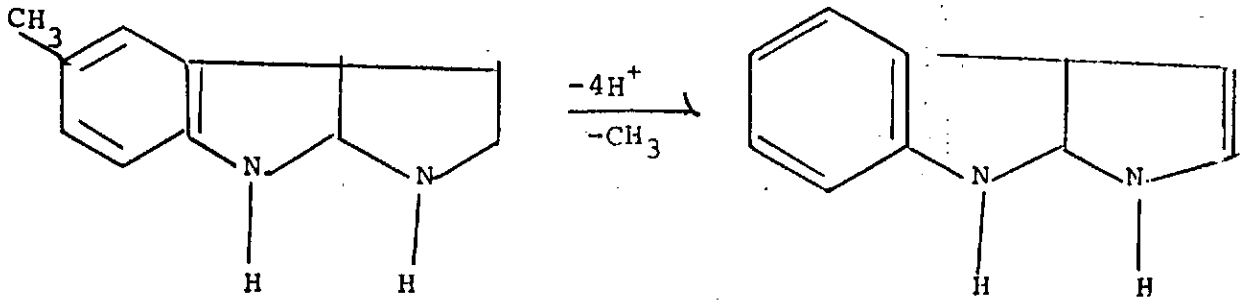


(120)

(122)

Ion $m|_z$ 171 (18.5%)

$C_{11}H_{11}N_2$



(123)

$m|_z$ 173 (12.9%)

$C_{11}H_{13}N_2$

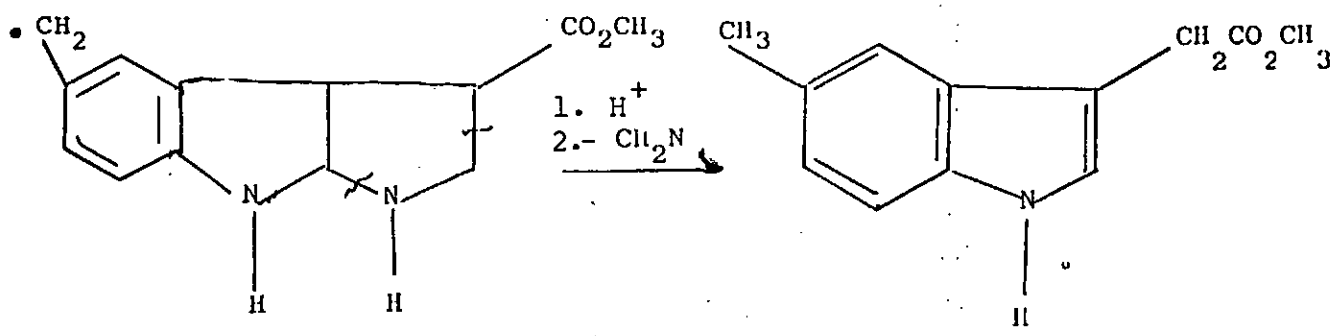
(124)

$m|_z$ 154 (16.5%)

$C_{10}H_6N_2$

The cleavage of ion $m|_z$ 59, $C_2H_3O_2$ from ion 120 gave ion 123 Protonation of the latter gave ion 123 which on dehydrogenation produced ion $m|_z$ 154. The above fragmentation indicated the position of the methyl carboxylate group.

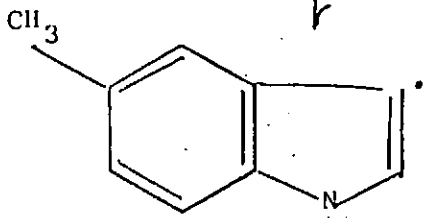
The other possible fragmentation of ion 120is shown below:



(120) (125)

m|z 203 (11.8%)

$C_{12}H_{13}NO_2$



(126)

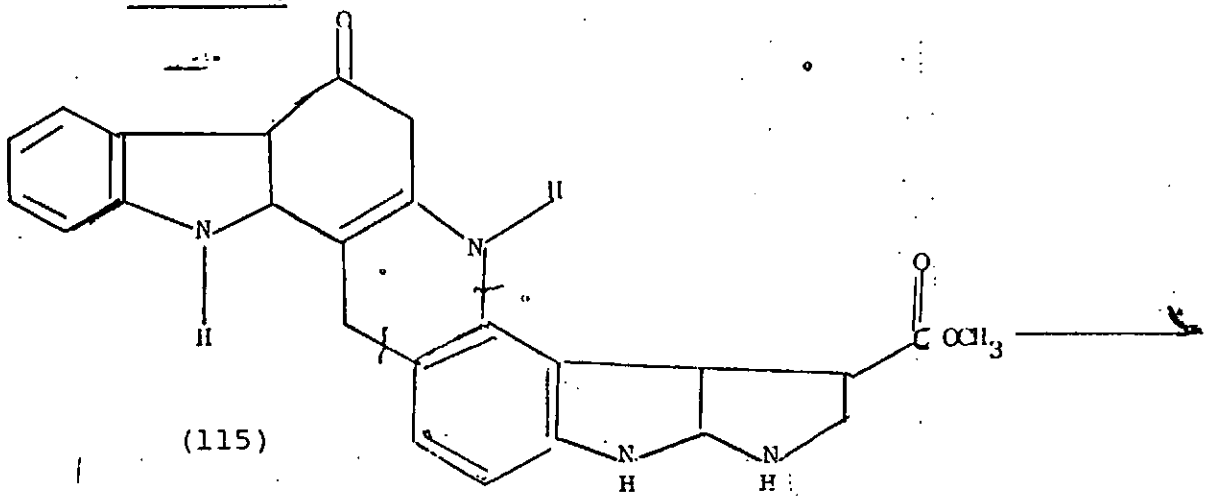
m|z 130 (17.5%) H

C_9H_8N

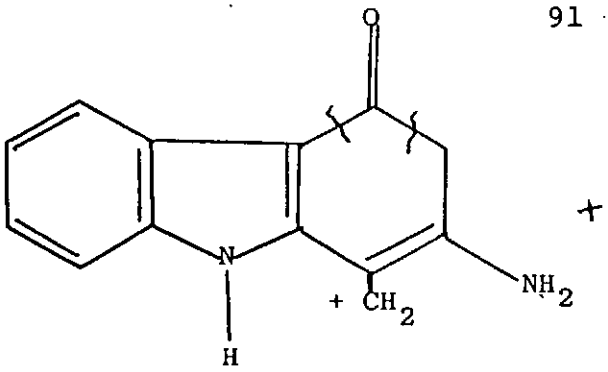
The production of ion 125 m|z 203 from ion 120 further confirmed the position of the ester.

The third possible cleavage of the C-C single bond between the two dimers of SSHU4 136, involved the bonds C10-C6^1 and N-C5^1.

Scheme 2c



(115)

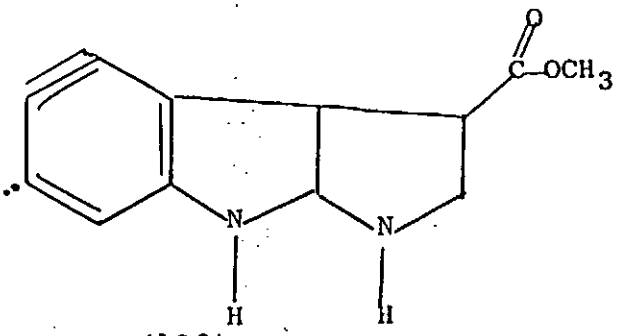


(127)

$m/_z$ 211 (35.2%)

$C_{13}H_{11}N_2O$

+

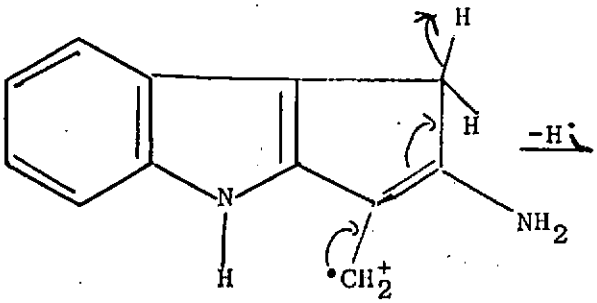


(128)

$m/_z$ 215

$C_{12}H_{11}N_2O_2$

-CO

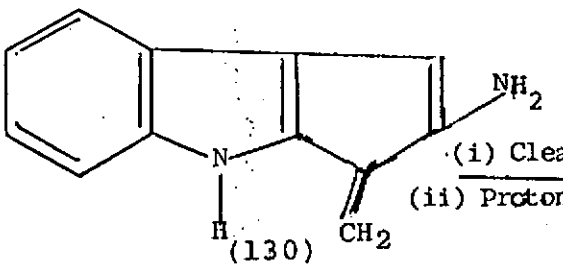


(129)

$m/_z$ 183 (11.4%)

$C_{12}H_{11}N_2$

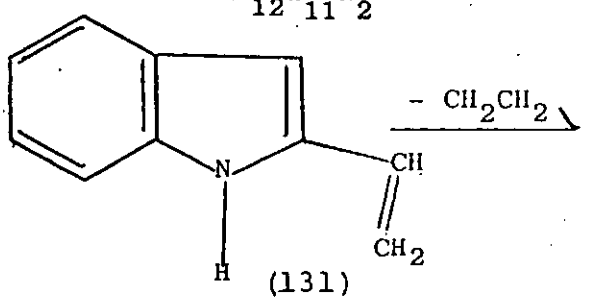
-H·



(i) Cleavage
(ii) Protonation

(130)

$m/_z$ 182 (35.40%)

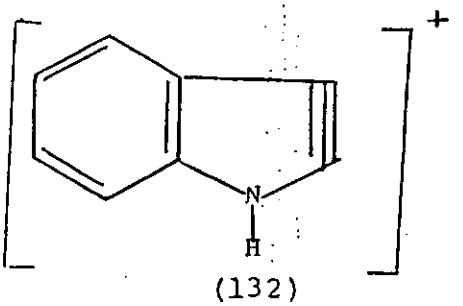


- CH_2CH_2

(131)

$m/_z$ 143 (12.3%)

$C_{10}H_9N$



(132)

$m/_z$ 115 (22.9%)

C_8H_5N

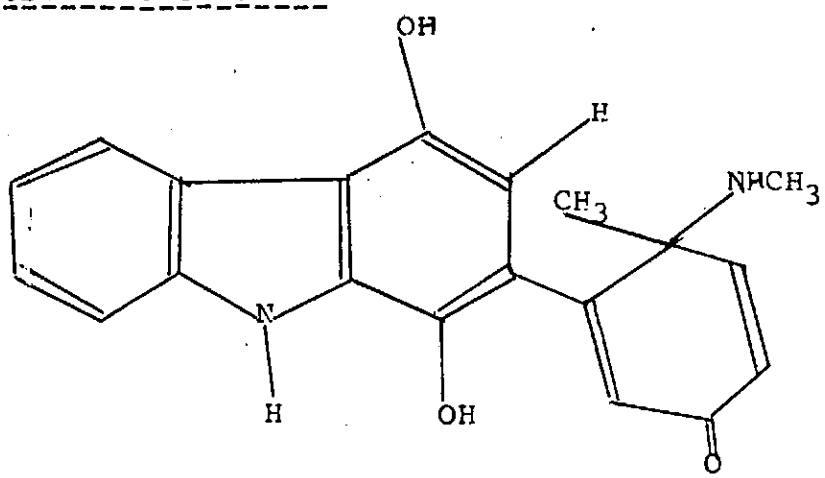
In scheme 2c, ion 127 $m|_z$ 211 was produced by the cleavage of the bonds N-C5[1] and C10-C6[1]. By loss of CO gas gave ion (129) $m|_z$ 183 $C_{12}H_{11}N_2$.

Rearrangement took place in the ion 129 and by loss of a proton, ion 130 $m|_z$ 182 $C_{12}H_{10}N_2$ was produced having an appreciable intensity of 35.4%. Cleavage of bonds and protonation of ion $m|_z$ 182 gave ion 131 $m|_z$ 143. Further loss C_2H_4 $m|_z$ 28 from ion 131 $m|_z$ 143 produced ion 132 $m|_z$ 115, C_8H_5N with relative intensity of 22.9%.

5.3 Water Soluble alkaloids from the leaves

The water soluble alkaloids from the leaves are more polar than those from the seeds. Attempts to separate them using the same procedure similar to those of the seeds failed. More polar solvent mixtures were used to elute from column and then thin layer from runing TLC. Four new compounds SLHU1, SLHU3, SLHU4, and SLHU5 were isolated.

Structure of SLHU1



(133) SLHU 1

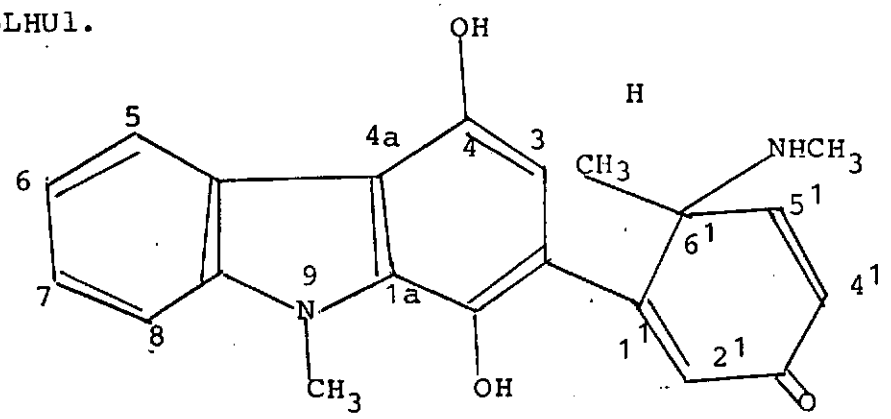
SLHU1 is a light brown solid and has R_f value of 0.18 (silica methanol; ammonia, 15;1). The UV absorption showed maxima at λ_{max} 251nm, 308nm and 319nm. In acid λ_{max} was at 251nm, 307nm, and 369nm. Thus there was a bathochromic shift of 50nm in the absorptions. This type of spectrum is also reminiscent of a carbazole nucleus.

The IR showed characteristic absorption at ν_{max} cm^{-1} 3584 (OH), 1706 (αβ-unsaturated Carbonyl). 1631 (benzene ring) and at 1213 (ArOH).

The ^{1}Hnmr data in CD_3OD are summarised in Table 4. P.154 The spectrum showed five aromatic protons. There were absorptions for one proton each as a triplet at δ8.5 J = $2H_z$ and doublet J = $2H_z$ δ8.4 respectively, for two protons as a doublet at δ7.8 J = $2H_z$. The less acidic H-3 absorbed as a singlet at δ7.5 and the expected low field signals for H-1 and H-4 were conspicuously absent. The UV spectrum confirmed the carbazole nucleus.

There were other absorptions at δ3.85 (1H, S), δ3.3 (3H, S), δ1.30 (3H, S), δ0.9 (3H, S), δ5.5 (1H, br), δ5.11 (1H, d), δ5.18 (1H, d), δ3.95 (1H, d), δ4.64 (1H, d), and δ2.0 (3H, broad).

The mass spectrum gave a molecular ion at $m/_z$ 348 with El spectrum and an ion $m/_z$ 349 in the corresponding Cl system. The molecular formular from spectra data was therefore $C_{21}H_{20}N_2O_3$. The double bond equivalent is $\overline{\overline{13}}$. From the above spectra data, structure 133 given below was proposed for SLHU1.



(133)

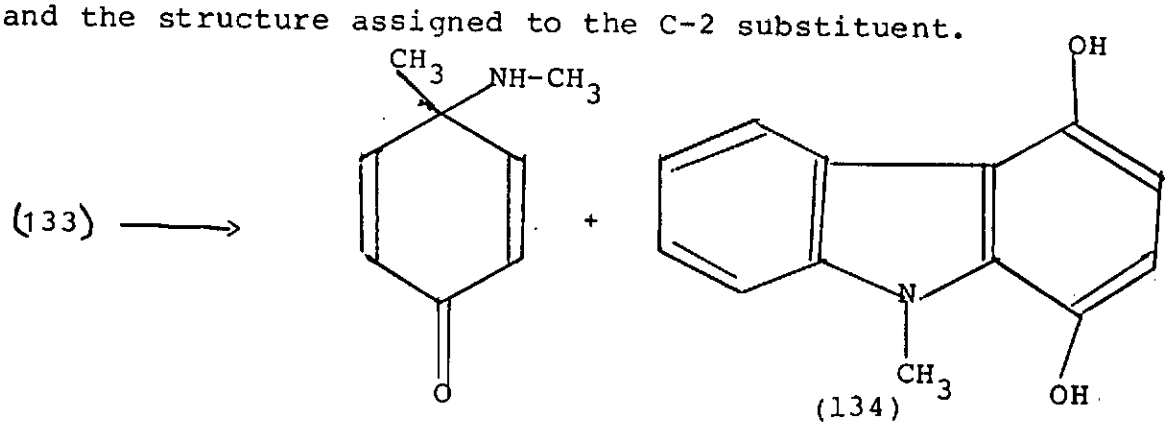
The aromatic protons were distributed in the following manner: the absorptions at δ8.5 was assigned to proton H-7, because of its high acidic nature due to its relative position to nitrogen, δ8.4 to H-5; δ7.8 to H-6, and δ7.8 H-8, and the

less acidic proton absorpting as a doublet at δ7.5 J = $2H_z$ to H-3.

Absorptions at δ3.9 and δ3.85 were due to the hydroxyl proton at C-1 and C-4. The expected low field signals for these two protons were conspicuously absent. The presence of two -OH groups was also confirmed by the IR spectrum.

C-2 was therefore assigned to the oxocyclohexadienyl residue since NH-9 and the C-1 hydroxyl group would have additively induced a more downfield signal to H-2 than to H-7. The UV λ_{max} at 369nm and IR λ_{max} 1706 cm^{-1} confirmed a dienone in the C-2 substituent.

The H-2^1 signal appeared as an olefinic doublet at δ5.26, H-5^1 as a doublet at δ5.11 J = $2H_z$ as a doublet at δ3.95. The C-6^1 methylamino hydrogen absorbed as a broad band at δ2.0. The presence of the N-9 methyl, 6^1-aminomethyl and 6^1 - methyl groups were established readily by I.R. and ^{1}Hnmr data. These absorbed for three protons each as a singlet at δ3.30, δ1.30, and 0.9 respectively. As usual, mass spectral fragmentation pattern outlined below corroborated the C-1 and C-4 siting of the hydroxyl groups, and the structure assigned to the C-2 substituent.

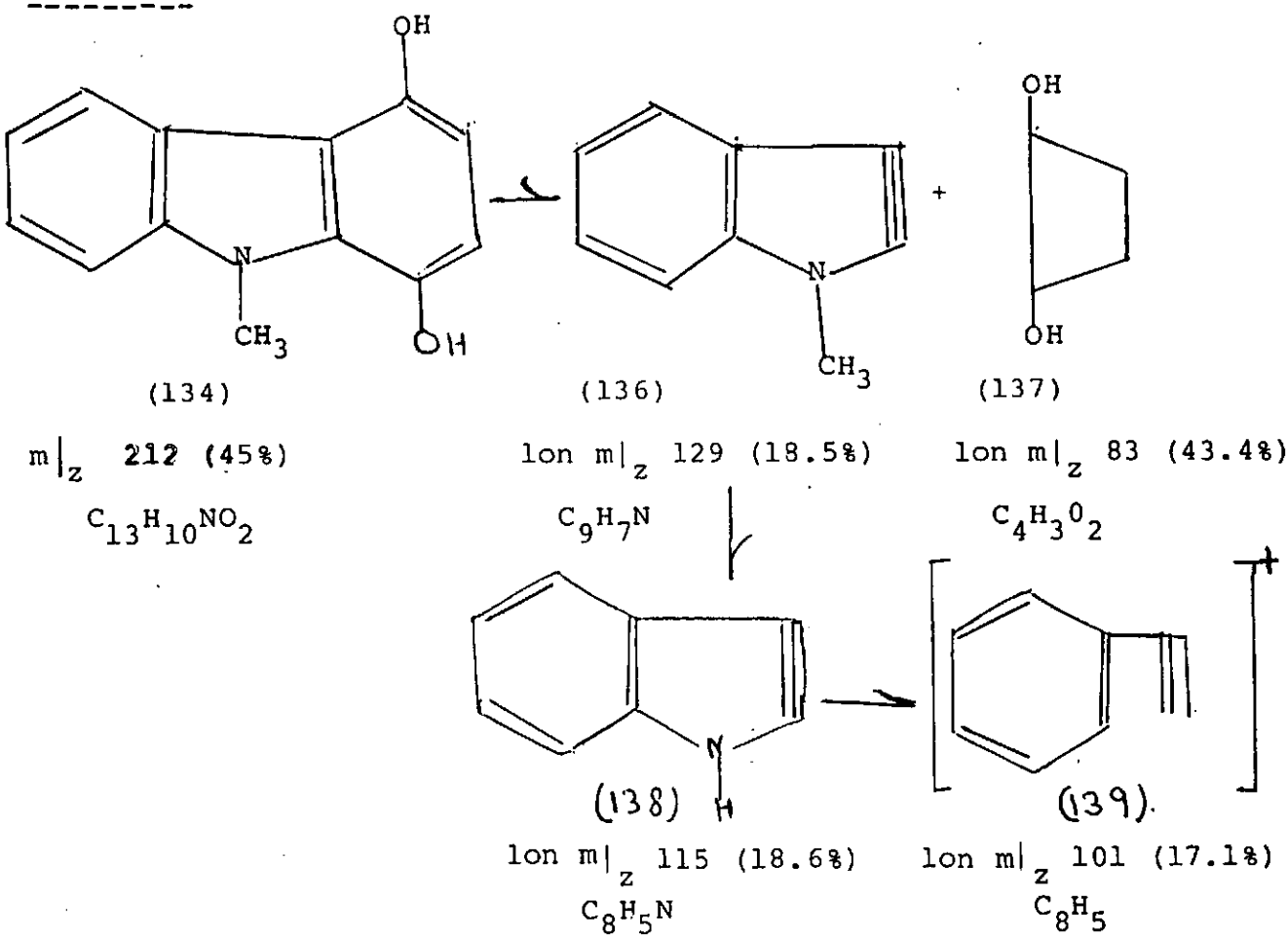


(135)
m|z 136
$C_8H_{10}NO$

(134)
m|z 212 (45%)
$C_{13}H_{10}NO_2$

The ions 134 $m|_z$ 212 $C_{13}H_{10}NO_2$ and ion 135 $m|_z$ 136 $C_8H_{10}NO$ were produced by the cleavage of bond C-2 and C-1[1]. The presence of these ions in the mass spectrum with high relative intensity confirmed the structure assigned to C-2. These ions further broke down as shown in the scheme below.

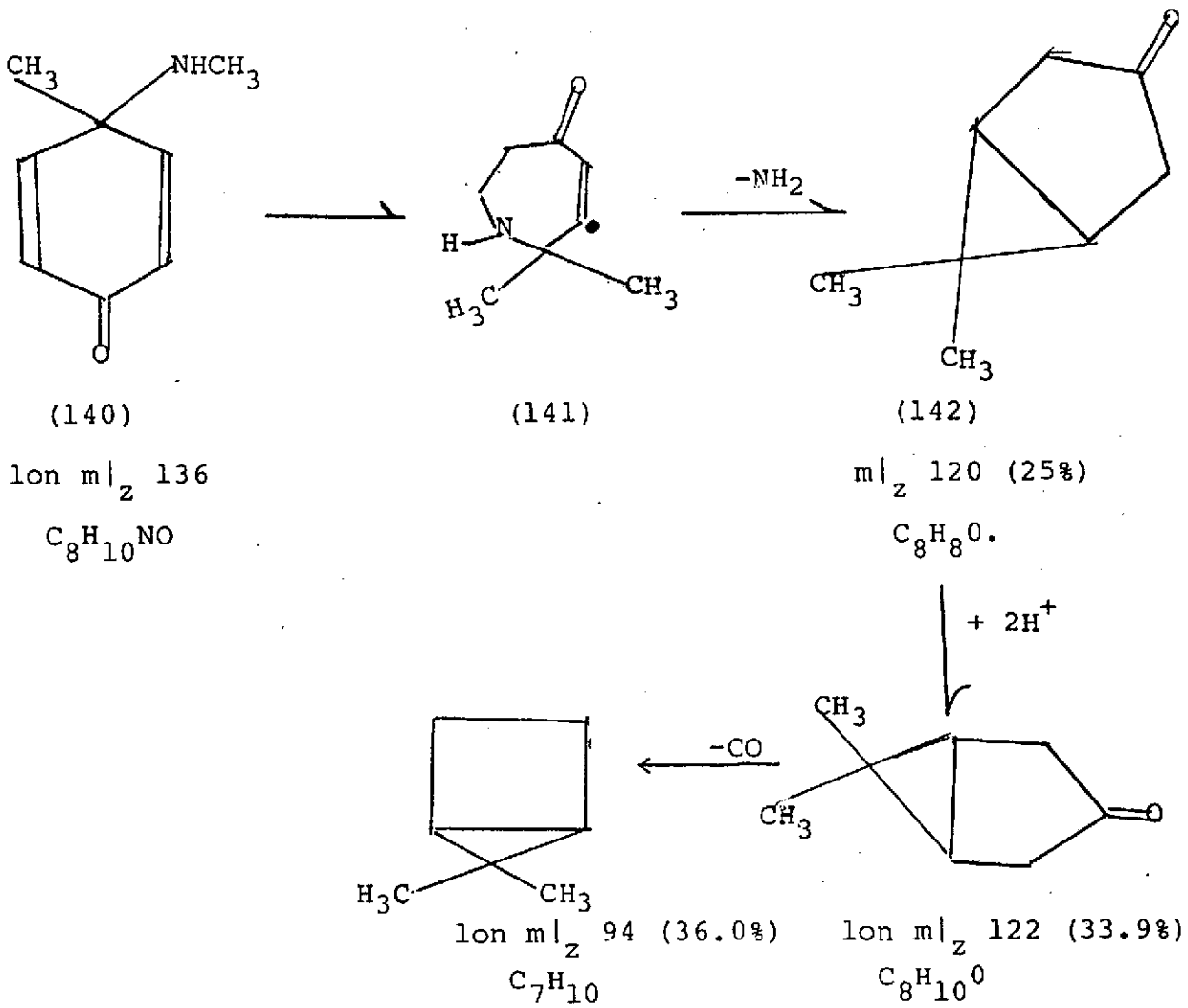
Scheme 7



The ions $m|_z$ 129 (136) and $m|_z$ 83 (137) were produced by the cleavage of the bonds C-1a and C-4a. The production of ion 137 $m|_z$ 83 which is 1, 2-dihydroxy cyclobutadiene further confirmed the C-1 and C-4 siting of the hydroxyl groups ion $m|_z$ 101 (139) is a phenyl acetylenium ion.

Scheme 8 shown below, gave other featural details of the C-2 substituent.

Scheme 8



(140) lon $m|_z$ 136 $C_8H_{10}NO$

(141)

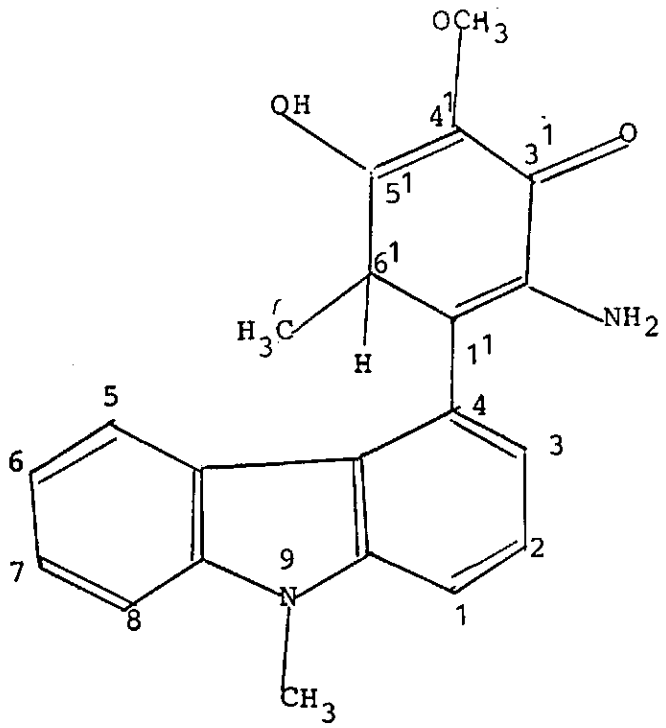
(142) $m|_z$ 120 (25%) C_8H_8O.

lon $m|_z$ 94 (36.0%) C_7H_{10}

lon $m|_z$ 122 (33.9%) $C_8H_{10}O$

Essentially ion $m|_z$ 136 (140) $C_8H_{10}NO$ ring expanded and extruded an amino group to give ion $m|_z$ 120 (142) C_8H_8O (25%). Decarboxylation of the latter gave ion $m|_z$ 94 (144) C_7H_{10} with relative intensity of 36.0%. The foregoing spectral evidence and fragmentations confirmed the structure assigned to SLHU1.

SLHU3



(145)

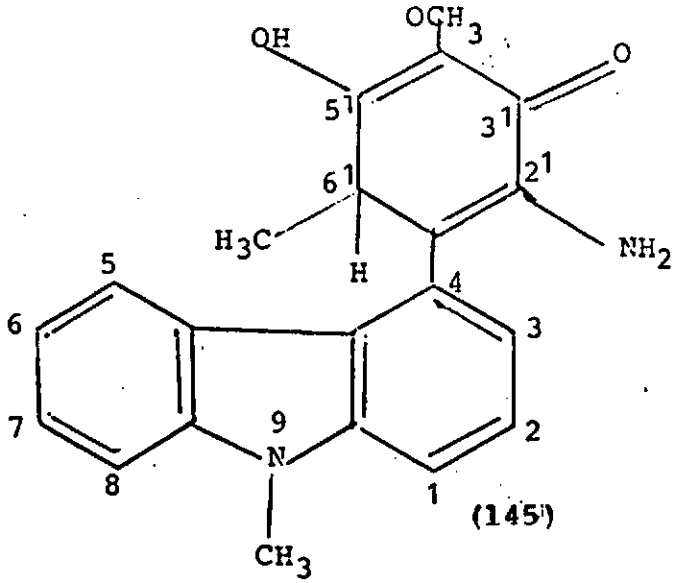
Structure of SLHU3

This glassy material had an R_f value of 0.4. The UV spectrum was very similar to that of SLHU1 viz $_{max}$ at 251nm, 308nm, 368nm, and 429nm. Unchanged in acid.

The IR spectrum showed absorptions at 1703 cm^{-1} ($\alpha\beta$-unsaturated carbonyl) and at 1633 cm^{-1} (benzene ring). These absorptions are typical and similar to that of SLHU1. There were absorptions at 754 cm^{-1} for an ortho disubstituted benzene. The 1, 2, 3-trisubstituted benzene might as well absorb in the same region.

The absorption pattern of the aromatic protons in the NMR was the same as that of SLHU1 but the numners of protons were different SLHU3 has seven aromatic protons. These protons showed signals assigned in conformity with electron density distribution over carbazole. The cyclohexadienyl residue was therefore linked to carbazole at C-4. An equally downfield signal obtained for H-1, H-5, and H-8 was conspicuously absent for H-4. The absorptions were δ8.5 (2H, 2d), δ8.4 (1H, d), δ7.8 (3H, m), δ7.5 (1H, d). Both SLHU1 and SLHU3 have five protons resonating at lower field. As in SLU1 there was a sharp methoxy resonance at δ3.8 ($OCH-4^1$). At high field, a distinct methyl doublet at δ1.45 J = $1H_2$ (CH_3-6^1) was present. There were also absorptions at δ2.5 (NH_2-2^1), δ4.70 ($OH-5^1$), δ1.09 (1H, S H - 6^1) and δ2.4 (3H, S N-9).

The mass spectrum gave a molecular ion at $m/_z$ 348, $C_{21}H_{20}N_2O_3$ with E.I. spectrum and an ion $m/_z$ 349 in the corresponding Cl spectrum. From the mass spectrum, SLHU1 and SLHU3 both have the same molecular weight but the breakdown of the molecules into ions are not the same. The double bond equivalent is also $\sqrt{13}$. From the above spectral data/ structure (145) was proposed for SLHU3.

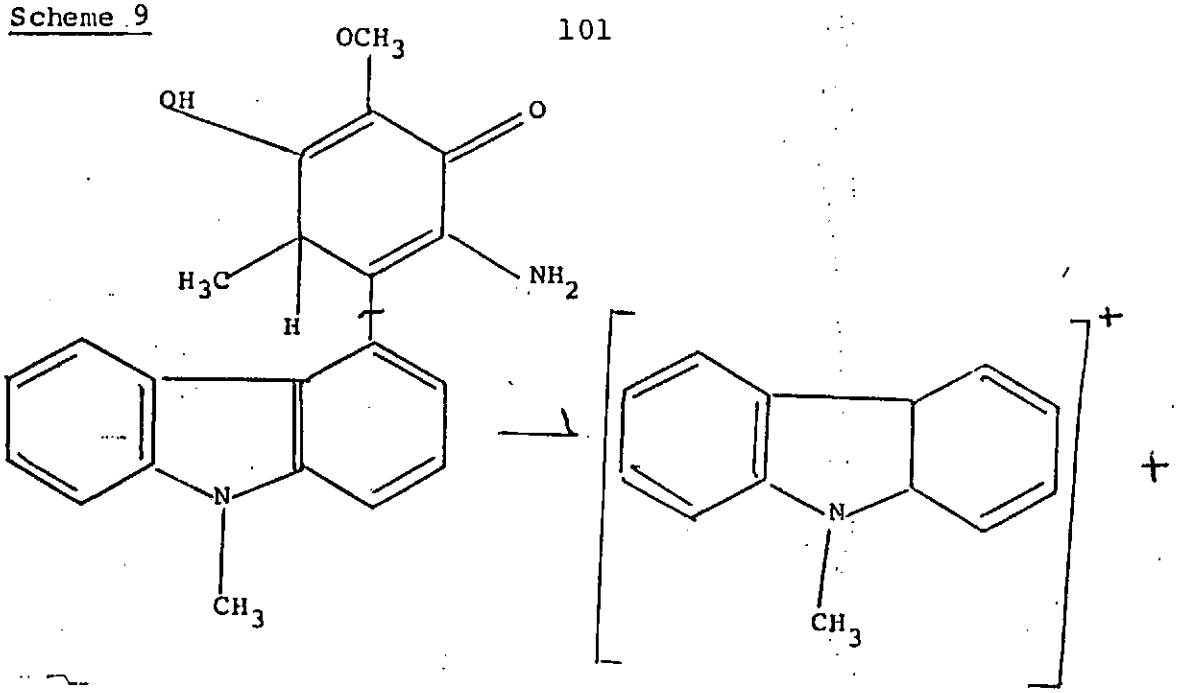


(145)

The corresponding aromatic protons were assigned as follows: δ8.5 absorption due to H-8 and H-1, δ8.4 H-5, δ7.8 H-6, H-2, and H-7, and δ7.5 H-3. The cyclohexadienyl residue was linked to the carbazole at C-4.

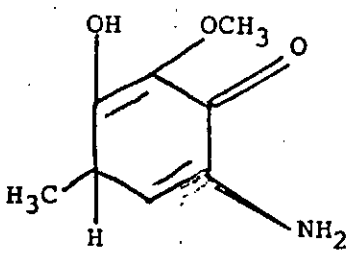
The pathway suggested for the production of the fragment ions observed in the mass spectrum is shown in the scheme below:

Scheme 9

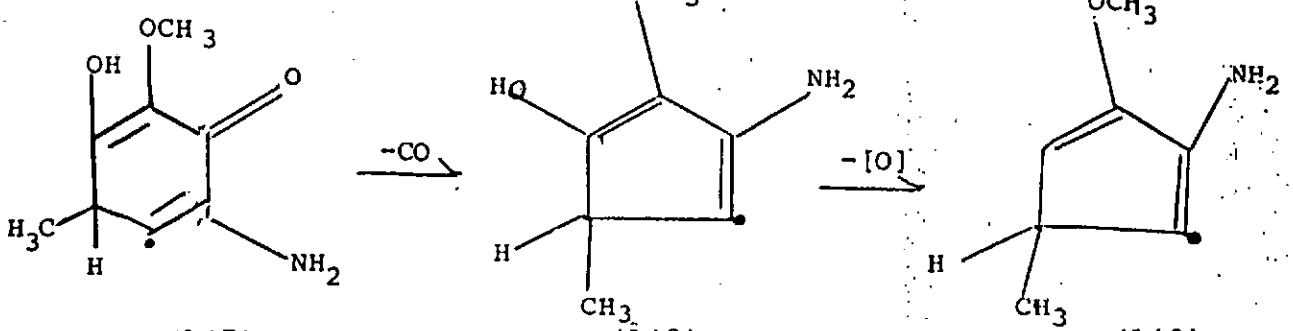


(145)

(146)
Ion m/z 166 (23.1%)
$C_{12}H_8N$.



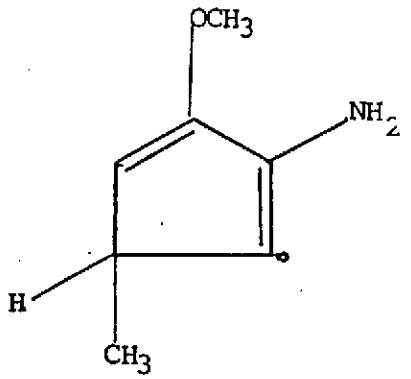
(147)
Ion m/z 168 (50.3%)
$C_8H_{10}NO_3$



(147)

(148)
Ion m/z 140 (31.3%)
$C_7H_{10}NO_2$

(149)
Ion m/z 124 (32.9%)
$C_7H_{10}NO$



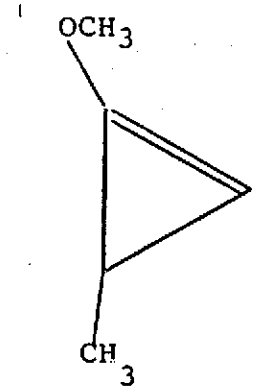
(149)

$C \equiv C - NH_2$

(150)

Ion $m|_z$ 40 (61%)

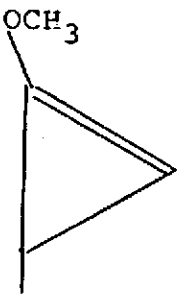
C_2H_2N



(151)

Ion $m|_z$ 85 (28%)

C_5H_9O



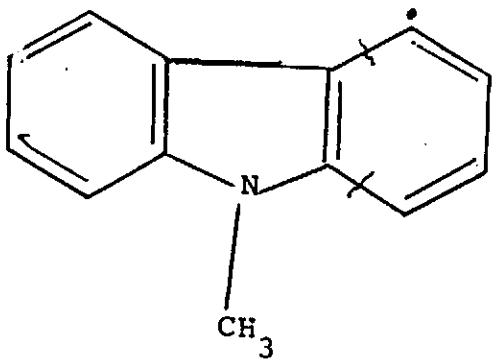
(152)

Ion $m|_z$ 69 (43%)

C_4H_5O.

The cleavage of bond C-4 and C-1' to give the oxocyclohexadienyl residue ion $m|_z$ 168 (147) $C_8H_{10}NO_3$ and ion $m|_z$ 166 (146) $C_{12}H_8N$ confirmed the substituent at C-4. By loss of carbon monoxide, ion $m|_z$ 168 (147) gave ion $m|_z$ 140 (148) $C_7H_{10}NO_2$. Formation of the latter confirmed the position of the oxogroup in the C-4 substituent. The siting of other functionalities were evident from a further fragmentation of ion $m|_z$ 140 (148) to ion $m|_z$ 124 (149) to ion $m|_z$ 40 (150) C_2H_2N by bond ruptures and ion $m|_z$ 85 (151) C_5H_9O to ion $m|_z$ 69 (152) C_4H_5O by loss of a methyl group.

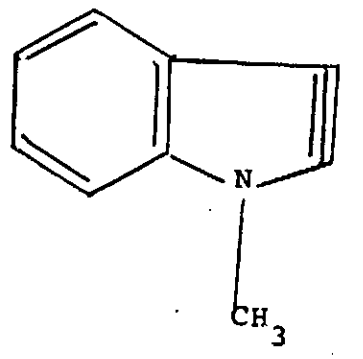
Scheme 10



(146)

lon $m|_z$ 166 (23.1%)

$C_{12}H_8N$



(153)

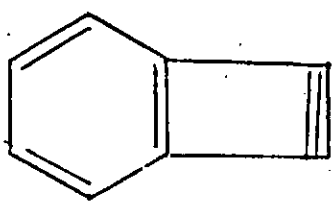
lon $m|_z$ 129 (15.4%)

C_9H_7N

(154)

lon $m|_z$ 51 (38.3%)

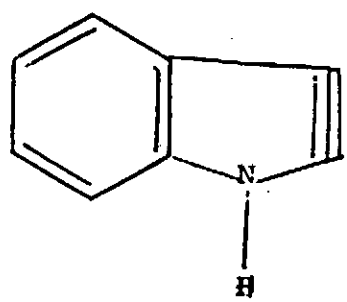
C_4H_3



(156)

lon $m|_z$ 100 (12.6%)

C_8H_4



(155)

lon $m|_z$ 115 (19.4%)

C_8H_5N

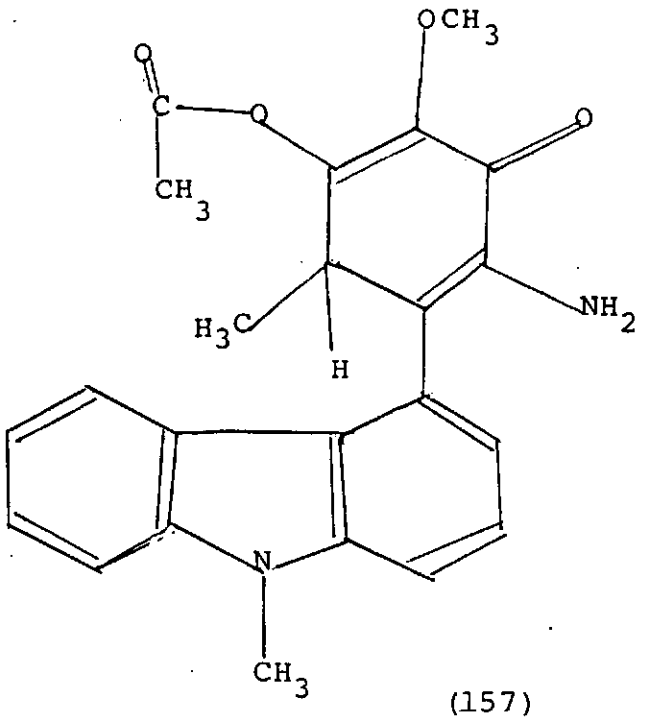
lon $m|_z$ 166 (146) further broke down as shown above to give ions $m|_z$ 129 (153) and $m|_z$ 51 (154) by cleavage of bond C-4a and C-1a. lon (153) further fragmented to give ions $m|_z$ 115 (155) which gave ion $m|_z$ 100 (156).

Acetylation of SLHU ESLHU3

SLHU3 was acetylated using acetic anhydride and pyridine. The UV of the product showed quite a different UV absorption with a shoulder at 340nm. In acid there were maxima at λ_{max} 306nm and 298nm. There was a significant hypsochromic shift of 34λ_{max}.

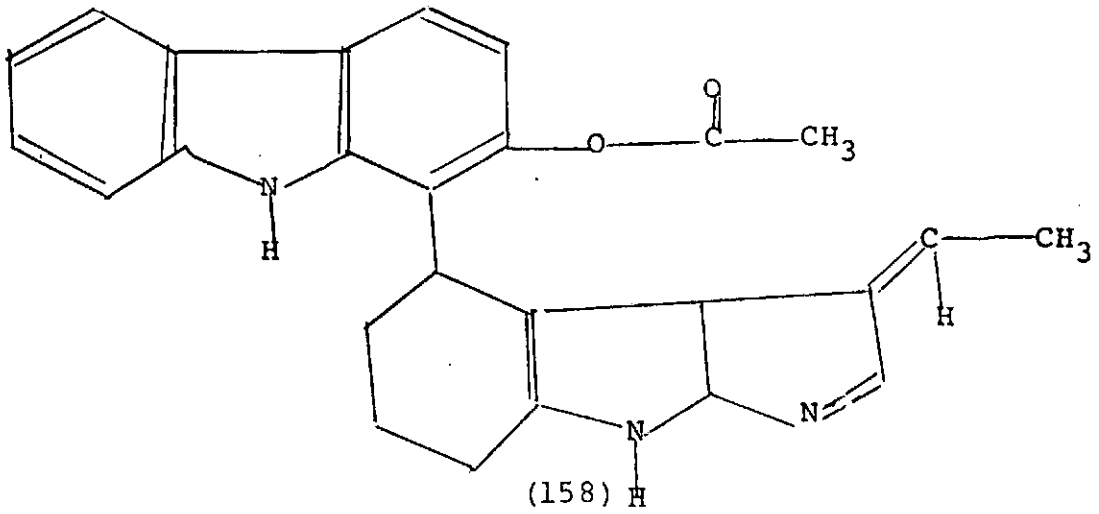
IR of ELSLHU3 gave absorption at 1741 cm^{-1} due to the formation of the acetate and 1679 cm^{-1}. The NMR was not well resolved. The mass spectrum and other spectra data confirmed the molecular formular as $C_{23}H_{23}N_2O_4$. The base peak is at $m/_z$ 43 in the El ' This confirms the presence of the acetate group. Other ions present in the fragmentation of the unacetylated molecule were also present e.g. $m/_z$ 166 (25.0%), $m/_z$ 168 (69.3%), $m/_z$ 140 (33.5%), $m/_z$ 168 (69.3%), $m/_z$ 140 (33.5%, $m/_z$ 124 (57.1%) and $m/_z$ 69 (53.5%).

The acetylated SLHU3 has the structure (157) shown below.



(157)

SLHU4



(158)

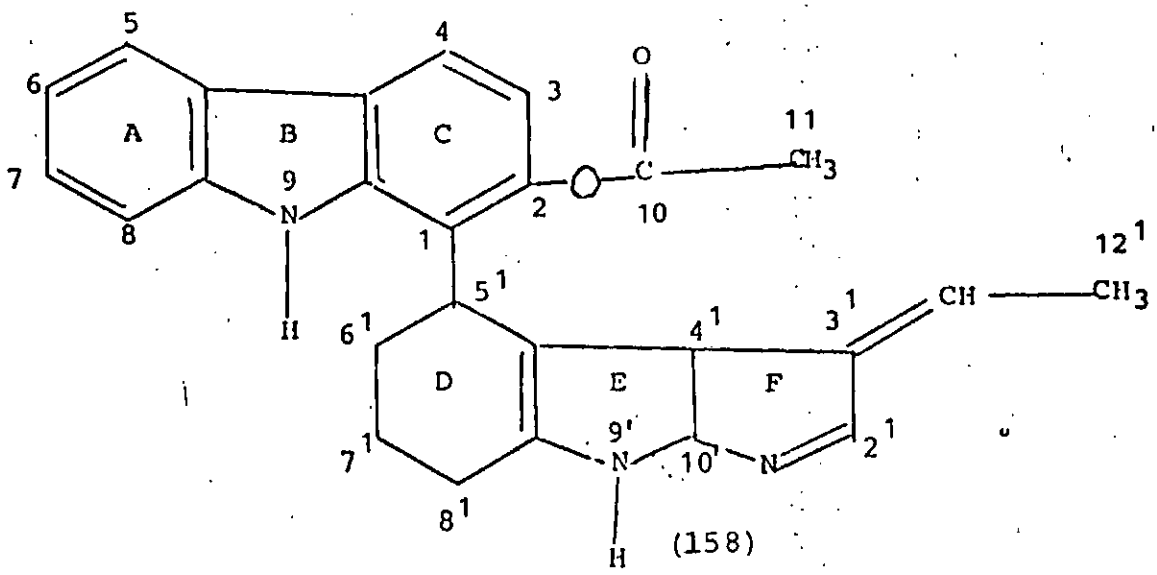
Structure of SLHU4

The glassy pale brown solid had R_f value of 0.52 on silica (methanol: ammonia, 15:1). The UV spectrum showed absorptions λ_{max} at 227nm, 265nm, 320nm, and 385nm. Appreciable hypsochromic shift was observed on acidification, λ_{max} being observed at 225nm, 264nm, 319nm, and 380nm. From the UV absorptions, the molecule is a dimeric indole alkaloid.

The IR spectrum showed an absorption at 1706 cm^{-1} for a ketone or an ester and at 1633 cm^{-1} for a benzene ring.

The NMR showed absorptions of six aromatic protons at δ8.5 (1H, d), δ8.4 (1H, d), δ7.75 (1H, s), δ7.55 (1H, m); δ7.1 (1H, d), and δ6.7 (1H, d). This confirmed the carbazole nucleus as indicated by the UV spectrum. There were NMR absorptions at δ4.0 (1H, d) and δ3.8 (3H, S). Other NMR absorptions included δ2.5 (1H, broad), δ1.45 (5H, d), δ1.9 (3H, S) and δ0.39 1H, broad).

The mass spectrum showed a relative molar mass of 411, $C_{26}H_{25}N_3.O_2$. From this the double bond equivalent was 16. The protons corresponding to the absorption above were assigned in the following manner. The structure proposed is (158).



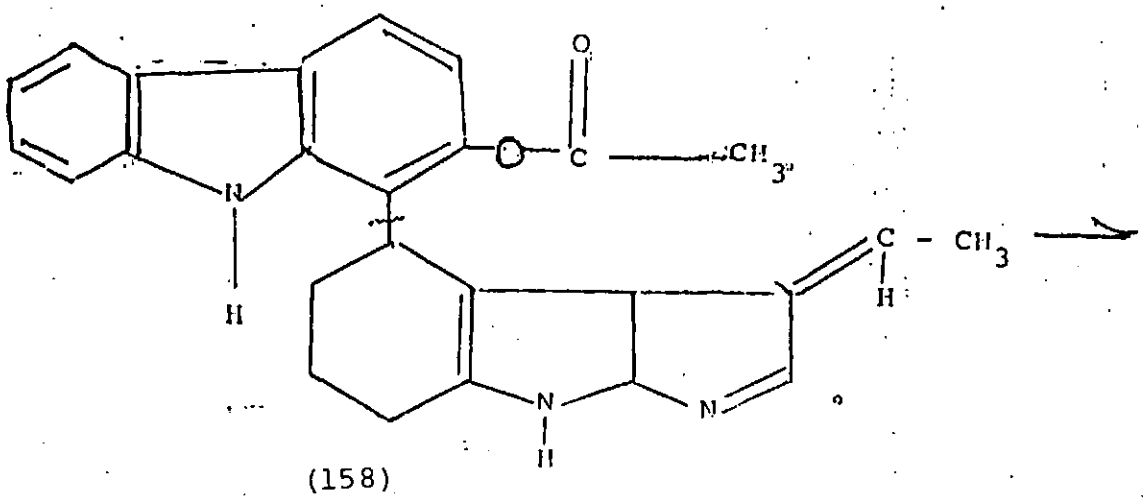
(158)

Proton H-8 absorbed at δ8.5, H-5 at δ8.4, H-3 at δ7.75; H-7 at δ7.6, H-4 at δ6.7, H-9 at δ4.0, H-11 at δ3.8, H-11^1 at δ4.6, H-9^1 at δ2.5, H-2^1 at δ2.3, H-1^1, H-4^1 H-5^1, H-6^1 and H-8^1 at δ1.45 each, H-12^1 at δ1.9 and H-10^1 at δ0.89.

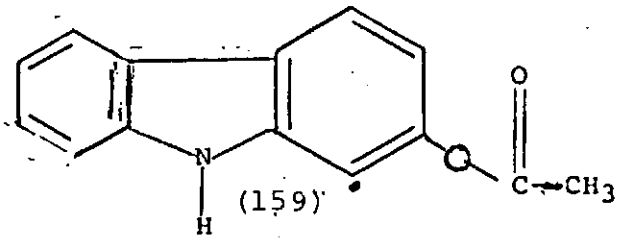
The breakdown of the molecule as shown by the mass spectrum is in support of the structure.

Scheme 11 outlined the fragmentation pattern.

Scheme 11



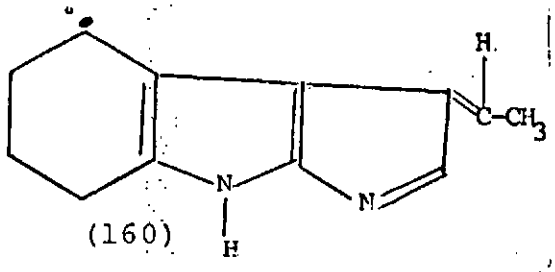
(158)



(159)

lon $m|_z$ 224 (20.4%)

$C_{14}H_{10}NO_2$

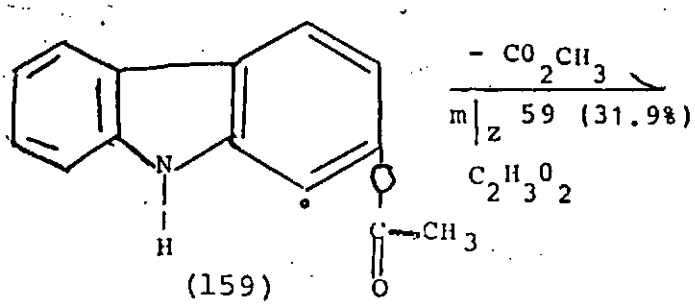


(160)

lon $m|_z$ 187

$C_{12}H_{15}N_2$

The lons $m|_z$ 224 (159) $C_{14}H_{10}NO_2$ and $m|_z$ 187 (160) $C_{12}H_{15}N_2$ were produced by the cleavage of bond C-1 and C-5[1]. The presence of these ions in the spectrum confimed the linkage of the carbazole moeity to the other unit of the molecule. lon (159), $m|_z$ 224, as usual, accounted neatly for the acetylated carbazole ring system.

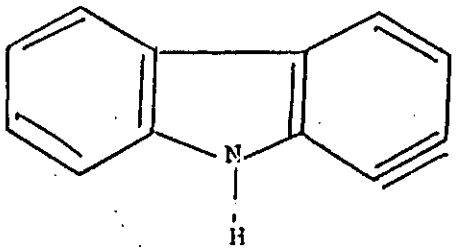


(159)

$- CO_2CH_3$

$m|_z$ 59 (31.9%)

$C_2H_3O_2$



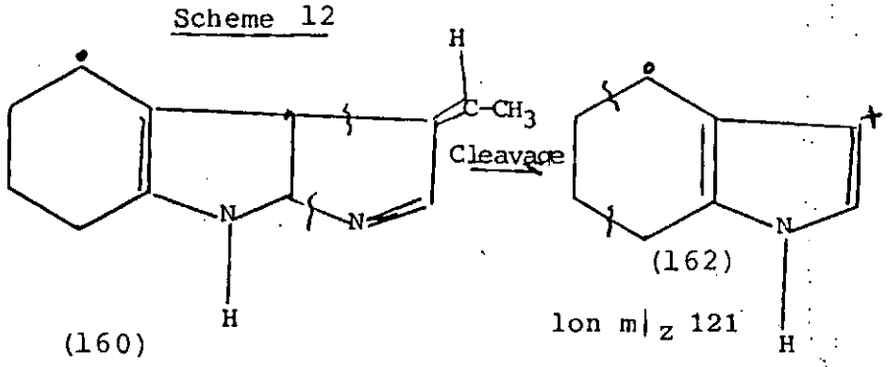
(161)

lon $m|_z$ 165 (6.7%)

$C_{12}H_7N$.

Loss of the acetate from ion (159) then afforded the Carbazolyne (161) of relatively low molar mass. The presence of this carbazolyne ion in the mass spectrum confirmed the siting of the acetate ion at C-2.

Scheme 12



(160)

(162)

lon m|z 121 (20.7%)

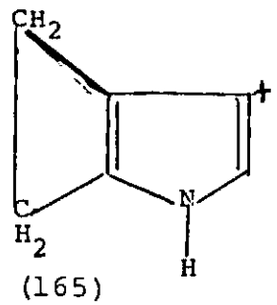
$C_8H_{11}N$

+ C_4H_5N.

(163)

lon m|z 67 (49.6%)

Cleavage



(165)

lon m|z 97 (31.3%)

C_6H_7N.

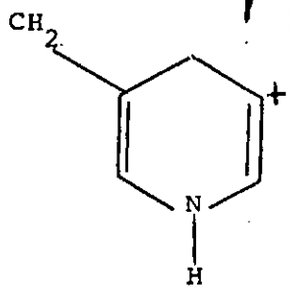
+

(164)

lon m|z 28 (8.6%)

C_2H_4

Ring expansion



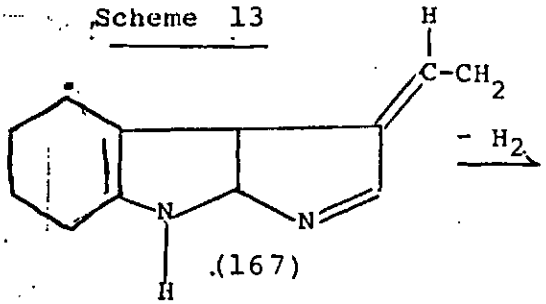
(166)

lon m|z 97 (31.3%)

C_6H_7N

Ions $m/_z$ 121 (162) and 67 (163) were produced by the cleavage of bonds $C10^1$ b and $C3^1$b. Further cleavage of single carbon-carbon bond in ion $m/_z$ 121 (162) $C_8H_{11}N$ produced ions $m/_z$ 28 (164) C_2H_4 and ion $m/_z$ 97 (165) C_6H_7N at high relative intensity. The foregoing cracking of the ion $m/_z$ 187 (160) $C_{12}H_{15}N_2$ confirmed the position of the ethylene side chain.

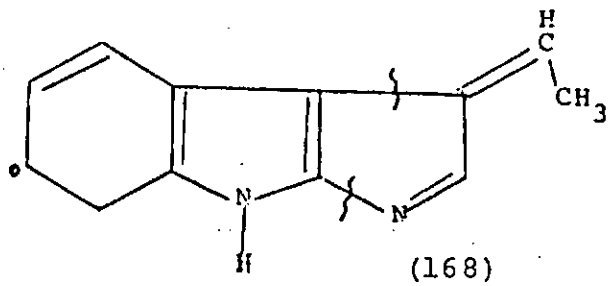
Scheme 13



(167)

Ion $m/_z$ 187

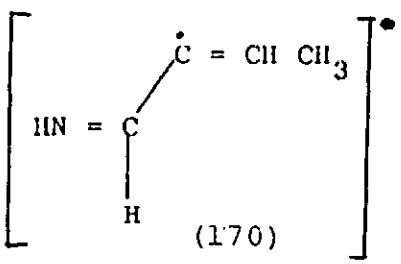
$C_{12}H_{15}N_2$



(168)

Ion $m/_z$ 185 (7.0%)

$C_{12}H_{13}N_2$

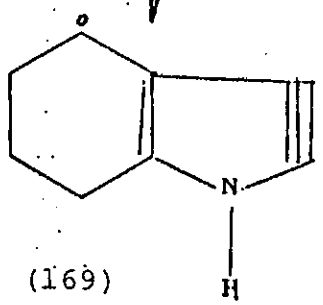


(170)

Ion $m/_z$ 68 (45%)

C_4H_6N

+

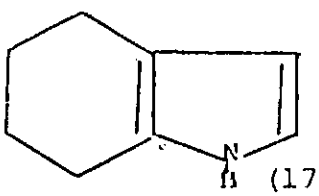


(169)

Ion $m/_z$ 118

C_8H_8N

+ $2H^+$



(171)

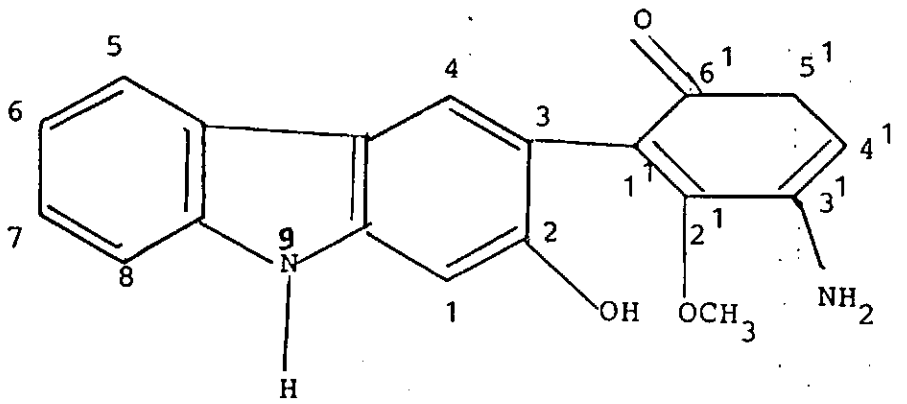
Ion $m/_z$ 121 (20.7%)

$C_8H_{11}N$.

The C1-C5^1 link between the monomers of SLHU4 was expected since a much down field absorption expected for H-1 as for H-5 or H-8 was conspicously missing. The low radical (168), derived from (167) by loss of two hydrogen radicals gave credence to the structure assigned to the monomer (167) The prominence of buta-3-dien-imino radical (170), confirmed the presence and position of the C-3^1 substituent. The alternative position of the double bond between rings C and D was excluded by the identification of ion (171), $m|_z$ 121m formed via bond cleavage and protonation of (168).

The above evidence further confirmed the structure assigned to SLHU4.

SLHU5



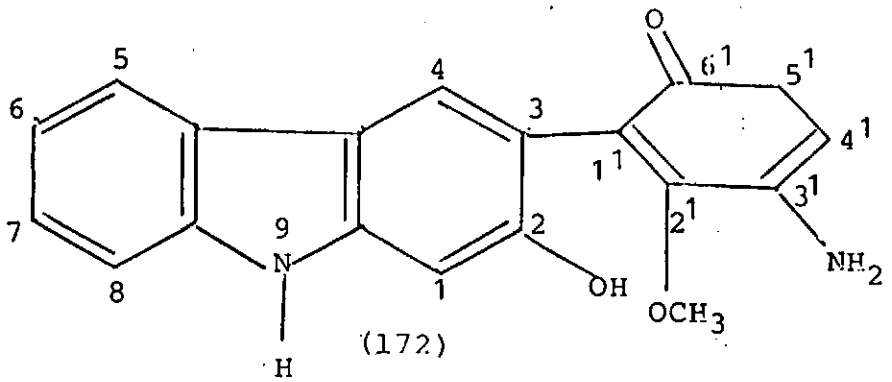
(172)

The R_f value of the brown glassy solid was 0.39. The UV spectrum showed λ_{max} at 220nm, 266nm, 321nm, and 384nm. In acid λ_{max} were 228nm, 265nm, 319nm, and 380nm. There was thus a slight hypsochromic shift of 4nm in the acid medium. Possibly there must be a considerable conjugation in the molecule as reflected in the UV λ_{max} 384nm compared to 369nm value of SLHU1.

The IR showed absorptions at 3600 cm^{-1} (OH), 1704 (αβ - unsaturated carbonyl), 1633 cm^{-1} (aromatic ring), 1339 cm^{-1} (= C-NH_2) 888 cm^{-1} (1, 2, 4, 5 - terrasubstituted benzene) and 780 cm^{-1} (ortho disubstituted benzene).

The ^1H nmr spectrum showed six aromatic protons at δ8.4 (2H, q) δ7.75 (1H, S), δ7.6 (1H, t), δ7.1(1H, d) and δ 6.7 (1H, d). There were other absorptions at δ4.6 (1H, d), δ4.0 (1H, broad OH), δ3.8 (3H, OCH_3), δ2.5 (broad), δ2.21 (1H, broad), δ1.09 (2H, d, NH_2)

The molar mass of 320gm and other spectral data led to the formular $C_{19}H_{16}N_2O_3$. The double bond equivalent was 13. From the above data structure 172 was proposed for SLHU5 and the NMR protons distribution was in support of this.



(172)

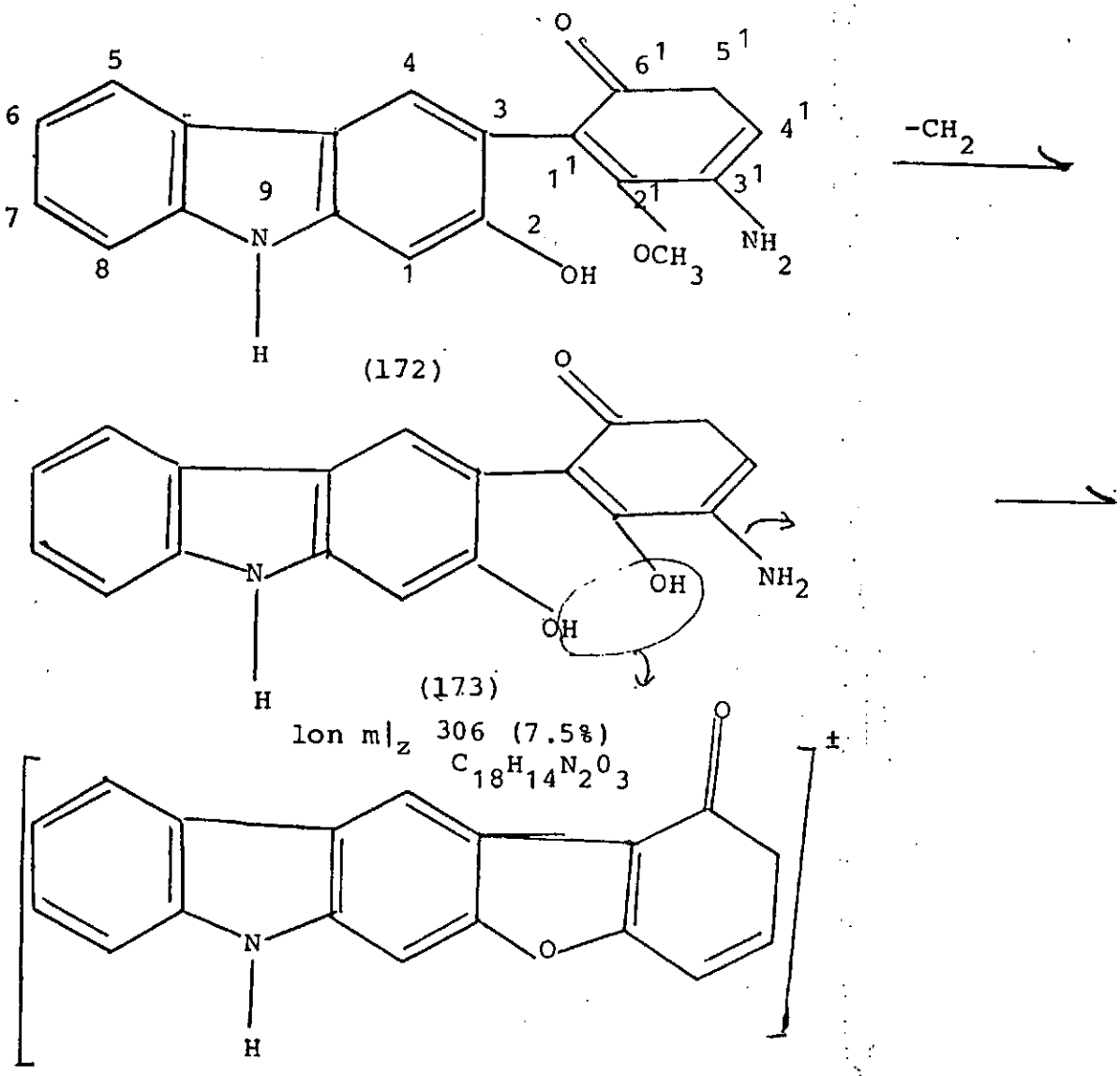
Thus δ8.4 was assigned to proton H-1, and H-8 δ7.8 to H-4, δ7.6 to H-5, δ7.1 to H-7, and δ6.7 H-6. The assignment was consistent with the expected splitting pattern of the signals and with an acceptable $\bar{\Lambda}$ - electron density measurement of carbazole. The siting of OH-2 which appeared as a broad signal or δ4.0 was therefore unambigous.

The α - β unsaturated carbonyl system was located in the C-3 cyclic side chain. The carbonyl extended the conjugation in the moeity since a higher UV λ_{max} absorption was observed for this compound as earlier mentioned above.

The ^{1}H NMR signal at δ 1.09 was assigned to NH_2-3^1 δ3.8 OCH_3 - 2^1, δ4.6; H-4^1, δ2.5 H-5^1 and δ2.1 NH-9.

The pathway suggested for the molecular ions observed in the mass spectrum is shown in scheme 14.

Scheme 14

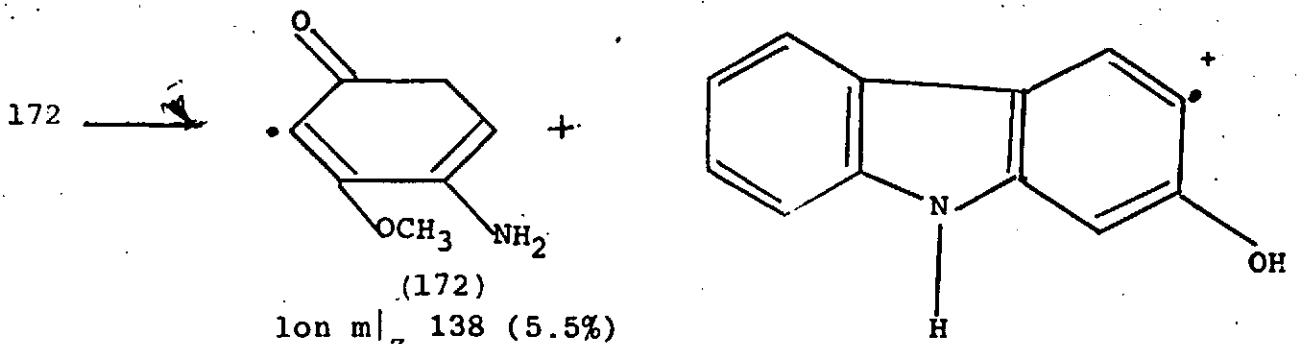


(174)

lon m/z 272 (35.3%)

$C_{18}H_{10}NO_2$

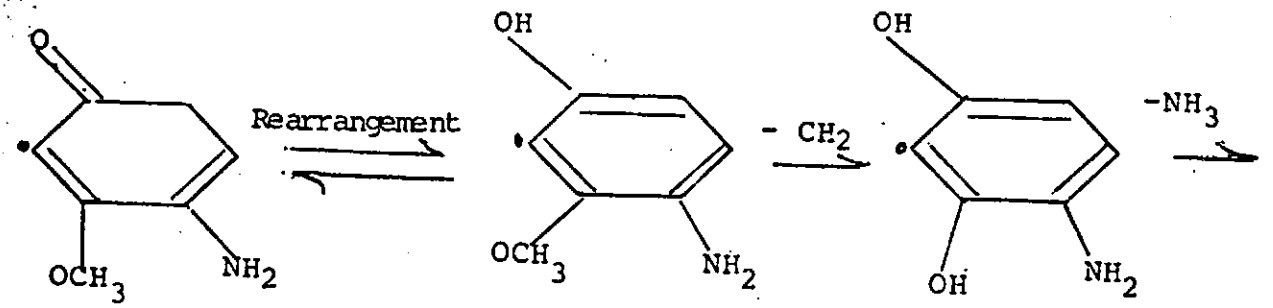
Fragmentation of 172 to the ion 174 confirmed the juxta positions of the OH-2 and OCH_3-2^1 relative to each other.



(172)
Ion $m|_z$ 138 (5.5%)
$C_7H_8NO_2$.

(176)
Ion $m|_z$ 182 (9.1%)
$C_{12}H_8NO$.

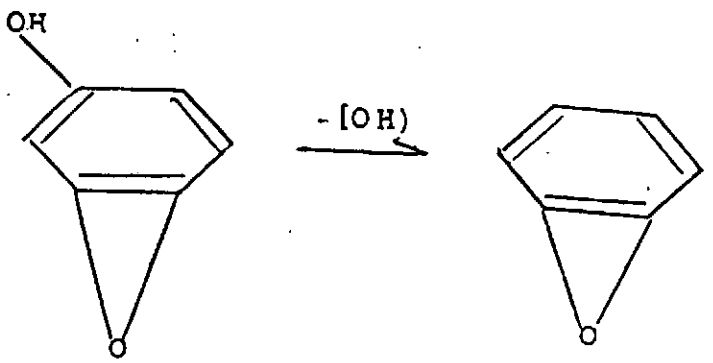
The cleavage of the bond C-3 and C-1^1 gave ions $m|_z$ 182 (176) $C_{12}H_8NO$, and ions $m|_z$ 138 (177) $C_7H_8NO_2$. Presence of these ions in the mass spectrum gave a complete identity of the C-3 cyclic side chain.



(177)

(178)

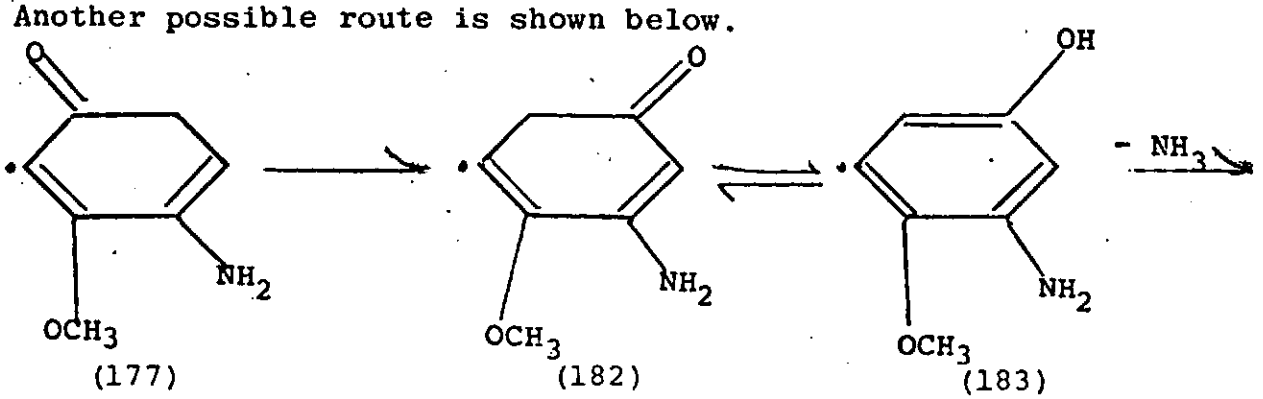
(179)
Ion $m|_z$ 124 (11.9%)
$C_6H_6NO_2$

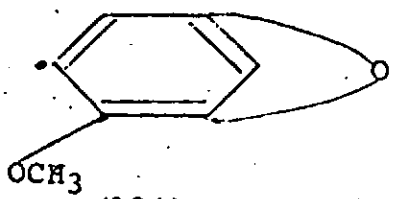


(180)
Ion $m|_z$ 107 (18.6)
$C_6H_3O_2$

(181)
Ion $m|_z$ 91 (32.5%)
C_6H_3O.

The breakdown of (178) to (181) via (179) to (180) established the Ortho positions of the OCH_3-2^1 and NH_2-3^1. Another possible route is shown below.





(184)

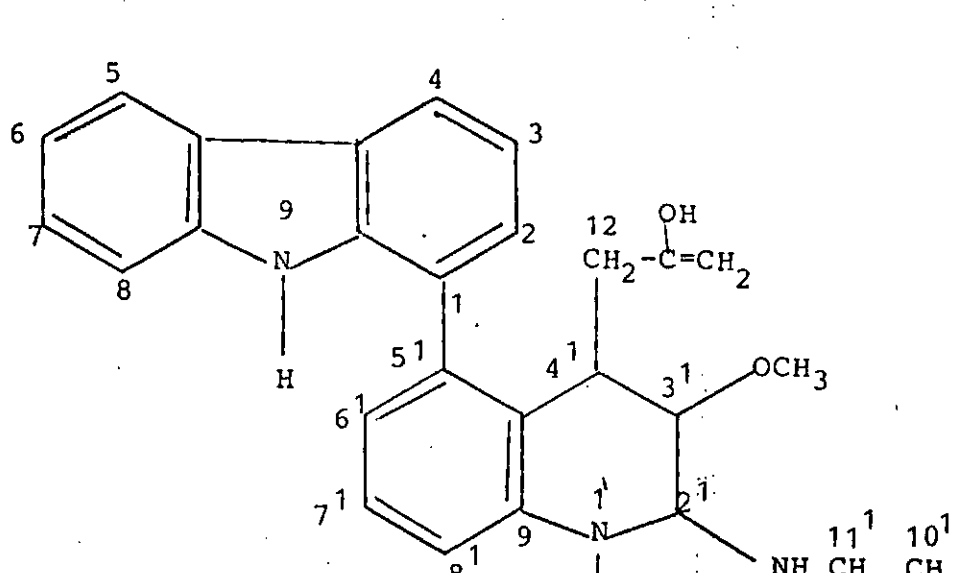
lon $m|_z$ 121

$C_7H_5O_2$

The ion peak for the cyclic ether (184) above was conspicuously absent in the M.S. and ruled out the alternative structure (182) that could be proposed for the C-3 cyclic side-chain. The above observations confirmed the structure assigned to SLHU5 as (172)

5.4 Water soluble alkaloids obtained from the bark

The water soluble alkaloids obtained from the bark of Hunteria umbellata were very difficult to separate due to the proximity of their R_f values. Four different alkaloids were isolated pure and were designated SBHU1, SBHU2, SBHU3, and SBHU4.



Structure of SBHU1 (185)

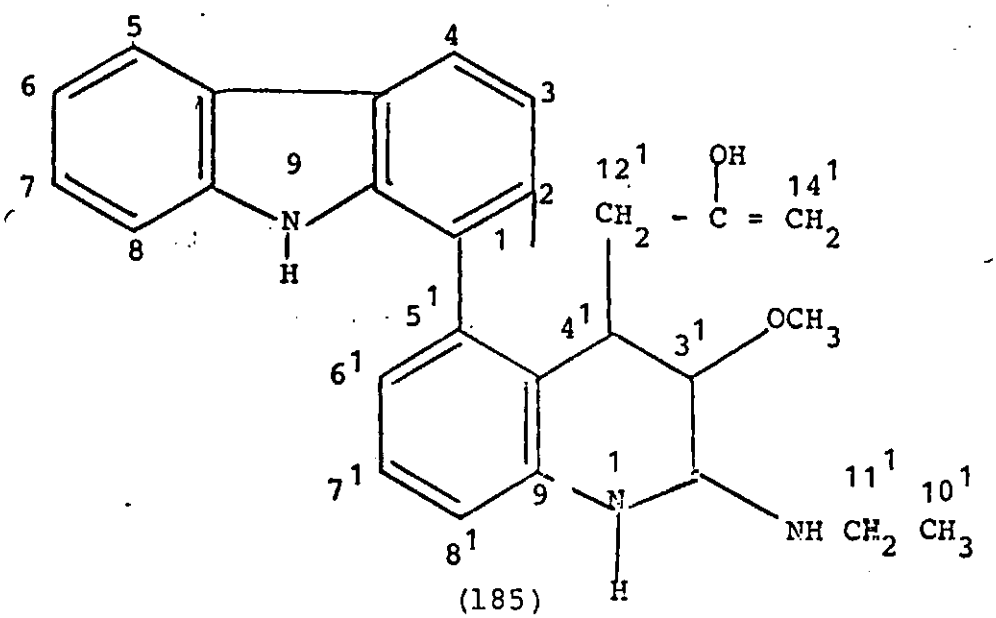
This is a brown glassy solid, R_f 0.22. The UV spectrum showed λ_{max} at 253nm, 307nm, and 367nm. In acid λ_{max} 253, 307, and 368nm were observed. No shift of absorption observed was therefore significant.

The IR showed characteristic absorption ν_{max} cm^{-1} at 3654 (OH), 1723 and at 1638 cm^{-1} (benzene ring).

The ^{1}Hnmr data showed ten aromatic protons. Apart from the carbazole nucleus predicted by the UV pattern, there must be another aromatic ring in the molecule. The absorptions

occurred at δ8.55 (2H, q) δ8.45 (3H, d), δ7.85 (3H, q), and δ7.5 (2H, m). Other absorptions were at δ 6.0 (1H, q, OH), δ5.45 (2H, dC-CH_2-C) δ5.4 (1H, d NH), δ5.2 (2H, d C = CH_2), δ3.8 (3H, OCH_3), δ3.6 (2H, m $NHCH_2$-), δ2.3 (1H - CH -), δ1.9 (1H, m) δ1.8 (1H, d NH), δ1.7 (1H q NH), and δ1.3 (3H, m - CH_3).

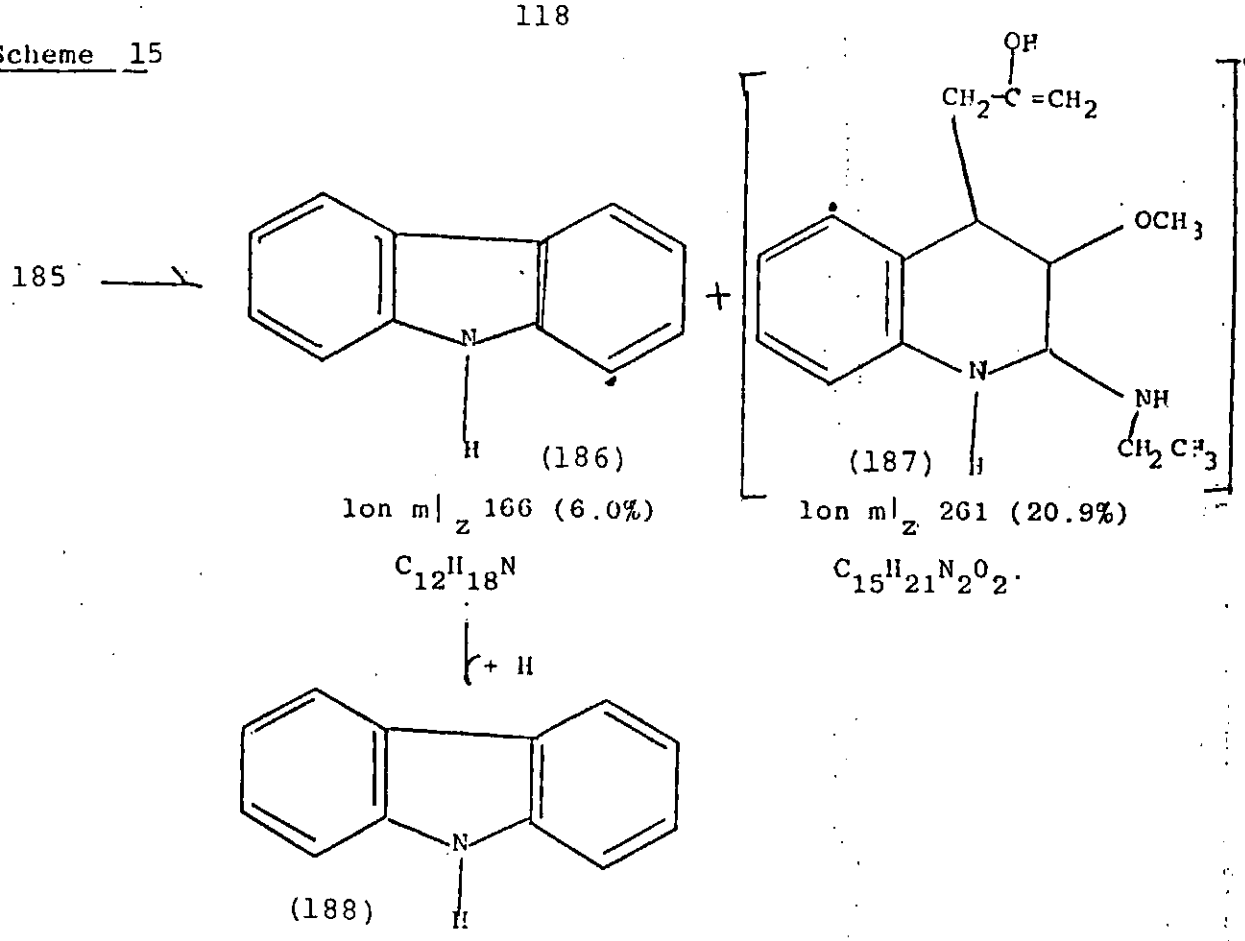
The mass spectrum showed a molecular ion 427 consistent with molecular formular $C_{27}H_{29}N_3O_2$ obtained from all the spectra data. The double bond equivalent is 16. The proposed structure for SBHU1 is (185).



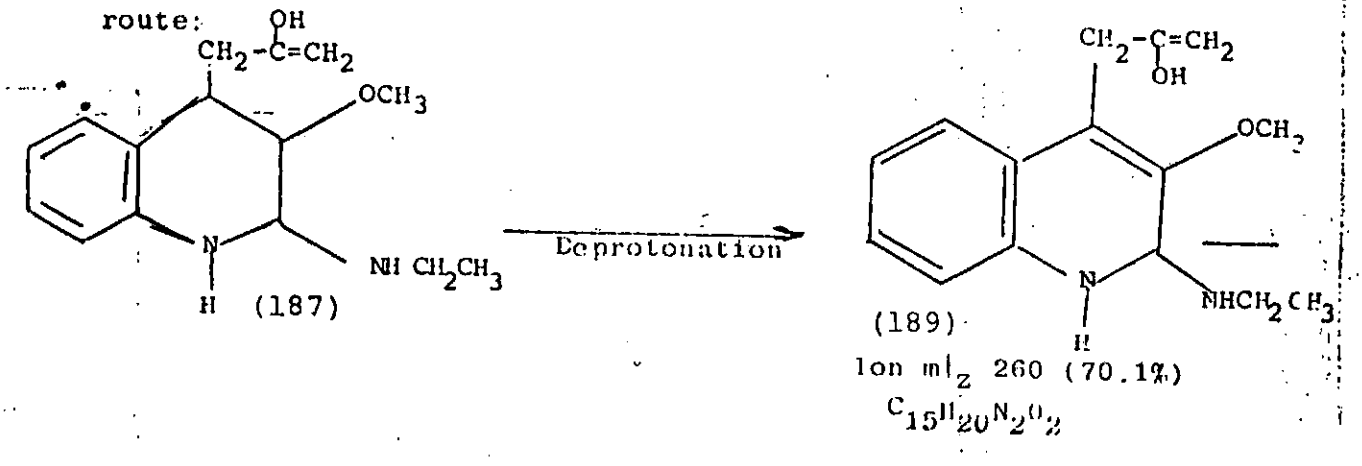
(185)

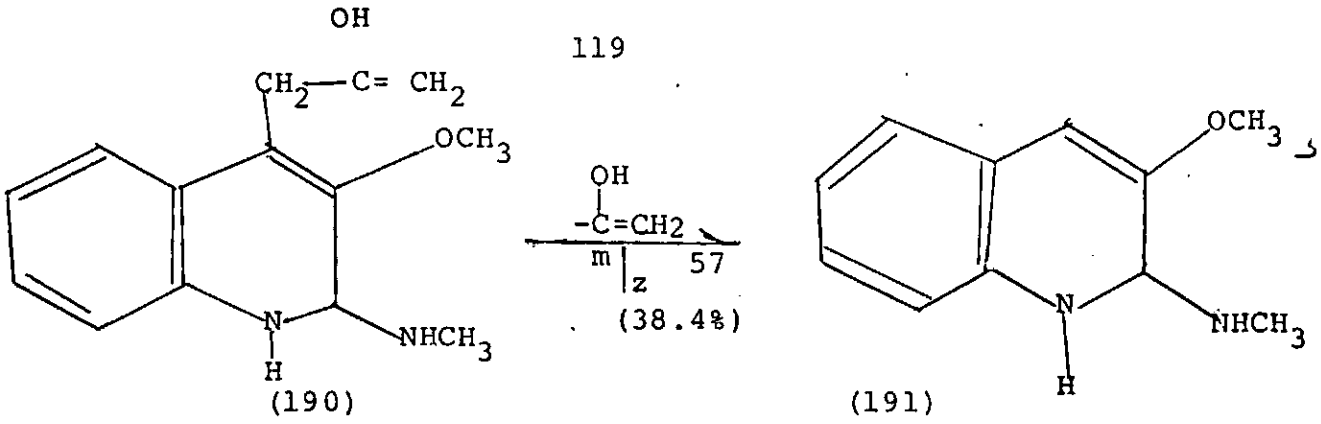
The protons were assigned as follows: δ8.55 H-8 and H-8^1, δ8.45 H - 4, H - 5 and H - 2, δ7.85 H - 6, H - 7 and H - 7^1, δ7.5 H - 3 and H - 6^1, δ6.0 OH - 13^1, δ5.45 H - 12^1, δ5.4 H - 1^1 δ5.2 H - 14^1, δ3.8 (-3^1 - OCH_3. δ3.6 H - 11^1 δ2.3 H- 4^1. δ1.9 H-2^1, δ1.8 NH-2^1 δ1.7 H-9^1 and δ1.3 H-10^1. The fragmentation pattern of the molecule confirmed the proposed structure as illustrated in the scheme below.

Scheme 15



The production of ion 186 m/z 166 $C_{12}H_8N$ and ion 187 m/z (261) $C_{15}H_{21}N_2O_2$ from SBHU1 showed that the molecule contained the carbazole nucleus linked up as shown above to another unit. The second monomer fragmented via the following route:





(190)

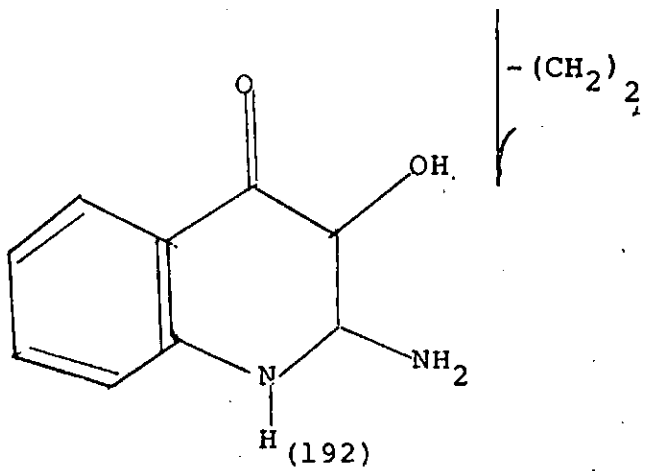
ion $m/_z$ 246 (41.5%)

$C_{14}H_{18}N_2O_2$

(191)

ion $m/_z$ 207 (5.7%)

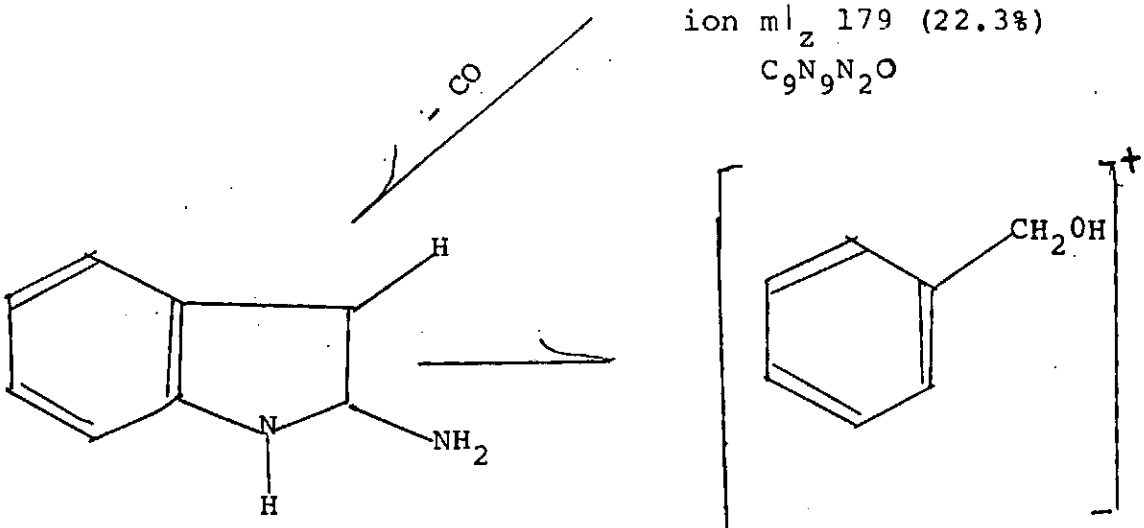
$C_{12}H_{16}N_2O_2$



(192)

ion $m/_z$ 179 (22.3%)

$C_9N_9N_2O$



(193)

ion $m/_z$ 151 (32.5%)

$C_8H_9N_2O$

(194)

ion $m/_z$ 107 (23%)

C_7H_7O

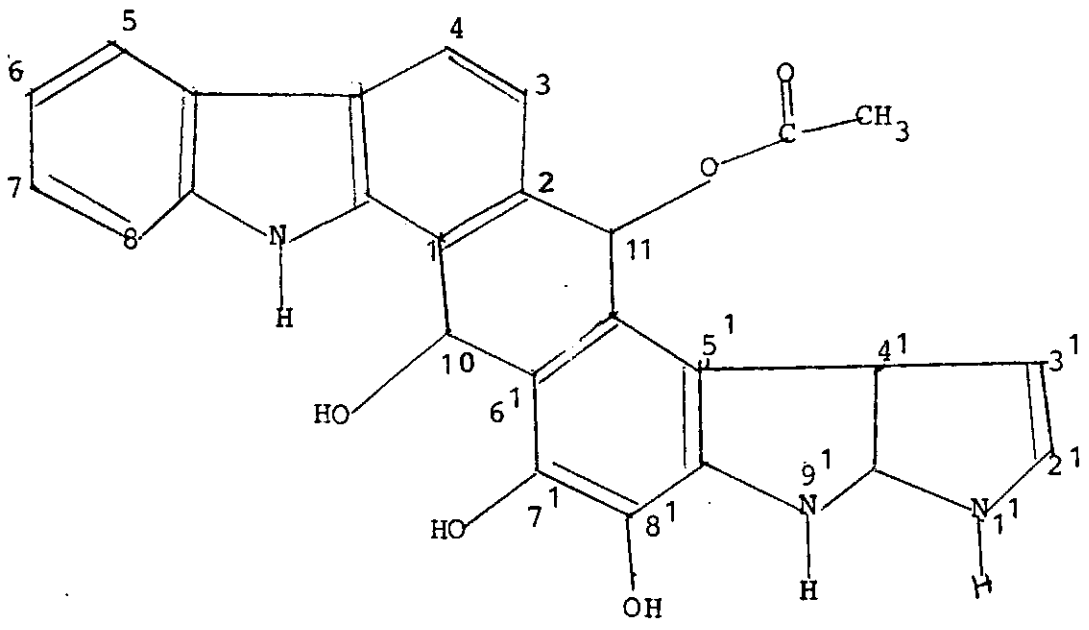
The quinolinium monomer (187), $m/_z$ 261 $C_{15}H_{21}N_2O_2$ after charge transfer followed by proton loss gave (189) $m/_z$ 260. Molecule (189) lost a C-2[1] methylene to give (190) $m/_z$ 246. Loss of C-4[1] hydroxyethylene afforded (191) $m/_z$ 220,

The loss of methylenes from C-2^1 ethyl amino, C-3^1 methoxy and C-4^1 pro-1-eno-2-ol residues gave ion (192) $m/_z$ 179 $C_9H_9N_2O$ after C-4^1 hydroxylation by hydroxyl group from the propl-en-2-ol residue. The presence of ion (191) $m/_z$ 207 confirmed the structure assigned to 261.

Ion (192) lost one carbon monoxide molecule to produce (193), 2-amino-3-2, 3-dihydro-quinoline.

Ion (193) was significant since its structure established the citing of the C-2^1, C-3^1 and C-4^1 substituents in SBHU1 ' The C-3^1 citing of the methoxy group had an additional support in the formation of benzyl alcoholic ion (194) $m/_z$ 107. The above observations confirmed the structure assigned to SBHU1 as (185).

SBHU2



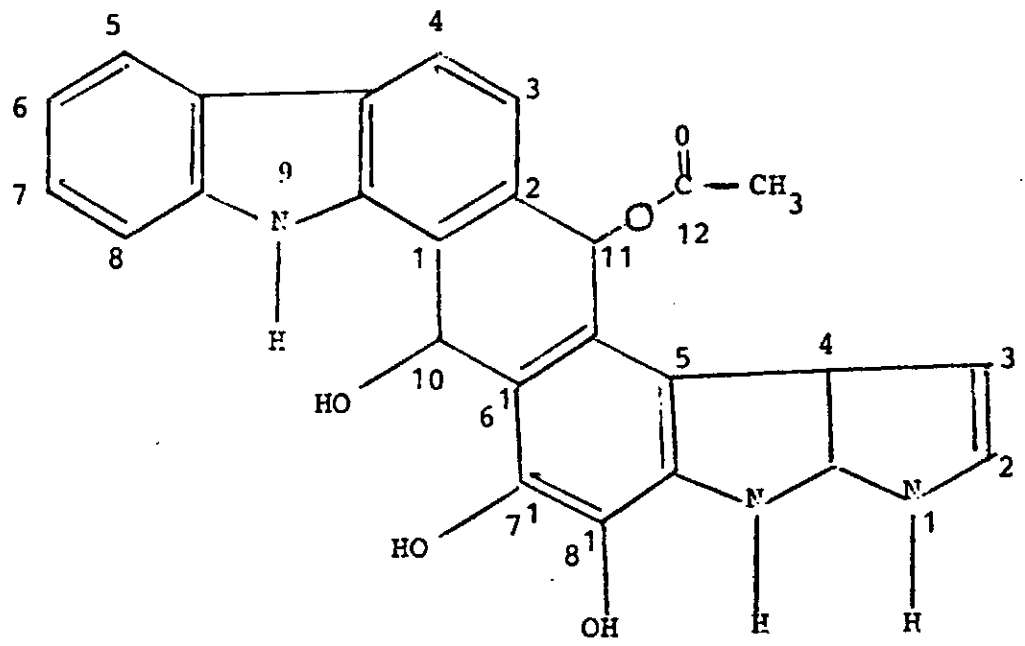
(195)

This compound has an R_f value of 0.82. It showed λ_{max} at 216nm and shoulder at 255, and 300nm in its UV spectrum. In acid medium λ_{max} is 304nm with shoulder at 255 and 215nm. There was a change of 7nm.

The IR showed absorptions at 3693 (NH), 3679(NH), 3652 (NH), 3632 (OH), 1726 (C = 0 acetate) and 1563 (benzene) cm^{-1}.

The NMR, though not well resolved showed six aromatic protons absorbing at δ8.6 (1H, S), δ7.8 (2H, S+t), δ7.7 (1H, t), δ7.4 (2H, m). Other absorptions were δ4.35 (1H, d), δ4.25 (1H, d), δ4.1 (1H, S, N-H), δ4.0 (1H, broad), δ3.75 (1H, S), δ3.55 (3H, S, OCH_3), δ2.27 (2H, S), δ2.37 (2H, t) and δ2.2 (1H, broad).

The mass spectrum showed a molecular weight of 455, $C_{26}H_{21}N_3O_5$. The double bond equivalent was 18. The structure therefore proposed was (195).



(195)

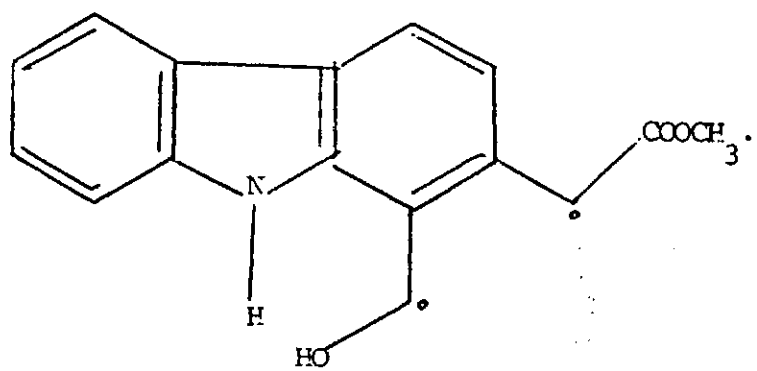
The protons corresponding to the absorptions above were assigned as shown in structure (195). Absorptions at δ8.6 were due to H-8, δ7.8 to H-4 and H-5, δ7.4 to H-3 and H-6, δ7.7 to H-7, δ4.35 $H\text{-}2^1$, δ4.25 $H\text{-}3^1$ the last two signals, imposed a double bond between $C\text{-}2^1$ and $C\text{-}3^1$ and precluded the possible location of the two hydroxyl groups at both sites but as only $OH\text{-}7^1$ and $OH\text{-}8^1$ absorbing at δ2.37 each.

The acetate methyl absorbed as a singlet at δ3.55 (3H) and H-12, at δ3.75 (CH-CO-OMe). Absorptions at δ4.1 was due to H-9, δ4.0 to $H\text{-}1^1$, $H\text{-}9^1$, and at δ2.27 to $H\text{-}4^1$ and $H\text{-}10^1$.

Scheme 16 shows the fragmentation pattern of the proposed structure.

Scheme 16

(195) Cleavage

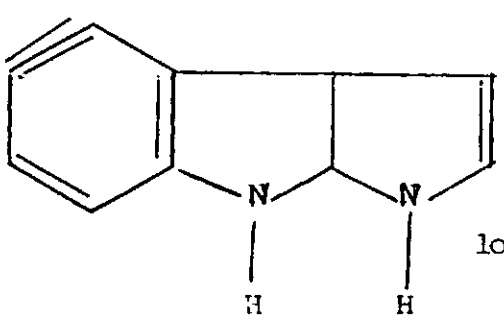


and

(196)

Ion m/z 267) (5.3%)

$C_{16}H_{13}NO_3$

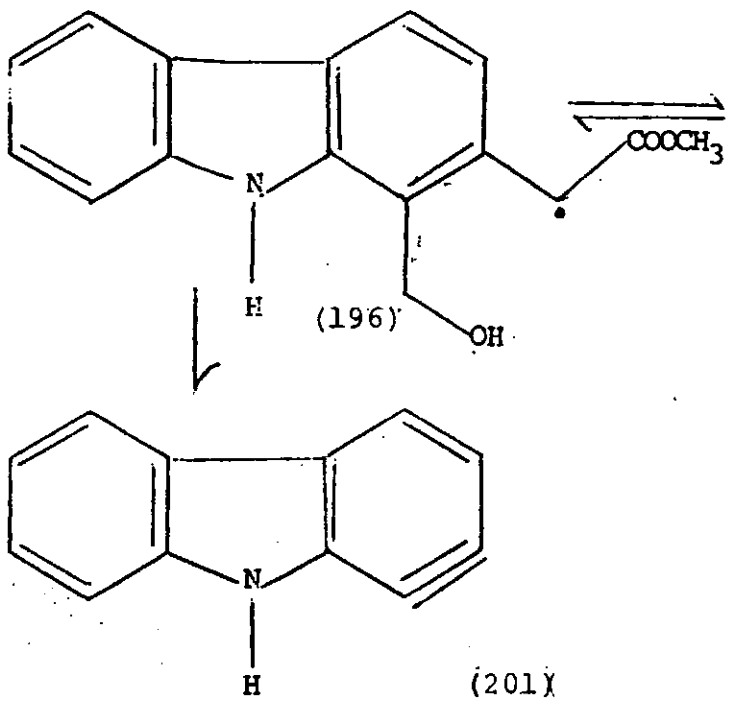


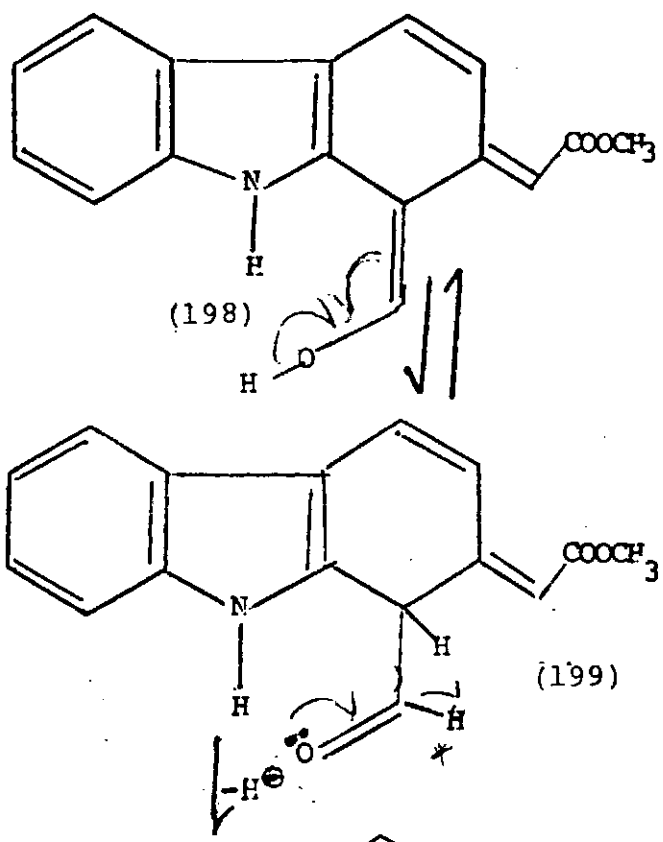
(197)

Ion m/z 188 (5.1%)

$C_{10}H_8N_2O_2$

Ions 196 and 197 were produced by the cleavage of bond C-11 and C-10.





lon $m|_z$ 151 (12.1%)

$C_{12}H_7$

+

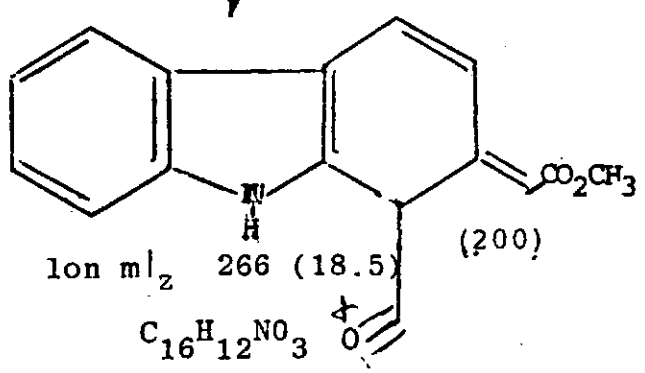
CH_2OH

CH_2

CO_2CH_3 (202)

lon $m|_z$ 104 (19.5%)

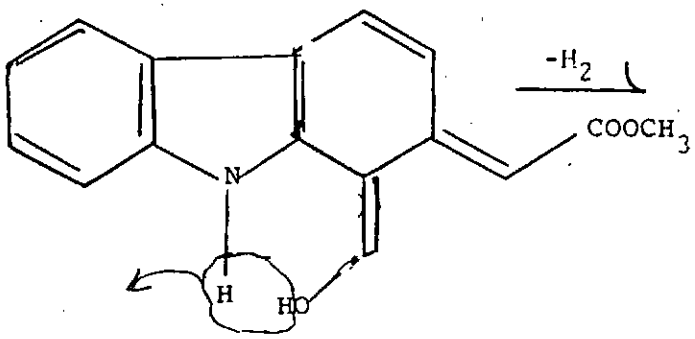
$C_4H_8O_3$



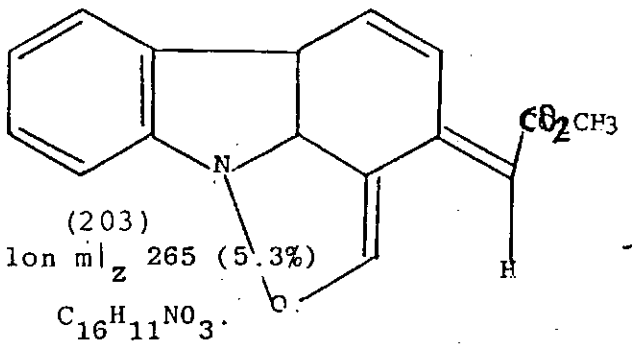
lon $m|_z$ 266 (18.5)

$C_{16}H_{12}NO_3$

lon (200) $m|_z$ 266 $C_{16}H_{12}NO_3$ was produced by rearrangement of ion (196) $m|_z$ 267 $C_{16}H_{13}NO_3$ followed by deprotonation. lon (201) $m|_z$ 151 $C_{12}H_7$ and ion (202) $m|_z$ 104 $C_4H_8O_3$ were produced by the cleavage of C-C single bond as shown above. The production of these ions confirmed the position of the side chain and the presence of the ester group.

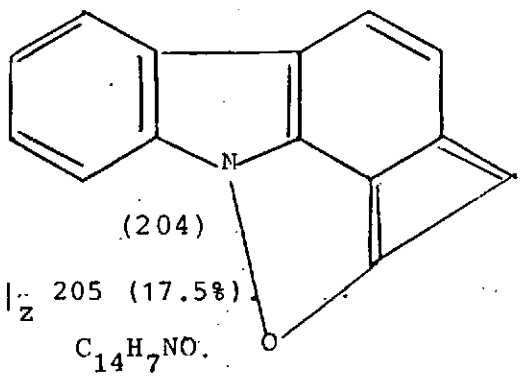


(199)



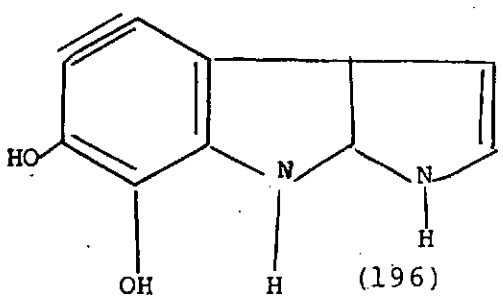
(203)
lon $m|_z$ 265 (5.3%)
$C_{16}H_{11}NO_3$.

- CO_2CH_3
- H^+



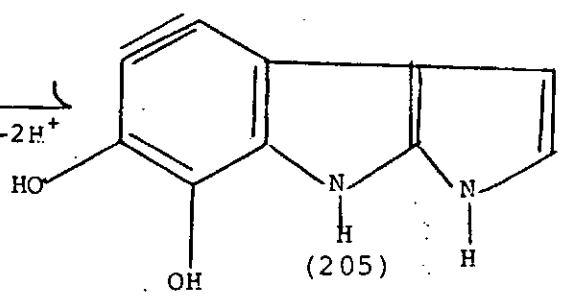
(204)
lon $m|_z$ 205 (17.5%)
$C_{14}H_7NO$.

The production of ions (203) $m|_z$ 265 and (204) $m|_z$ 205 further confirmed the structure of SBHU2 to be as proposed.



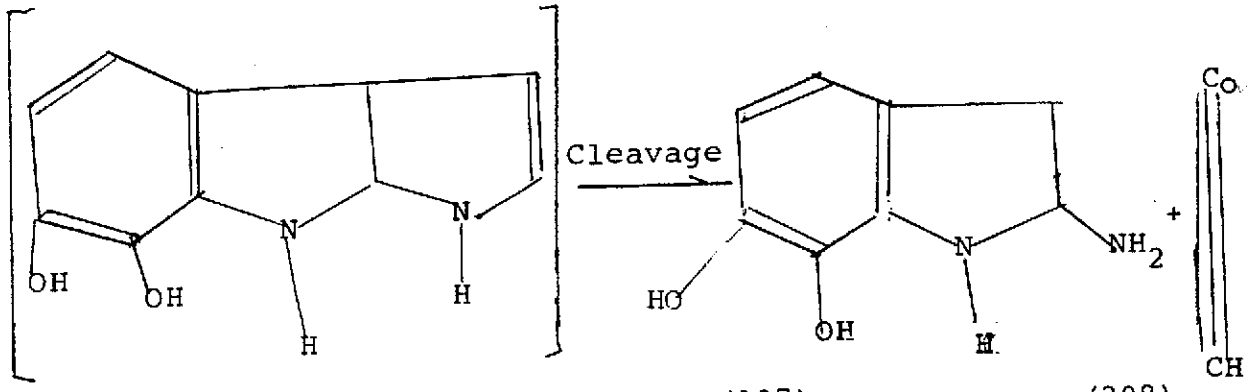
(196)
lon $m|_z$ 188 (5.1%)
$C_{10}H_8N_2O_2$

$-2H^+$



(205)
lon $m|_z$ 186 (6.7%)
$C_{10}H_6N_2O_2$

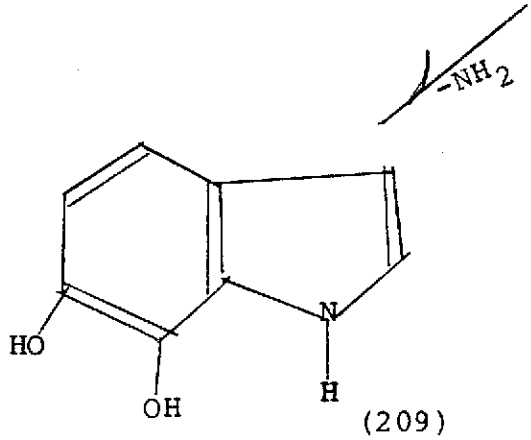
+ $2H^{\bullet}$
+ H^+



(206) (207) (208)

Ion $m/_z$ 189 (13.4%) $m/_z$ 166 (11.9%) $m/_z$ 26

$C_{10}H_{10}N_2O_2$ $C_8H_{10}N_2O_2$ C_2H_2

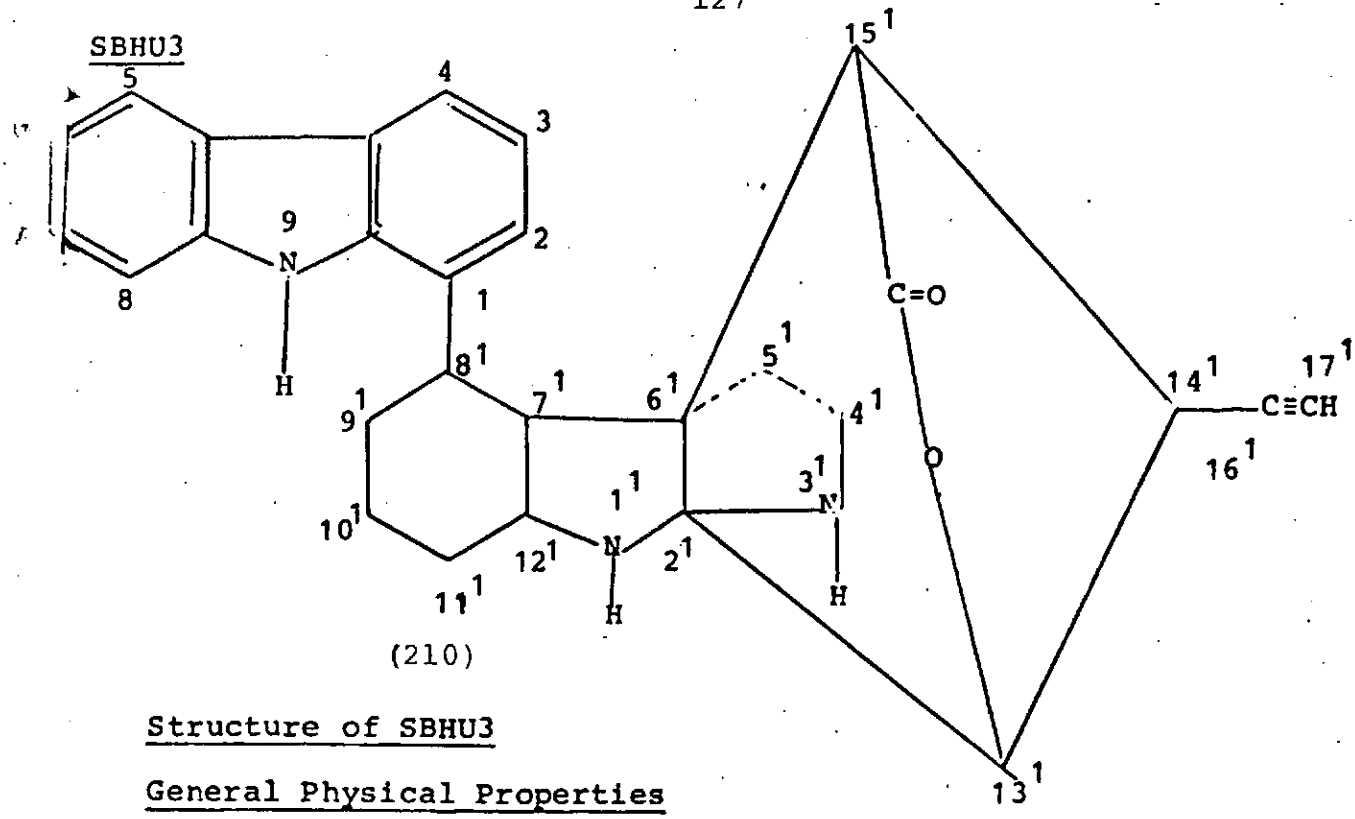


(209)

$m/_z$ 149 (100%)

$C_8H_7NO_2$

Deprotonation of ion (196) $m/_z$ 188 $C_{10}H_8N_2O_2$ gave ion (205) $m/_z$ 186 $C_{10}H_6N_2O_2$ followed by protonation, C-C single bond cleavage and removal of ammonia yielded ion (209) $m/_z$ 149 $C_8H_7NO_2$ at 100% relative intensity. The production of these ions with relative high intensity further confirmed the presence of the side chains and their position in the molecule. Ions (207) $m/_z$ 166 and (209) $m/_z$ 149 clearly established the Siamese fusion of the two pyrroline rings present in the monomer. The foregoing evidence confirmed the proposed structure.



(210)

Structure of SBHU3

General Physical Properties

This was obtained as a brown amorphous solid with R_f value of 0.09 and a yield of 18.6mg. UV gave λ_{max} 218nm, 252nm, 307 and 366 nm-in ethanol and in acid λ_{max} 218nm, 253, and 369nm. It has only changed slightly. This is a bathochromic shift of 3nm. The UV showed the presence of a carbazole nucleus.

The IR data showed no hydroxyl or acetate carbonyl absorptions. There were absorptions at 1766cm^{-1}, indicative of presence of a lactone carbonyl, at 1629cm^{-1} and at 2250 cm^{-1} (C≡CH).

^{1}HNMR spectrum gave eight downfield signals, seven as aromatic and the eighth as a diarylimino hydrogen. They were δ8.5 (1H, d), δ8.3 (3H, q), δ7.79 (1H, d), δ7.6 (1H, m), δ7.3 (2H, m). There were six methine protons absorbing at δ1.4, other absorptions were δ1.3 (2H, m), δ1.7 (1H, S), δ1.1 (2H, S) and δ0.9 (2H, q).

Acetylation of SBHU3

About 6mg of SBHU3 was acetylated using pyridine and acetic anhydride. The UV of the acetylated compound gave absorptions quite different from those of SBHU3. λ_{max} was at 289nm, and in acid λ_{max} was 299nm. There was a shift similar to that observed earlier.

The IR spectrum showed absorptions at 763 cm^{-1} and 736 cm^{-1}. These were indicative of 1, 2 - and 1, 2, 3 -trisubstitution patterns of carbazole benzene rings. In the acetate, there was a displacement of the acetylenic bond absorption to a higher value of 2262 cm^{-1}. This was a reflection of steric hinderance imposed on the ethylinic bond by the newly-formed carboxyl group in the acetate.

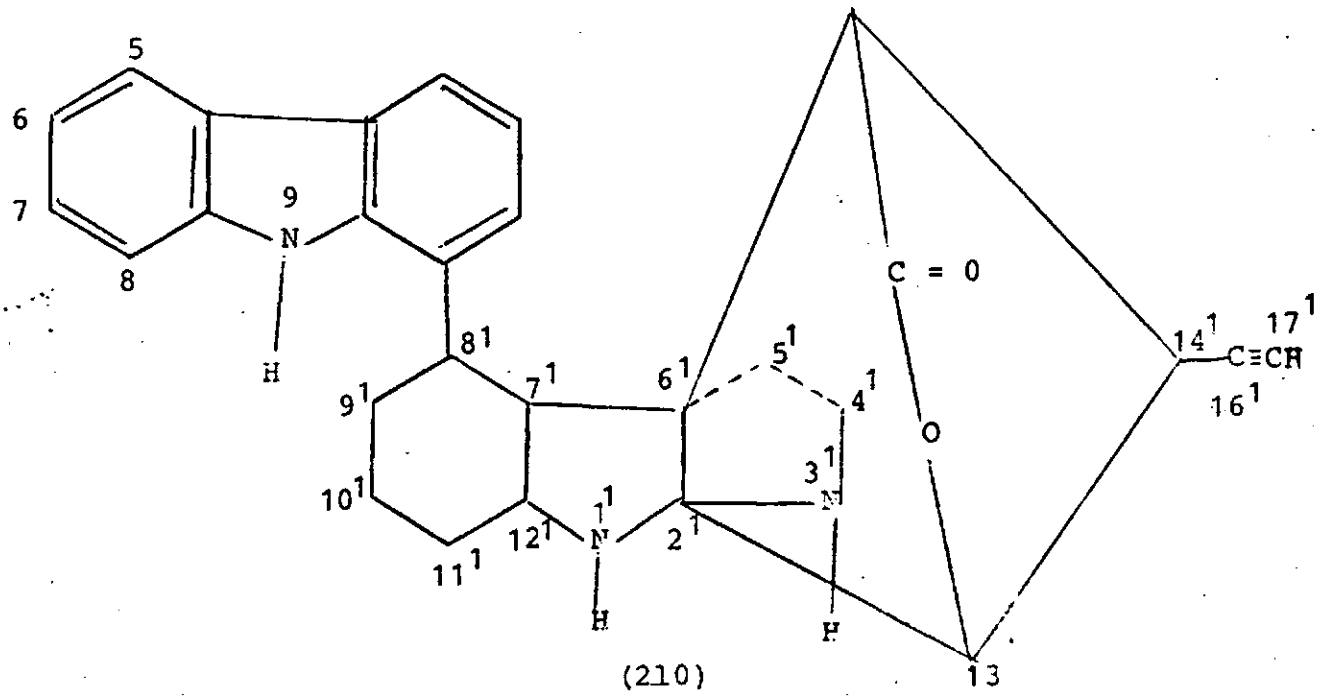
It also showed strong absorption peaks at 1709 cm^{-1} (ester), 1634 cm^{-1} and at 1621 cm^{-1}. There was a great difference in the IR spectrum here compared to that of SBHU3. The acetylated product contained no peak at 1766 cm^{-1}

The NMR was not well resolved. When spotted along side the original sample, i.e. SBHU3, the R_f 0.35 of the acetylated product was higher and showed a completely different colour under the UV lamps.

The MS of SBHU3 gave non-detectable molecular ion peak but that of a fragment of the dimer. Its acetate however, gave the accurate molecular mass of 498. A comparison of the IR spectrum of SBHU3 with that of its acetate, coupled with the data obtained from their ^{1}HNMR spectra led to a molecular formular of $C_{28}H_{27}N_3O_2$ for SBHU3.

The acetate of SBHU3 originated from the acetylation of the hydroxyl group resulting from ring opening of the lactone bridge in the ring during reaction.

The molecular weight of SBHU3 was 437. From the above spectra data structure (210) was proposed for SBHU3 and the protons assigned as follows:



(210)

Three aromatic protons absorbing at δ8.3 were assigned as H-4, H-5, and H-8 respectively. The relatively downfield NH-9 was assigned the value δ8.5, δ7.79 to H-2, δ7.6 to H-7, δ7.3 to H-6, and H-3. There was a missing downfield absorption value expected for H-1. This fixed the link for a second molecule to the carbazole nucleus at C-1.

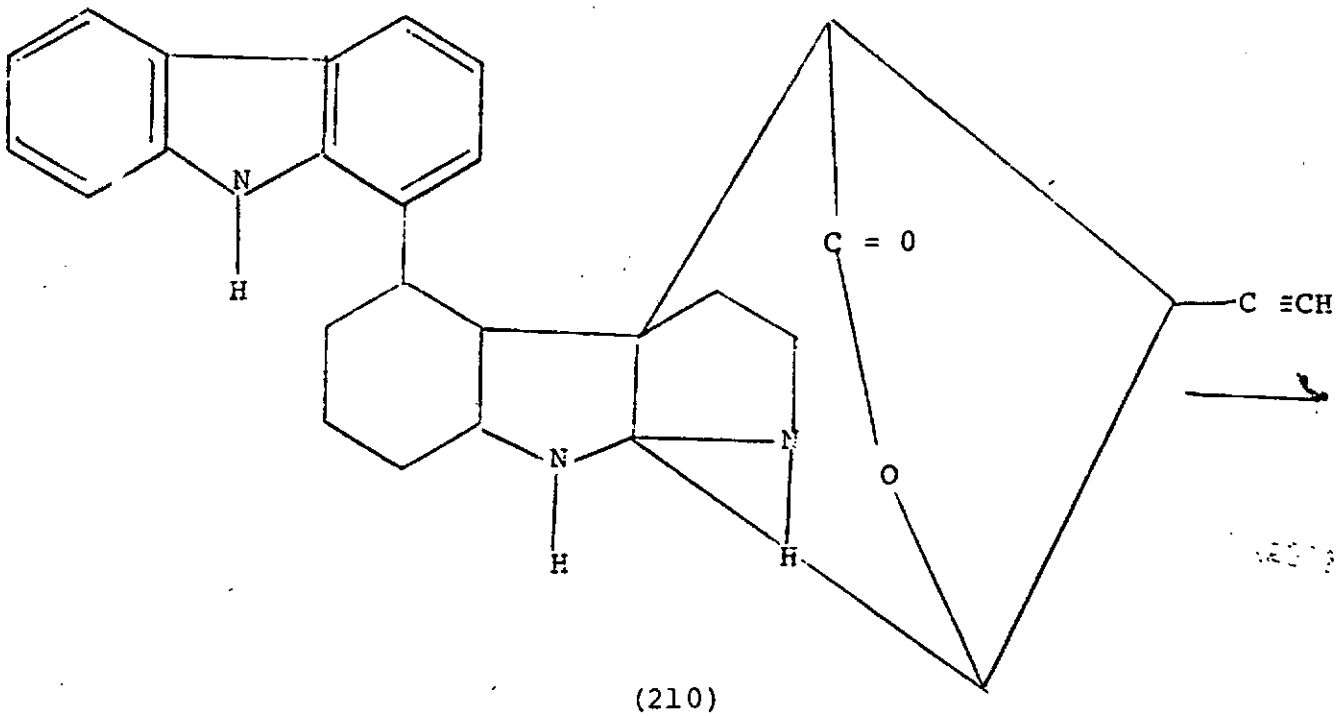
The signals for the five methine protons were $H\text{-}7^1$, $H\text{-}8^1$, $H\text{-}13^1$, $H\text{-}14^1$, and $H\text{-}15^1$. On the other hand absorptions for the five methylene protons characteristic of $H\text{-}4^1$, $H\text{-}5^1$, $H\text{-}9^1$, $H\text{-}10^1$ and $H\text{-}11^1$ were observed. The $NH\text{-}1^1$ and $NH\text{-}3^1$

signals overlaped at δ5.2 while the terminal acetylene H-17[1] appeared as a singlet at δ1.7.

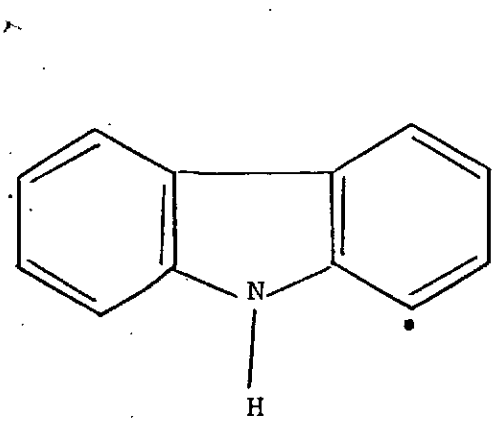
Based on the above spectral data, an N-dimethylated hexahydroiso corymine nucleus consisting of only rings A,B,C and a five-membered ring D with the lactone bridge retained was proposed for the monomer attached to C-1. The monomer, obviously carried the terminal acetylenic linkage at C-14[1].

In the mass spectra of the acetate there was peak $m|_z$ 43 $CH_3C\overset{+}{O}$ of relative intensity 100%. This confirmed the presence of the acetate in the molecule. Scheme 17 (below) shows the fragmentation pattern of the monomer joined to the carbazole nucleus at C-1.

Scheme 17



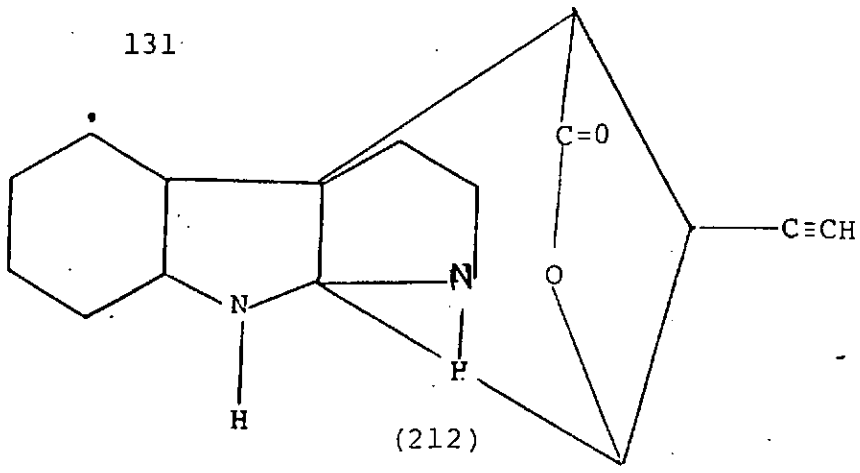
(210)



(211)

lon $m|_z$ 166 (4.8%)

$C_{12}H_8N.$



(212)

lon $m|_z$ 259 (6.0%)

$C_{16}H_{19}N_2O_2$

$\downarrow +H^+$

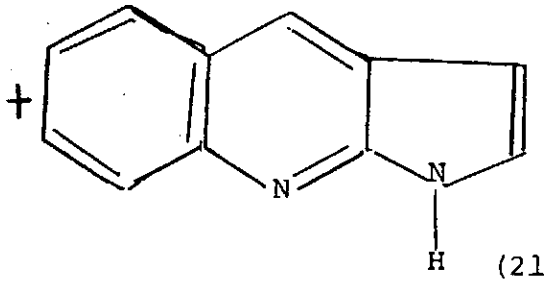
(213)

lon $m|_z$ 260 (22.8%)

$C_{16}H_{20}N_2O_2$

$\downarrow -CO_2$ $m|_z$ 44

(83.0%)



(215)

lon $m|_z$ 168 (17.0%)

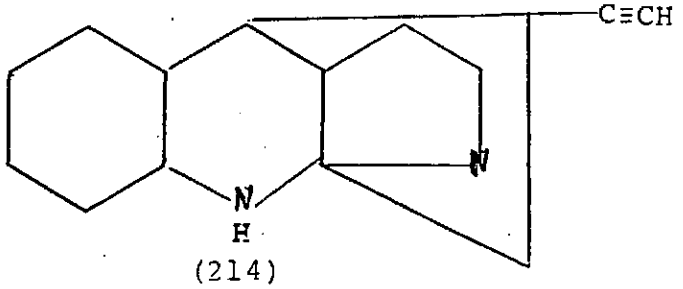
$C_{11}H_8N_2$

+

lon $M|_z$ 60 (57.5%)

C_4H_{12}

(216)



(214)

lon $m|_z$ 224

$C_{15}H_{20}N_2$

$\downarrow$

$\overset{H}{C} = \overset{H}{C} - C \equiv CH \xrightarrow{+ 2H^+}$

(217)

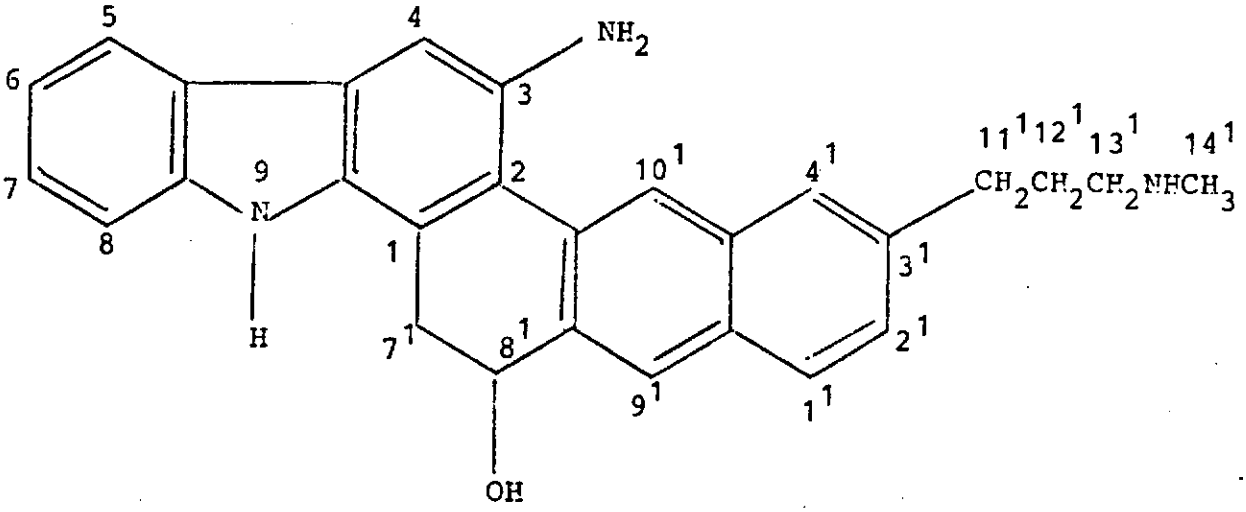
lon $m|_z$ 51 (15.0%)

C_4H_3

The fragmentation of ion (214) to minor ion (217), all present in the MS of SBHU3 confirmed the identity and the C-14 position of the terminal acetylenic linkage.

SBHU4

Structure of SBHU4



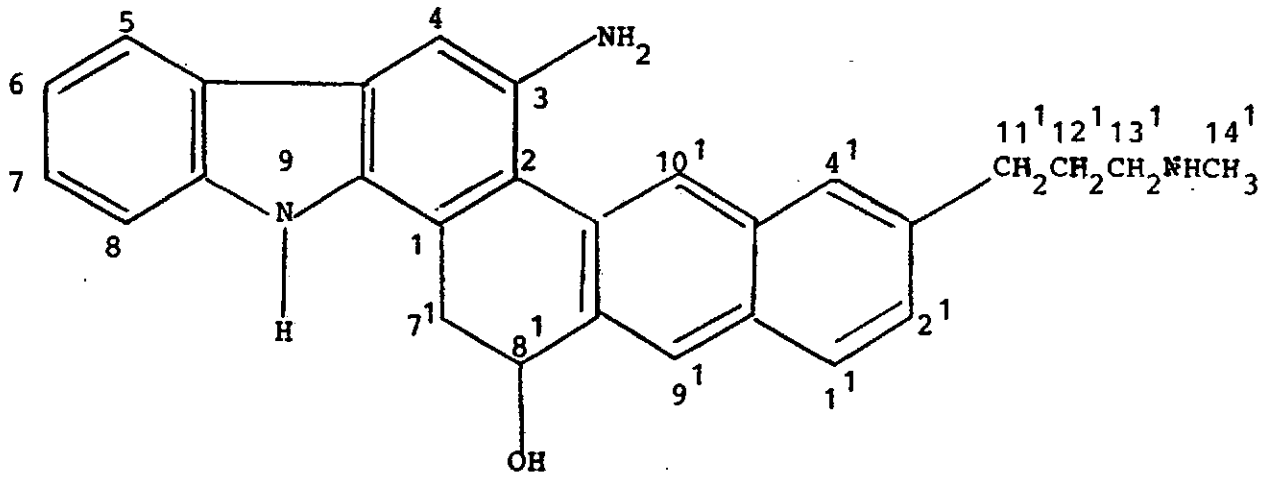
(217)

This amorphous glassy compound has an R_f 0.44. The UV spectrum showed absorptions at λ_{max} 250nm, 307nm, and 360nm. In acid λ_{max} absorptions occured at 250nm, 307nm, and 361nm. There was a slight bathochromic shift of 1nm. The absorptions are typical of that of carbazole.

The IR showed absorptions at 3300 alongside 621cm^{-1} indicating presence of aromatic primary amine i.e. the C-3 substituent. Absorption at 3692cm^{-1} was due to the OH-8^1 while that at 3450 cm^{-1} originated from the presence of N-9-hydrogen bond. There was no ester or lactone carbonyl absorption.

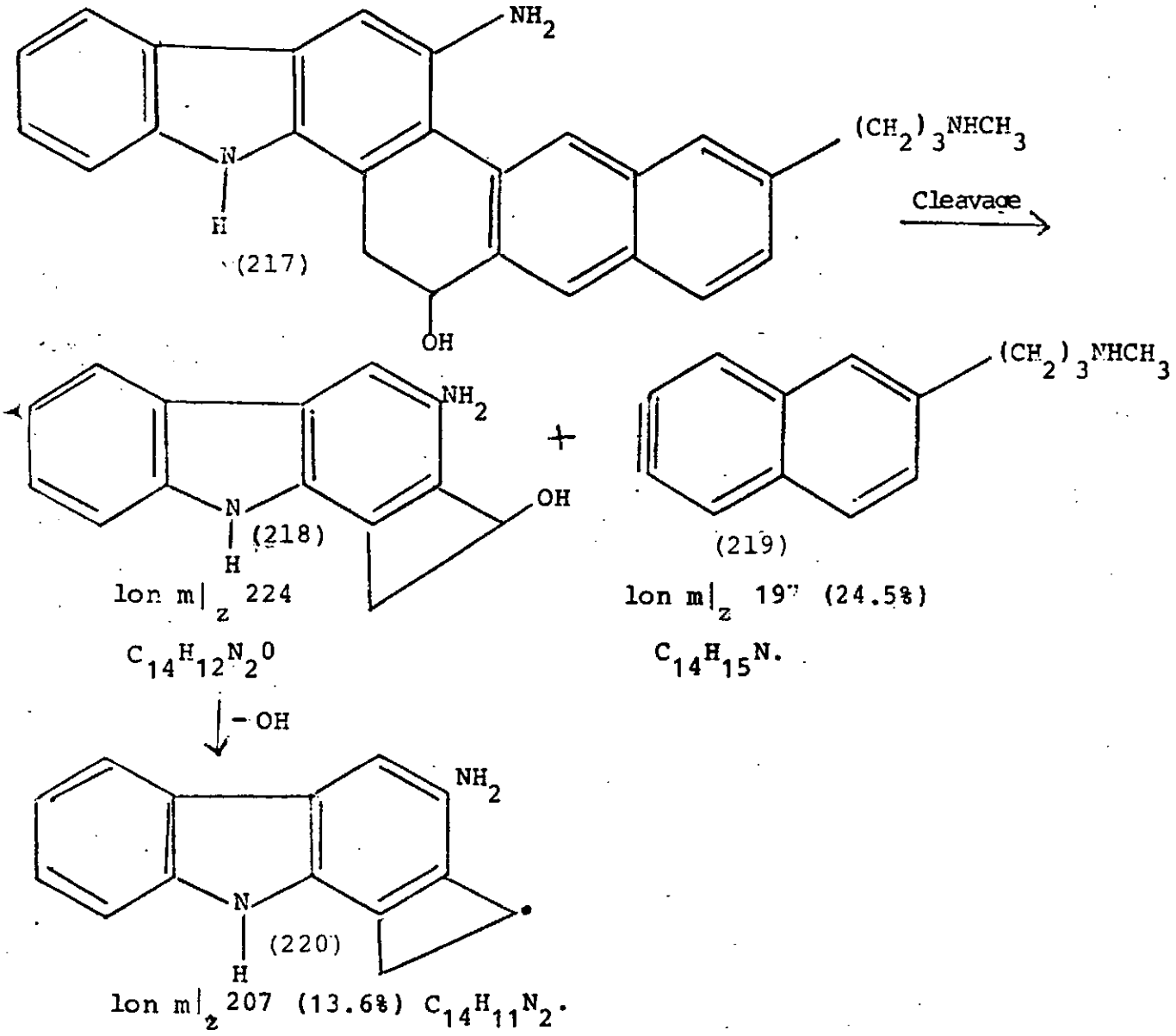
^{1}H NMR showed ten aromatic protons viz at δ8.6 (1H, d), δ8.45 (2H, dm), δ8.3 (1H, d), δ8.1 (1H, m), δ7.75 (4H, d), and δ7.6 (1H, d). There were other absorptions at δ7.5 (1H, S NH), δ7.4 (2H, d NH_2), δ3.5 (1H), δ3.0 (1H, S-NH), δ2.95 (2H, S), δ2.7 δ2.55, δ2.2 (1H, S, broad), δ1.5, and δ0.9 (S).

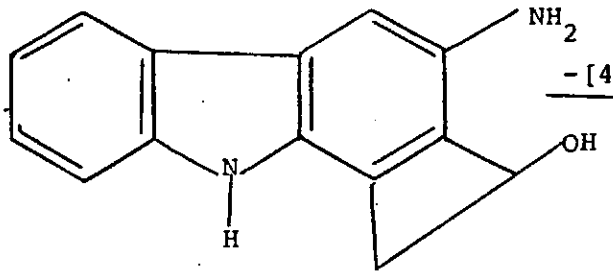
The mass spectrum showed molecular weight of 421 $C_{28}H_{27}N_3O$. The double bond equivalent was $\sqrt{17}$. From the above spectra data structure (212) was proposed for SBHU4. The proton absorptions were assigned as follows



(217)

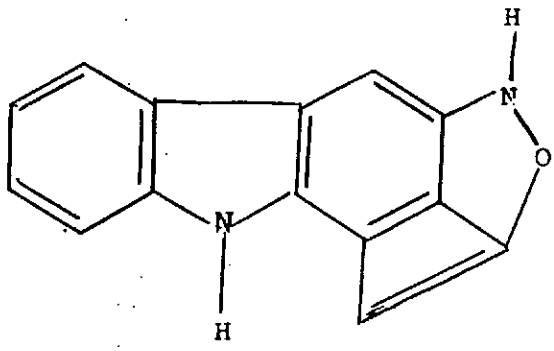
δ8.6 was due to H-8, δ8.45 H-4, H-5, δ8.3 H-7, δ7.75 H-1^1, H-2^1, H-10^1 and H-4^1, δ7.6 H-9^1, δ7.5 H-9, δ7.40 NH_2-3, δ3.5 H-8^1, δ3.0 NH-13^1, δ2.95 H-7^1, δ2.70 H-11^1, δ2.55 H-13^1, δ2.2 OH-8^1, δ1.5 H-12^1, and δ0.9 H-14^1. The fragmentation pattern of the molecule is shown below:





(218)

Ion $m/_z$ 224 $C_{14}H_{12}N_2O$

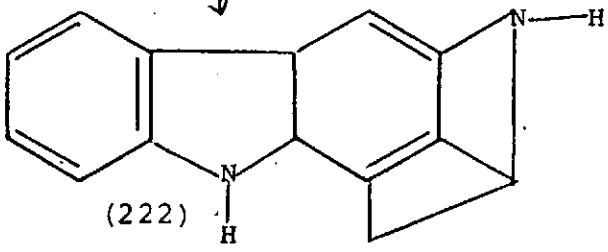


(221)

Ion $m/_z$ 220 (84.2%)

$C_{14}H_8N$.

$-H_2O$

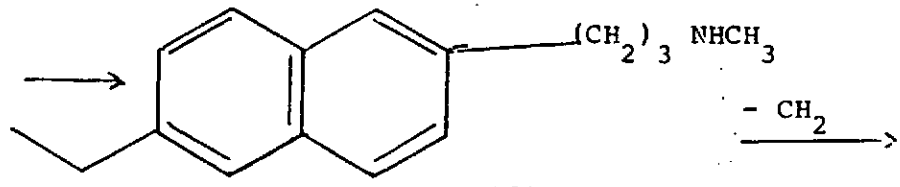


(222)

Ion $m/_z$ 206 (14.3%)

$C_{14}H_{10}N_2$.

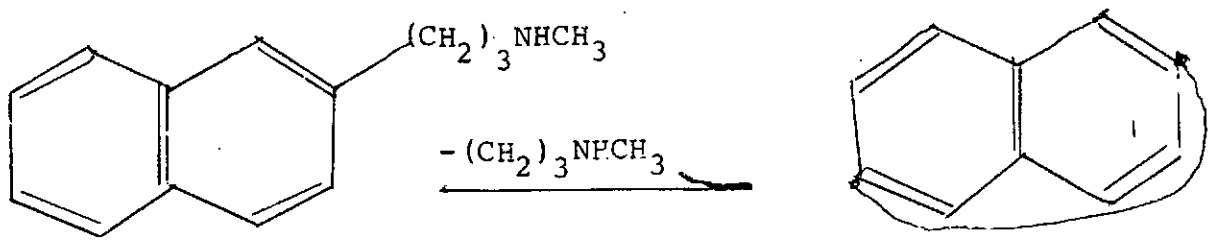
SBHU4 contained ion 218 , which by loss of hydroxyl group afforded 220 $m/_z$ 207. The ready conversion of 218 to 222 an isoxazole by loss of protons confirmed the C-3 amino substituent, the C-8^1 position of the hydroxyl group and the position of linkage of the anthracene to the carbazole moeity.

217 

(223)

Ion $m/_z$ 225. (19.0%)

$C_{16}H_{19}N$.



(224)

Ion $m|_z$ 211 (53.3%)

$C_{15}H_{17}N$

(225)

Ion $m|_z$ 126 (16%)

$C_{10}H_6$

By another route, 223 $m|_z$ 225 originated from (217). By loss of methylene radical, (217) dehomologated to ion (224) $m|_z$ 211 and this by a total loss of both the methylamino-n-propyl and methylene residues left charges on sites C-2 and C-6 of the naphthalene residue leading to the tricyclic hydrocarbon (225) $m|_z$ 126. The presence of (225) confirmed the C-2 and C-6 as sites for substituents in rings E and F. Here again, NMR data were supportive. All the expected aromatic signals were prominent. The $C\text{-}3^1$ N-methyl, unexpectedly absorbed at δ0.9, a little up-field. The Olefinic $C1^1$ - $C2^1$ bond protons as expected adsorbed at, δ7.5 for two hydrogens as doublet. The above evidence thus confirmed the structure assigned to SBHU4.

CHAPTER 6

CONCLUSION

The method previously described by Adegoke et al[99] applied and improved in this report had revealed that the different parts of the plant Hunteria umbellata has a very brilliant future in Nigerian folk medicine.

From the seeds, up to date not less than seventeen alkaloids have been isolated; in the present work however only two new ones are reported. From the back, literature appears to be scanty on the number of alkaloids from this source.

The present work had also uncovered four new dimers, and the acetate of one of them, which helped to successfully revive the actual molecular mass of the alkaloid. Work on the leaves appears to have come from this work only except the non-water soluble ericine. In particular the mass fragmentation pattern had helped to reveal the structures proposed for the alkaloids. They are all carbazole alkaloids existing either as monomers or dimers.

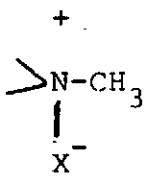
The possible uses of these alkaloids for the cure of most tropical diseases become evident from its importance in traditional medicine for the cure of diabetics, stomach ulcer etc. Unfortunately, as useful as these alkaloids are, they are found present in the plant in trace amounts. Therefore further work e.g. absolute configuration inclusive of

stereo-chemistry for each of the ten compounds isolated may take a long time to accomplish before natural seal on the structure via synthesis could be achieved. Money spent in the direction of a thorough examination of this plant will in future certainly pay a great dividend in both orthodox and traditional medicine.

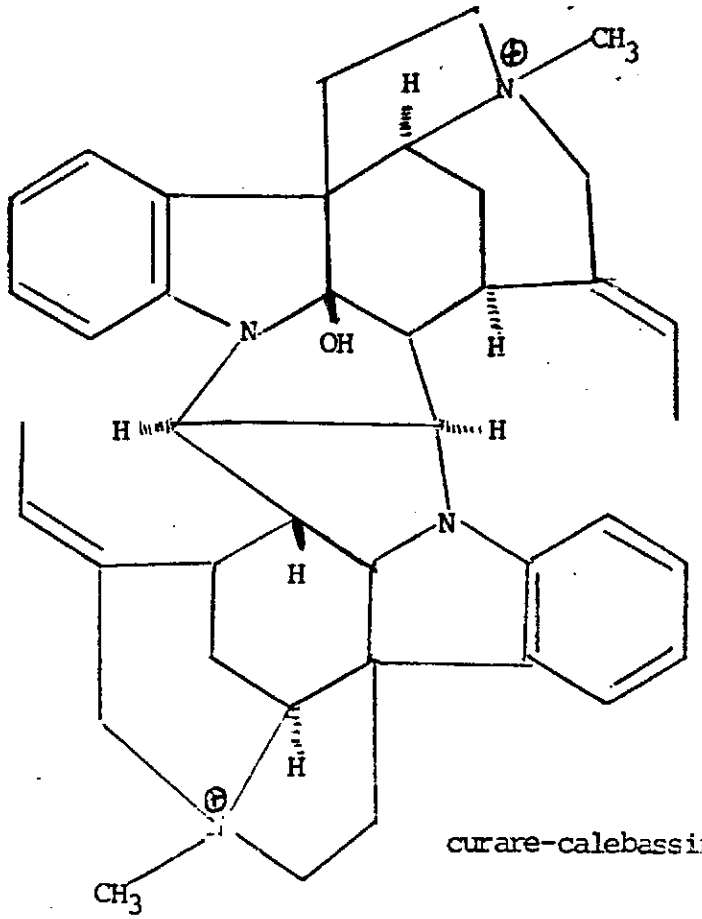
6.2 Why are they water soluble?

Many of the samples isolated were as one would expect from the method of their isolation, soluble in water. This suggests that there are many hydroxyl groups and or NH groups present in the compounds. This prompted an acetylation of two of the samples. The acetylated products were much less polar and therefore very readily soluble in chloroform, which further confirmed that the polarity of the original compound must have diminished.

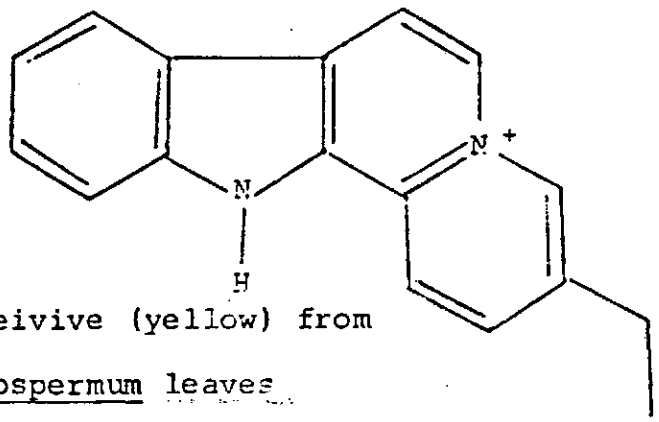
An alternative explanation for the solubility of some of these compounds in water might arise from the existence in some of them of hydrogen-bonding groups. There are two types of quaternary ammonium indole alkaloids- one containing the unit such as:



in C-calebassine (226) from curare (South American arrow poison), and the other type is of the form $-\overset{+}{N}=\langle$ usually within a ring system such as in flavopereivive (yellow) (227) from Geissospermum leave.



curare-calebassine (226)



(277) flavopereivive (yellow) from

Geissospermum leaves

CHAPTER 7

EXPERIMENTAL

Introduction

Infra-red absorption spectra of samples were ran in spectrometer model 1710 FT- neat and potassium bromide discs. The Proton Magnetic resonance spectra were recorded in CD_3OD solution (otherwise stated) with a varian 300MH_z instrument (Dimethylsilicon oxide DMSO) as internal reference. The spectra were obtained on DS-55 Mass spectrometry data system at the department of chemistry, University of Manchester, England.

Extraction

Fat components of the seeds, leaves and the bank were first extracted with hot petroleum ether (40 - 60^{o}).

The materials were collected by the author directly from the tree (pictures attached at the back of thesis) and were identified in the Botany Department. These materials were thoroughly air-dried for three weeks. The dried pulverized material (leaves, seeds, or bark) was refluxed with petroleum ether for 48 hours. During grinding, care was taken not to inhail the dust to prevent burning sensation in the throat and general weakness.

The petroleum ether extract gave no alkaloid for each of the different plant parts. The crude material (not less than 400 gms in each case) in the soxhlet thimble was then

filtered. The filterate contained both the water-soluble and water-insoluble alkaloids. The filterate gave a positive test for alkaloids, using either Mayer's reagent or Dragendoff reagent.

Aqueous sodium bicarbonate solution (2%) was added to the filtrate until it was basic. The turbid solution was extracted with chloroform to remove the water insoluble alkaloid. The basic aqueous solution which contained the water-soluble alkaloids was tested with Mayer's reagent. It gave a yellow precipitate.

The basic aqueous solution was made acidic using 2% HCL and this was followed by the addition of excess Mayer's reagent. A light yellow precipitate was formed. The precipitate was filtered and the aqueous layer discarded. The precipitate, which contained the water soluble alkaloids as complexes, was left to dry at room temperature for two days.

The water soluble alkaloids formed complexes with the mercuric iodide in Mayer's reagent. The precipitate in ethanol gave a negative result when tested with either Mayer's reagent or Dragendoff reagent.

To release the water soluble alkaloid from the complex, the precipitate was allowed to stand for not less than six weeks on the bench in ethanol at room temperature. Decomposition of the complex really started after five weeks. This was confirmed by testing for presence of alkaloids in the ethanol layer. Occasional agitation of the mixture by shaking also aided the rate of decomposition of the complex.

The ethanol layer was removed by filtering off the remaining undecomposed complex. Hydrogen sulfide gas was passed into the ethanol solution to precipitate out the remaining mercury as mercuric sulfide. The black precipitate obtained was filtered off, and the ethanol solution allowed to stand and then concentrated. This was then passed through an amberlite resin in 10% Kl (50 gms for each gm of extract) several times to remove any traces of mercury 'Evaporation of the ethanol gave the crude mixture of water soluble alkaloids from the seeds, leaves or bark extract.

After separation of the crude mixtures, pure samples obtained were all glassy but gummy materials and so no melting point was recorded. ^{1}H NMR spectra were determined in CD_3OD or DMSO at 300mH_z. In each case tetramethylsilane was used as the internal reference. IR spectra were taken in NaCℓ or KB_r pellets and ELMS at 70eV. All the UV spectra were run in ethanol and each run repeated after an addition of one drop of concentrated HCL. All the UV and IR spectra were carried at the department of Chemistry of Chemistry, University of Manchester, England. The NMR and mass spectra were run by the technicians in the same University.

The Seeds

From 2,400gm of seed, only 14.6gm of water soluble alkaloids were obtained. This value is about 0.61% of the dried plant material. The water insoluble alkaloid obtained

from this extract was 10gm, about 0.42% of the dried plant material. The water insoluble alkaloid crystallized out as a brown powder using petroleum ether 40 - 60^{o} while the water-soluble alkaloid gave a black solid after evaporating off the ethanol.

The black solid, water soluble alkaloid was tested with water: it dissolved. A small portion of it was added to 2% Hcl this solution with drops of Dragendoff reagent, gave an orange precipitate. A little of the solid was added to a mixture of methanol and concentrated nitric acid: a red colour was obtained, which faded away on standing. A solution of the solid alkaloid was tested with ferric chloride reagent and it gave a yellow precipitate. The foregoing tests confirmed that the black solid was an alkaloid soluble in water.

A little of the black solid was dissolved in methanol spotted on a thin layer chromatography plate and then developed in a tank containing mixture of n-butanol, ammonia and water in the ratio (15:1:0.5). This was the only polar solvent mixture that gave good separation of the components of the water soluble alkaloids of the seed. Four spots having various R_f values were obtained.

A narrow column for chromatography was prepared using neutral alumina (60-70) mesh). This alumina was first deactivated using 5% glacial acetic acid. To every 90gms of alumina, slurry 10ml of 5% glacial acetic acid was added in a dry bottle and shaken for sometime. Deactivation reduced the basicity of the alumina used.

At the bottom of the column was placed glass wool, followed by sand. The deactivated alumina was poured inside the thin colum as a slurry in petroleum ether. As the mixture of alumina and petroleum ether was poured inside the column slowly the tap was also opened to allow the petroleum ether to drop. Occasionally the column was tapped for smooth package. The column was 2/3 filled with alumina. Then it was covered with thin layer of sand. The level of solvent was always maintained above that of the sand.

The black solid 14.6gm from the seed was only soluble in methanol giving greenish yellow solution. After dissolving in the minimum amount of solvent (10ml of methanol), it was poured on the column. The mixture was absorbed in the column then eluted with diethylether nothing was obtained, with ethyl acetate nothing was eluted, with methanol many fractions were collected but these were found to be mixtures when spotted on thin layer plates.

Similar fractions, after spotting were merged and put on preparative thin layer plates for separation using a mixture of n-butanol concentrated ammonia and water in ratio (15:1:0.5). Commercial thin layer plates were used and others prepared. The latter were obtained by mixing silica gel with water and spreading this quickly and uniformly over a rectangular thin plate.

Three different compounds were detected using UV lamps and plate isolated from the TLC by scrapping bands off and extracting the alkaloid off the silica using methanol. The methanol extract after evaporation gave the alkaloids together with some silica. The latter was removed by redissolving in chloroform and adding a drop of methanol.

SSHU1 is a Light brown R_f 056

I.R. in Chloroform

Absorption at 1728 cm^{-1} (COOH); 1677 cm^{-1} (C = 0) and 1596 cm^{-1} benzene.

Yield of the guming product was 3.5mg.

MS $m|_z$ (rel. Int.) 418 (M^+) $C_{27}H_{18}N_2O_3$, 211 (56.6) $C_{14}H_{13}NO$, 210 (88.6) $C_{14}H_{12}NO$, 157 (8.4) $C_{10}H_7NO$, 209 (88.5) $C_{13}H_7NO_2$, 165 (4.6) $C_{12}H_7N$, 166 (3.6) $C_{12}H_8N$, 140 (44.1) $C_{11}H_8$, 109 (11.5) $C_6H_5O_2$, 65(20.7) C_5H_5 and 70 (62.1) $C_3H_2O_2$.

SSHU3

This was found to be mercuric lodide from the mass spectrum. Pure mercuric lodide was spotted along side with the compound. They both gave the same R_f value of 0.8 and the same colour under UV lamps. The UV spectrum of mercuric iodide was run in ethanol, and was found almost identical to that of SSHU3. There is protonation when a drop of concentrated hydrochloric acid was added to the ethanolic solution of SSHU3 and the UV run. But in the case of mercuric lodide there was no protonation. This shows that there was a trace of organic compound in SSHU3 but the majority was mercuric lodide.

The mercuric iodide must have come in from the reagent used in precipitating the complex. Removal of mercuric iodide from the other subsequent extract from the leaves and back were overcome by scraping off all those bands having R_f value of 0.8 and the colour of mercuric iodide under the UV lamps.

SSHU4 is a Light Yellow glassy solid, the yield was 1.5mg.

IR in Chloroform

Absorptions at 1635 cm^{-1} ($-OCH_3$ ester), 1660 cm^{-1}

NMR in CD_3OD See Table 2.

MS $m|_z$ (rel. Int) 426 (M^+) $C_{25}H_{22}N_4O_3$. 195 (44.7) $C_{13}H_9NO$, 28 (32.5)CO, 167 (34.3) $C_{12}H_9N$, 203 (11.8) $C_{12}H_{13}NO_2$, 167 (34.3) $C_{12}H_9N$, 203 (11.8) $C_{12}H_{13}NO_2$, 173 (12.9) $C_{11}H_{13}N_2$, 154 (16.5) $C_{10}H_6N_2$, 211 (35.2) $C_{13}H_{11}N_2O$, 182 (35.4) $C_{12}H_{10}N_2$, 145 (29.2) $C_{10}H_{11}N$, 115 (22.9) C_8H_6N and 29 (48)C_2H_5.

Table 1

UV. data for SSHU1 & SSHU4 IN ETHANOL

SSHU1 λ_{max}	SSHU 1 with one drop of HCl λ_{max}	SSHU 4 λ_{max}	SSHU 4 with one drop of HCl λ_{max}
220			
264	264	260	261
271	271		
284	289		
		386	436

^{1}HNMR data for SSHU1 and SSHU4 Table 2

	SSHU1 (DMSO) δ	SSHU4 (CD_3OD) δ
H-2		
H-3	1.7d J = $2H_z$	2.5 br.
H-4		
H-5	7.6q.	
H-6	7.6q	7.1t J = $2H_z$
H-7	7.6m. J = $2H_z$ and $2H_z$	7.36d J = $2H_z$
H-8	7.8d J = $2H_z$	7.96s
H-9	7.1s	5.6br.
H-10	3.6s	2.90s
H-11	3.9d J = $2H_z$	3.7s
H-1^1	1.1s	3.7
H-2^1		
H-3^1	3.1t J = $2H_z$ and $6H_z$	3.62
H-4^1	1.4t J = $2H_z$ and $2H_z$	1.60d
H-6^1		
H-7^1	7.35s	6.8d J = $2H_z$
H-8^1	7.8d J = $2H_z$	7.42d J = $2H_z$
H-9^1		4.0 br
H-10^1		2.85d J = $4H_z$
H-11^1		

The Leaves

From 400grams of the pulverized leaves 5.6 grams of water soluble alkaloids were obtained as green powder. This amount is about 1.4% of the starting material. The extract from the leaves was the last examined. As experienced from the separation of the seed and bark water soluble alkaloids, the compounds in the leave extract were polar. The only solvent mixture that gave good separation was methanol and amonia in the ratio 15:1.

All preliminary tests for solubility in water and all tests for alkaloid mentioned above for the seed extract were carried out. They all gave positive results. TLC in methanol: concentrated ammonia (ratio 15:1) indicated six, well separated spots. Like the seed, the sixth spot was identical to mercuric iodide. A pure sample of which was spotted along the original alkaloid mixture. Good separation could not be achieved by column chromatography so the leave extracts were dissolved in methanol (5 mg in 2ml of methanol) and put on TLC plates. Each plate was spotted with 500mg of the extract. The plates were eluted in a tank of methanol and concentrated ammonia (15:1). Six different bands were obtained.

After scraping each silica band was extracted with chloroform, and then filtered. Five different but similar compounds from each plate viewed under the UV lamps (360nm, and 254nm) were obtained. Similar components were recombined and each spotted. They all gave single spots of different R_f values with little impurities. Each fraction was put on

a small column, the size of a small test-table.

In the column was cotton wool, sand and kiese'ghur 60, 230-400 mesh. For every 1mg of compound, five times the value of kieselghur was used. The eluting solvent was a mixture of methanol and concentrated ammonia (15:1). The compound was also dissolved in this mixture. To every 20mg, 0.5ml of solvent was used. This method of separation gave very pure compounds. The physical data of the five different compounds obtained are as given below.

SLHU1

This is water soluble leave Hunteria umbellara 1. Yield was 9.1mg, R_f = 0.18. It is a brown solid.

IR In Chroloform

Absorption at 3705 cm^{-1} (OH), 3584 cm^{-1} (OH), 1706 cm^{-1} (αβ-unsaturated carbonyl), 1631 (benzene ring) and at 1213 cm^{-1} (ArOH).

MS m/z (rel. Int) 348 (M^+) $C_{21}H_{20}N_2O_3$, 212 (45) $C_{13}H_{10}NO_2$, 129 (18.5) C_9H_7N, 83 (43.4) $C_4H_3O_2$, 115 (18.6) C_8H_5N, 101 (17.1) C_8H_5, 122 (33.9) $C_8H_{10}O$, 94 (36) C_7H_{10}

SLHU3

This is water soluble leave alkaloid Hunteria umbellata 3. Yield was 18.1mg R_f = 0.4. It is brown in colour.

The quantity obtained was relatively high. The compound was acetylated using 6.8mg of the alkaloid. The whole SLHU3 was dissolved in methanol and few drops were transferred by means of a dropping pipette into a weighed dry round bottom flask. The methanol was evaporated off using rotary evaporator. The flask was reweighed with the sample in it. The weight of SLHU3 in the flask was 6.8mg. 1ml of a mixture of acetic anhydride and pyridine (1:1) was added, and the solution shaken and allowed to stand at room temperature for 12 hours. All the compound dissolved giving a brown solution.

The acetic anhydride and pyridine were evaporated off without heating using a high vacuum pump at Cg. 0.5mm Hg. The resulting brown solid was worked up by adding 5ml of distilled water and a small quantity of potassium bicarbonate (solid). The potassium bicarbonate was added to neutralise the acetic acid formed in the reaction. The acetylated alkaloid was extracted with chloroform. The chloroform layer was washed twice with distilled water, and dried over anhydrous magnesium sulfate. The chloroform was evaporated off. The ester was spotted along side with the starting material. It gave the same R_f value but different colour under the UV lamps. The starting material was light blue but the ester gave a deep blue colour. The UV spectrum was run in ethanol and in dilute hydrochloric acid, and the spectrum compared with that of the starting material. There was a big difference. The IR

of the ester showed absorption corresponding to the new ester. The foregoing data showed that SLHU3 was acetylated.

IR In Chloroform

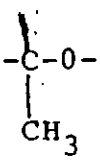
Absorptions at 1703 cm^{-1}, 1633 cm^{-1} typical and similar to that of SLHU1, at 754 cm^{-1} (an ortho-disubstituted benzene).

MS $m|_z$ (rel. Int) 348 M^+ ($C_{21}H_{20}N_2O_3$. 168 (50.5) $C_8H_{10}NO_3$, 140 (13.3) $C_7H_{10}NO_2$, 124 (32.9) $C_7H_{10}NO$, 40 (61) C_2H_2N, and 69 (43) C_4H_5O.

ESLHU3

This is ester of water soluble leave Hunteria umbellata 3. Yield was 12.3mg, $R_f = 0.4$

IR in Chloroform

Absorption at 1055 cm^{-1} (), 1674 cm^{-1}.

NMR was not well resolved. MS $m|_z$ (ref. Int) 391 (M^+) $C_{23}H_{23}N_2O_4$, 43 (100) C_2H_3O, 166 (25), 168 (69.3), 140 (33.5), 124 (57.1) and 69 (53.5).

SLHU4

This is water soluble leave Hunteria umbellata 4. Yield was 6.2 mg, R_f = 0.52. It is glassy pale brown solid.

IR In Chloroform

Absorption at 1706 cm^{-1} (c = 0), and 1633 cm^{-1} (benzene)

MS $m|_z$ (rel. Int) 411 (M^+) $C_{26}H_{25}N_3O_2$, 224 (20.4) $C_{14}H_{10}NO_2$, 59 (31.9) $C_2H_3O_2$, 165 (6.7) $C_{12}H_7N$, 121 (20.7) $C_8H_{11}N$, 185 (7.0) $C_{12}H_{13}N_2$, 68 (45) C_4H_6N.

SLHU5

This is water soluble leave Hunteria umbellata 5. Yield 3.1mg, R_f = 0.39 it is brown glassy solid.

IR In Chloroform.

Absorption at 3788 cm^{-1} (OH), 2851 cm^{-1} (OCH_3), 1704 cm^{-1} (αβ-unsaturated carbonyl), 1633 cm^{-1} (aromatic ring), 1339 cm^{-1} (= C - C - NH_2), 1064 cm^{-1} (OCH_3) 88cm^{-1} (1,2,4,5, tetra substituted benzene), and 780cm^{-1} (ortho disubstituted benzene).

MS $M|_z$ (rel. Int.) 320 (M^+) $C_{19}H_{16}N_2O_3$.

272 (35.3) $C_{18}H_{10}NO_2$, 124 (11.9) $C_6H_6NO_2$, 107 (18.6) $C_6H_3O_2$, and 91 (32.5) C_6H_3O,

Table 4: UV data for SLHU1, SLHU3, SLHU4, SLHU5 IN EETHANOL

SLHU1 (Ethanol) λ'_{max}	SLHU 1 with one drop of HCℓ λ_{max}	SLHU3 λ_{max}	SLHU3 with one drop of HCℓ λ_{max}	SLHU4 λ_{max}	SLHU4 with one drop of HCℓ λ_{max}	SLHU 5 λ_{max}	SLHU 5 with one drop of HCℓ λ_{max}	SLHU 3 Acetate λ_{max}	SLHU3 Acetate with one drop of HCℓ λ_{max}
251	251	251	251	227	225	220	228		
				265	264	266	265		298
308	307	308	308	320	319	321	319		306
319	369	368	368					340	
				385	380	384	380		
		429	430						

Table 4: ^{1}HNMR data for SLHU1, SLHU3, SLHU4 and SLHU5

	SLHU 1 (CD_3OD) δ	SLHU3 δ	SLHU4 (CD_3OD) δ	SLHU5 δ
H-1		8.50d J=2H_z		8.4q J=1H_z, 6H_z and 1H_z
OH-1	3.8			
H-2		7.8d J=1H_z		
OH-2				4.0 br
H-3	7.5d J=2H_z	7.5t J=1H_z, and 1H_z	7.75s	
H-4			6.7d J=2H_z	7.75s
OH-4	3.85			
H-5	8.4d J=2H_z	8.4d J=2H_z	8.4d J=2H_z	7.6t J=2H_z and 2H_z
H-6	7.8d J=2H_z	7.8d J=1H_z	7.09d J=2H_z	6.7d J = 2H_z
H-7	8.5t J=2H_z and 2H_z	7.8d J=1H_z	7.55m J=2H_z and 2H_z	7.1d J = 2H_z
H-8	7.8d J=2H_z	8.50d J=2H_z	8.5d J=2H_z	8.4q J=1$_z$, 6H_z and 1H_z
H-9		2.4s	4.0 br	2.21 br
NCH_3-9	3.8s			
CH_3-9		2.40s		
H-11			3.8s	
H-2^1	5.35		2.3 br	
NH_2-2^1		2.5 br		1.09d
OCH_3-3^1				3.8
H-4^1	5.5 br		1.45d J=2H_z	4.6m J = 4H_z
OCH_3-4^1		3.8		
H-5^1	3.95		1.45d J=2H_z	2.5 br
OH-5^1		4.70 br		
H-6^1		1.3	1.45d J=2H_z	
CH_3-6^1	3.8s	1.45d J=1H_z		
CH-6^1		1.09s		
NH-6^1	2.0 br			
NCH_3-6^1	1.30s			
H-7^1				1.45m
H-8^1				1.45m
H-9^1				2.5s
H-10^1				0.89d J=2H_z
H-11^1				4.6d J=2H_z
H-12^1				1.9s

The Bark

From 784 grams of the pulverized bark, 6.17 grams of water soluble alkaloids were obtained. 1.75 grams of water insoluble alkaloids were also obtained. The water soluble alkaloid was 0.79% of the starting material while the insoluble one was 0.22%. On the whole the water soluble alkaloids present were in greater quantity in all the three different parts (seeds, leaves and bark) of the plant than the water insoluble alkaloids.

The water soluble alkaloids from the bark was a light yellow powder, obtained after evaporating off the ethanol. Preliminary tests for alkaloid and solubility test were carried out as earlier described. The light yellow powder gave positive tests for alkaloid and it was readily soluble in water.

Good separation of the components was achieved on a TLC plate by eluting with a mixture of 1% triethyl amine in methanol and ethnol (i.e. methanol: Ethanol: triethylamine (49.5: 49.5:1). On TLC, it gave five different spots. The one with the highest R_f value was also found to be mercuric iodide. The extract from the bark was also passed through a column of deactivated Alumina. It is quite interesting to note that a lot of compound was eluted with diethylether unlike that of the seed and no compound was eluted from column with ehyl acetate.

Fractions from column came out to be mixtures which were separated on TLC using the solvent mixture of 1% triethyl-amine, methanol and ethanol. After extraction from

silica, similar fractions from different plates were spotted together before marging. Four different compounds were isolated.

SBHU1

This is water soluble, extract from the bark Hunteria umbellata 1. Yield 5.6mg, R_f = 0.22.

IR In Chloroform

Absorptions at 3756 cm^{-1} (OH), 1723 cm^{-1}, 1638 cm^{-1} (aromatic ring).

MS m/z (rel. Int). 427 (M^+) $C_{27}H_{29}N_3O_2$, 166 (6.0) $C_{12}H_{18}N$, 167 (44.8) $C_{12}H_9N$, 261 (20.9) $C_{15}H_{21}N_2O_2$, 260 (70.1) $C_{15}H_{20}N_2O_2$, 246 (41.5) $C_{14}H_{18}N_2O_2$, 220 (20.2) $C_{12}C_{16}N_2O_2$, 177 (23.3) $C_9H_oN_2O_2$, 149 (32.5) $C_8H_{10}N_2O$ and 107 (23) C_7H_7O.

SHBU2

This is water soluble bark extract from the bark of Hunteria umbellata 2.

IR In Chloroform

Absorption at 3446 cm^{-1} (OH), 2924 cm^{-1} (CH_3)

MS m|z (rel. Int) 455 (M^+) $C_{26}H_{21}N_3O_5$.

205 (17.5) $C_{14}H_7NO$, 188 (5.1) $C_{10}H_8N_2O_2$, 266 (18.5) $C_{15}H_{12}NO_3$,

186 (6.7) $C_{10}H_6N_2O_2$, 189 (13.4) $C_{10}H_9N_2O_2$, 166 (8.1) $C_8H_{10}N_2O_2$,

and 149 (5.4) $C_8H_7NO_2$.

SBHU3

This was the water soluble extract from the bark of Hunteria umbellata and is hereby named SBHU3. Yield 18,6mg R_f = 0.09.

IR In Chloroform

Absorptions at 1766 cm^{-1} (lactone bridge carbonyl 1629 cm^{-1} (benzene), 2250 cm^{-1} (C ≡ CH).

MS m|z (rel. Int.)

168 (17.0) $C_{11}H_8N_2$, 44 (83.0) CO_2, 53 (12.7) C_4H_5,

55 (46.3) C_4H_7, 57 (43) C_4H_9

The quantity obtained was relatively large so acetylation was attempted as described for SLHU3, 5.8mg was used for the acetylation. The acetylated product when spotted along side the starting material gave one spot, different in colour from the starting material. The starting material was light blue under the two UV lamps while the acetylated product was brown. The R_f value of the acetylated product was different from that of the starting material.

ESBHU3

This was the ester of water soluble extract from the bark of Hunteria umbellata and is hereby named ESBHU3. Yield was 12.3mg R_f = 0.35 that of starting material was 0.09.

1R In Chloroform

Absorption at 1709 cm^{-1} (acetate) and 1621 cm^{-1} (benzene).

SBHU4

This is water soluble bark Hunteria umbellata 4. Yield was 6.8m , R_f = 0.44. It is amorphus glassy compound.

1R In Chloroform

Absorptions at 3406 cm^{-1} (OH), 1585 cm^{-1} (benzene when further conjugated).

MS $m|_z$ (rel. Int). 421 M^+ $C_{28}H_{27}N_3O$. 223 (18.3) $C_{14}H_{11}N_2O$, 207 (13.6) $C_{14}H_{12}N_2$, 220 (84.2) $C_{18}H_8N_2O$, 211 (53.3) $C_{14}H_{17}N$, and 126 (16) $C_{10}H_6$.

Table 5: UV data for SBHU1, SHBU2, SBHU3, SBHU3, SBHU3 Acetate SBHU4

IN ETHANOL

SBHU 1 λ_{max} (ethanol)	SBHU1 with one drop of HCℓ λ_{max}	SBHU 2 λ_{max}	SBHU 2 with one drop of HCℓ λ_{max}	SBHU 3 λ_{max}	SBHU3 with one drop of HCℓ λ_{max}	SBHU 3 Acetate λ_{max}	SBHU 3 Acetate with one drop of HCℓ λ_{max}	SBHU 4 λ_{max}	SBHU 4 with one drop of HCℓ λ_{max}
		216	215	218	218				
253	253	255	255	252	253			250	250
307	307		304	307	369	289	299	307	307
367	368			366				360	361

Table 6: ^{1}HNMR data for SBHU1, SBHU2, SBHU3, SBHU3 Acetate and SBHU4

	SBHU 1 (CD,OD)δ	SBHU 2 (CD,OD)δ	SBHU 3	SBHU 3 Acetate	SBHU4 (DMSO)δ
H-2	8.45d J=2H_z		7.75d J=8H_z	7.74d	
H-3	7-5d J=2H_z and 6H_z	7.4m	7.30m J=8H_z	7.30m	
NH_2-3				7.40q	
H-4	8.45d J=2H_z	7.8t	8.30q	8.3q	8.45m
H-5	8.45d J=2H_z	7.8t	8.3q	8.3q	8.45d
H-6	7.8q	7.4m	7.3m	7.3m	8.10m
H-7	7.8q	7.7t	7.6m	7.6m	8.3d
H-8	8.55q J=8H_z	8.6s	8.3q	8.3q	8.6d
H-9	5.40q	4.1br	8.5d J=6H_z	8.55	7.5d
)H-10		2.2br			
H-11		3.75s			
H-12		3.35s			
H-1^1	5.4d J=2H_z	4.0 br	5.15d		7.75q
H-2^1	1.9m	4.35q			7.75q
NH-2^1	1.8d				
H-3^1	3.8m	4.25d	5.15m J=12H_z		
H-4^1	2.3	2.27s	2.4		7.75q
H-5^1			1.4d J=6H_z	1.6d	
H-6^1	7.5m				
H-7^1	7.8q		1.4d J=6H_z	1.6d	2.95s
OH-7^1		2.37t			2.25 br
H-8^1	8.55q		1.4d J=6H_z	1.6d	3.5
OH-8^1		2.37t			2.25br
H-9^1	1.7d J=6H_z	4.0d	1.1s	1.1s	7.60s
H-10^1	1.3q	2.27	0.9q	0.9q	7.75q
H-11^1	3.6t		1.3m	1.3m	2.70
H-12^1	5.4d J=2H_z				1.5
H-13^1	-				2.55
OH-13^1	6.0q				
NH-13^1					3.0m
H-14^1	5.2d J=6H_z		1.4d J=6H_z	1.6q	0.9s
H-15^1					
H-17^1			1.7s	1.7s	

SUMMARY

Yields

SSHU 1	3.1mg	5.3mg from original
SSHU 2	0.7	
SSHU 4	1.5	

14.6gm crude water-soluble alkaloid.

SLHU 1 -	9.1 mg	41 mg from an original 5.6 gm crude water-soluble alkaloid.
SLHU 2	4.5 mg	
SLHU 3	18.1	
SLHU 4	6.2	
SLHU 5	3.1	

SBHU 1	5.8 mg	35.3 mg from an original 6.17 gm. crude water soluble alkaloid.
SBHU 2	4.1	
SBHU 3	18.6	
SBHU 4	6.8	

The highest yield (1.4% of dry weight) was obtained from the leaves compared with 0.42% from the seed and 0.22% from the bark. It seems likely that with improved techniques, considerably larger quantities of pure alkaloids could now be obtained from the crude mixture. However it has now been shown clearly that several, separable alkaloids can be obtained by the methods described in this work.

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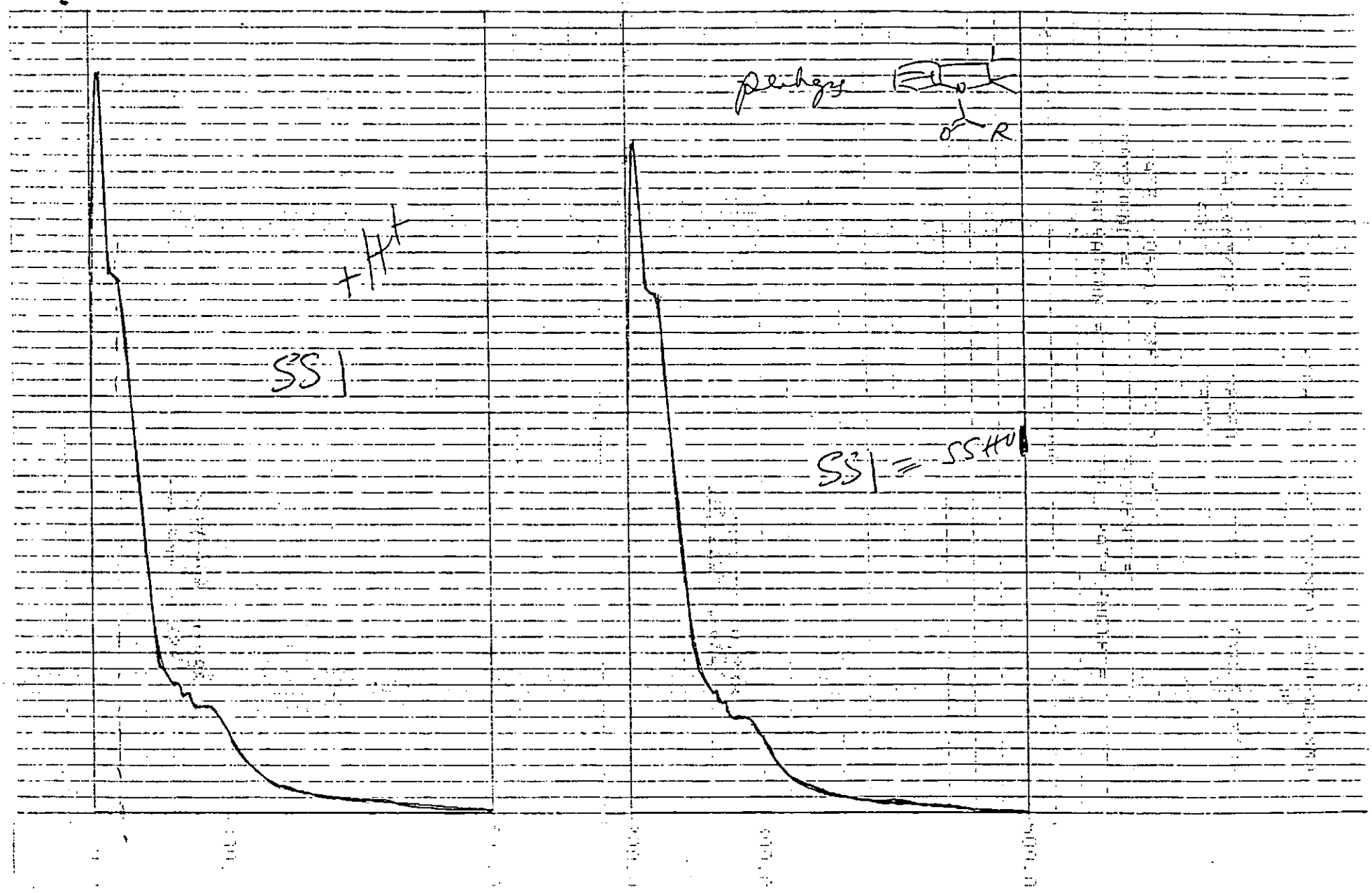
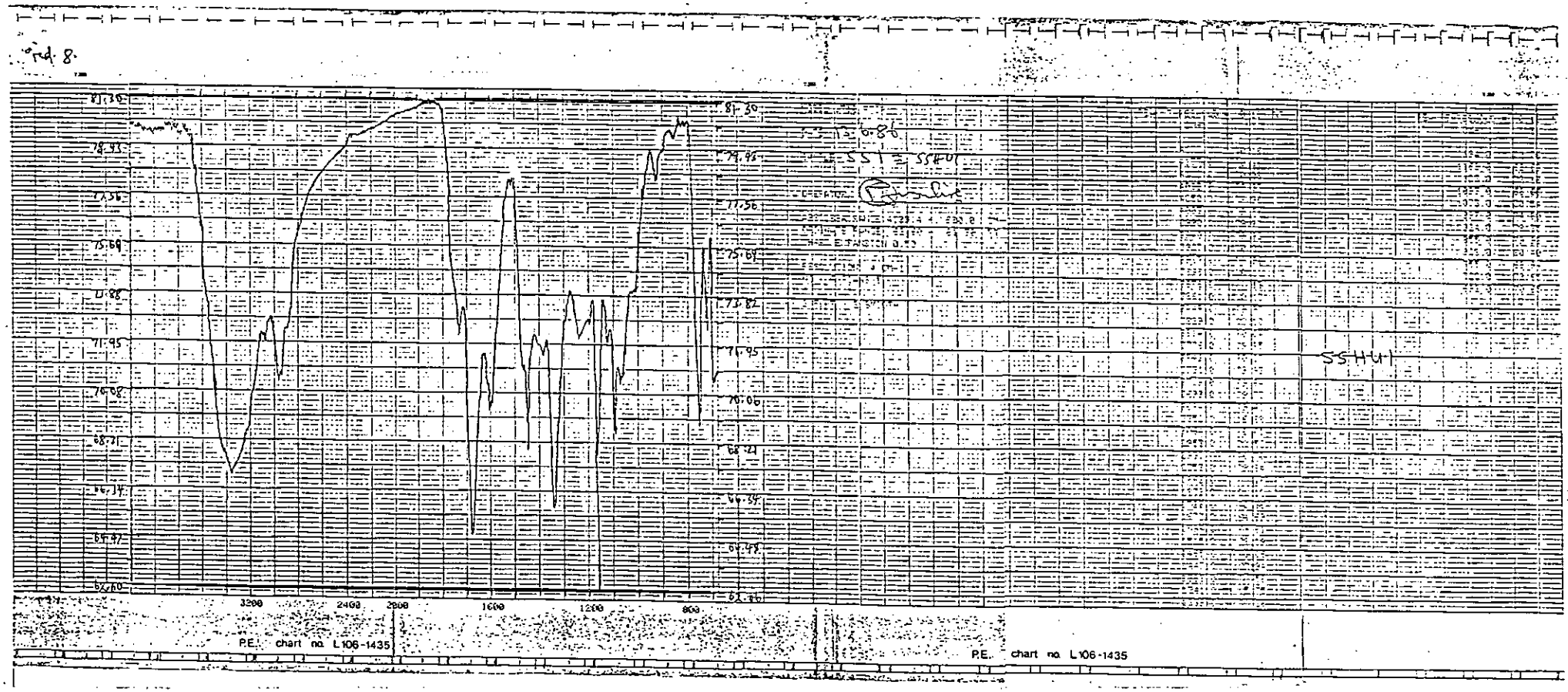


SMD. CR. CHART 200-91522

Fig. 8.



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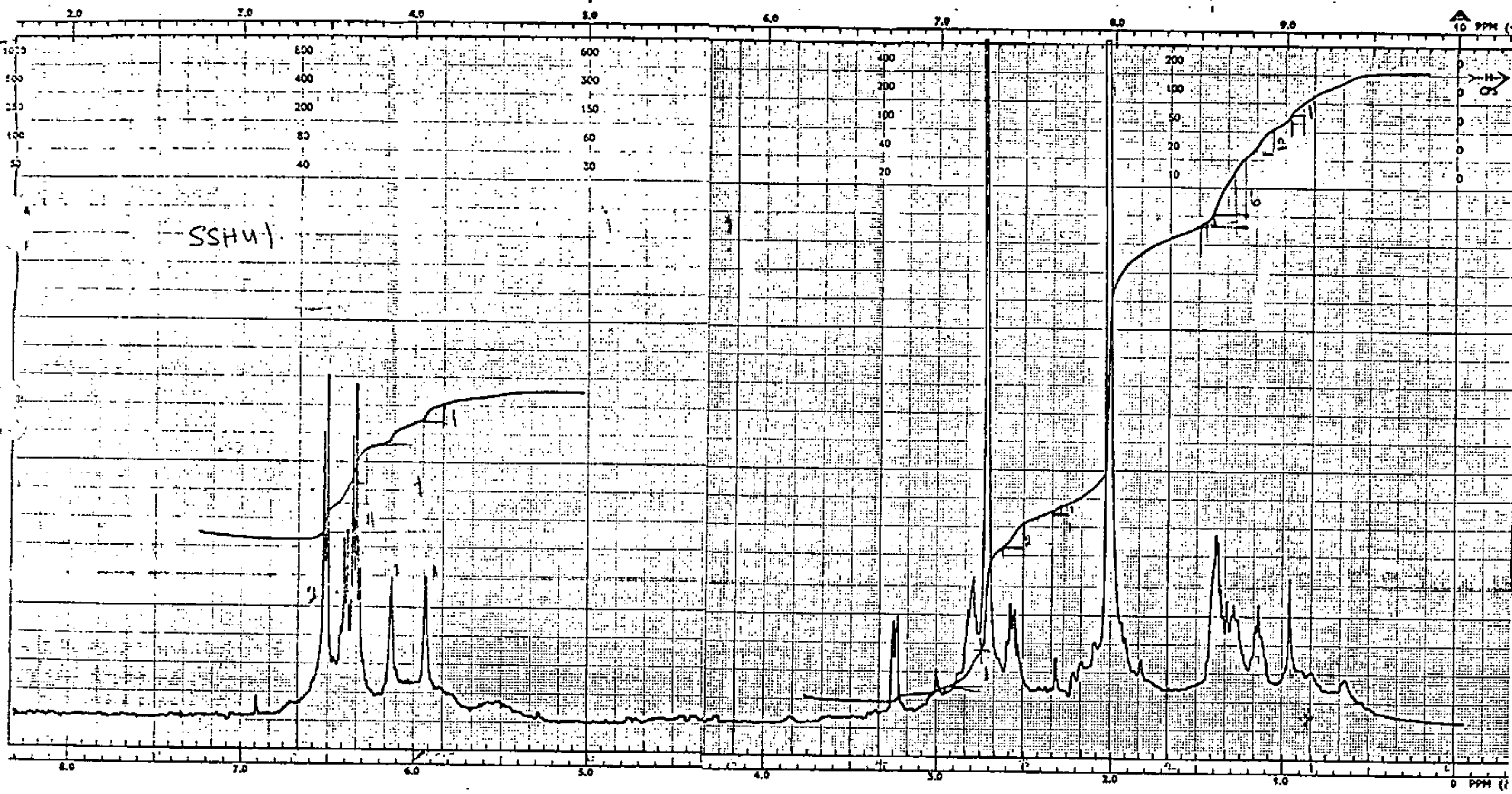


SC300 SPECTRUM NO. 75

^{1}H 300 MHz ☑ ^{13}C 75 MHz ☐
^{19}F 282 MHz ☐ OTHER ____ ☐
^{31}P 121 MHz ☐

SAMPLE SS1 SSHU).

SOLVENT d₆ DMSO
TUBE O.D. 5 mm SPIN RATE 25 Hz
TEMP. 23 °C GAS FLOW 10 CFH

LOCK
^{1}H ☐ ^{2}H ☑
RF LEVEL ____ dB RF GAIN ____

OBSERVE
CW ☐ FT ☑ LF ☐ HF ☑
RF FREQUENCY 300.247433 MHz
SWEEP/SPECTRAL WIDTH 4.2 Hz
SWEEP/ACQUISITION TIME 4.0 sec
RF LEVEL/PULSE WIDTH 5.5 dB/μsec
PULSE DELAY 20 sec FILTER C Hz
NO. SCANS/TRANSIENTS 100
RF GAIN 8.2 SPECTR AMPL 100
END OF PLOT 500 Hz WIDTH OF PLOT 2? Hz
VERT. SCALE 1000 TIME CONSTANT -1 sec

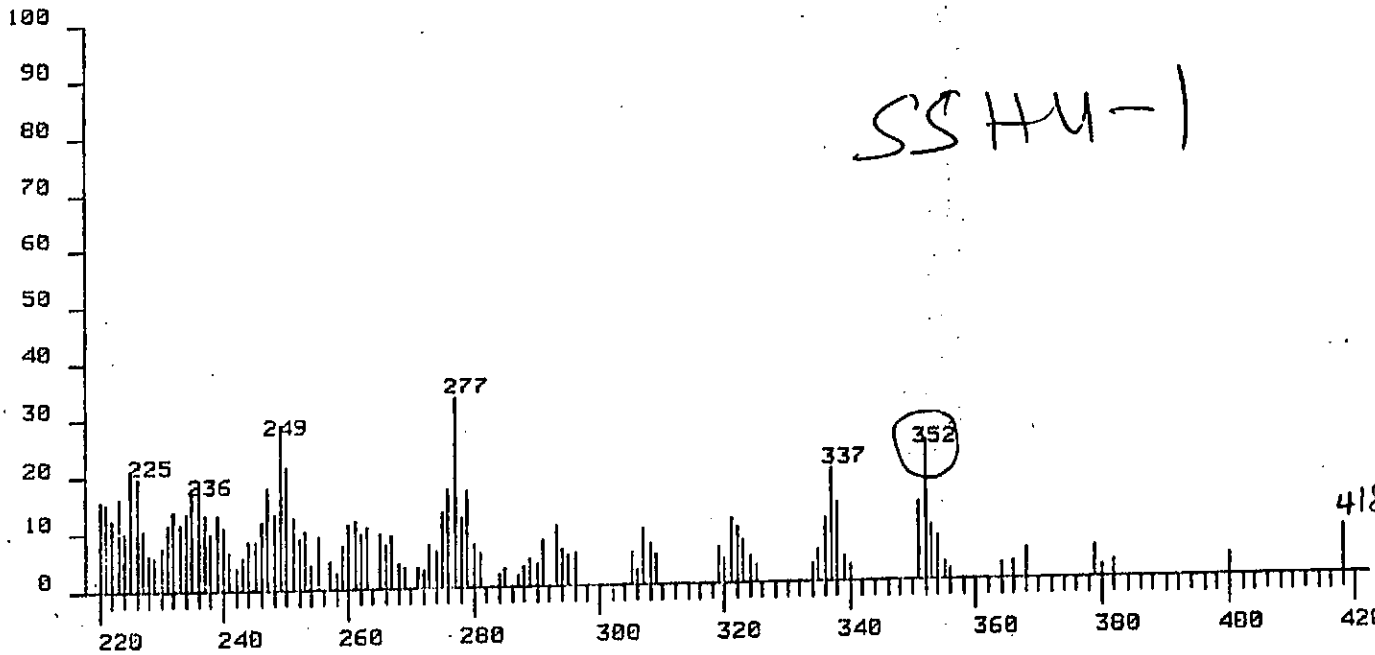
DECOUPLE
RF FREQUENCY ____ MHz
HOMO ☐ INDOR ☐ LEVEL ____ dB
HETERO ☐ LEVEL ____ BANDWIDTH ____ KHz

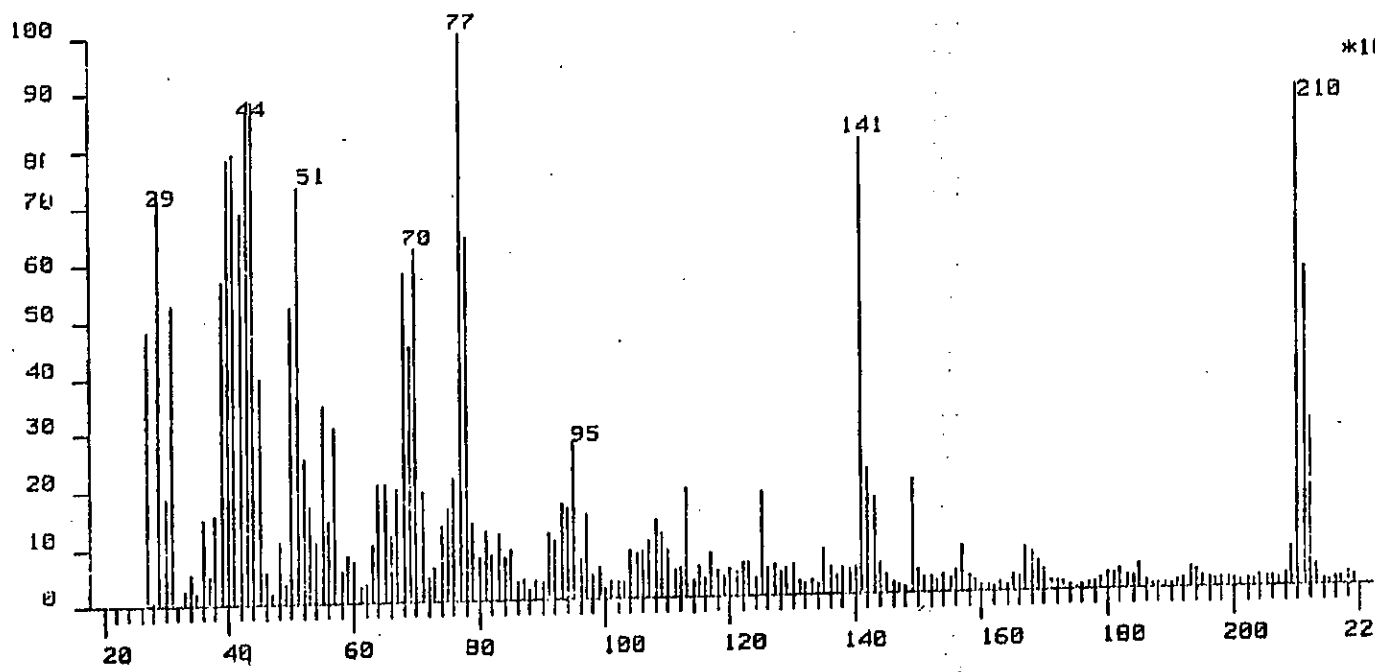
OPERATOR Heather Kol DATE 17/6/?

CHART SC300 varian

17LR11.50 [TIC=22452224, 100%=7962721] EI

SSHH-1





PEAK NO	MEASURED MASS	% INT. NREF	
1	418	0.8	
11	354	0.8	
12	353	1.0	
13	352	2.5	
14	351	1.4	
17	338	1.4	
18	337	2.0	
19	336	1.1	
24	323	0.7	
25	322	1.0	
26	321	1.1	
28	319	0.6	
30	308	0.7	
31	307	1.0	
36	294	0.6	
37	293	1.1	
38	291	0.8	
49	277	3.3	
74	249	2.9	
110	213	3.9	
111	212	29.3	
112	211	56.6	*
113	210	88.6	*
114	209	6.5	*
131	193	3.9	
139	185	4.4	
155	169	5.5	
156	168	6.7	
157	167	7.8	
167	157	8.4	
174	150	3.9	
175	149	20.2	
180	144	5.4	
181	143	17.0	
182	142	21.9	
183	141	80.0	
185	140	4.9	
186	139	4.4	
187	138	4.7	
189	136	4.8	
190	135	8.1	
195	130	5.4	
196	129	5.0	
197	128	4.2	
198	127	5.6	
199	126	4.9	
200	125	18.5	
203	123	6.0	
204	122	6.1	
206	121	4.2	
207	120	4.9	
209	118	4.7	
210	117	7.8	
212	115	5.3	
214	113	19.1	

SSHMI EI

SSHMI EI

PAGE 2

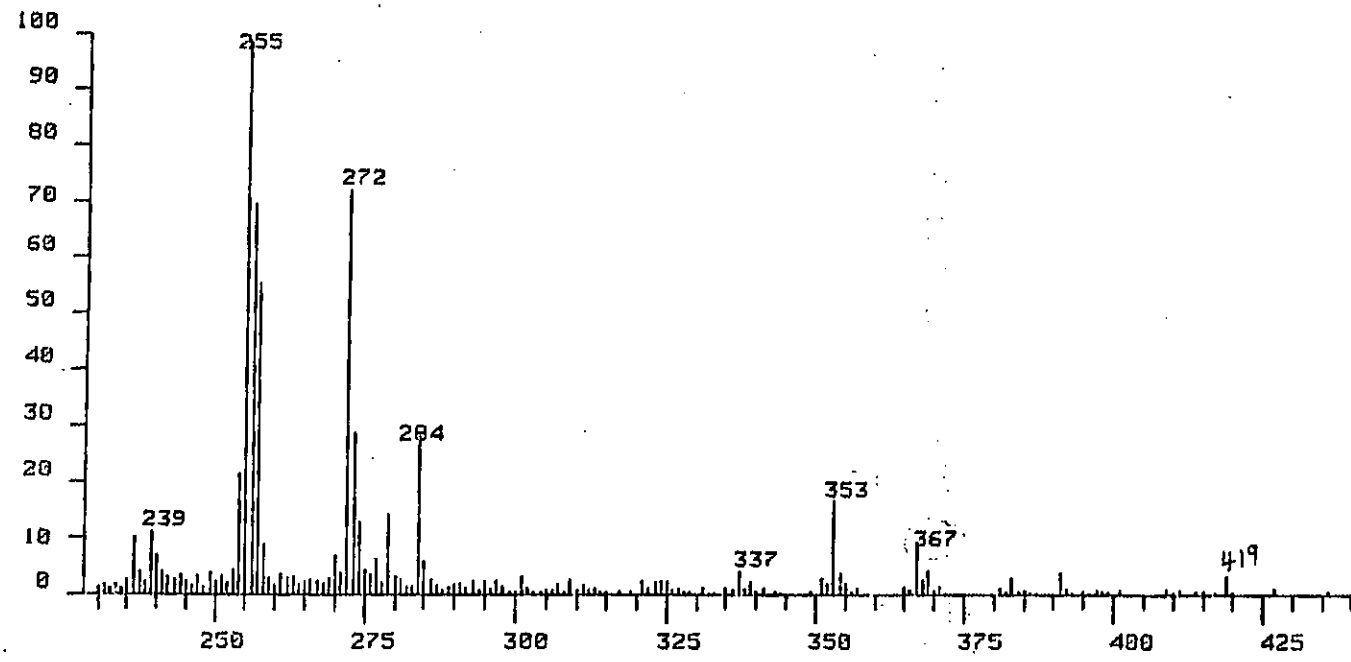
PEAK NO.	MEASURED MASS	% INT NREF	
215	112	5.1	
216	111	4.9	
217	110	8.5	
219	109	11.5	
220	108	13.6	
221	107	10.4	
222	106	8.4	
223	105	8.1	
224	104	8.6	
230	99	5.6	
231	98	4.7	
232	97	14.9	
234	96	7.0	
236	95	27.0	*
237	94	16.1	*
238	93	16.9	
239	92	10.7	
240	91	11.7	
244	87	3.7	
246	85	8.9	
247	84	7.8	
249	83	11.7	
251	82	8.1	
252	81	12.3	
253	80	7.7	
254	79	13.8	
255	78	44.3	
256	77	100.0	
257	76	21.4	
258	75	16.2	
259	74	13.3	
260	73	6.0	
261	72	4.2	
262	71	19.2	
264	70	62.1	
265	69	45.2	
266	68	58.1	
267	67	19.9	
268	66	11.8	
269	65	20.7	
270	64	20.7	
271	63	10.2	
274	60	7.5	
275	59	8.3	
276	58	5.8	
277	57	30.7	
278	56	14.3	
279	55	34.7	
280	54	10.8	
281	53	14.8	
282	52	25.1	
283	51	73.3	
284	50	52.3	
285	49	8.8	
286	48	11.0	

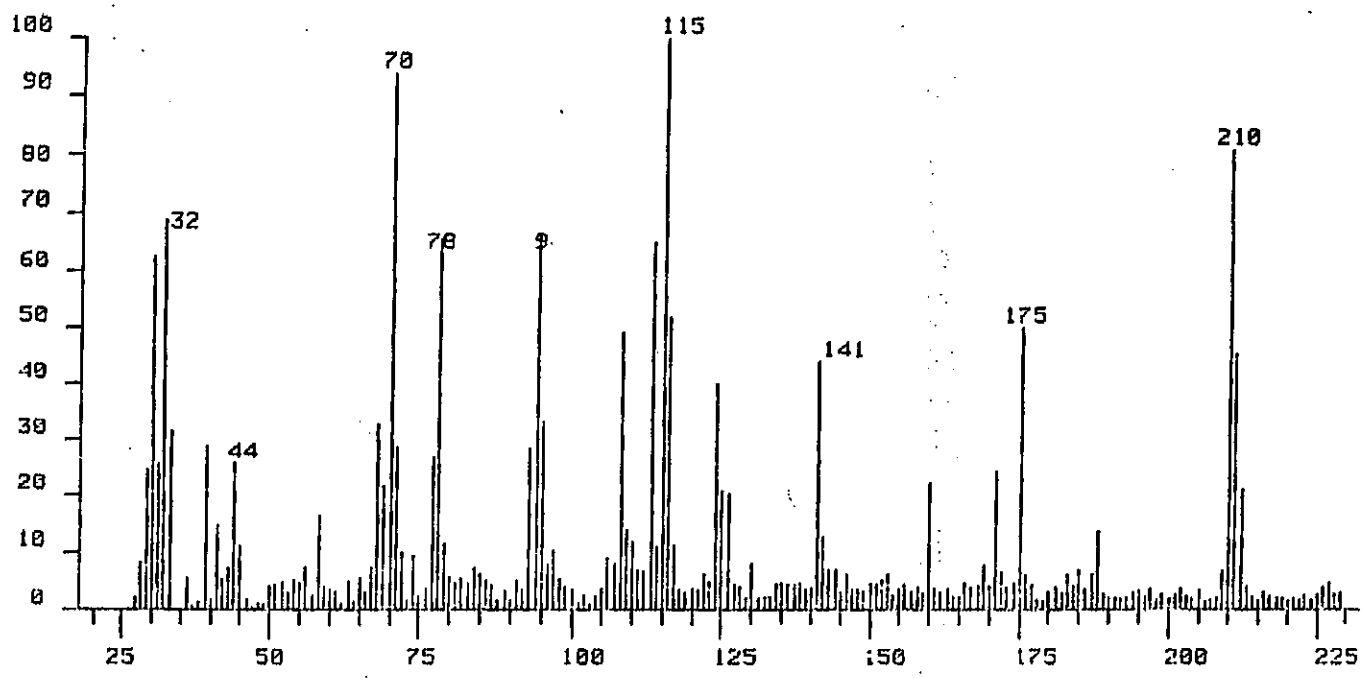
PAGE 3

PEAK NO	MEASURED MASS	% INT NREF	
288	46	5.8	
290	45	39.7	
291	44	88.5	
292	43	52.9	*
293	43	34.2	*
297	40	47.8	
299	41	79.4	
300	40	78.5	
301	39	57.0	
302	38	15.5	
304	37	5.2	
305	36	14.9	
307	34	5.4	
311	31	53.1	
312	30	18.7	
313	29	71.5	
316	27	48.3	

SSH41

17LR11.32 [TIC=24282112, 100%=8437761 +VE CI, REAGENT:AMMONIA





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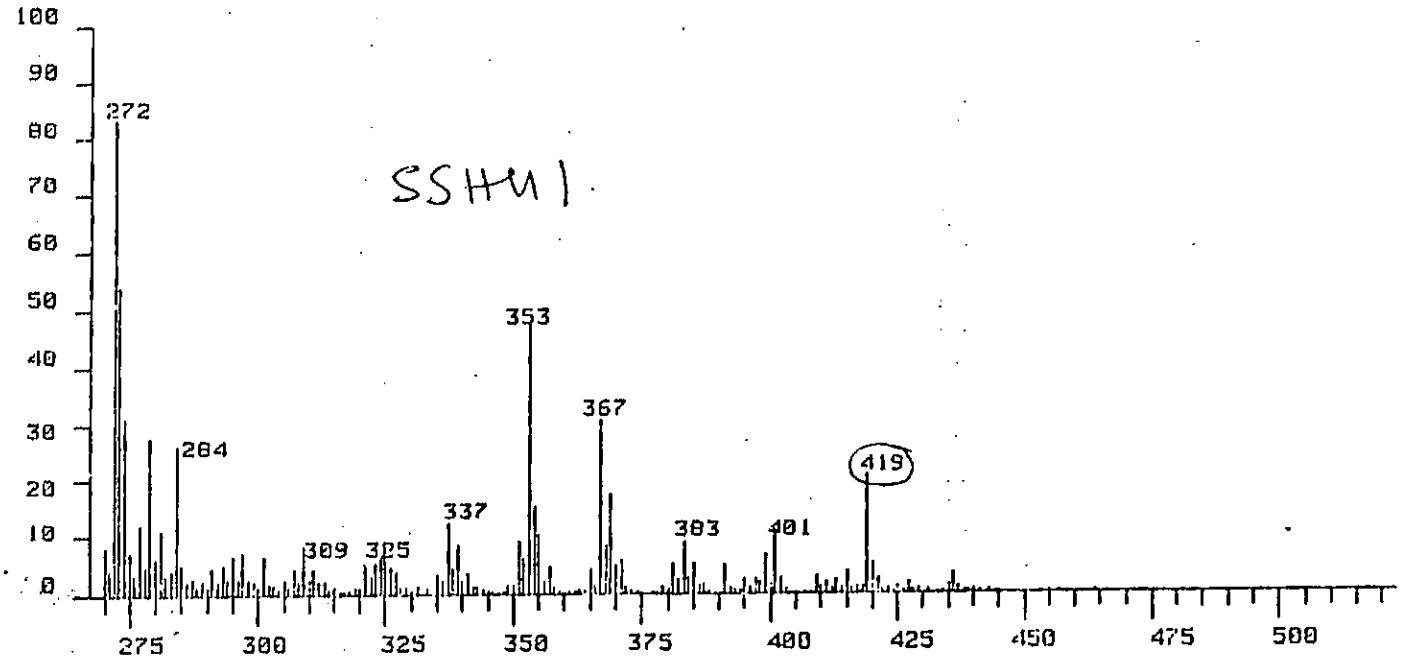

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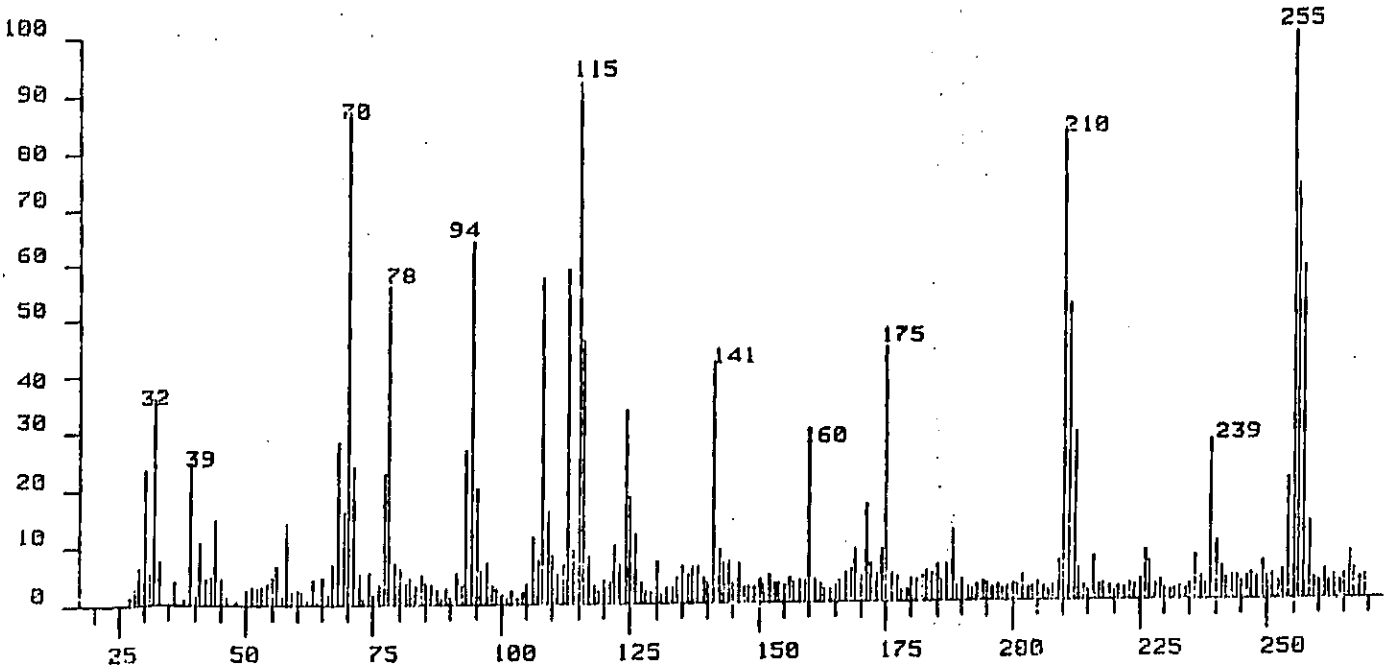


SSHM1 C1

SSHM1C1

PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF	
2	427	1 1	
5	419	3 2	
20	397	1 1	
21	391	3 8	
26	383	2 9	
28	381	1 2	
30	371	1 4	
32	369	4 1	
33	368	2 7	
34	367	9. 1	
36	365	1. 3	
39	355	2 0	
40	354	3 7	
41	353	16 8	
47	337	2. 1	
43	351	3. 0	
48	341	1. 2	
50	339	2 2	
51	338	1. 2	
52	337	4. 0	
57	331	1. 1	
60	327	1. 3	
62	325	2. 5	
63	324	2 2	
64	323	2. 3	
65	322	1. 1	
66	321	2. 3	
93	301	3 3	
99	285	5. 8	
100	284	28 3	
104	280	3. 2	
105	279	14. 4	
107	277	6. 2	
108	276	3. 4	
109	275	4. 2	
110	274	13. 0	
111	273	29. 0	*
112	272	72 4	*
113	271	3. 8	*
114	270	6. 9	*
123	261	3 4	
126	258	3 8	*
127	257	55 3	*
128	256	62. 8	*
129	255	98 7	*
130	254	21 5	*
131	253	4. 4	*
133	251	3. 2	
135	249	3. 7	
138	247	3 2	
142	244	3 5	
144	242	3 2	*
145	241	4 0	*
146	240	7. 0	*
147	239	11. 3	*

PAGE 2

PEAK NO.	MEASURED MASS	% INT. NREF
149	237	4 0
150	236	9. 9
159	227	4 9
160	226	3 9
173	213	4 5
174	212	20. 9
175	211	45 6
176	210	81 2
177	209	6. 8
181	205	3. 6
184	202	3. 7
189	197	3 6
191	195	3 4
192	194	3. 2
198	188	13 5
199	187	6 2
200	186	3 7
201	185	6 9
202	184	4 3
203	183	6 0
204	182	3 2
205	181	4 0
209	177	4 5
210	176	6 1
211	175	50. 0
213	174	4. 6
214	173	4 0
216	172	6. 4
218	171	24. 0
219	170	4 5
220	169	7. 7
221	168	4 3
222	167	3 9
223	166	4 6
226	163	3 6
227	162	3 2
228	161	3 7
229	160	21. 9
231	158	4 0
232	157	3 2
233	156	4 2
234	155	3 8
236	153	6 2
237	152	5 3
238	151	4 6
239	150	4 7
240	149	3 2
241	148	3 8
242	147	3 4
243	146	6 3
245	144	7 7
246	143	7 3
247	142	12 6
248	141	44 1
249	140	3 9

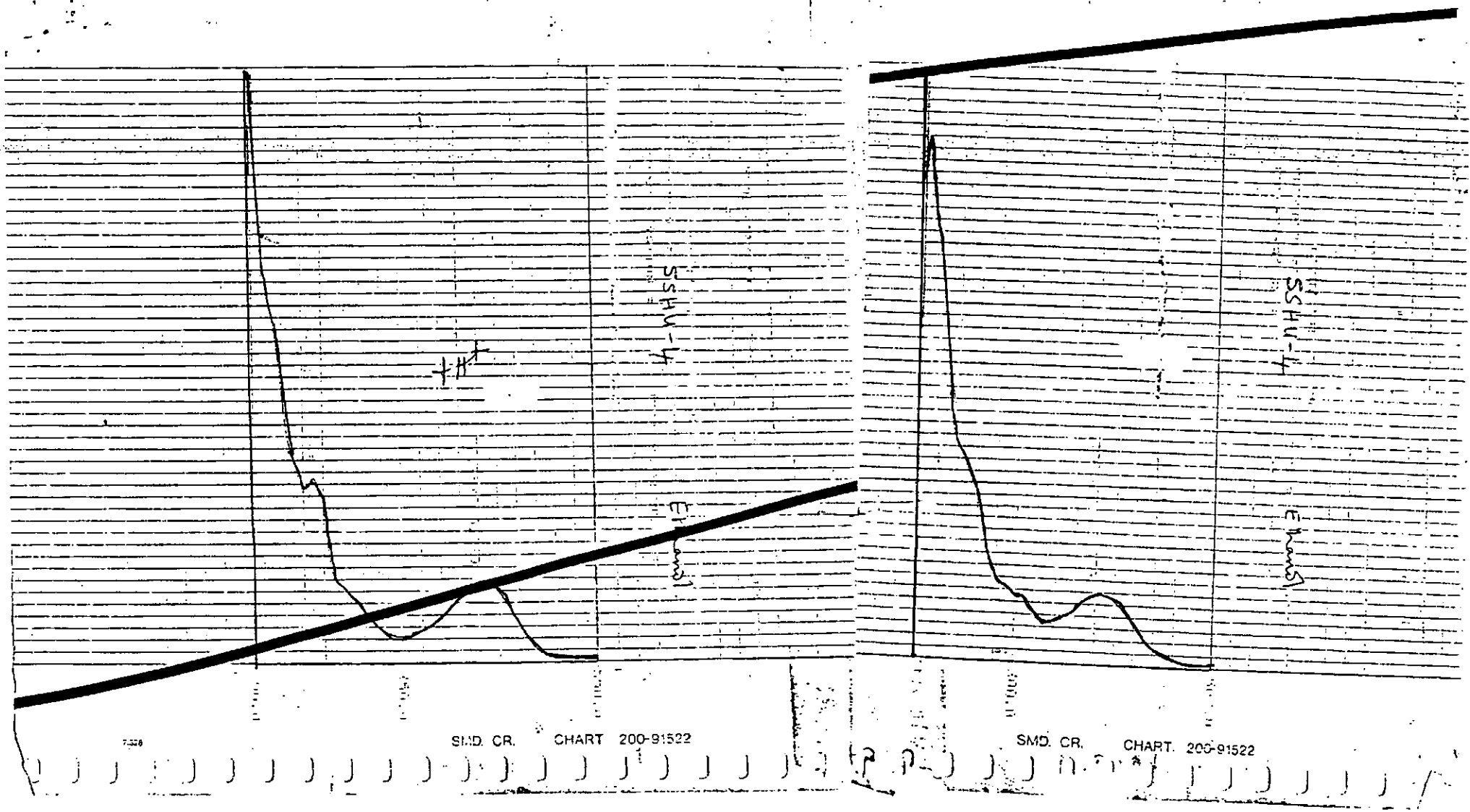
PAGE 3

PEAK NO.	MEASURED MASS	% INT. NREF
250	139	3 9
251	138	4 7
252	137	4. 7
253	136	4. 6
254	135	4. 7
255	134	4 7
259	130	8 0
261	128	3. 9
262	127	4 6
263	126	20. 1
264	125	20. 6
265	124	40. 1
266	123	5. 0
267	122	6 5
268	121	3. 4
269	120	3. 7
270	119	3 3
271	118	3. 4
272	117	11. 3
273	116	51. 9
275	115	100. 0
277	114	10. 8
278	113	65. 3
279	112	6 8
280	111	7. 0
281	110	11. 8
282	109	13. 8
283	108	49. 1
284	107	8 0
285	106	9 0
286	105	3 8
291	100	3. 5
292	99	4. 1
293	98	5 4
294	97	10. 4
295	96	7 9
296	95	33 3
297	94	66 3
298	93	28 4
299	92	3 4
300	91	5 1
302	89	3 4
304	87	4 3
305	86	5 3
306	85	6 4
307	84	7. 5
308	83	4. 5
309	82	5. 4
310	81	4 9
311	80	5 9
312	79	11 4
313	78	65. 7
314	77	27. 0
315	76	3. 9
317	74	9. 3

SSHMICI.

PAGE 4

PEAK NO.	MEASURED MASS	% INT. NREF
319	72	10 0
320	71	28 6
321	70	93. 7
322	69	21. 8
323	68	32. 7
324	67	7. 5
325	66	3. 2
326	65	5 8
328	63	5. 1
331	60	3. 9
332	59	3. 9
333	58	16. 5
335	56	7. 5
336	55	4 8
337	54	5. 3
338	53	3. 2
339	52	4. 8
340	51	4. 2
341	50	4. 4
346	45	11 2
347	44	25. 9
348	43	7. 6
349	42	5 4
350	41	14. 7
352	39	28. 9
355	36	5. 8
357	33	31. 5
358	32	68. 8
359	31	25. 7
360	30	62. 7
361	29	24. 6
362	28	8. 2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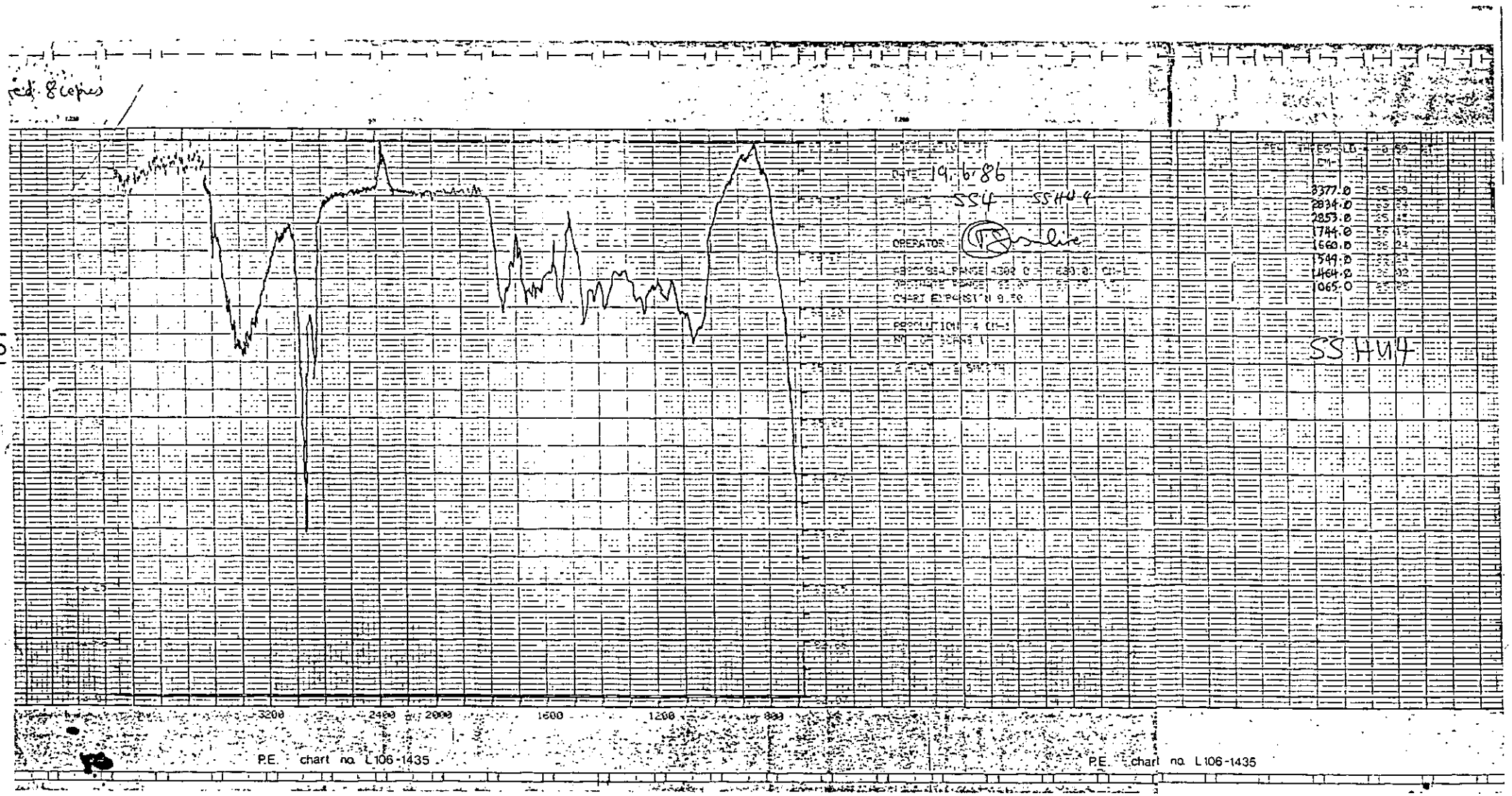
E1 and 2
SMD. CR. CHART 200-91522
SSHU-H
E1 and 2
SMD. CR. CHART. 200-91522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SSH
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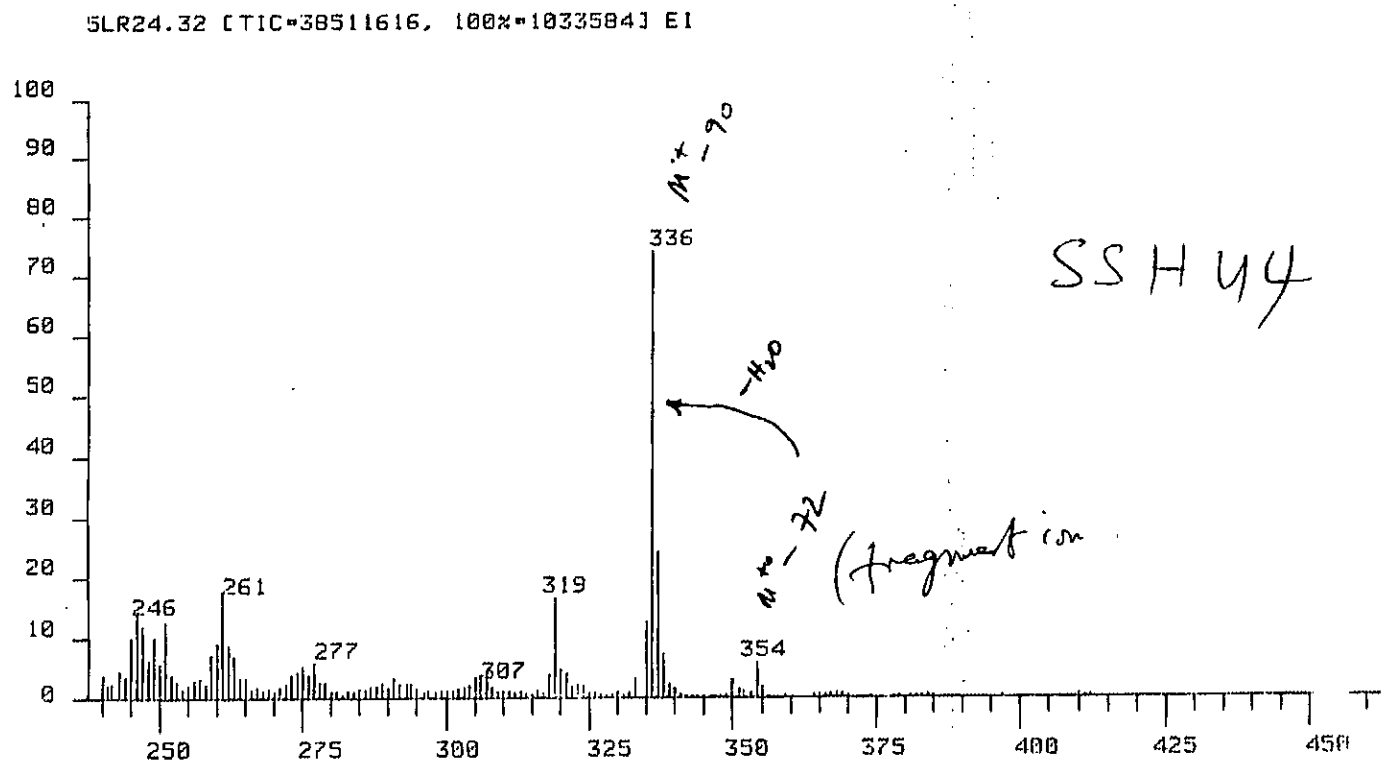
P.E. chart no. L106-1435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100
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10
0
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M+-90
-H2O
M+-72
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246
277
307
354
250
275
300
325
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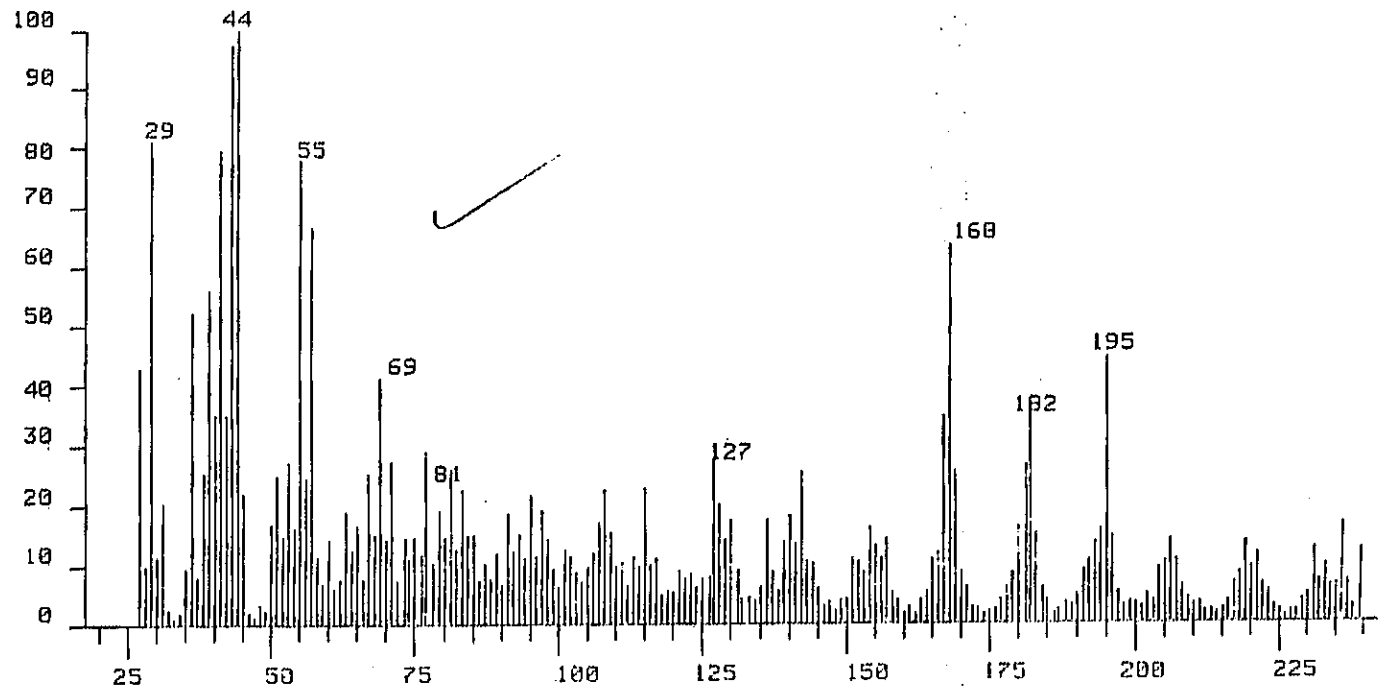
400
425
450

SSH44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150
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200
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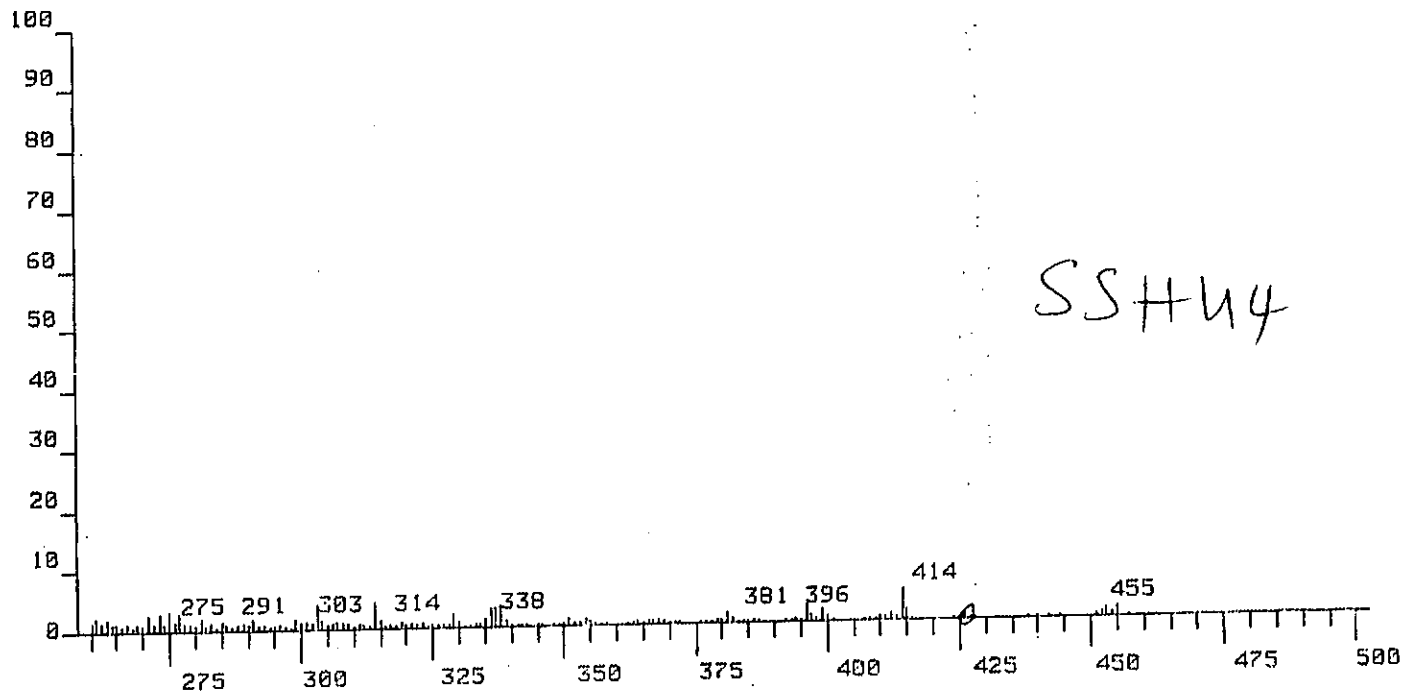

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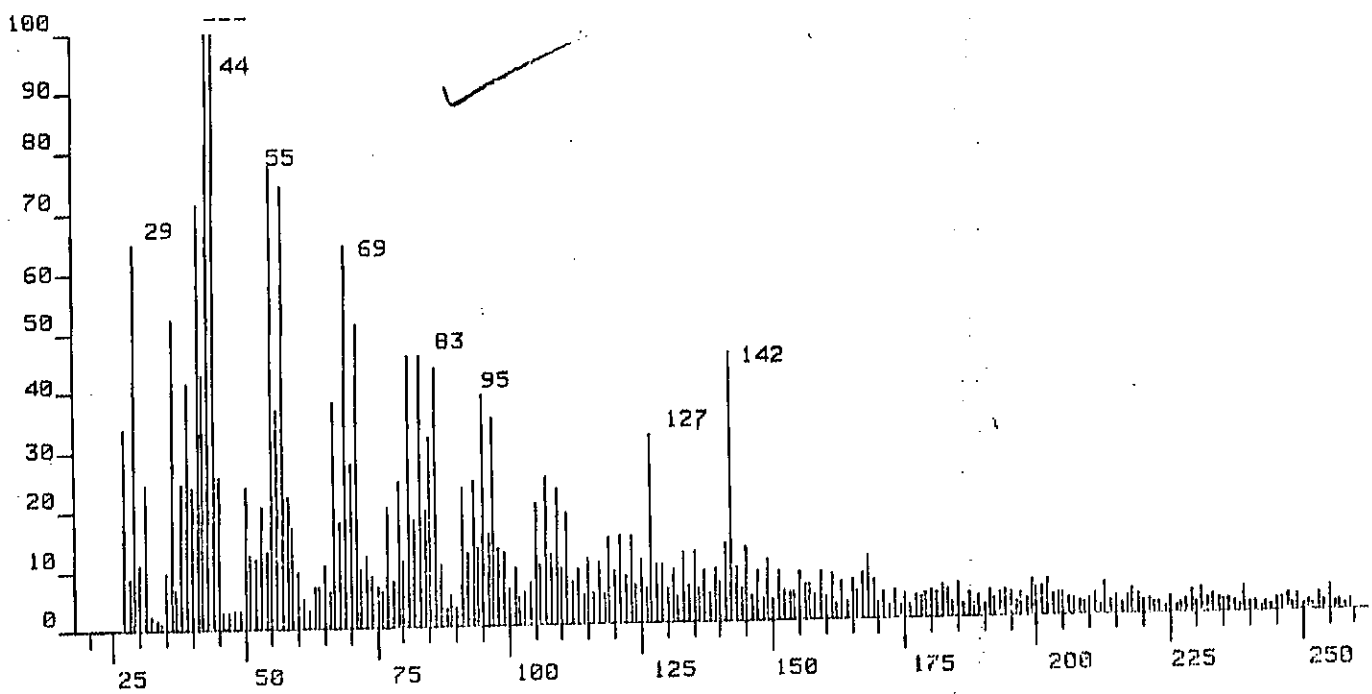

SSH44

H	N	O	DEV	MEAS MASS	EPTS	%INT
30		5	10			
23	18	3	0	-3.1	336.1470	0	0.00
25	20	0	1	-4.4		
18	18	5	2	1.0		
20	20	2	3	-0.4		
15	20	4	5	3.6		
17	22	1	6	2.3		
11	22	5	7	-4.9		
13	24	0	8	5.0		

C	H	N	O	DEV	MEAS MASS	EPTS	%INT
30		5	10				
23	20	3	1	-1.7	354.1589	0	0.00
25	22	0	2	-3.1			
18	20	5	3	2.3			
20	22	2	4	1.0			
15	22	4	6	5.0			
17	24	1	7	3.6			
11	24	5	8	-3.6			
13	26	2	9	-4.9			

PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF	
31	354	5.6	
47	338	7.2	
48	337	24.4	*
49	336	74.1	*
50	335	12.6	*£
64	320	4.7	
65	319	16.7	
107	277	5.6	
109	275	5.2	
121	263	6.8	
122	262	8.7	
123	261	17.8	
151	236	16.7	
196	195	44.7	
199	194	15.7	
215	182	35.4	
216	181	26.4	
218	180	16.2	
230	169	25.5	
231	168	63.5	
232	167	34.3	
251	154	16.5	
269	142	25.4	
272	140	18.3	
277	136	17.4	
287	130	17.5	
292	128	19.8	
294	127	16.1	*
317	115	22.9	
329	108	22.6	
331	107	17.1	
347	97	19.2	
352	95	21.8	
361	91	18.4	
377	83	22.5	
381	81	25.6	
386	79	18.9	
391	77	28.9	
401	71	27.2	
406	69	41.3	
409	67	25.2	
412	65	16.6	
418	63	19.0	
427	57	66.8	
429	56	24.6	
431	55	77.6	
433	54	16.4	
434	53	27.2	
437	51	24.9	
439	50	16.8	
444	45	22.0	
445	44	100.0	
447	43	55.2	*
448	43	41.1	*
451	42	35.0	

SSHH4E1.

PAGE 2

PEAK NO.	MEASURED MASS	% INT. NREF	
453	41	78.7	
456	40	22.1	*
457	39	55.4	
459	38	25.6	
461	36	51.8	
469	31	20.1	
472	29	47.6	*
473	29	32.5	*
478	27	43.0	

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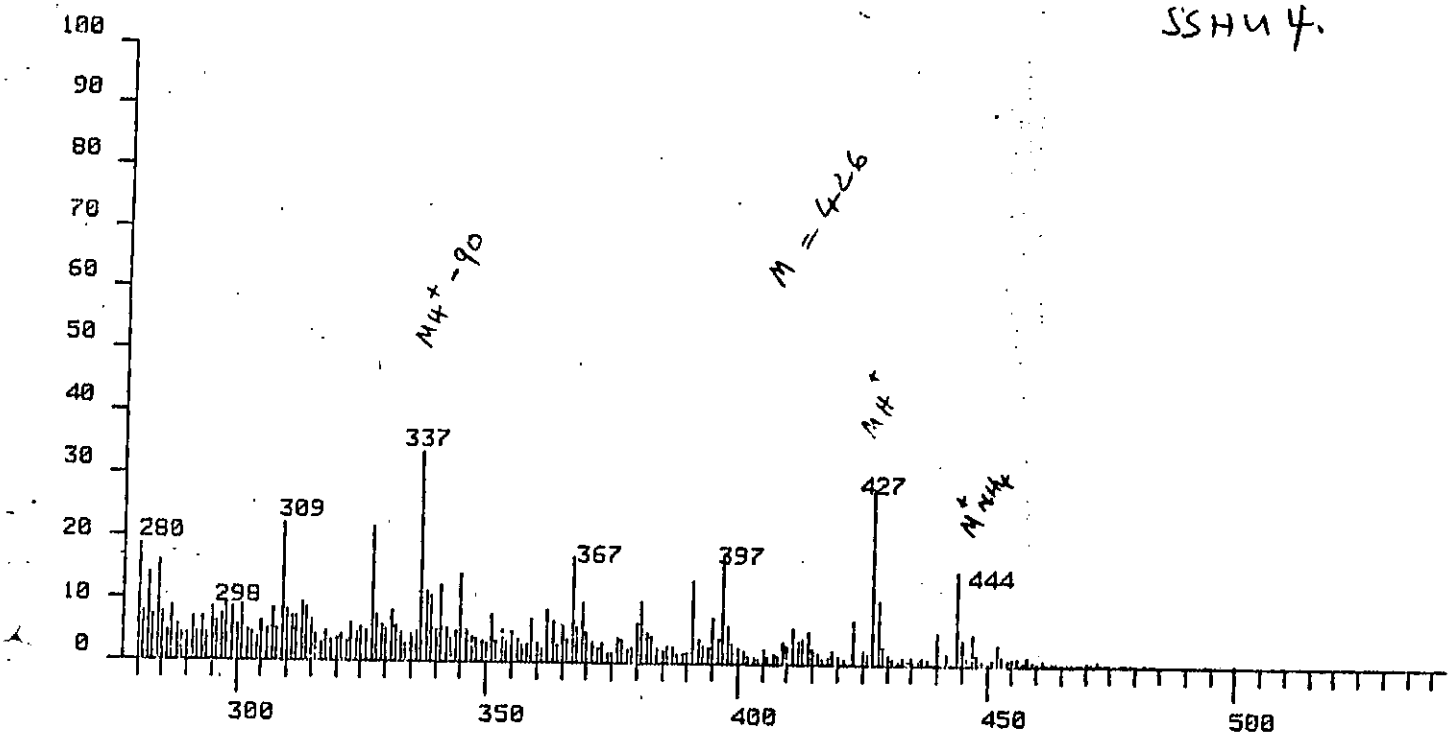

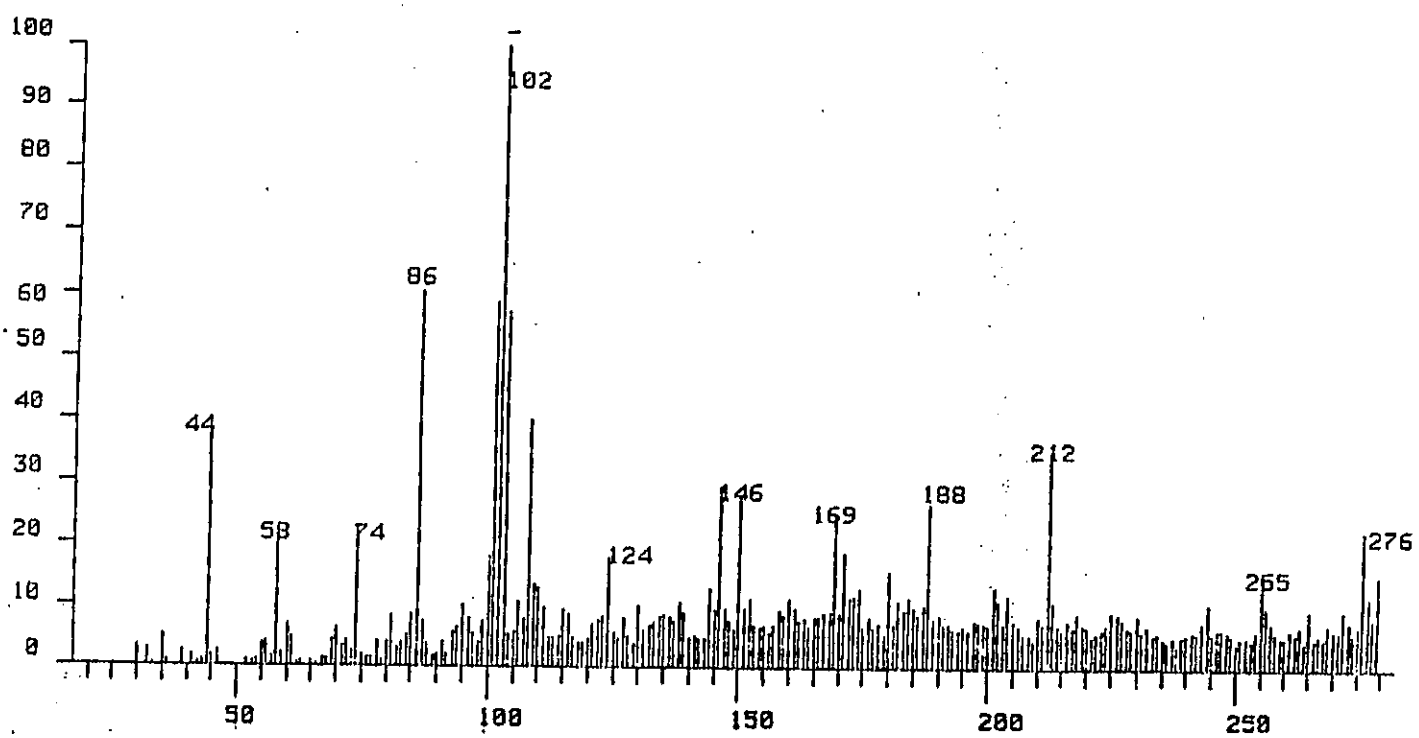

PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF	
41	452	3.1	
46	447	4.8	*
48	445	4.0	
49	444	14.8	
51	440	5.3	
61	429	2.8	
62	428	10.2	*
63	427	28.1	*
65	425	2.3	*
66	423	6.9	*
69	419	2.4	
73	415	2.5	*
74	414	5.3	*
75	413	4.1	
76	412	3.7	*
77	411	5.8	*£
78	410	2.8	*
79	409	3.5	*
83	405	2.2	
87	401	2.2	
91	397	17.2	*
97	391	13.2	
121	367	16.9	
143	345	14.1	*
147	341	12.2	*
149	339	10.7	*
150	338	11.5	*
151	337	33.4	*
161	327	21.5	*
180	309	21.9	*
205	284	16.0	
207	282	14.1	
209	280	18.6	
210	279	15.1	
212	277	11.8	
213	276	22.3	
234	255	15.3	
248	244	10.6	
288	213	10.8	
289	212	35.2	
297	204	11.8	
299	202	10.9	
300	201	13.2	
313	188	26.5	
314	187	10.4	
317	184	11.4	
319	182	11.0	
321	180	15.6	
327	174	12.9	
328	173	11.8	
329	172	11.4	
330	171	18.5	
332	169	25.4	
346	160	10.8	
362	152	11.1	

PAGE 2

PEAK NO.	MEASURED MASS	% INT. NREF
365	150	28.1
372	146	29.2
376	144	12.9
385	138	10.1
413	124	17.7
434	110	12.8
435	109	13.5
436	108	40.1
438	106	10.5
441	103	57.4
443	102	100.0
445	101	58.9
446	100	18.1
451	95	10.2
460	86	60.8
475	74	22.7
495	58	20.9
507	44	38.1

SSH44 C1

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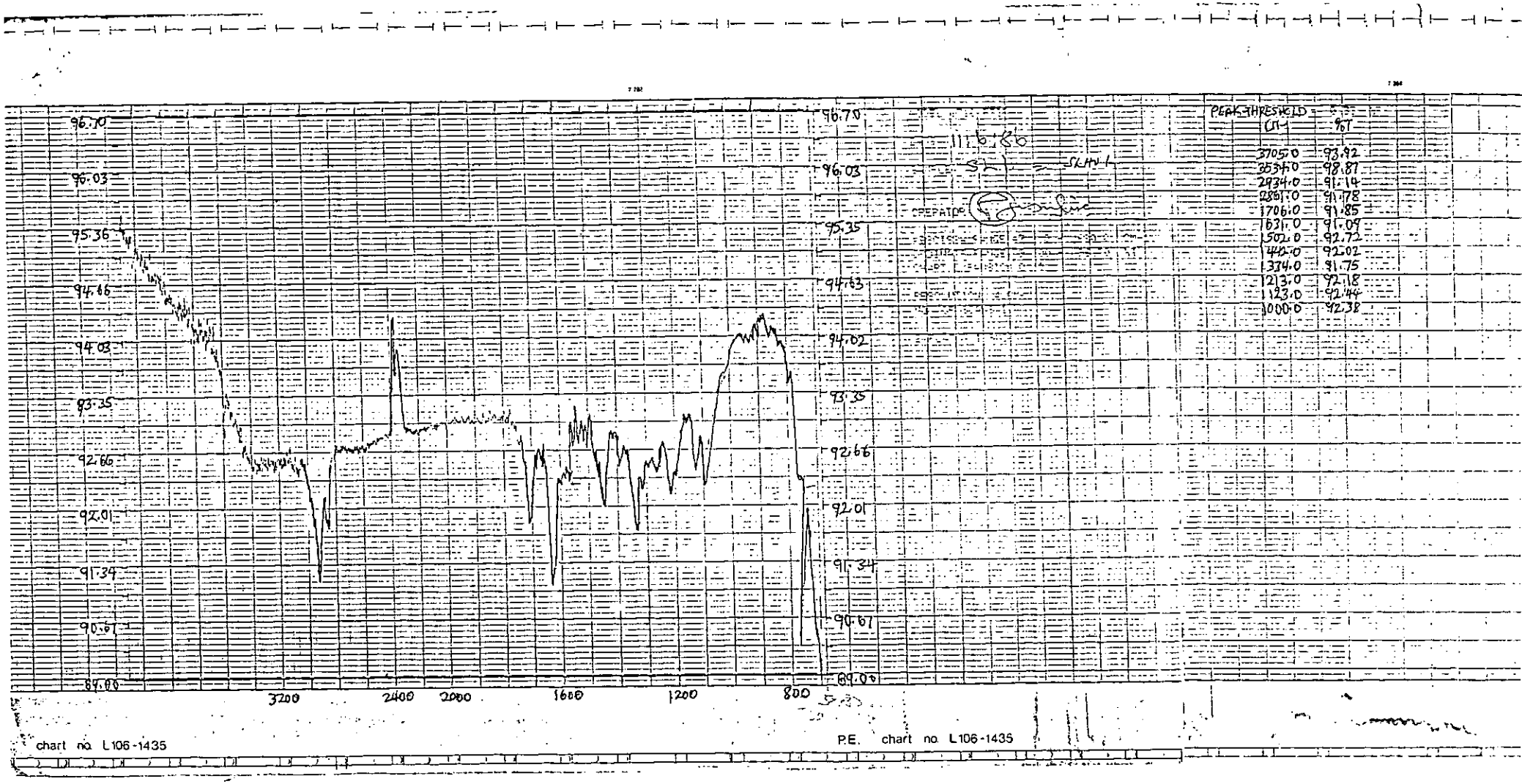
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94.63
94.02
93.35
92.66
92.01
91.34
90.67
89.00
3200
2400
2000
1600
1200
800
PEAK THRESHOLD
CH
%T
3705.0 93.92
3534.0 93.87
2934.0 91.14
2880.0 91.78
1706.0 91.85
1631.0 91.09
1502.0 92.72
1440.0 92.02
1334.0 91.75
1213.0 92.18
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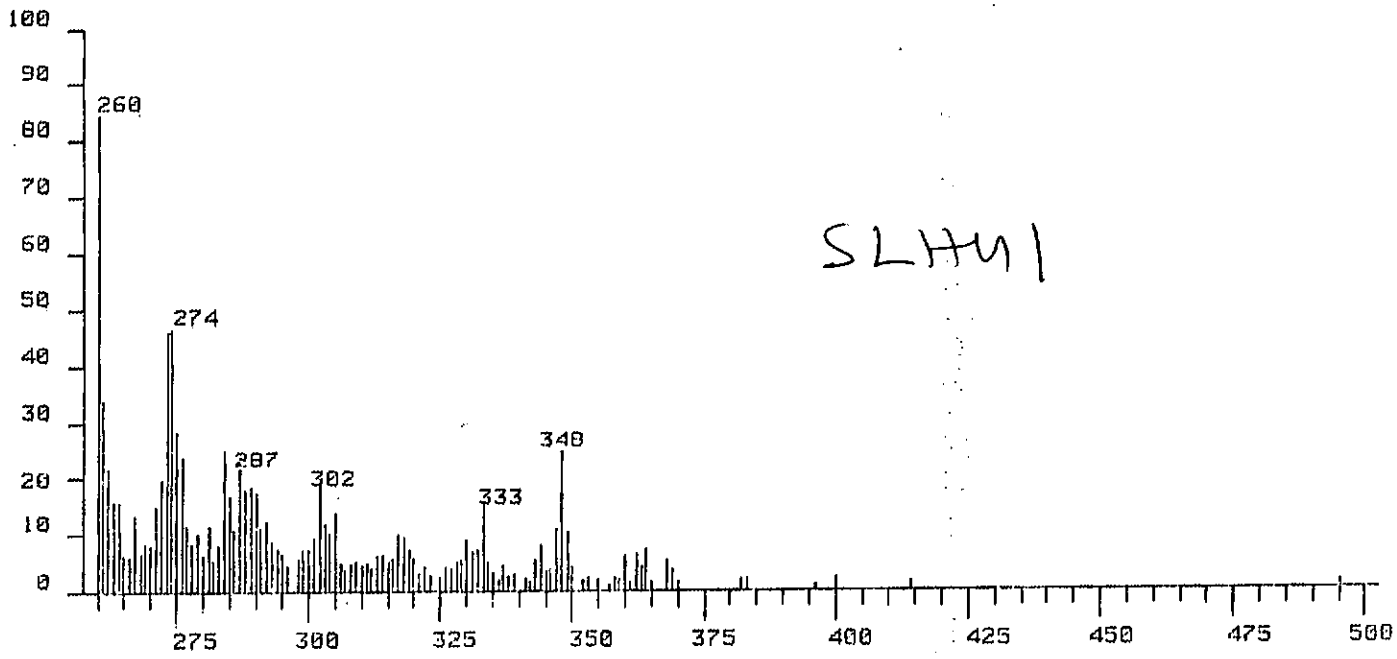
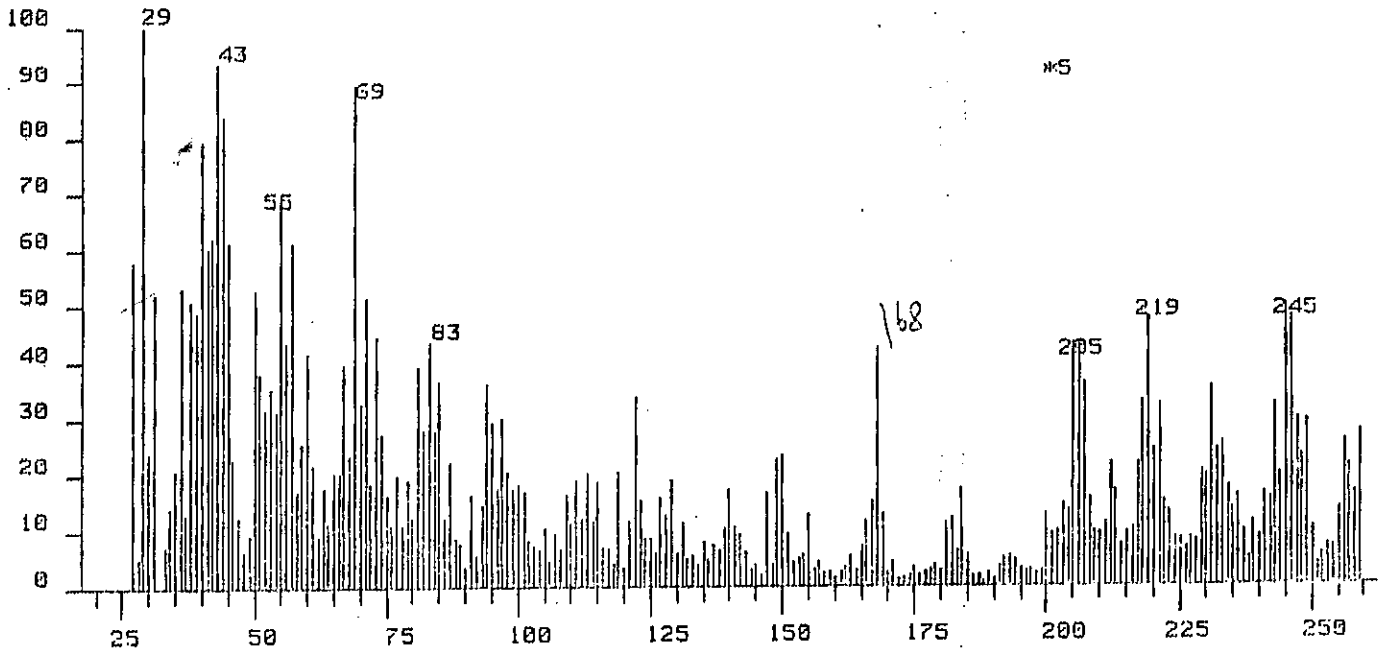
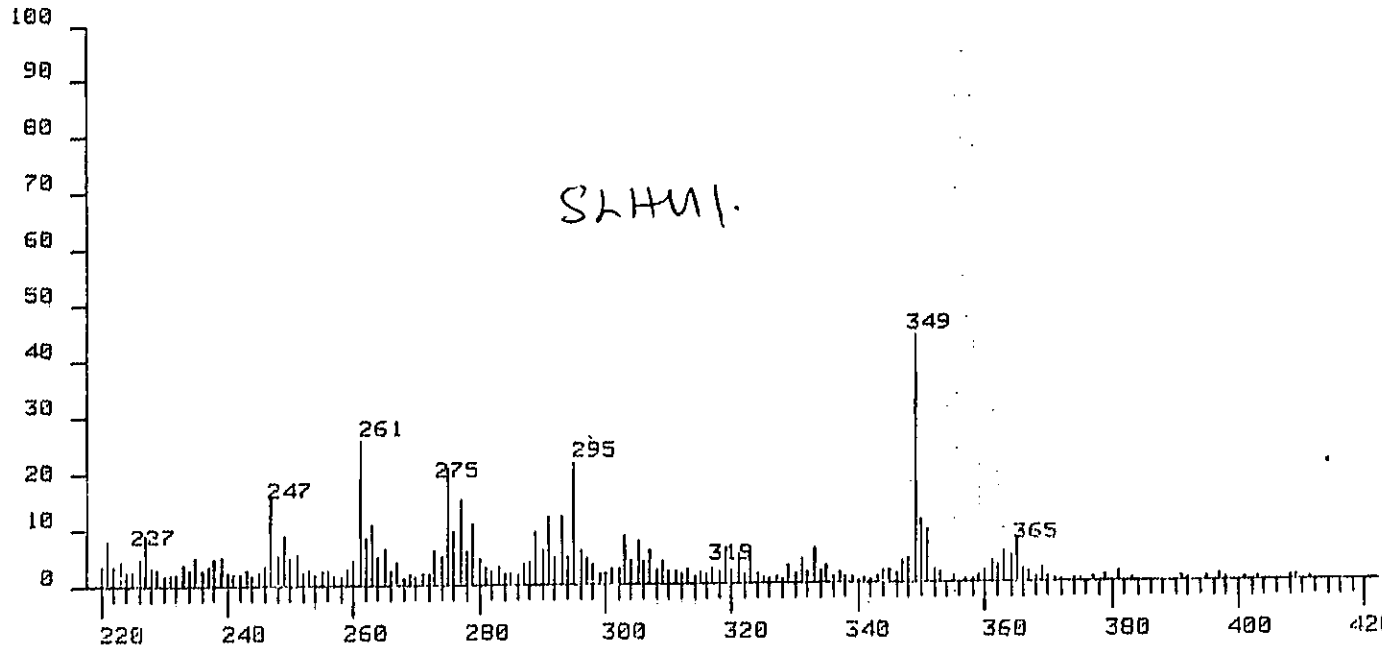
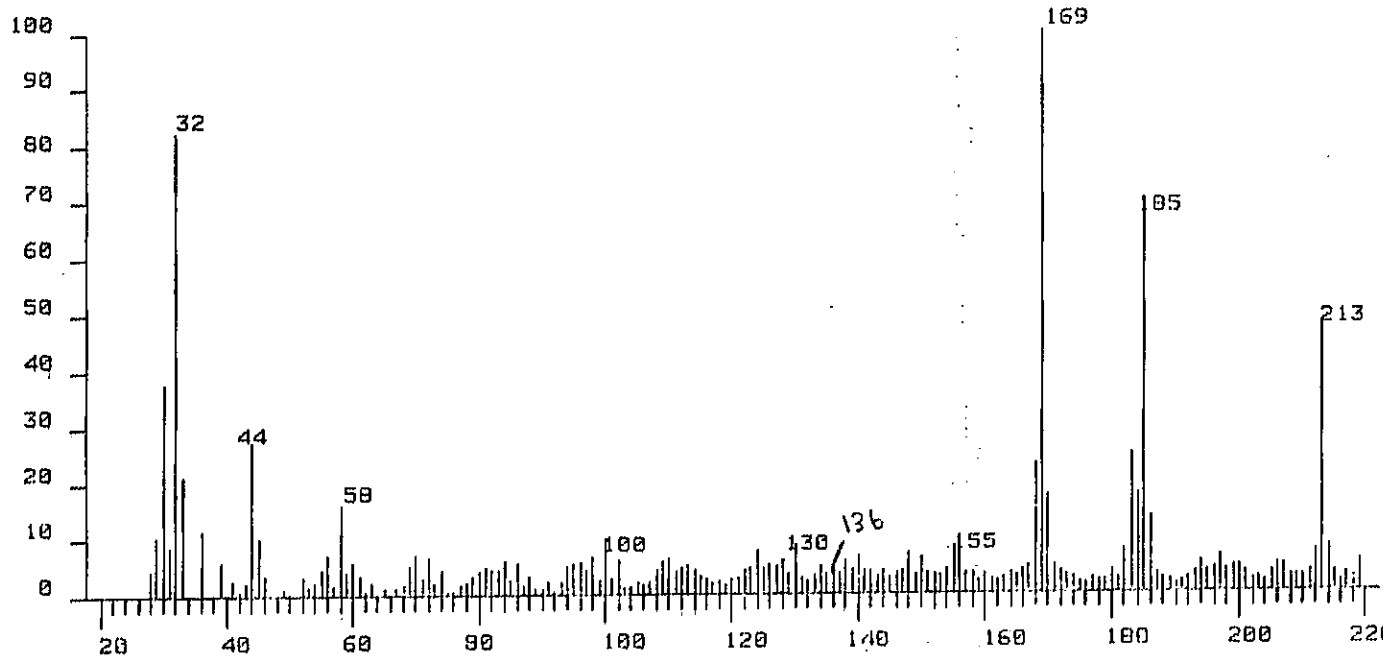


SLHM1



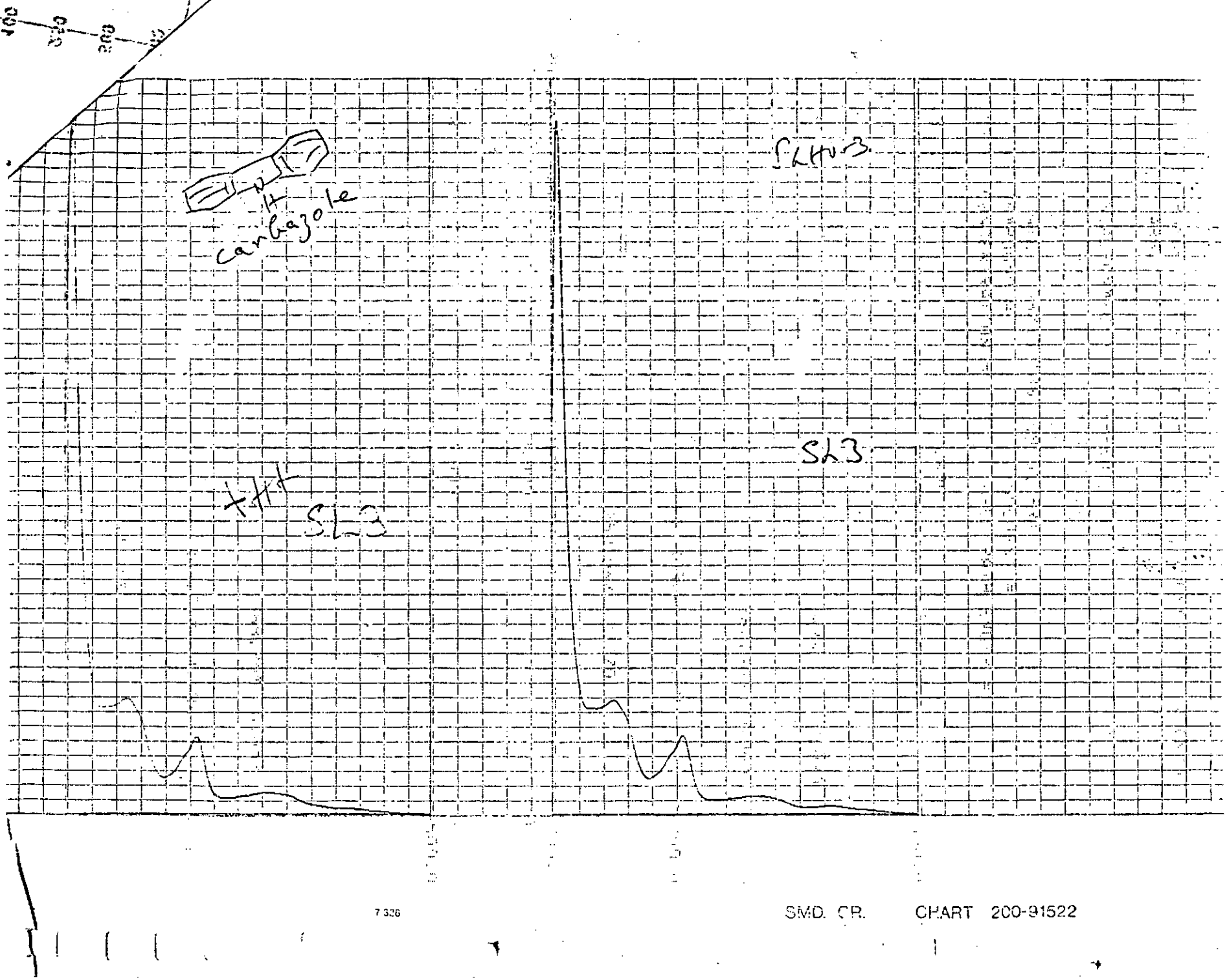
16LR16.22 [TIC=11034112, 100%=3972491] +VE CI, REAGENT:AMMONIA





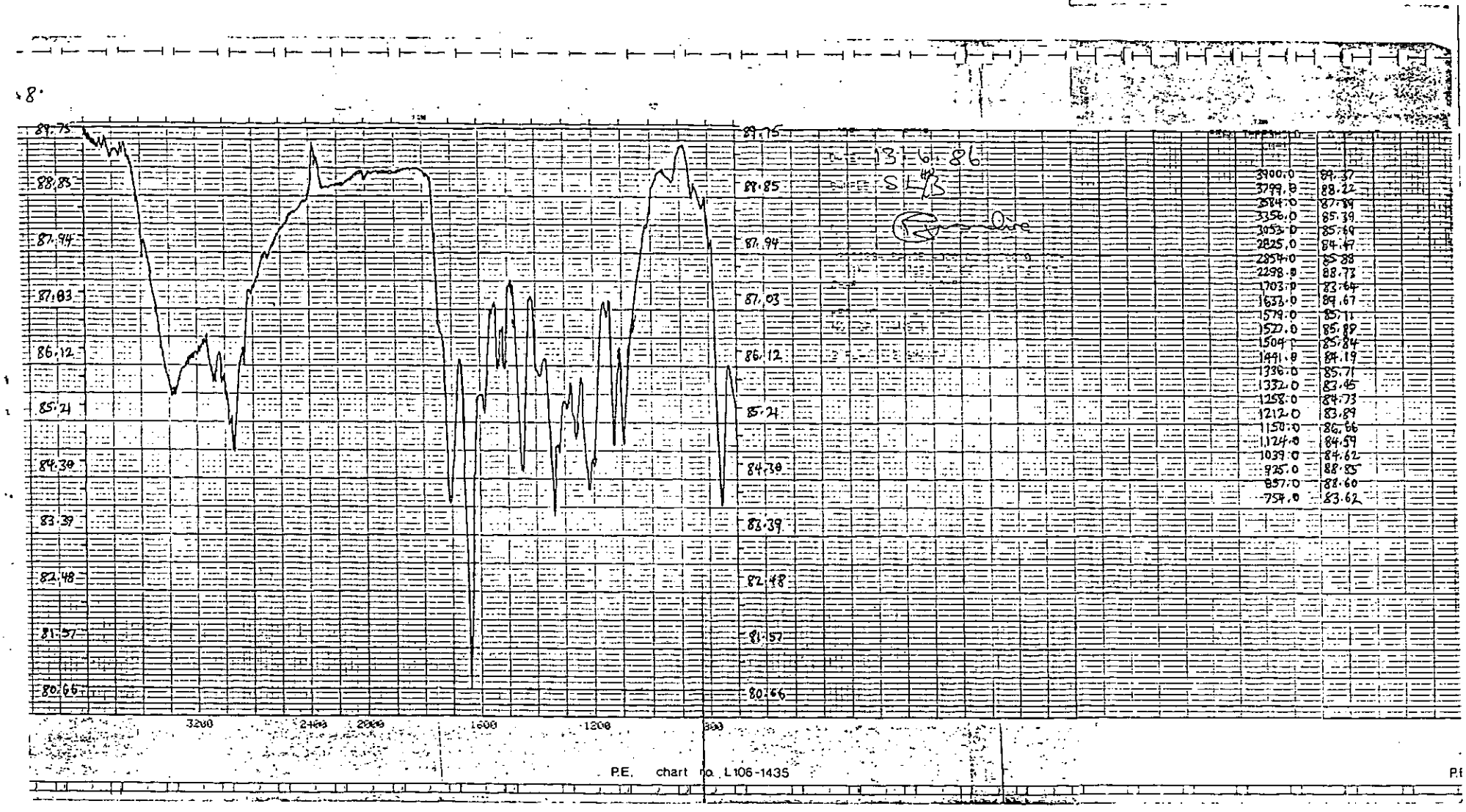
PAGE 1

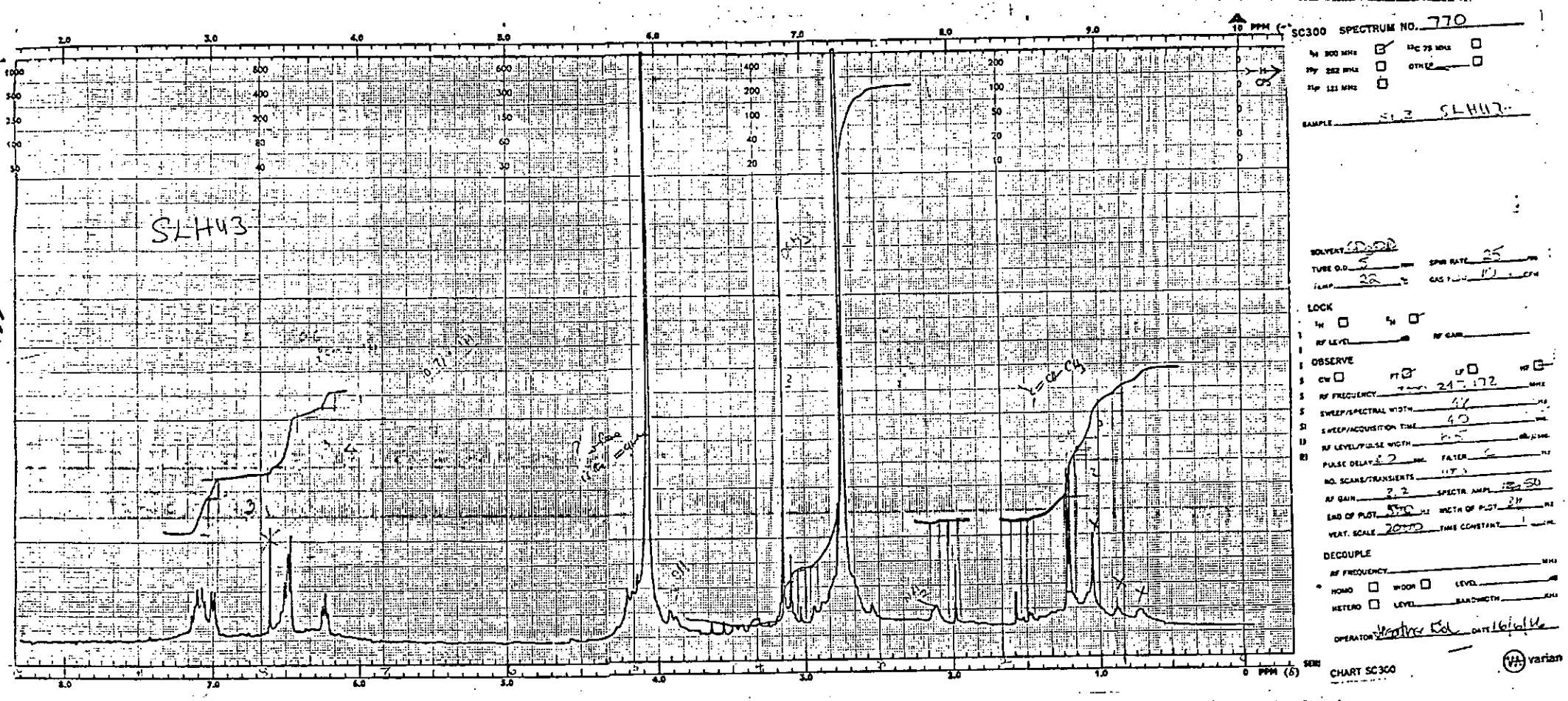
PEAK NO.	MEASURED MASS	% INT. NREF
38	349	44.1
126	241	25.9
174	213	47.4
202	185	67.4
204	183	75.0
218	169	100.0
338	44	27.4
346	32	82.5
348	30	37.8



7 326

SMD. CR. CHART 200-91522


89.75
88.85
87.94
87.03
86.12
85.21
84.30
83.39
82.48
81.57
80.66
3200
2400
2000
1600
1200
800
P.E. chart no. L106-1435
3900.0 89.37
3799.0 88.22
3584.0 87.89
3356.0 85.39
3053.0 85.69
2825.0 84.47
2854.0 85.88
2298.0 88.73
1703.0 83.64
1633.0 89.67
1579.0 85.11
1527.0 85.89
1504.0 85.84
1491.0 84.19
1336.0 85.71
1332.0 83.45
1258.0 84.73
1212.0 83.89
1150.0 86.66
1124.0 84.59
1039.0 84.62
935.0 88.85
857.0 88.60
754.0 83.62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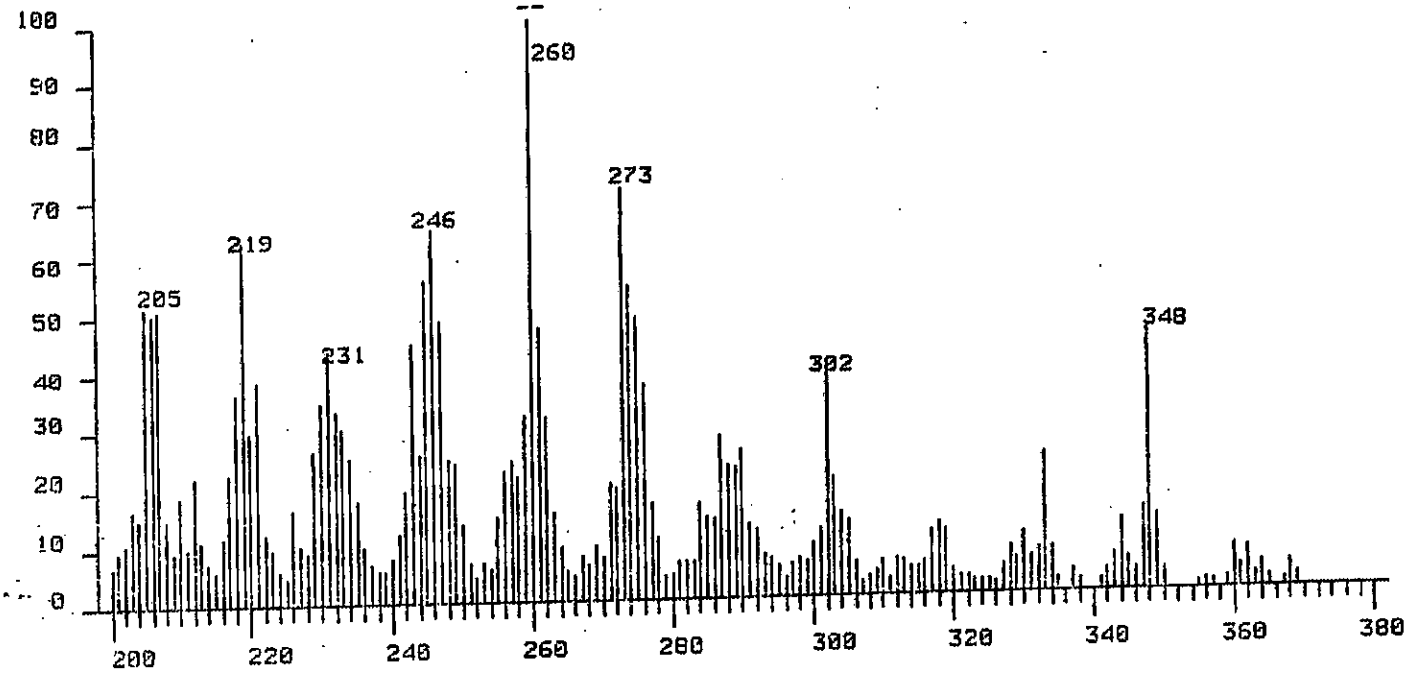
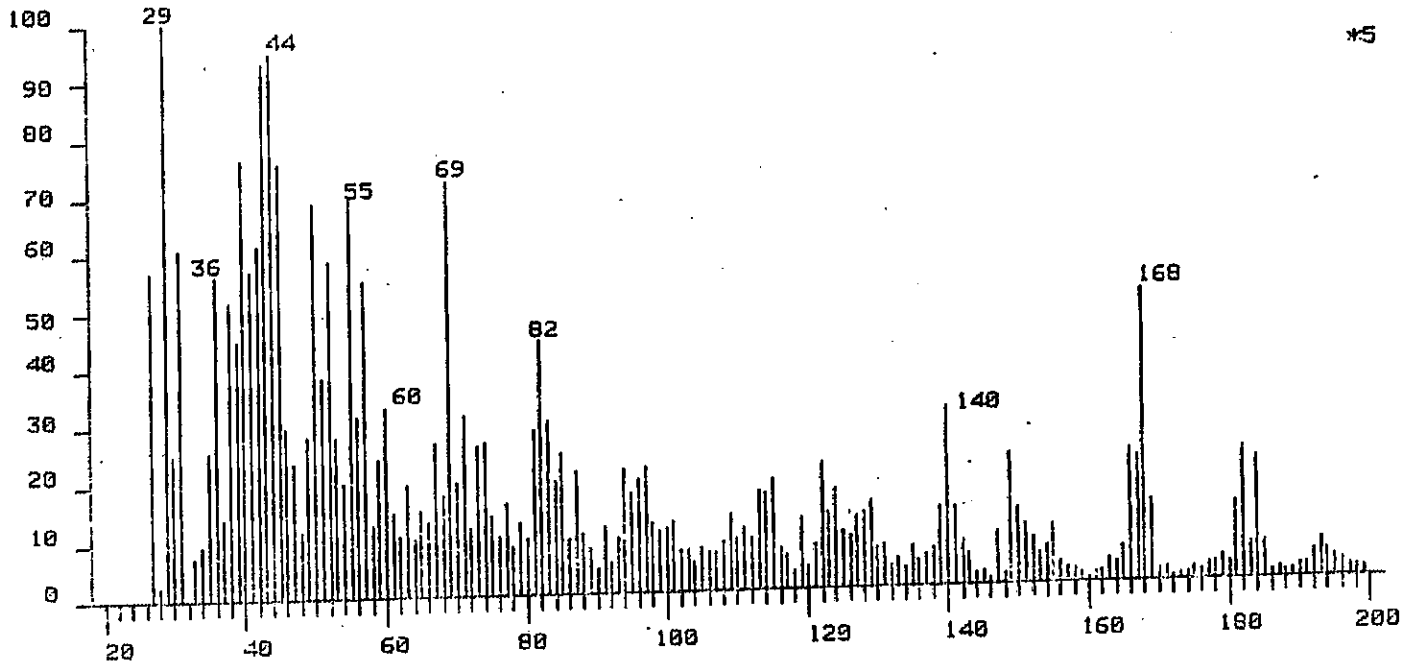
SAMPLE SLH43
SLH43
SOLVENT CDCl3
TUBE O.D. 5
SPIN RATE 25
LOCK
OBSERVE
RF FREQUENCY
SWEEP/SPECTRAL WIDTH 4K
SWEEP/ACQUISITION TIME 4.0
PULSE DELAY 2.0
NO. SCANS/TRANSIENTS 117
RF GAIN 7.2
END OF PLOT 5500
VERT. SCALE 2000
DECOUPLE
OPERATOR Heather Kiel
CHART SC300
varian
PPM (δ)

SLHH3.

16LR17.21 [TIC=30838272. 100%=9662401] EI





PEAK NO.	MEASURED MASS	% INT. NREF
7	362	1.5
9	360	1.6
15	349	2.7
16	348	9.1
17	347	2.9
20	344	2.5
27	334	1.6
28	333	4.8
29	332	1.5
31	330	2.1
33	328	1.7
42	319	2.3
43	318	2.5
44	317	2.2
56	305	2.7
57	304	2.9
58	303	4.1
59	302	8.1
60	301	2.4
71	290	5.1
72	289	4.5
73	288	4.6
74	287	5.6
85	276	7.5
86	275	9.8
87	274	10.9
88	273	14.2
99	262	6.3
100	261	9.5
101	260	20.8
103	259	6.4
104	258	4.3
105	257	4.9
106	256	4.5
114	249	4.8
115	248	4.9
116	247	9.7
117	246	12.9
118	245	11.1
119	244	5.1
120	243	9.0
130	234	5.0
131	233	6.0
132	232	6.6
133	231	8.8
134	230	6.9
135	229	5.3
143	221	7.7
144	220	5.9
145	219	12.5
146	218	7.3
147	217	4.5
152	212	4.4
157	207	10.2
158	206	10.1

SLHY3 E1

SLHM3E1.

PAGE 2

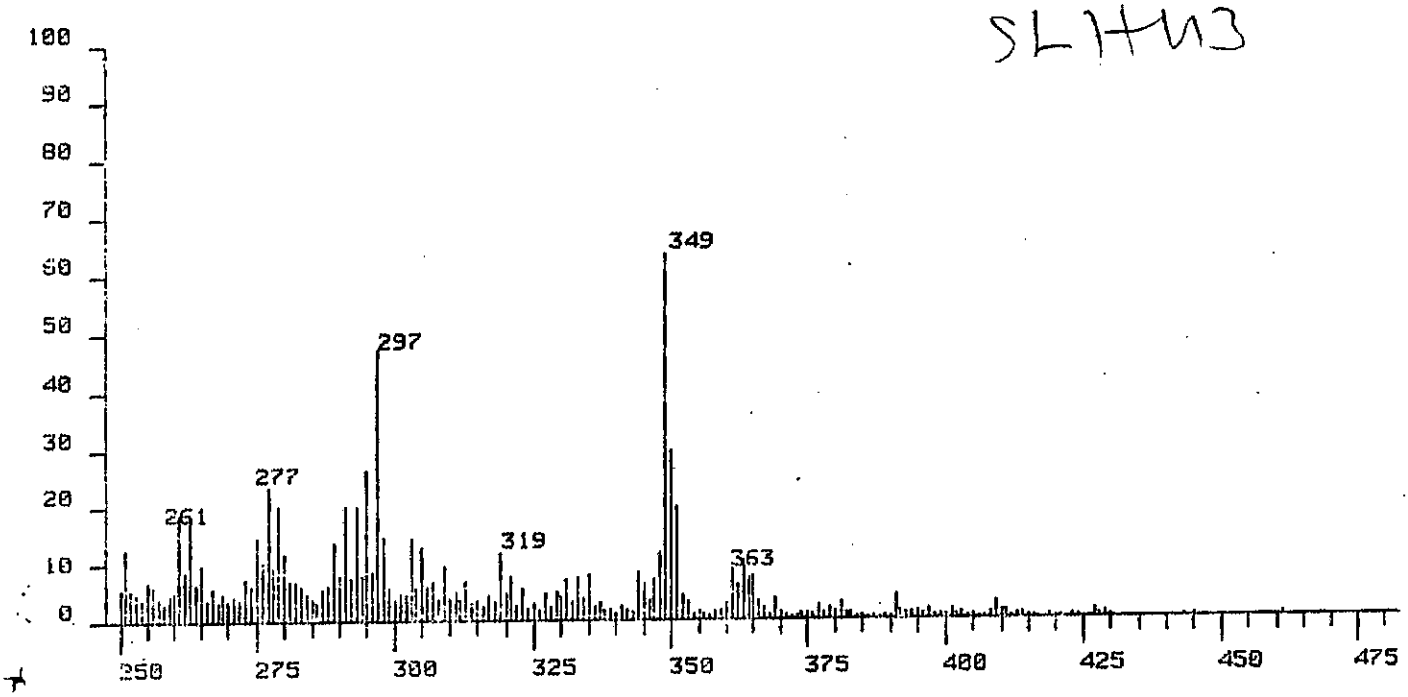
PEAK NO.	MEASURED MASS	% INT. NREF	
159	205	10.3	
170	194	5.1	
171	193	6.8	
172	192	5.0	
179	185	6.5	
180	184	21.4	
181	183	6.4	
182	182	23.1	
183	181	13.8	
186	179	4.4	
196	169	14.4	
198	168	50.5	
200	167	22.1	
202	166	23.1	
203	165	6.3	
221	150	13.5	
222	149	22.9	
233	141	14.1	
235	140	31.3	
236	139	14.1	
252	129	15.4	
254	128	12.8	
257	127	12.6	
261	124	17.5	
263	123	13.6	
265	122	21.9	
270	119	12.5	
278	115	19.4	
280	114	17.2	
282	113	17.5	
288	109	13.9	
301	101	12.6	
306	97	21.9	
312	94	21.6	
322	87	21.5	
324	85	24.5	
328	83	30.4	
330	82	44.5	
332	81	28.5	
343	74	24.7	
344	73	26.2	
346	71	31.4	
350	69	43.0	*
351	69	29.3	*
353	67	24.6	
361	60	33.0	
362	59	24.2	
365	57	55.3	
367	56	31.7	
368	55	69.6	
370	53	27.8	
371	52	58.6	
372	51	38.3	
373	50	68.9	
375	49	28.2	

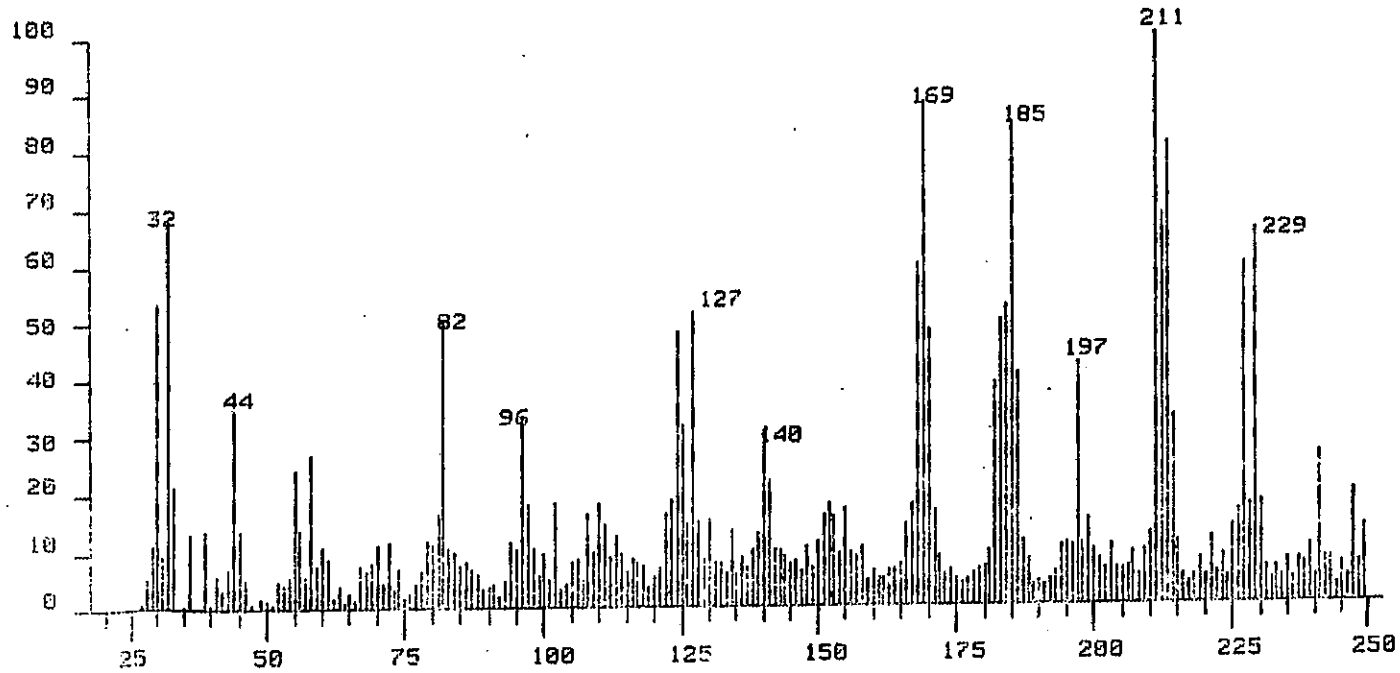
PAGE 3

PEAK NO.	MEASURED MASS	% INT NREF	
377	47	23.4	
378	46	29.5	
379	45	75.9	
381	44	94.5	
382	43	93.0	
383	42	61.9	
384	41	56.8	
386	40	61.0	*
387	39	44.9	
388	38	51.9	
390	36	56.1	
391	35	25.4	
395	31	61.0	
396	30	25.1	
397	29	100.0	
403	27	57.0	

16LR17.11 [TIC=39203840, 100%=860480] +VE CI, REAGENT:AMMONIA

SL1443





UNIVERSITY OF MANCHESTER
DEPARTMENT OF CHEMISTRY

DS-55 MASS SPECTROMETRY DATA SYSTEM
RELEASE 3.20

F 0 08UNSUL1KF 787

SL2>

DP0:[6]R17.MS
SCAN: 11, 6/16/86 14:41

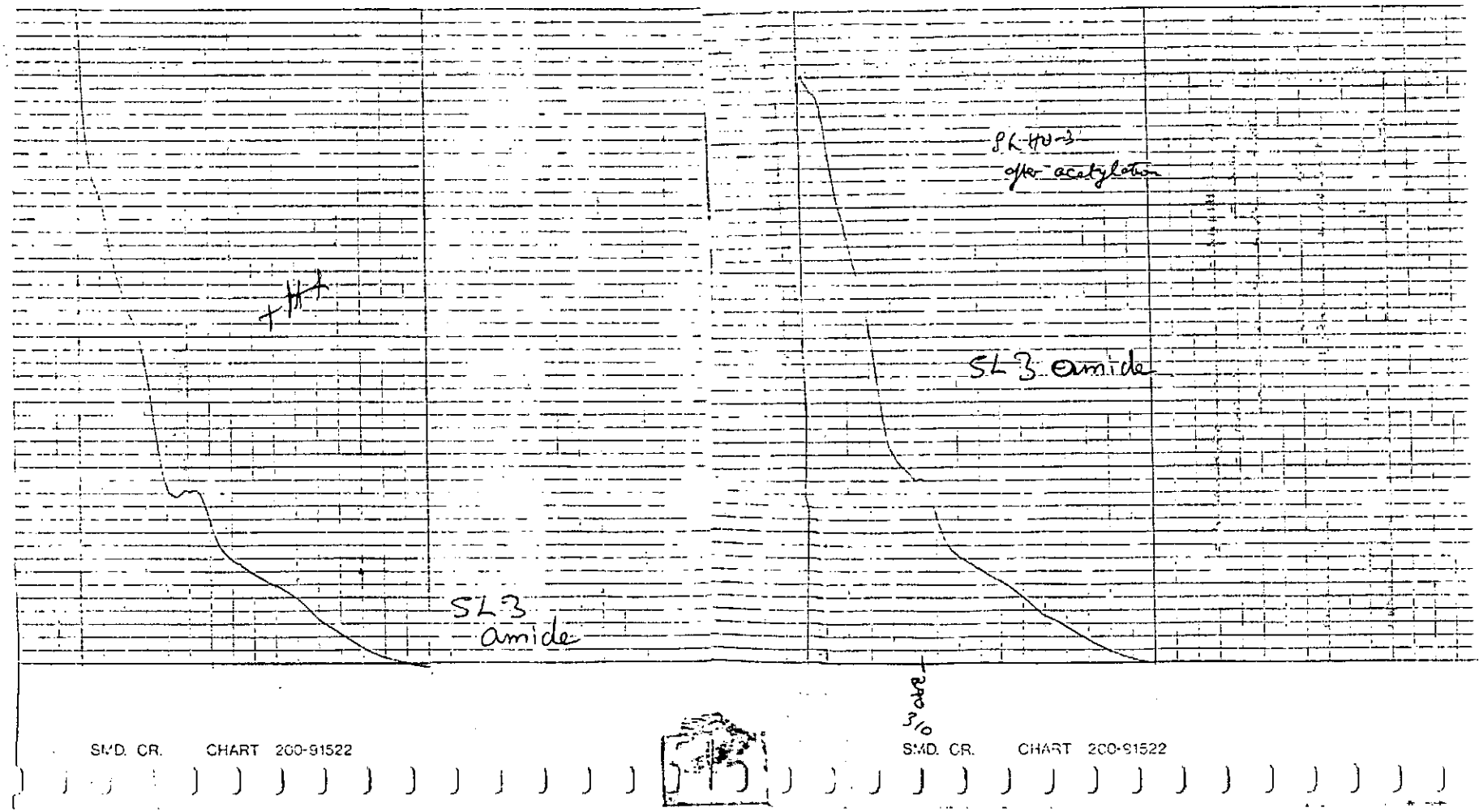
IONISATION: +VE CI REAGENT: AMMONIA

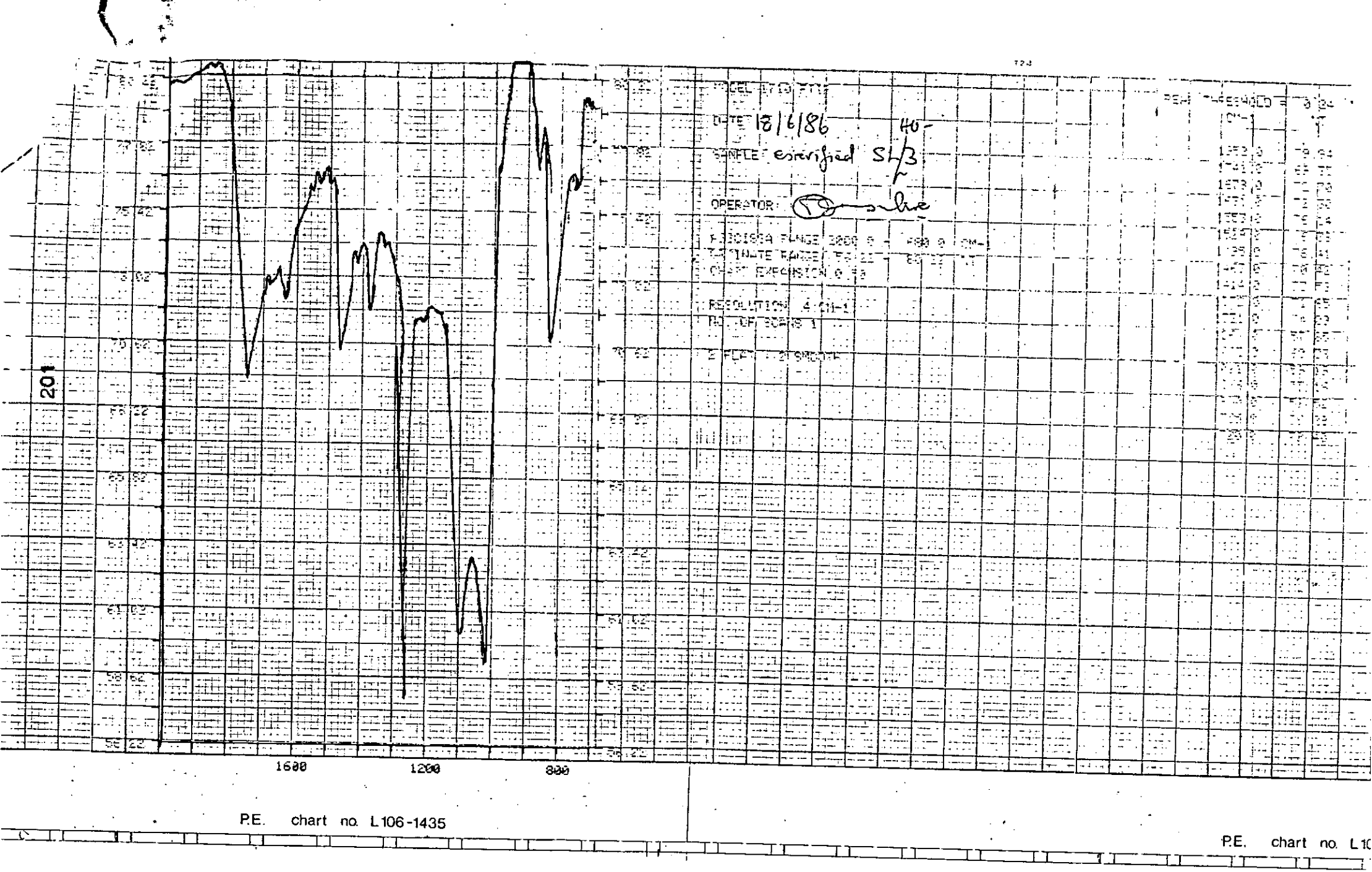
NO. PEAKS: 426
BASE/NREF INT: 2908224. / 860480
TIC: 39203840.
MASS RANGE: 27 - 461
RETN TIME/MISC: 0:28/ 823/ 0/ 3

PEAK NO.	MEASURED MASS	% INT. NREF	
87	350	29.9	*
88	349	63.5	*
135	297	47.6	*
203	299	65.4	*
205	227	59.6	
218	214	32.6	
219	213	80.6	*
220	212	68.4	*
221	211	100.0	*
235	197	42.0	
247	186	40.5	
248	185	84.3	*
249	184	52.5	*
250	183	50.0	
251	182	38.6	
263	170	48.1	
264	169	88.2	
265	168	59.9	
294	140	30.2	
307	127	51.6	
309	125	31.6	
310	124	48.1	
338	96	32.9	
352	82	50.4	
390	44	34.4	
401	32	68.4	
403	30	53.6	

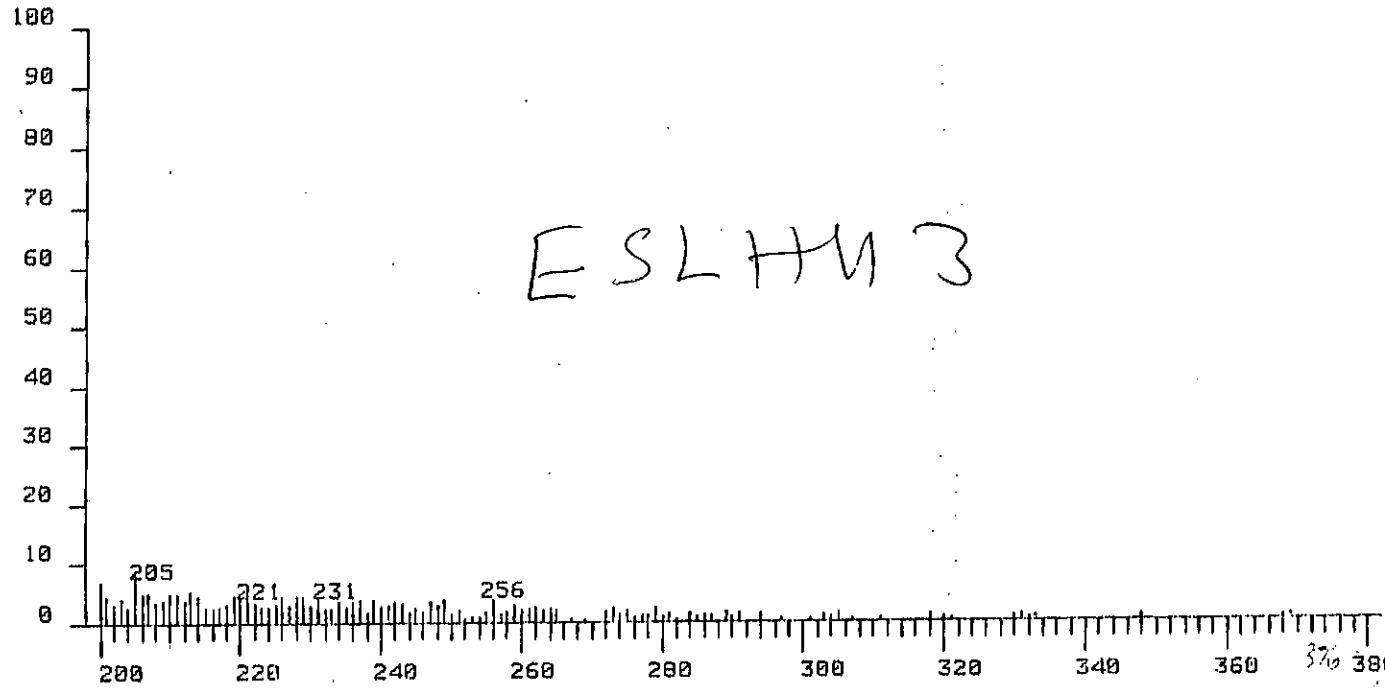
S2H4

S2H3Cl

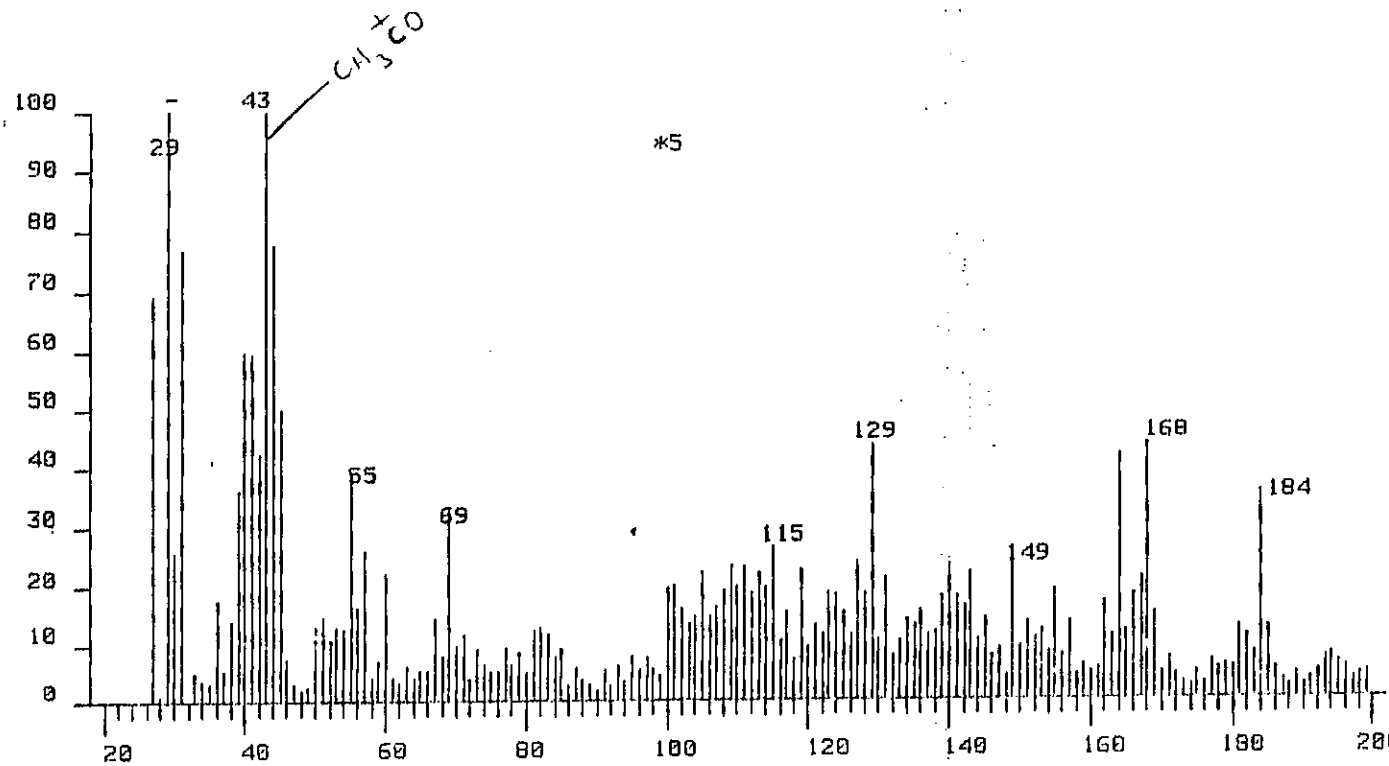

SL HW-3
after acetylation
SL 3 amide
SL 3
amide
SMD. CR. CHART 200-91522
SMD. CR. CHART 200-91522


DATE 18/6/86
SAMPLE: Enriched SL/3
OPERATOR:
RESOLUTION 4 CM-1
NO. OF SCANS 1
1600
1200
800
P.E. chart no. L106-1435
P.E. chart no. L1

27LR2.19 [TIC=26910720, 100%=1421120] EI



ESLHM3



ESL-HY3.

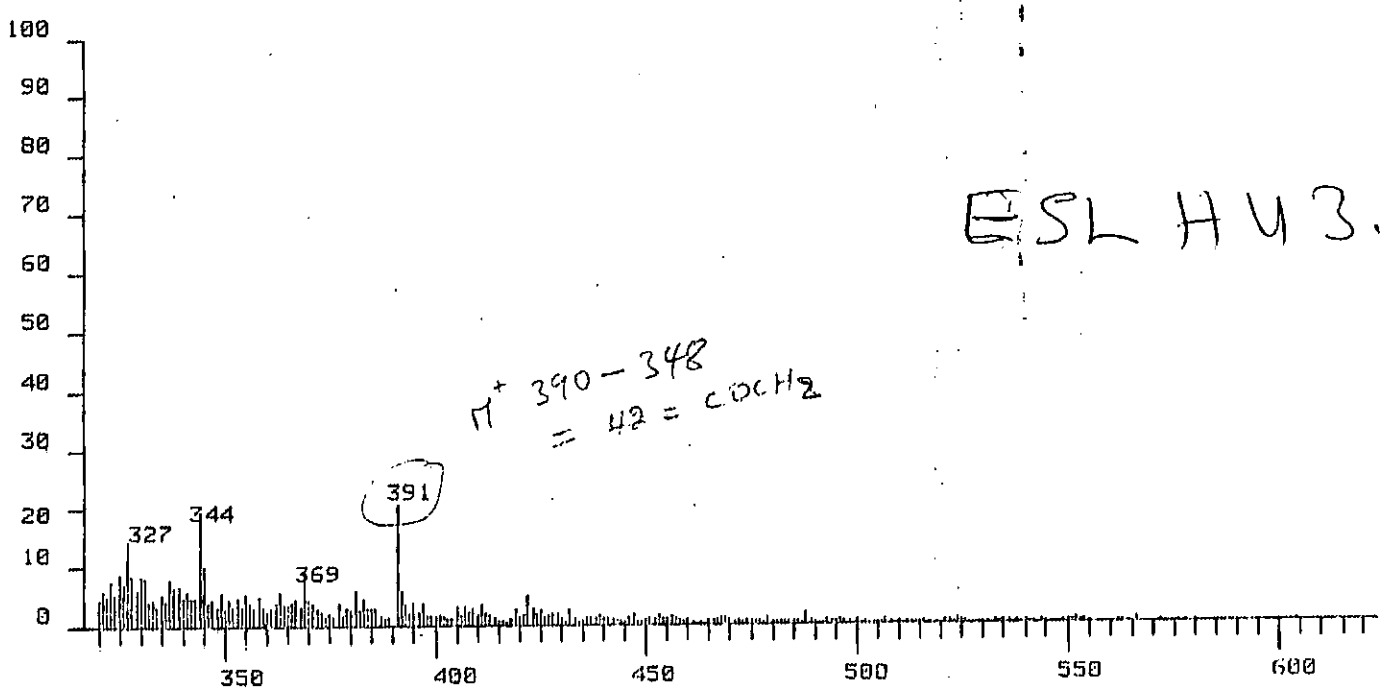
PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF	
99	205	1.8	
110	194	1.6	
119	185	2.5	
120	184	7.1	
121	183	1.7	
122	182	2.3	
123	181	2.5	
136	168	8.8	
140	164	8.4	
175	129	8.8	
207	97	7.7	
209	95	8.1	
211	93	6.4	
217	87	6.0	
219	85	9.1	
220	84	8.1	
222	83	11.8	
223	82	12.9	
224	81	12.4	
226	79	8.7	
227	78	6.6	
228	77	9.5	
232	74	6.5	
233	73	9.2	
235	71	11.8	
237	70	9.8	
238	69	18.4	*
239	69	14.3	*
240	68	8.2	
241	67	14.2	
245	63	8.3	
248	60	22.1	
249	59	7.1	
252	57	26.2	
253	56	16.5	
254	55	38.9	
255	54	12.6	
256	53	13.0	
257	52	10.9	
258	51	14.5	
259	50	13.3	
263	46	7.5	
264	45	50.1	
265	44	78.0	
266	43	100.0	
267	42	42.3	
268	41	59.7	
269	40	11.6	*
270	40	48.1	*
271	39	36.0	
272	38	7.1	*
273	38	7.0	*
275	36	17.4	
280	31	77.1	
281	30	25.9	

PAGE 2

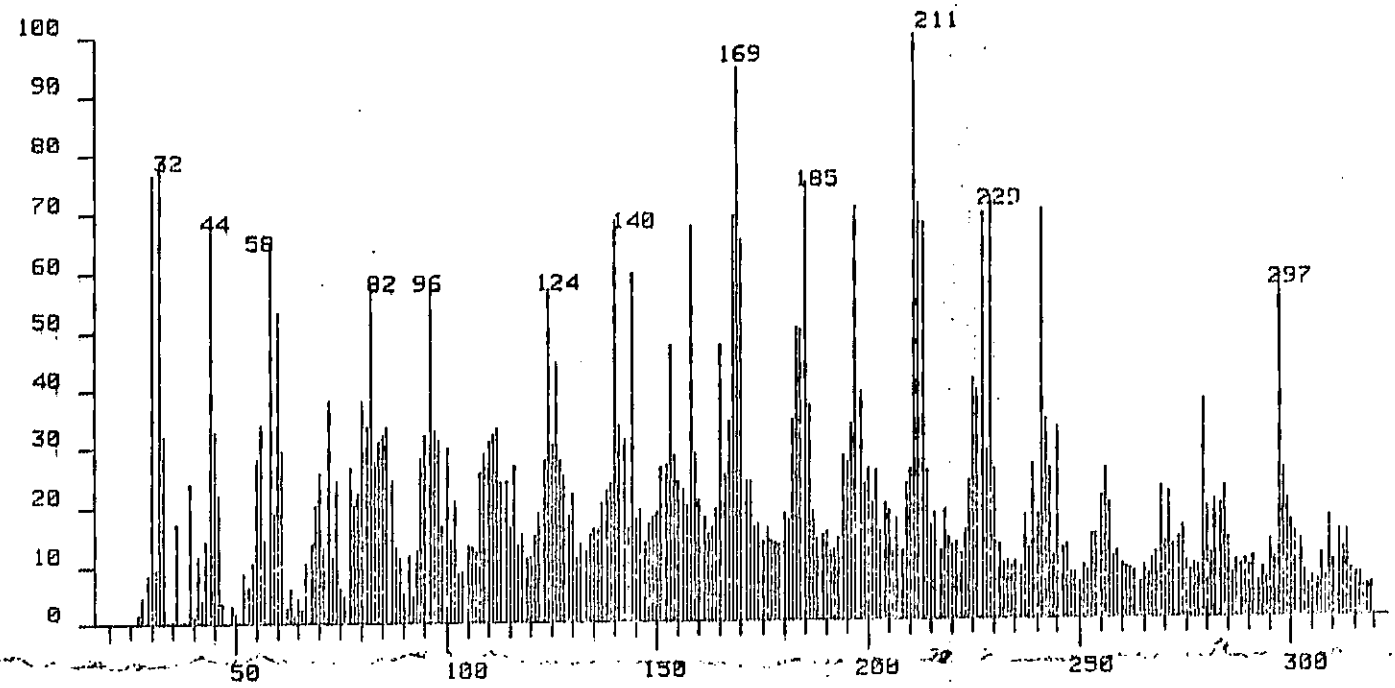
PEAK NO.	MEASURED MASS	% INT. NREF	
282	29	29.8	*
283	29	83.4	*
289	27	69.3	

27LR2.13 [TIC=102424576, 100%=1479808] +VE CI, REAGENT:AMMONIA



ESL H43.

M^+ 390 − 348 = 42 = $CCOCH_2$

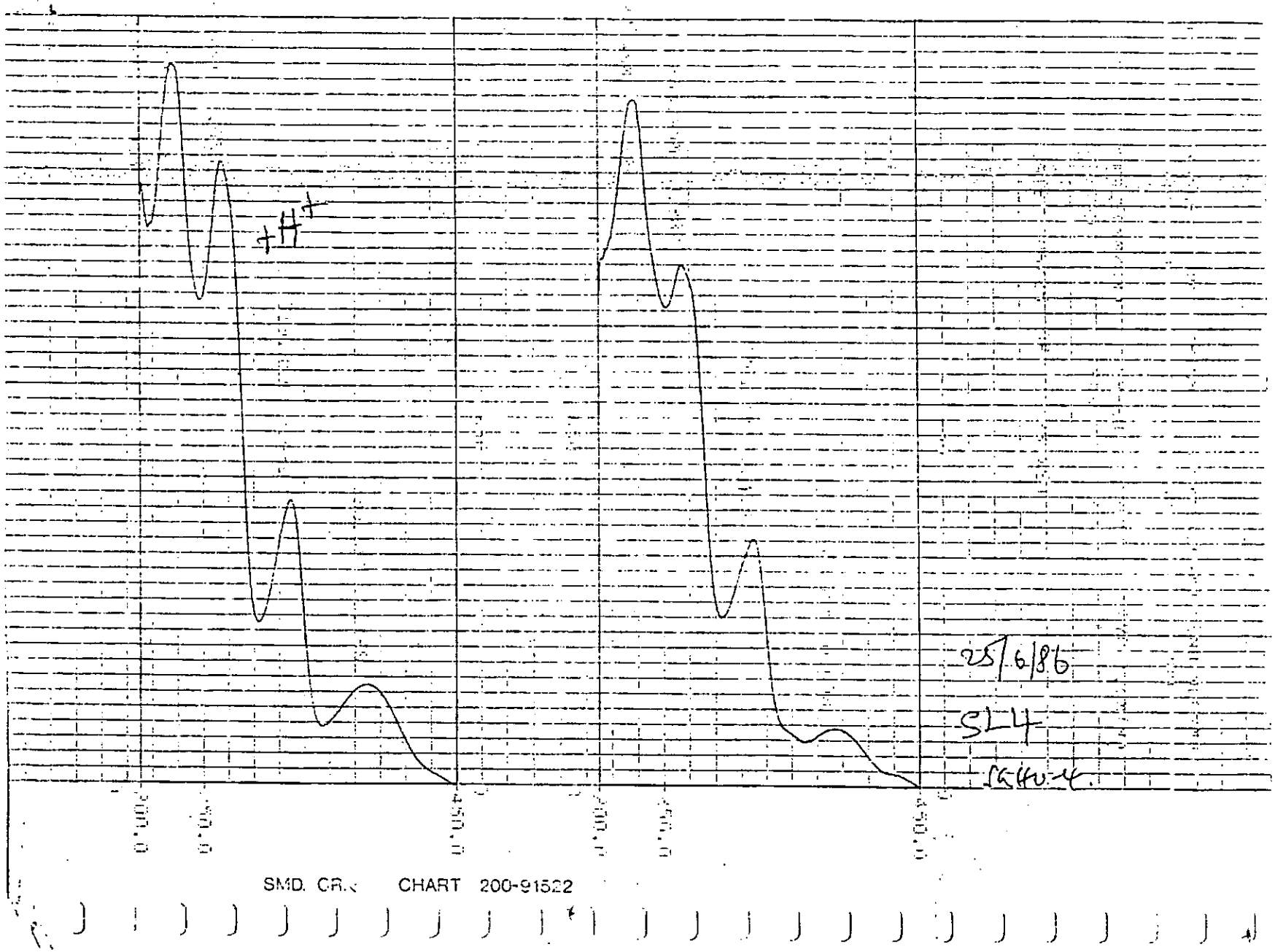


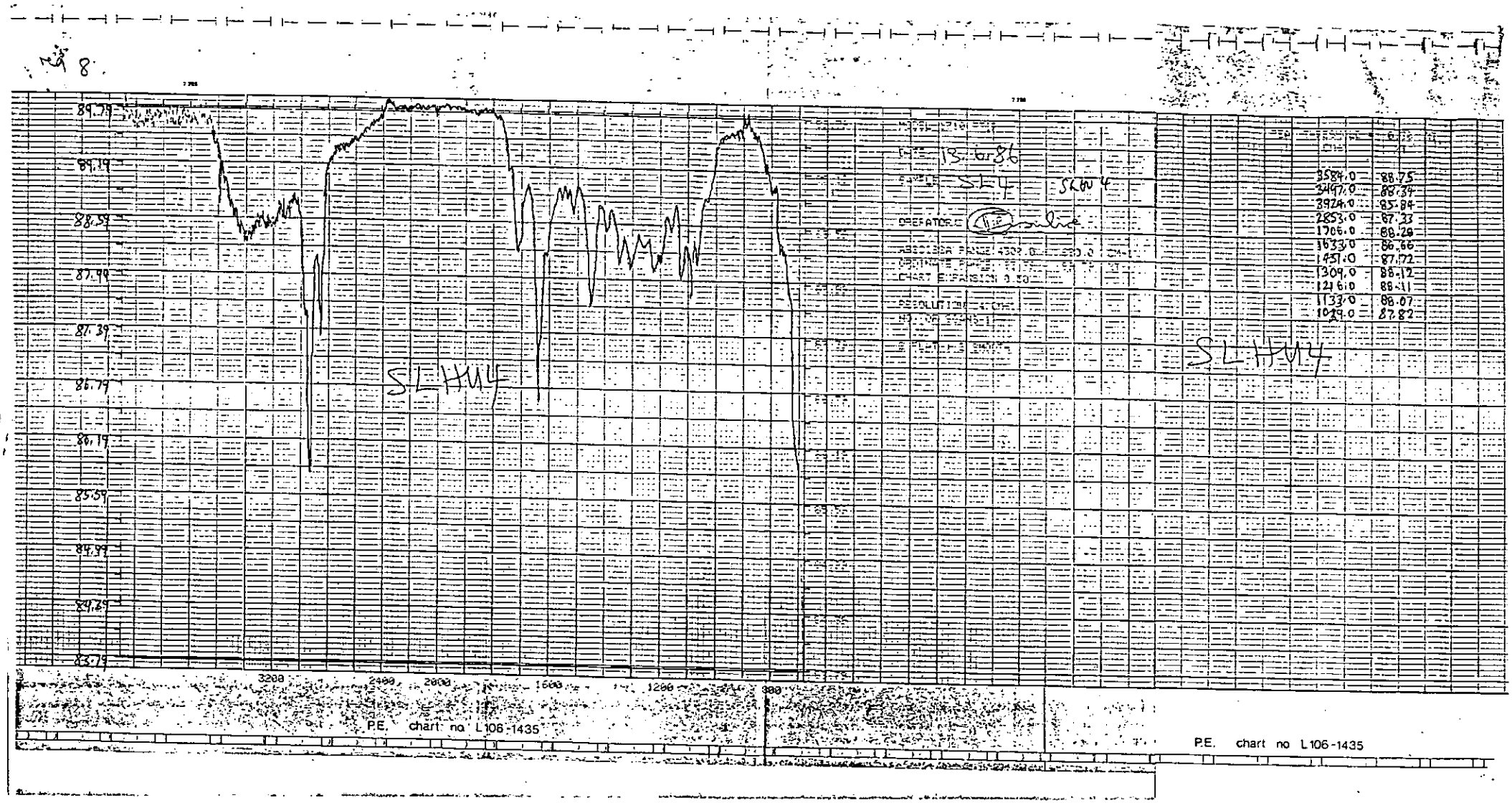
PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF	
185	391	20.6	*
232	344	19.3	
249	327	14.3	*
263	313	14.9	*
265	311	14.9	
267	309	17.3	
274	302	13.4	
275	301	14.7	
278	298	25.5	
279	297	58.3	
298	279	37.4	
321	256	25.8	
333	245	32.6	
336	243	25.8	
337	242	34.0	*
338	241	70.0	*
340	239	26.7	
351	230	25.9	*
352	229	72.5	*
353	228	28.7	*
355	227	69.6	
356	226	39.4	
357	225	41.2	
368	214	25.2	
370	213	67.7	*
371	212	71.3	*
372	211	100.0	*
373	210	25.8	*
382	202	25.7	
385	200	26.2	
388	198	39.1	
390	197	70.7	
392	196	33.3	
393	195	26.8	
395	194	28.2	
404	186	36.7	
405	185	74.9	
406	184	50.0	
407	183	50.3	
409	182	34.3	
423	170	65.3	*
424	169	94.5	*
425	168	69.3	*
426	167	34.2	*
428	166	25.0	
429	165	47.6	
435	159	28.6	
437	158	67.6	
441	154	28.2	
442	153	47.5	
443	152	27.0	
444	151	26.4	
452	144	59.7	
454	142	31.1	
455	141	33.5	

ESLHU

ESLHU3 Cl.


+H+
25/6/86
SL4
SMD. CR.
CHART 200-91522


89.79
89.19
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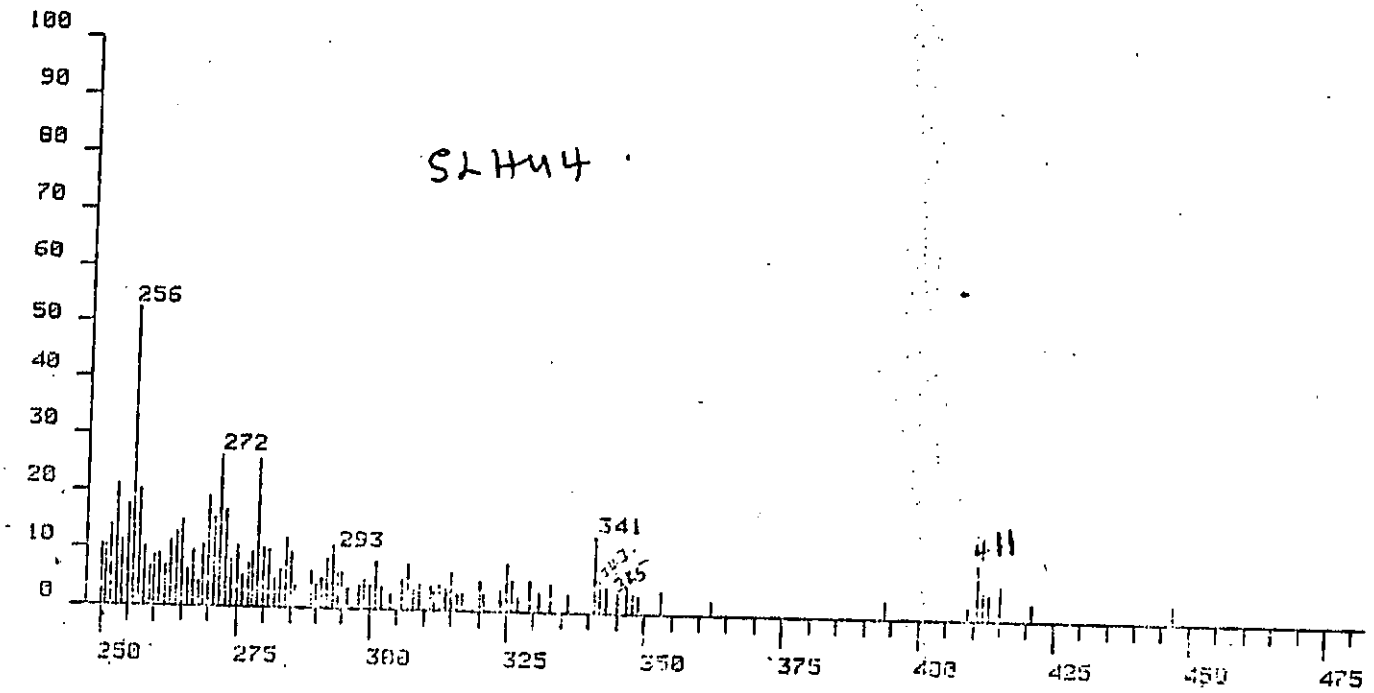
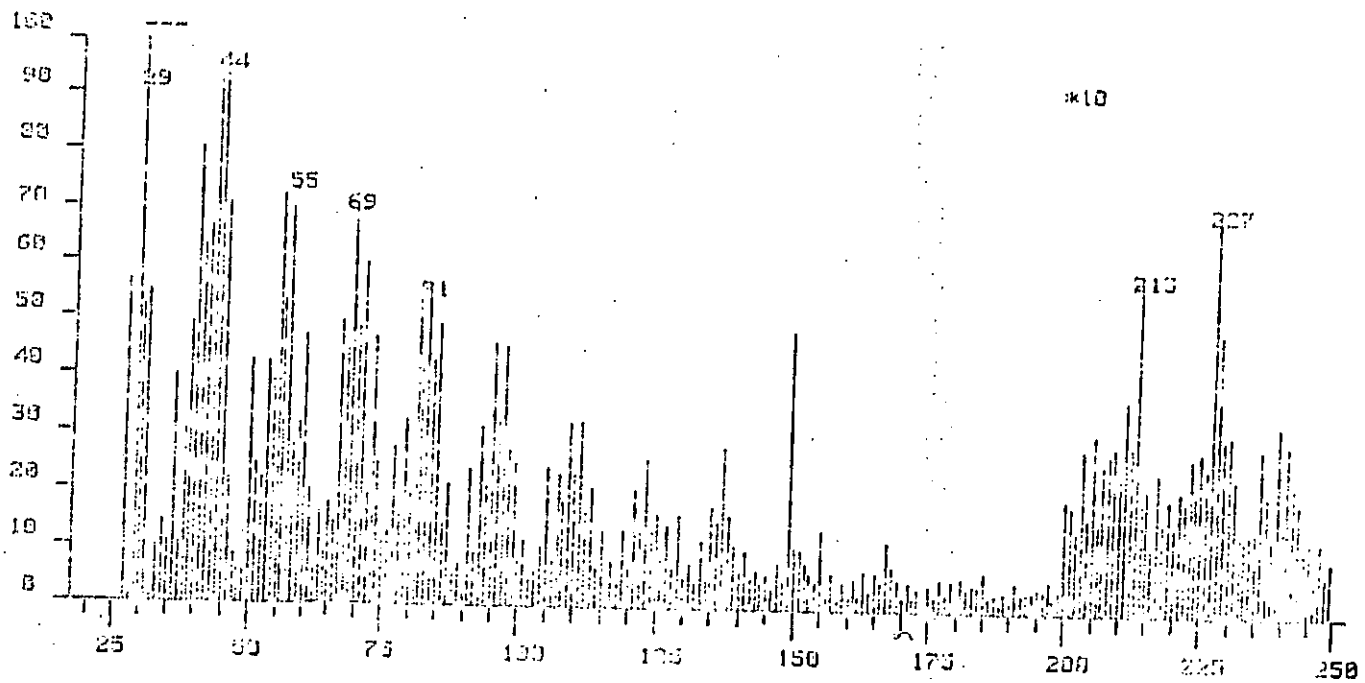
87.99
87.39
86.79
86.19
85.59
84.99
84.29
83.79
SLHW4
SLHW4
SLHW4
DATE 13 Oct 86
3584.0 88.75
3497.0 88.34
3424.0 85.84
2853.0 87.33
1706.0 88.29
1633.0 86.66
1451.0 87.72
1309.0 88.12
1216.0 88.11
1133.0 88.07
1029.0 87.82
3200
2400
2000
1600
1200
800
P.E. chart no L106-1435
P.E. chart no L106-1435

17LR8.40 [TIC=43208704, 100%=11017601] EI



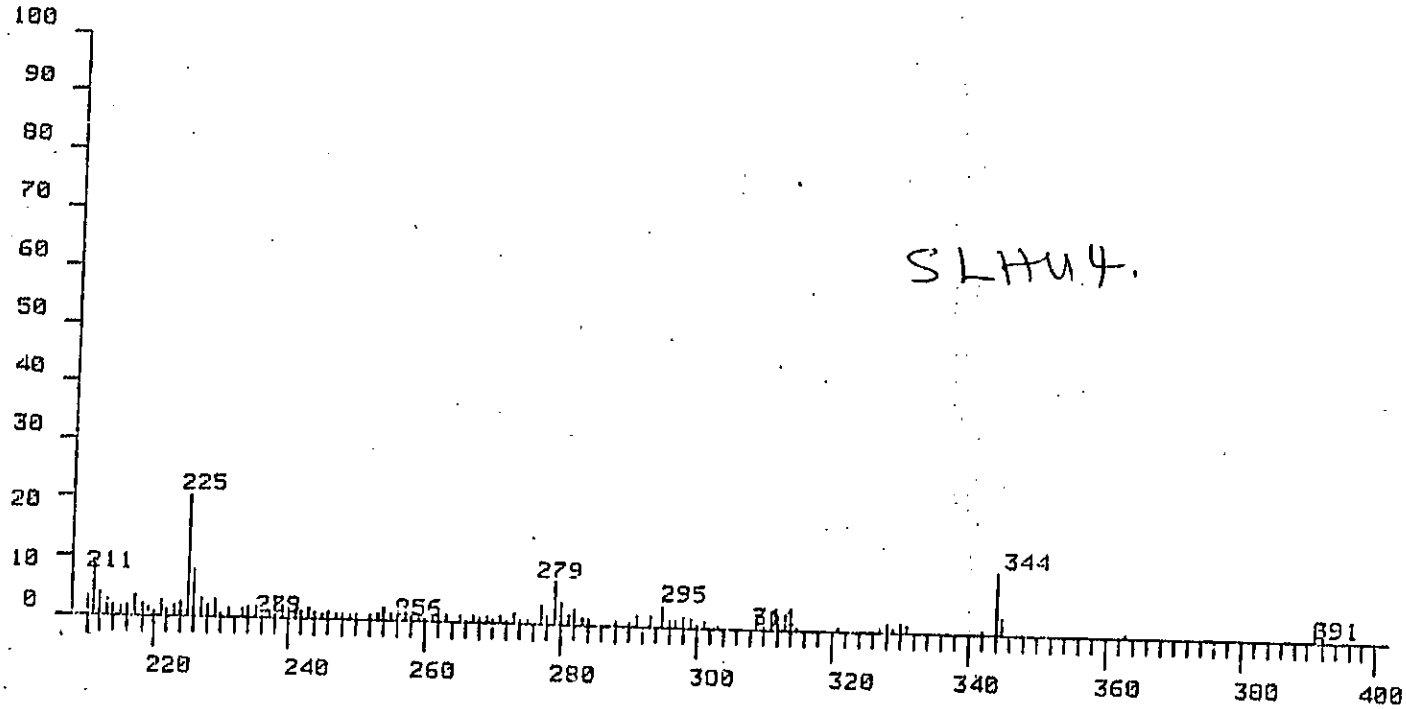
S2H44



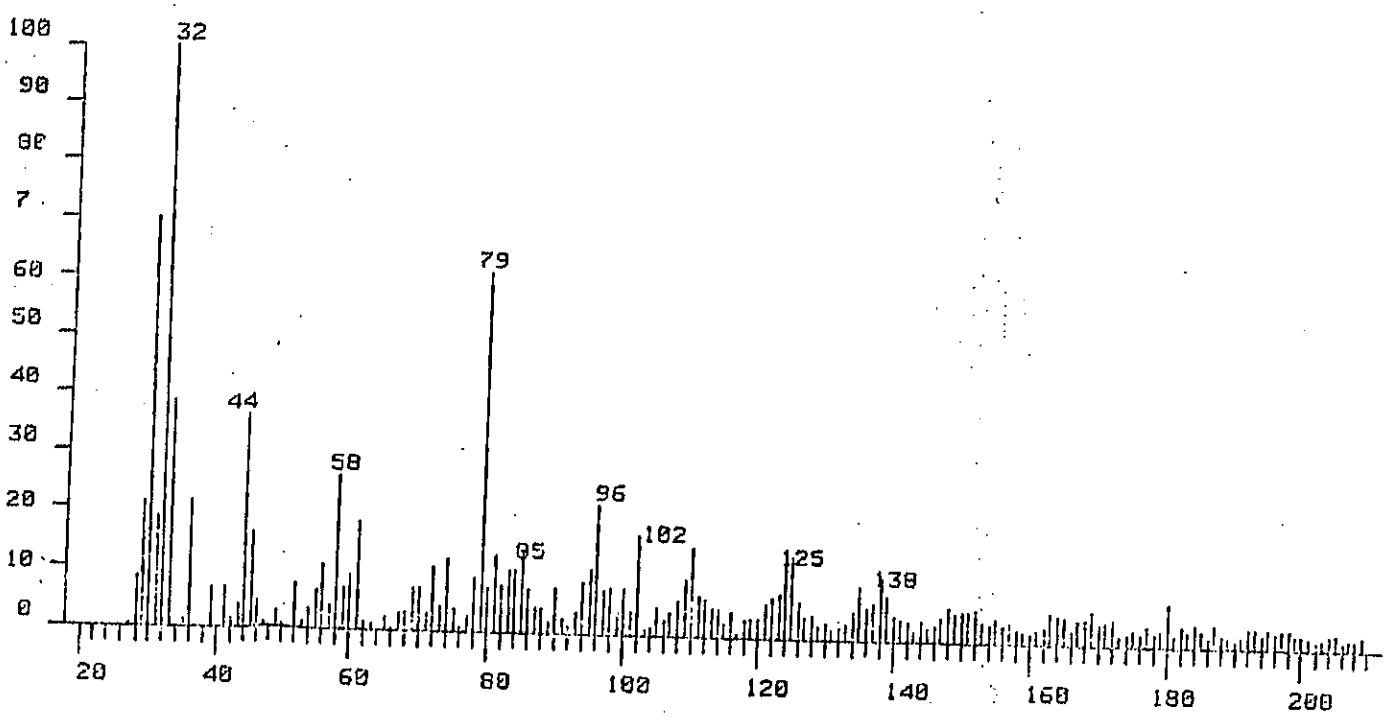
SLHH4 E

PEAK NO.	MEASURED MASS	% INT. NREF
73	279	2 6
79	273	1. 7
80	272	2 6
81	271	1. 5
82	270	1 9
87	265	1 5
95	257	2. 0
96	256	5. 2
97	255	1. 8
99	253	2 1
100	252	1. 4
125	227	7. 2
139	213	5 9
154	197	5. 4
168	185	7. 0
172	181	5. 6
185	168	7. 4
186	167	12 0
188	165	6 7
190	163	6. 9
192	161	5. 5
196	157	6. 3
198	155	13. 7
200	153	6. 4
201	152	8. 0
202	151	10. 4
203	150	10 5
204	149	48. 5
206	147	8 2
208	145	5 9
210	143	6 5
211	142	5. 6
212	141	10 0
217	136	28 3
230	123	24 0
232	121	20. 7
240	113	20. 8
242	111	22 [illegible]
243	110	23 3
244	109	32 4
247	107	23 1
250	105	24. 3
258	99	25. 1
259	98	27 5
260	97	45 5
261	96	31 4
262	95	46. 7
263	94	20. 7
265	93	31 3
268	91	24 0
273	87	21 7
275	85	49 1
276	84	12. 8
278	83	50. 2
280	82	43 5

17LR8.34 [TIC=9200384, 100%=5855841 +VE CI, REAGENT:AMMONIA



SLHU4.



SLH44C

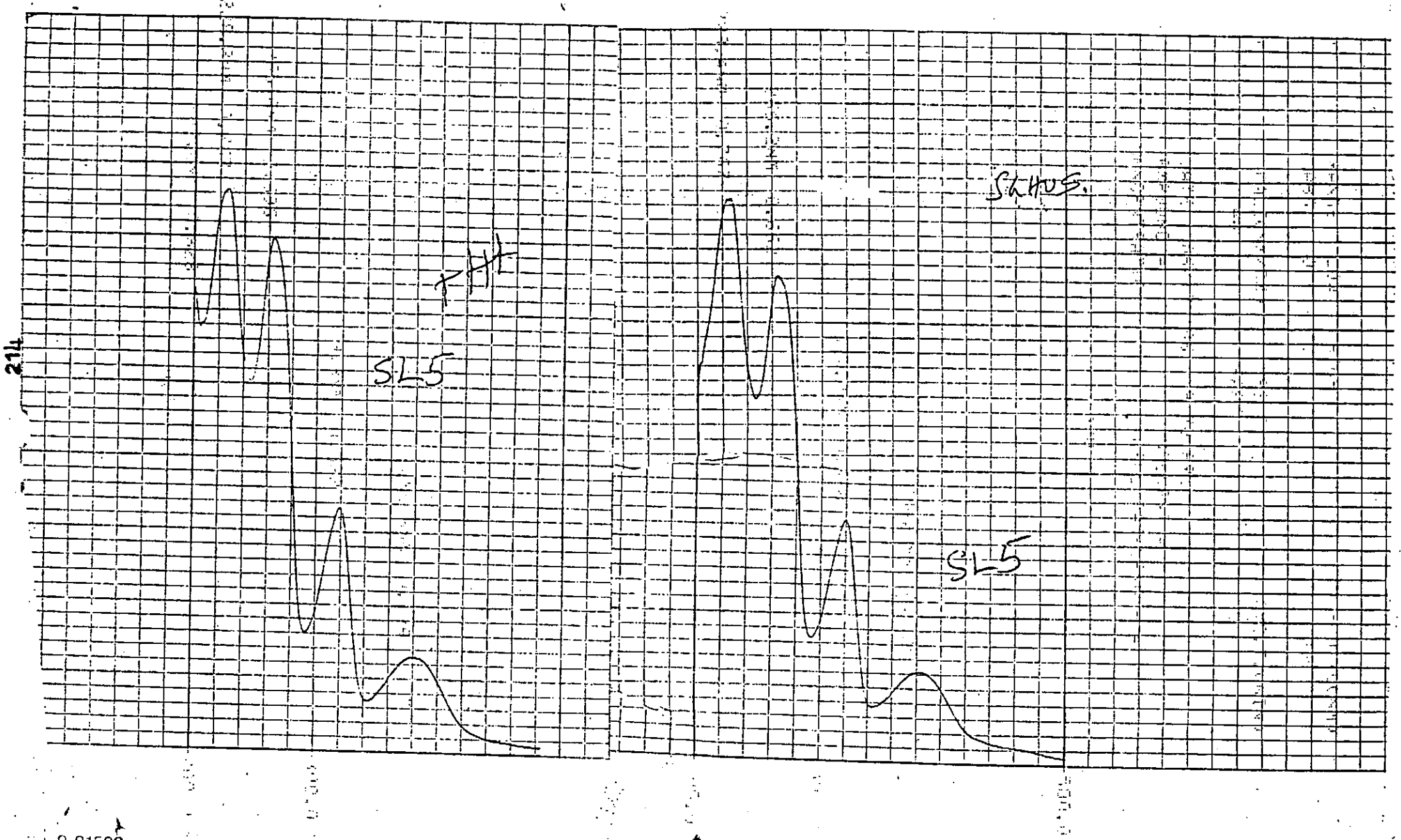
SLH44Cl.

PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF
0	391	3.0
5	345	2.6
6	344	10.4
16	313	2.7
17	312	2.4
18	311	3.1
20	309	2.7
25	298	1.8
28	295	3.6
29	293	1.8
30	291	1.8
35	282	2.5
36	281	1.9
37	280	3.7
38	279	7.2
40	277	3.2
59	256	2.8
61	254	2.1
87	226	7.8
88	225	20.4
102	211	8.5
133	180	7.1
144	169	5.8
150	163	5.3
161	152	5.5
162	151	5.3
163	150	5.2
165	148	5.6
174	139	7.4
175	138	10.7
176	137	6.4
177	136	5.4
178	135	8.9
187	126	6.5
188	125	13.9
189	124	13.3
190	123	7.5
191	122	6.9
192	121	5.9
201	112	6.5
202	111	6.8
203	110	15.1
204	109	9.3
205	108	6.1
211	102	17.2
213	100	7.9
215	98	8.0
216	97	7.5
217	96	22.1
218	95	11.3
219	94	8.8
223	90	7.7
227	86	7.3
228	85	13.9
229	84	10.5

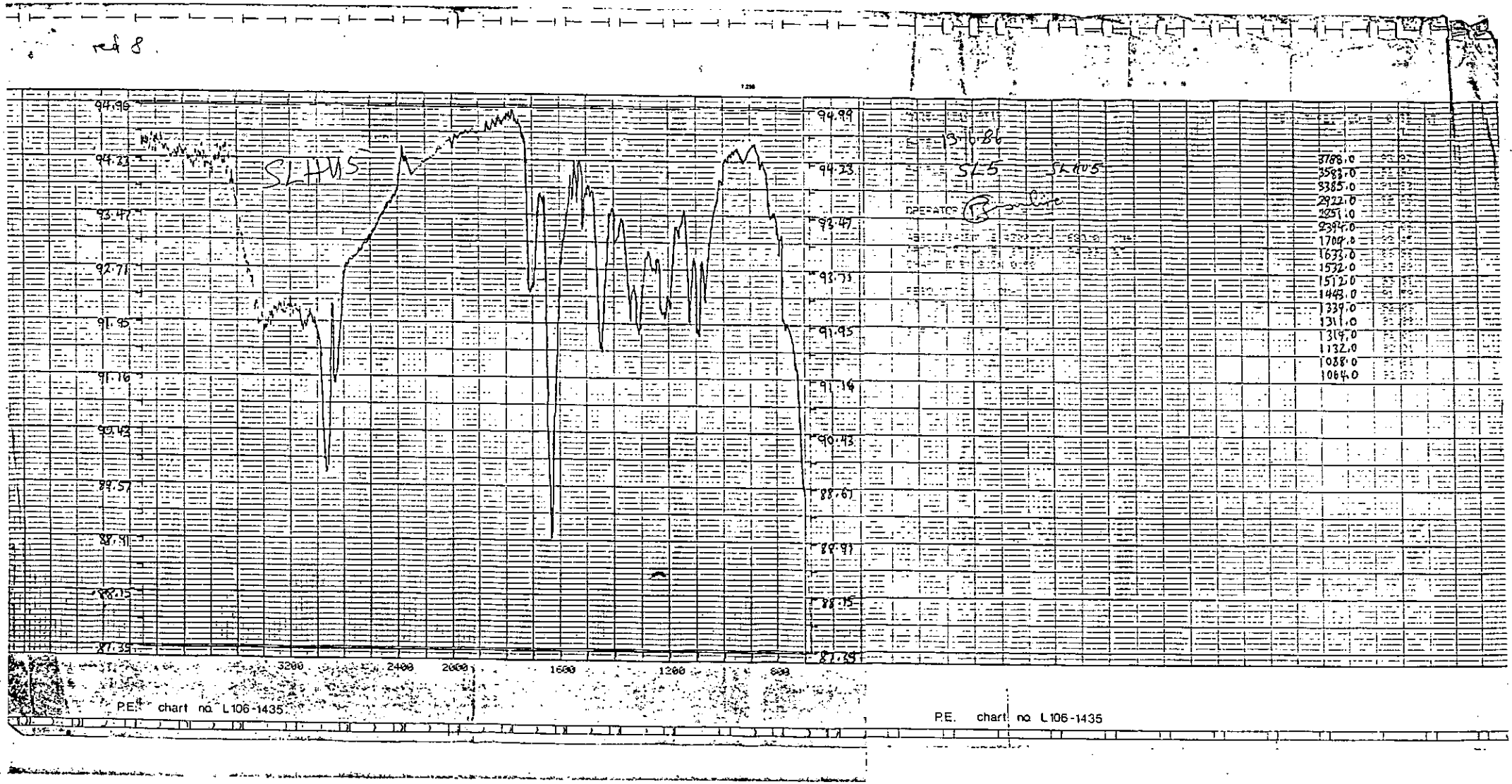
PAGE 2

PEAK NO.	MEASURED MASS	% INT. NREF
230	83	10.6
231	82	8.2
232	81	12.9
233	80	7.4
234	79	61.5
235	78	9.2
239	74	12.2
241	72	11.0
243	70	7.5
244	69	7.1
251	61	18.4
252	60	9.3
253	59	7.2
254	58	26.2
256	56	10.8
257	55	6.5
260	52	7.4
265	45	16.1
266	44	36.3
269	41	6.8
270	39	7.0
271	36	21.5
275	33	38.6
276	32	100.0
277	31	19.0
278	30	70.2
279	29	21.3
280	28	8.6



214

9-91522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SLHW5
3-6-86
SL5
SL405
OPERATOR
94.99
94.23
93.47
93.73
91.95
91.14
90.43
88.67
88.91
88.15
87.39
3788.0
3583.0
3385.0
2922.0
2851.0
2394.0
1704.0
1633.0
1532.0
1512.0
1448.0
1339.0
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1314.0
1132.0
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1200
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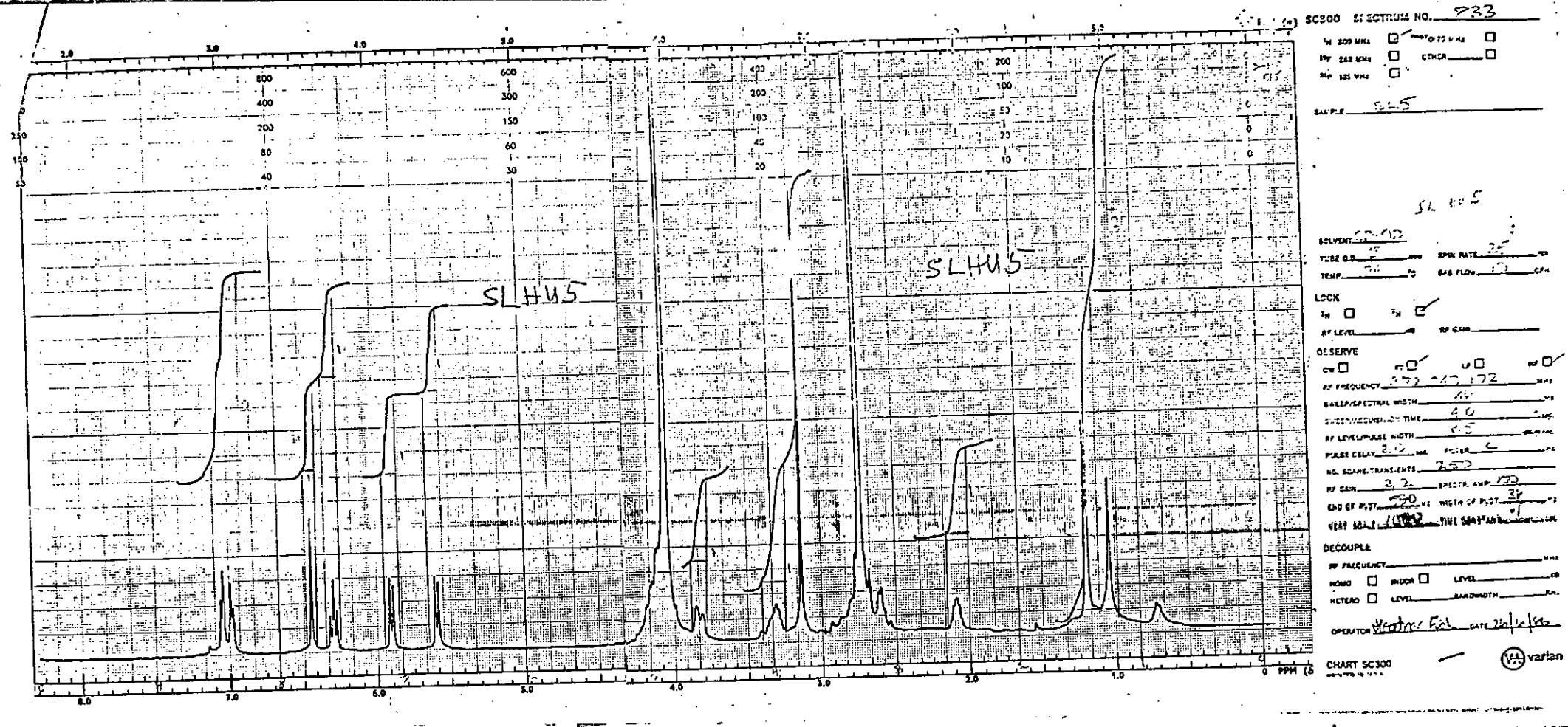
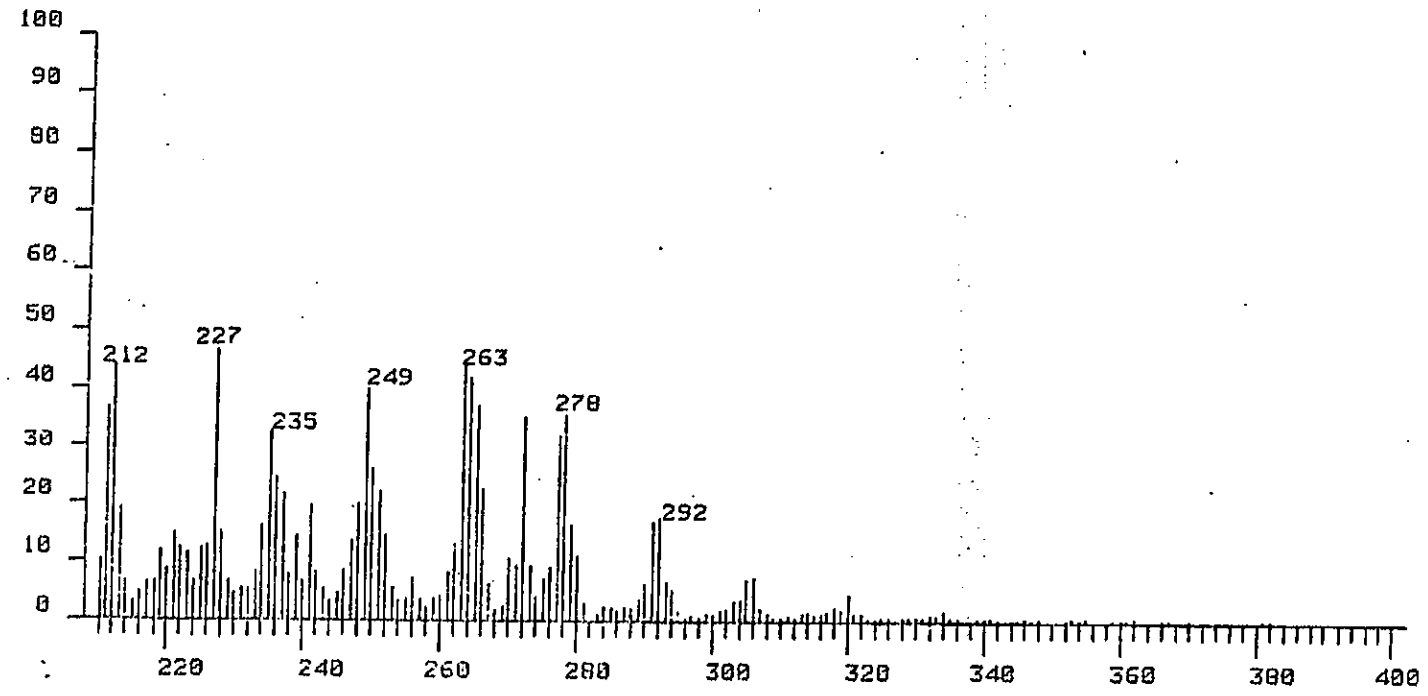
P.E. chart no. L106-1435

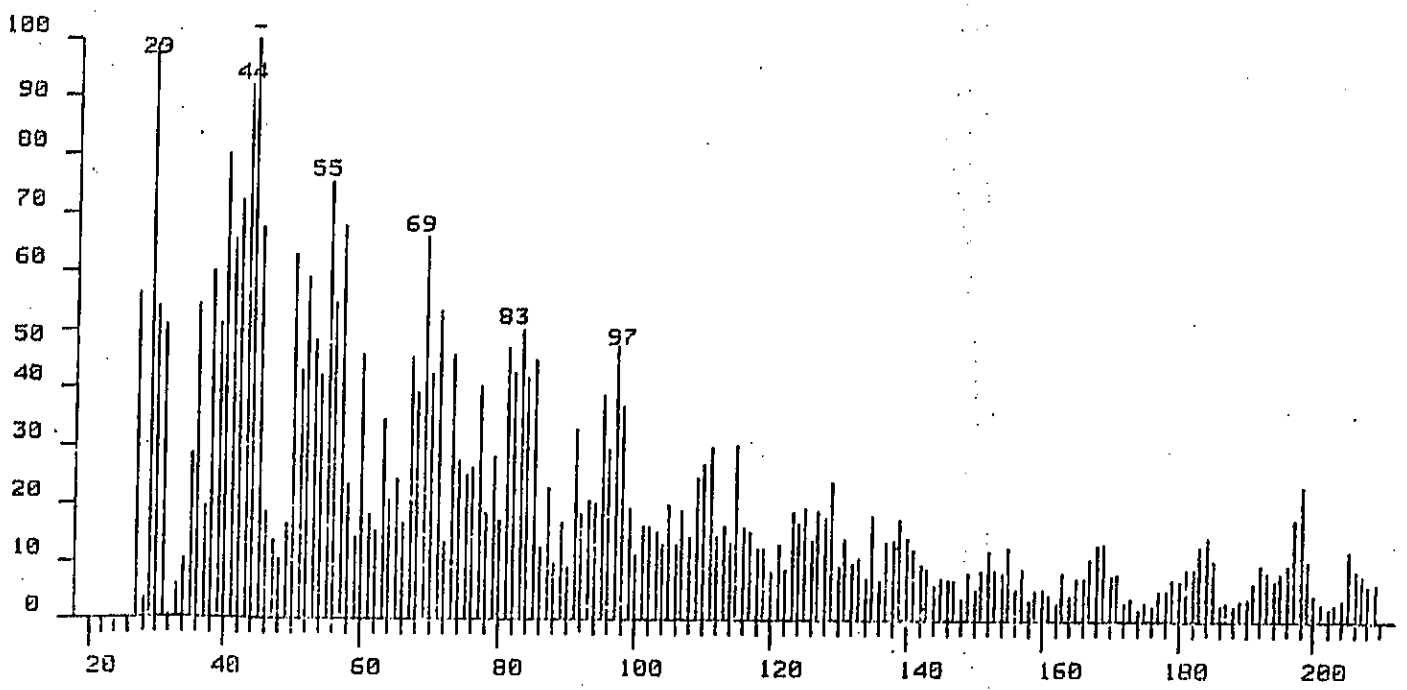

SL HH5
SL HH5
SC300 SPECTRUM NO. 933
SAMPLE SL-5
SL #5
SOLVENT CDCl3
SPIN RATE 25
TEMP
LOCK
OBSERVE
RF FREQUENCY 172
PULSE DELAY 3.0
NO. SCANS/TRANSIENTS 250
RF GAIN 3.2
SPECTR. AMP 120
DECOUPLE
OPERATOR Heather Fish DATE 2/1/88
CHART SC300
varian
PPM

216

SL5

17LR9.39 [TIC=50561536. 100%=1062656] EI





SLHMS

PEAK NO.	MEASURED MASS	% INT. NREF	
42	320	4.5	
56	306	7.5	
57	305	7.1	
58	304	3.7	
59	303	3.6	
69	293	7.0	
70	292	17.8	
71	291	17.0	*
72	290	6.3	*
82	280	11.3	
83	279	16.2	
84	278	35.5	✓
85	277	32.0	* ✓
86	276	9.2	*
87	275	7.1	
89	273	9.4	*
90	272	35.3	* ✓
91	271	9.3	
92	270	10.6	
96	267	6.2	
97	266	22.5	* ✓
98	265	37.2	* ✓
99	264	42.2	* ✓
100	263	45.1	* ✓
101	262	12.9	* ✓
102	261	8.4	*
107	256	7.4	
111	252	14.7	
112	251	22.1	*
113	250	26.1	*
114	249	40.0	* ✓
115	248	20.0	* ✓
116	247	13.6	*
117	246	8.6	
121	242	8.2	
122	241	19.9	
123	240	7.0	
124	239	14.2	
125	238	7.9	
126	237	21.7	*
127	236	24.6	*
128	235	32.6	*
129	234	16.0	*
130	233	8.3	*
135	229	6.7	
136	228	15.1	
137	227	46.6	*
138	226	12.6	*
139	225	12.0	
141	224	7.0	
142	223	11.4	
143	222	12.4	
144	221	14.8	
145	220	8.9	
146	219	11.3	

SLHMS

PEAK MEASURED % INT.

PAGE 2

PEAK NO.	MEASURED MASS	% INT. N'REF	
148	218	6.8	*
149	217	6.4	*
153	214	6.6	
154	213	19.1	
155	212	44.2	*
156	211	36.7	*
157	210	10.4	*
158	209	6.6	*
159	208	6.3	
160	207	7.9	
161	206	8 9	
162	205	12 2	
170	199	10.3	
171	198	23.5	
172	197	17.2	
174	196	9.6	
175	195	7.9	
177	194	7.2	
178	193	8.6	
179	192	9.7	
180	191	6 6	
186	185	10.6	
187	184	14 7	
189	183	12 9	
191	182	9.1	
192	181	8.7	
194	180	7.0	
195	179	7.3	
204	171	7.9	
205	170	8.2	
206	169	13.5	
208	168	13 2	
209	167	10.5	
210	166	7.3	
211	165	7 5	
213	163	8.2	
221	157	8.9	
225	155	12.6	
226	154	8.2	
227	153	8.8	
229	152	11.9	
231	151	8.6	
273	129	24.1	
277	127	19.1	
281	125	19.5	
285	123	18.8	
303	115	30.2	
311	111	30.0	
313	110	27.0	
315	109	24.7	
320	107	18.6	
325	105	20.1	
338	99	19.5	
340	98	37.0	
342	97	47.1	

PEAK MEASURED % INT

3

SLHMS EI

PEAK NO.	MEASURED MASS	% INT. NREF
345	96	29.6
347	95	39.0
349	94	20.2
350	93	20.7
352	92	18.4
355	91	32.5
363	87	22.9
367	85	44.8
369	84	41.6
371	83	50.5
374	82	42.6
376	81	47.1
379	79	28.4
381	78	18.7
384	77	39.8
387	76	26.2
391	75	25.3
392	74	27.9
394	73	45.9
398	71	53.6
400	70	42.6
402	69	66.1
405	68	38.8
406	67	45.6
411	65	74.3
416	63	34.7
421	60	45.?
426	58	23.4
428	57	67.9
430	56	54.7
431	55	75.2
434	54	42.2
435	53	48.4
436	52	58.8
438	51	43.2
440	50	62.?
444	46	18.7
445	45	67.7
448	44	100.0
449	43	92.0
451	42	72.4
453	41	65.5
455	40	79.7
456	39	50.?
458	38	60.1
461	36	54.5
462	35	28.5
467	31	50.?
469	30	53.8
470	29	98.7
480	27	56.3

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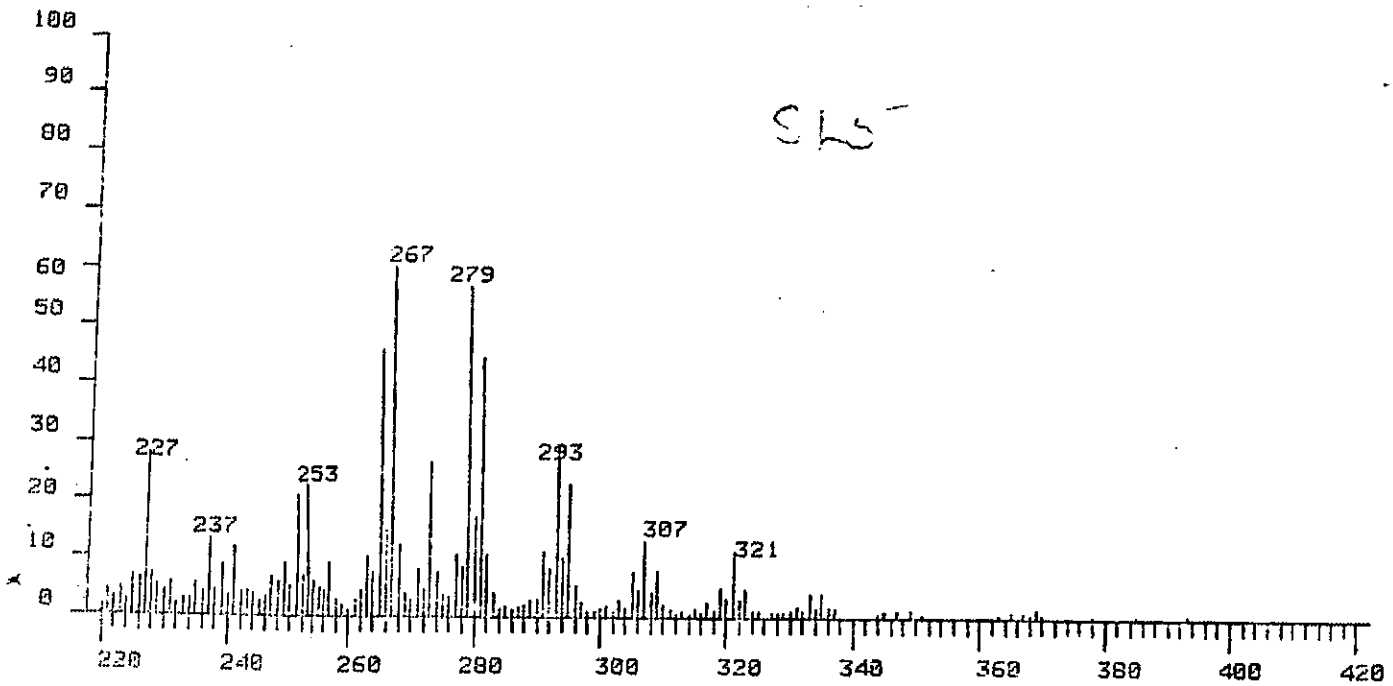
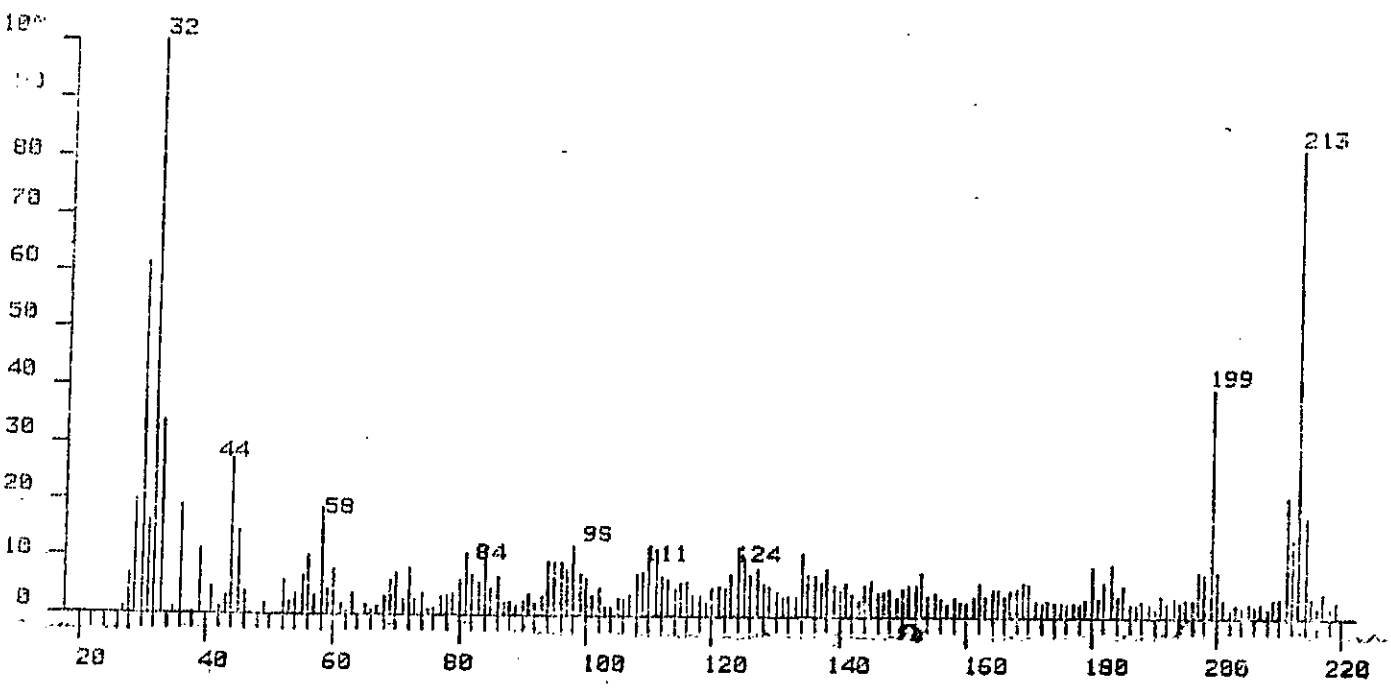




SLHM5 Cl.

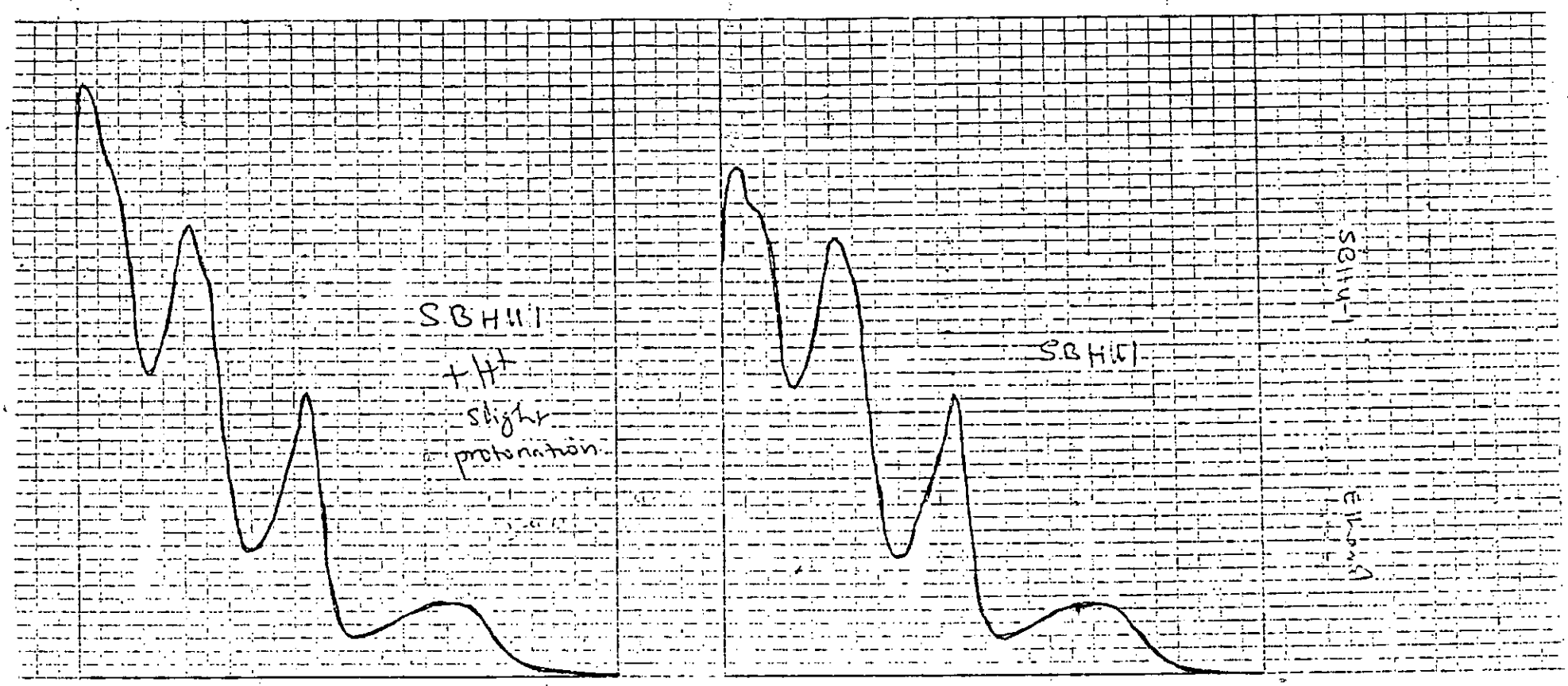
PEAK NO.	MEASURED MASS	% INT. NREF	
17	336	1.8	
18	335	3.9	
20	333	4.0	
22	331	2.0	
29	323	4.8	
30	322	3.2	
31	321	11.6	
32	320	3.1	
33	319	5.3	
35	317	2.7	
42	310	2.4	
43	309	7.8	
44	308	4.2	
45	307	13.1	
46	306	4.6	
47	305	7.8	
48	304	1.8	
49	303	3.9	
51	301	2.1	
55	297	2.6	
56	296	5.5	
57	295	23.3	
58	294	10.1	
59	293	29.9	
60	292	8.3	
61	291	11.2	
62	290	3.2	
63	289	2.0	
64	288	2.1	
65	287	1.8	
69	283	4.3	
70	282	10.7	
71	281	45.3	
72	280	17.2	
73	279	57.2	*
74	278	8.1	*
75	277	10.7	
76	276	8.3	
77	275	2.9	
78	274	7.4	
79	273	26.9	
80	272	4.5	
81	271	8.2	
82	270	2.8	
83	269	4.0	
84	268	12.4	
85	267	60.7	
86	266	14.9	
87	265	46.2	
88	264	7.4	
89	263	10.3	
90	262	4.0	
91	261	2.9	
94	258	2.9	
95	257	2.1	

PEAK NO.	MEASURED MASS	% INT. NREF
96	256	4.4
97	255	4.9
98	254	6.0
99	253	22.8
100	252	7.0
101	251	20.8
103	249	9.2
104	248	5.7
105	247	6.7
111	241	11.7
113	239	8.6
115	237	13.3
117	235	5.4
121	231	5.8
123	229	5.3
124	228	7.3
125	227	28.4
126	226	6.5
127	225	6.8
138	214	17.5
139	213	87.3
140	212	13.5
141	211	21.1
152	200	7.8
153	199	40.1
154	198	7.3
155	197	7.8
167	185	5.3
169	183	8.9
170	182	5.9
172	180	8.5
182	170	5.8
183	169	6.0
190	162	5.7
199	153	7.6
200	152	5.4
201	151	5.6
207	145	6.3
208	144	5.4
211	141	5.7
213	139	6.5
214	138	7.9
215	137	6.1
216	136	6.8
217	135	7.2
218	134	10.9
224	128	5.6
225	127	8.2
226	126	7.2
227	125	10.1
228	124	11.9
229	123	7.2
236	116	6.1
237	115	5.6
239	113	6.4

PAGE 3

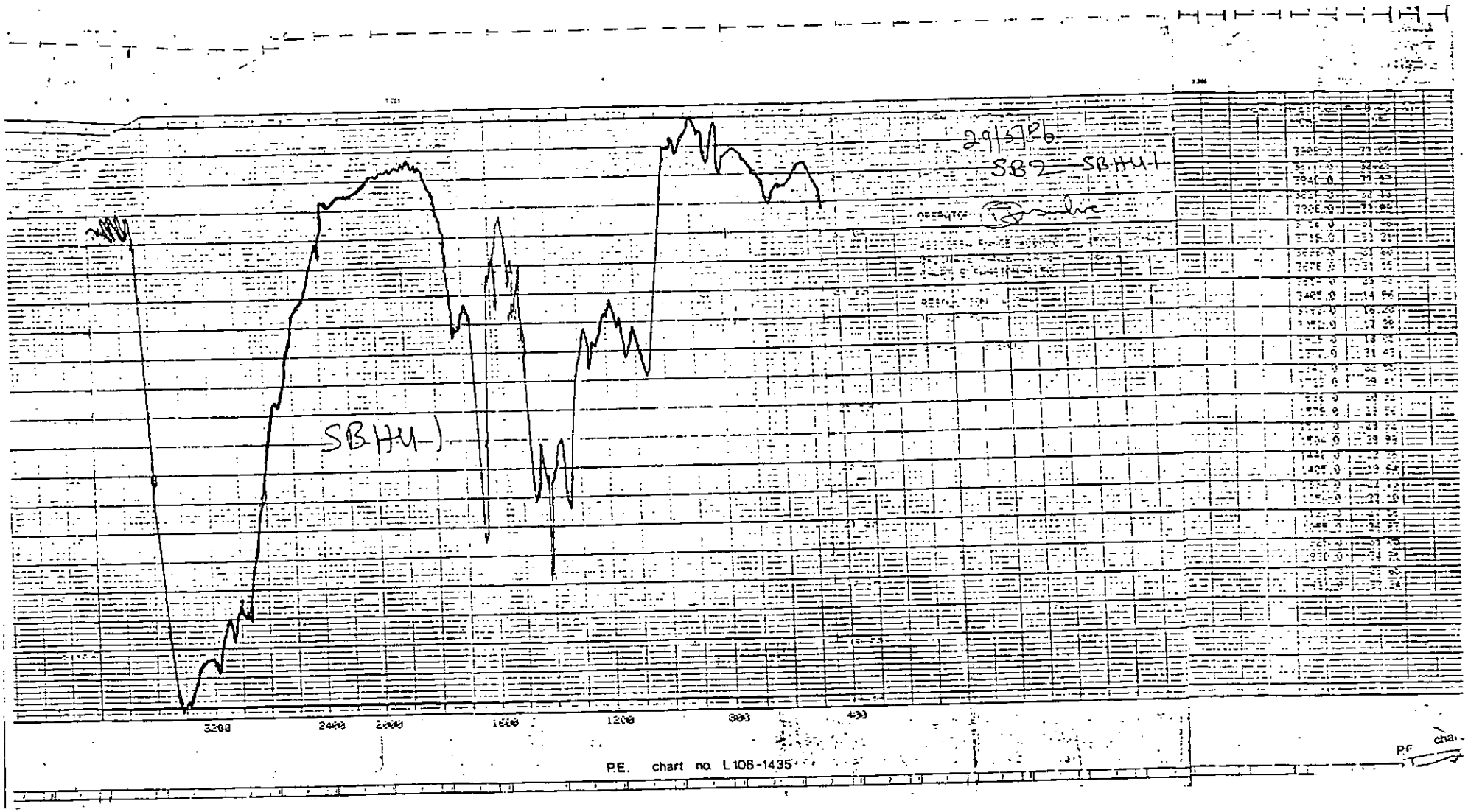
SLHM 5 C1.

PEAK NO.	MEASURED MASS	% INT. NREF
240	112	6.9
241	111	11.6
242	110	11.5
243	109	7.5
244	108	7.3
252	100	6.4
253	99	7.1
254	98	11.9
255	97	7.8
256	96	9.1
257	95	9.1
258	94	9.3
266	86	6.7
268	84	11.9
269	83	5.7
270	82	6.7
271	81	10.6
272	80	6.1
280	72	8.0
282	70	7.1
283	69	6.0
291	60	7.7
293	59	13.4
295	57	9.9
296	56	6.9
298	54	6.1
302	45	14.7
303	44	27.6
307	40	10.8
309	38	18.9
312	35	33.8
313	34	100.0
314	31	16.2
315	30	61.6
316	29	19.9
317	28	7.0



SMD. CR. CHART 200-01522

SMD. CR.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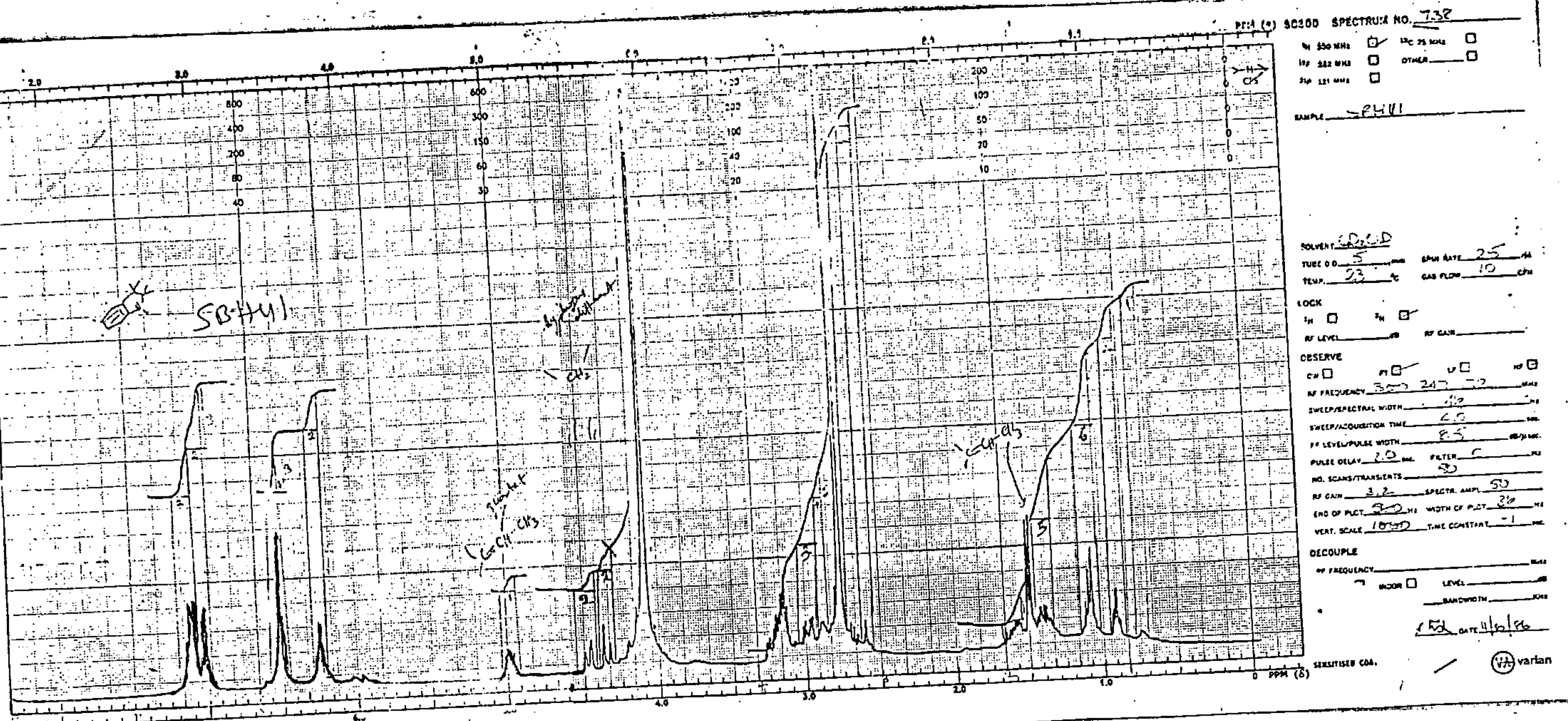
SBHH-1
3200
2400
1200
P.E. chart no. L106-1435

227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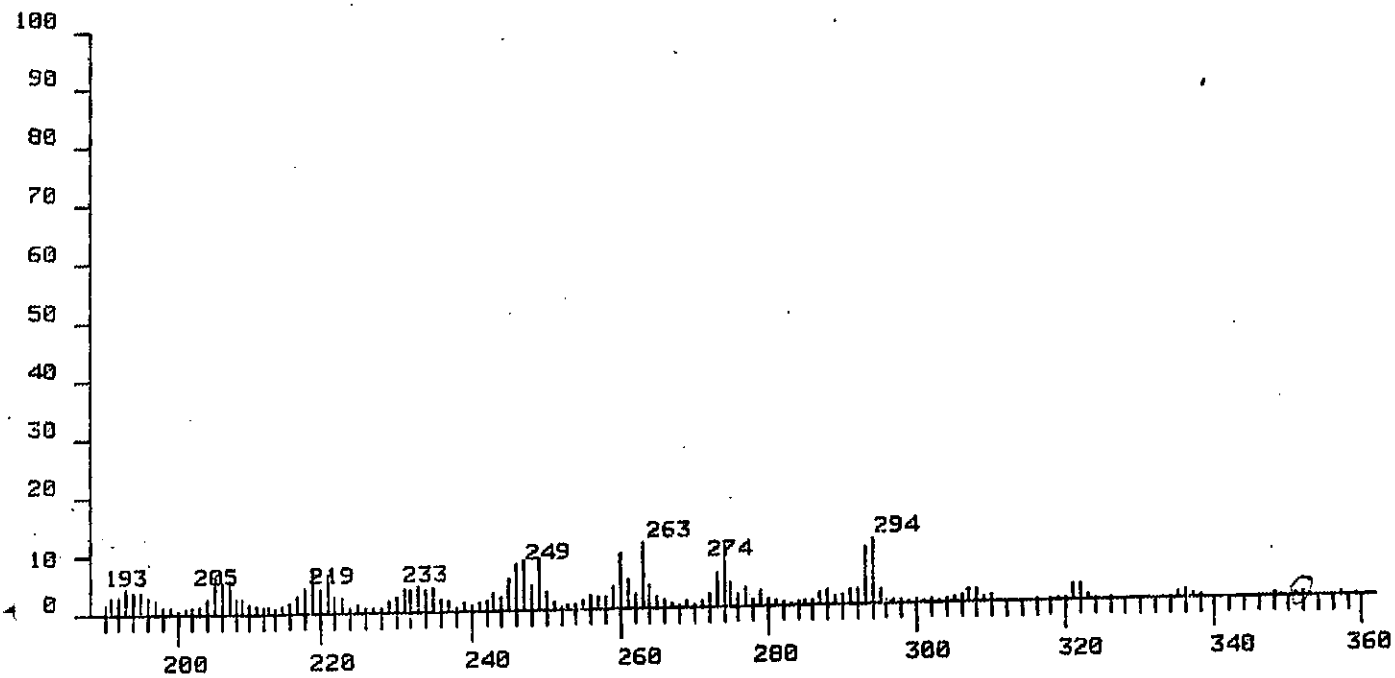
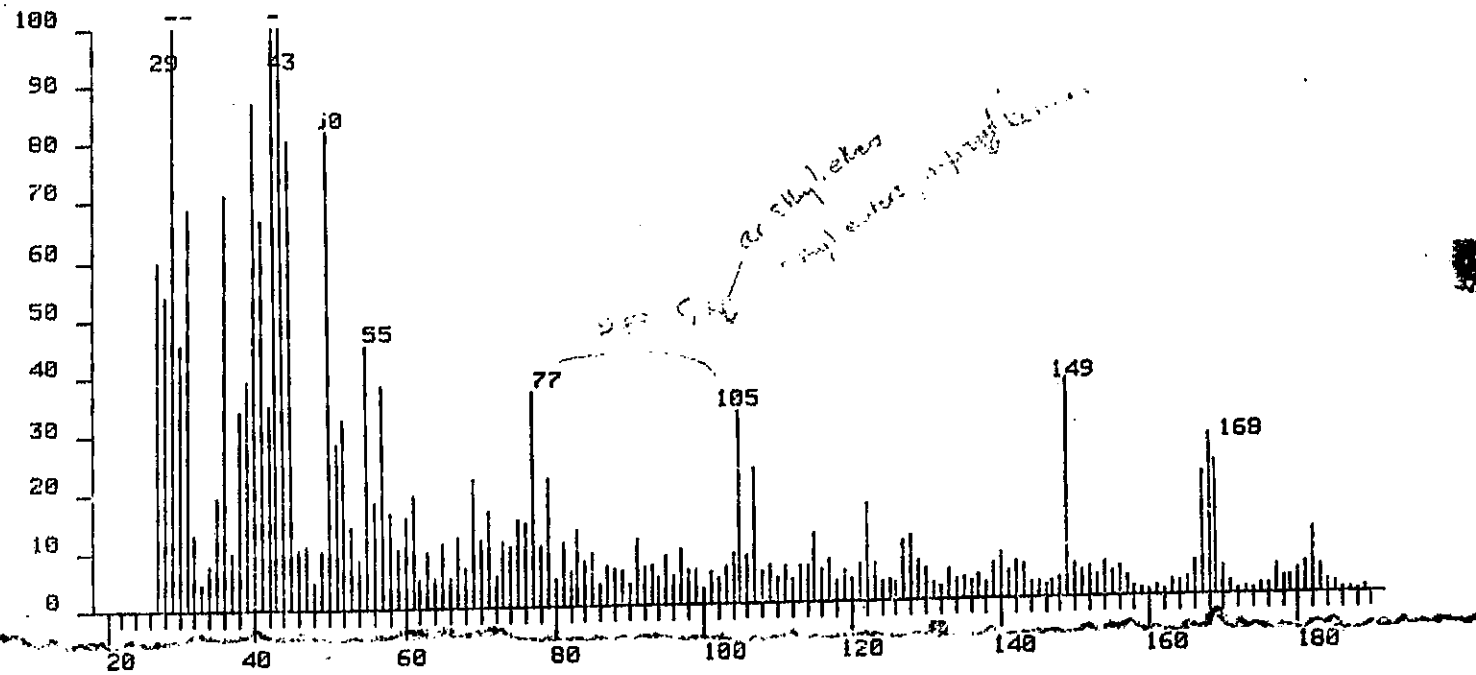
SAMPLE
SOLVENT
TUBE O.D.
SPIN RATE
TEMP.
GAS FLOW
LOCK
RF LEVEL
RF GAIN
OBSERVE
RF FREQUENCY
SWEEP/SPECTRAL WIDTH
SWEEP/ACQUISITION TIME
RF LEVEL/PULSE WIDTH
PULSE DELAY
FILTER
NO. SCANS/TRANSIENTS
RF GAIN
SPECTR. AMPL.
END OF PLOT
WIDTH OF PLOT
VERT. SCALE
TIME CONSTANT
DECOUPLE
RF FREQUENCY
LEVEL
BANDWIDTH
DATE
SENSITISED CAL.
PPM (δ)
varian

SR1H41.

6LR11.28 [TIC=34445312, 100%=1122880] EI





PEAK NO.	MEASURED MASS	% INT. NREF	
43	294	11.1	
44	293	9.8	£
64	274	10.5	
65	273	5.8	
75	263	11.2	
78	260	9.5	
89	249	8.8	
91	247	8.6	
92	246	7.9	
117	221	6.7	
119	219	7.1	
131	207	5.7	
133	205	6.3	
156	182	11.4	
170	169	22.9	
171	168	27.7	
173	167	31.1	
175	166	6.0	
195	149	37.5	
256	107	23.2	
258	105	33.0	
299	79	21.9	
303	77	36.7	
318	69	22.0	
336	61	19.4	
345	57	38.4	
349	55	45.3	
357	52	32.5	
359	51	28.5	
361	50	81.8	
367	45	78.2	
369	44	99.8	
372	43	100.0	
374	42	33.9	
376	41	64.8	£
379	40	76.3	*
381	39	37.8	
384	38	33.6	
388	36	70.7	
390	35	19.2	
397	31	67.4	
399	30	45.2	
401	29	43.1	*
402	29	79.4	*
406	28	54.1	
407	27	60.2	

SBHM1 E

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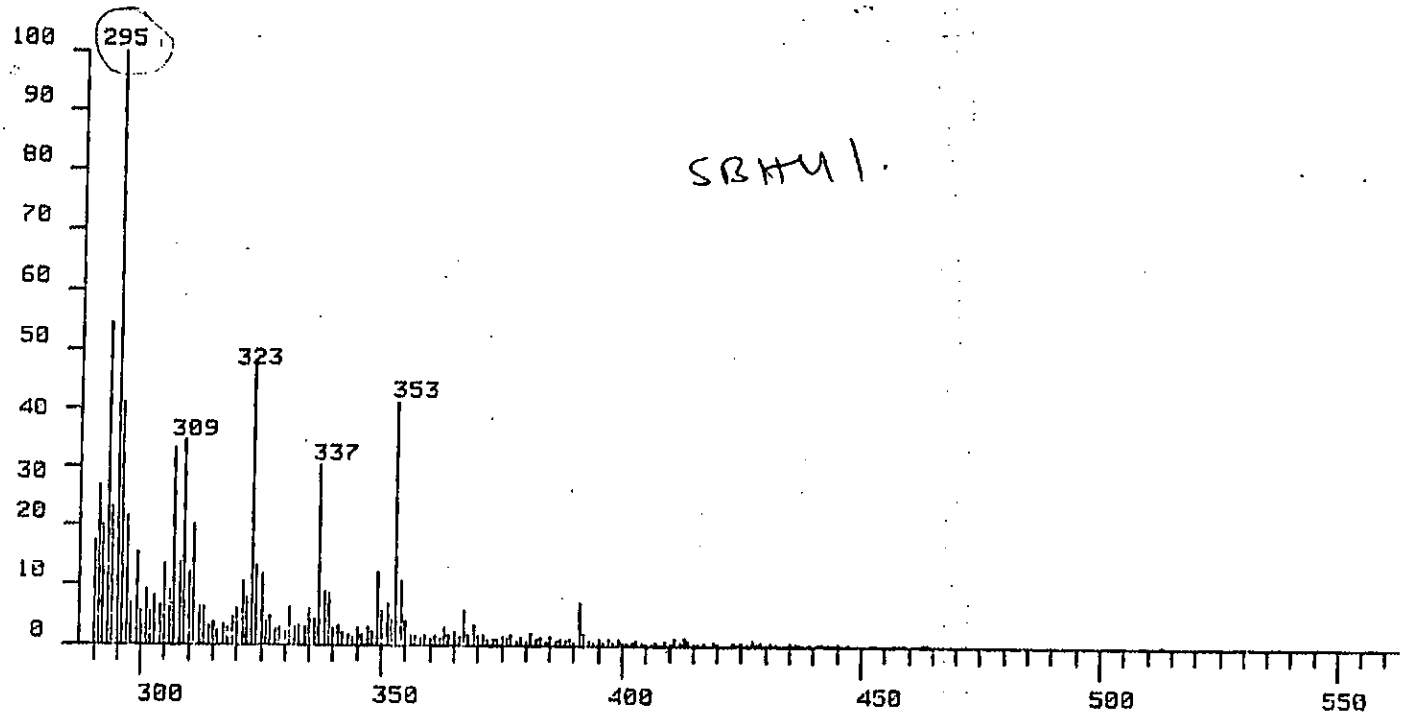

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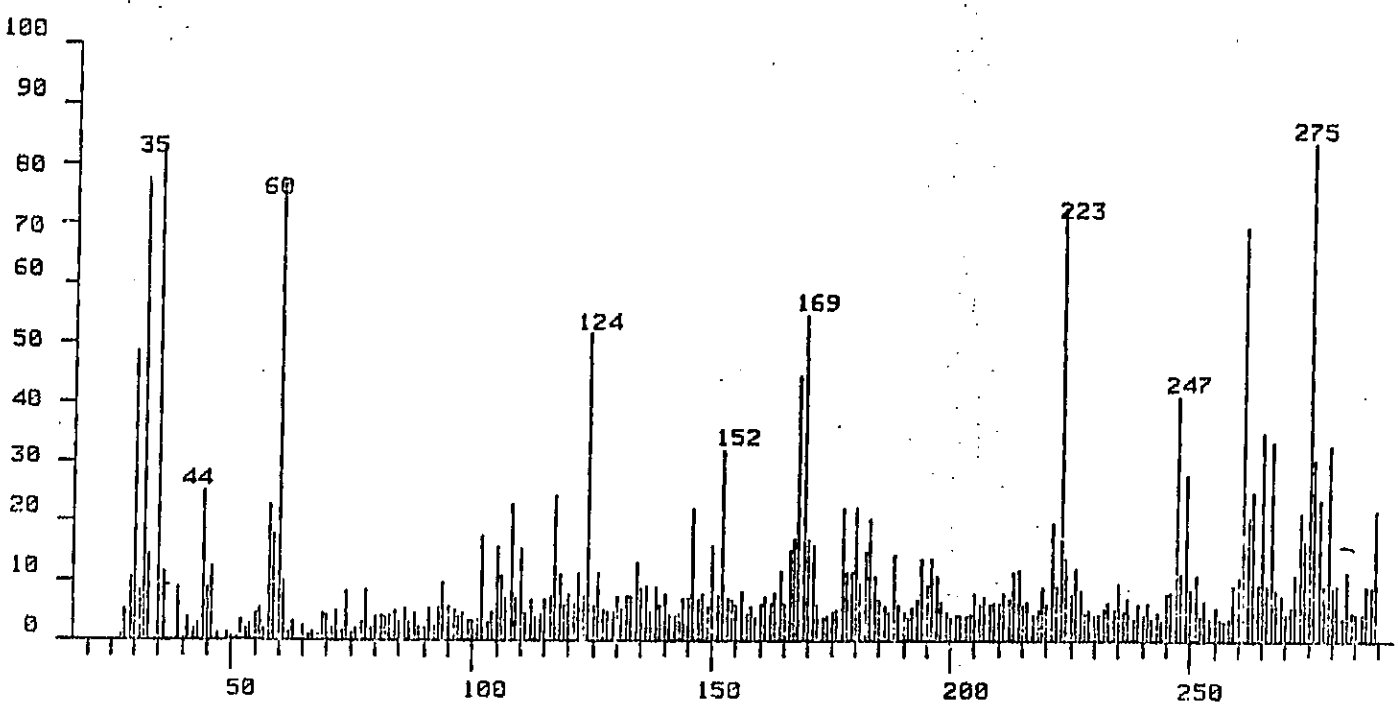

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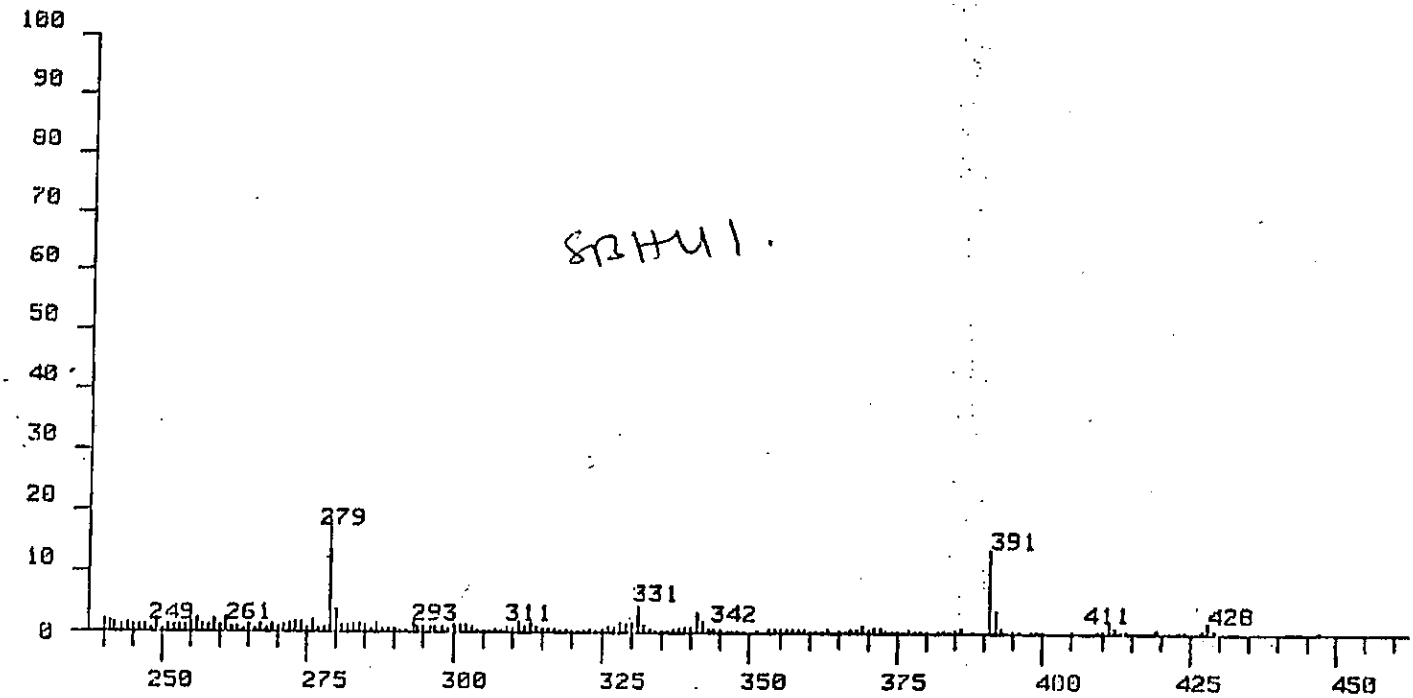

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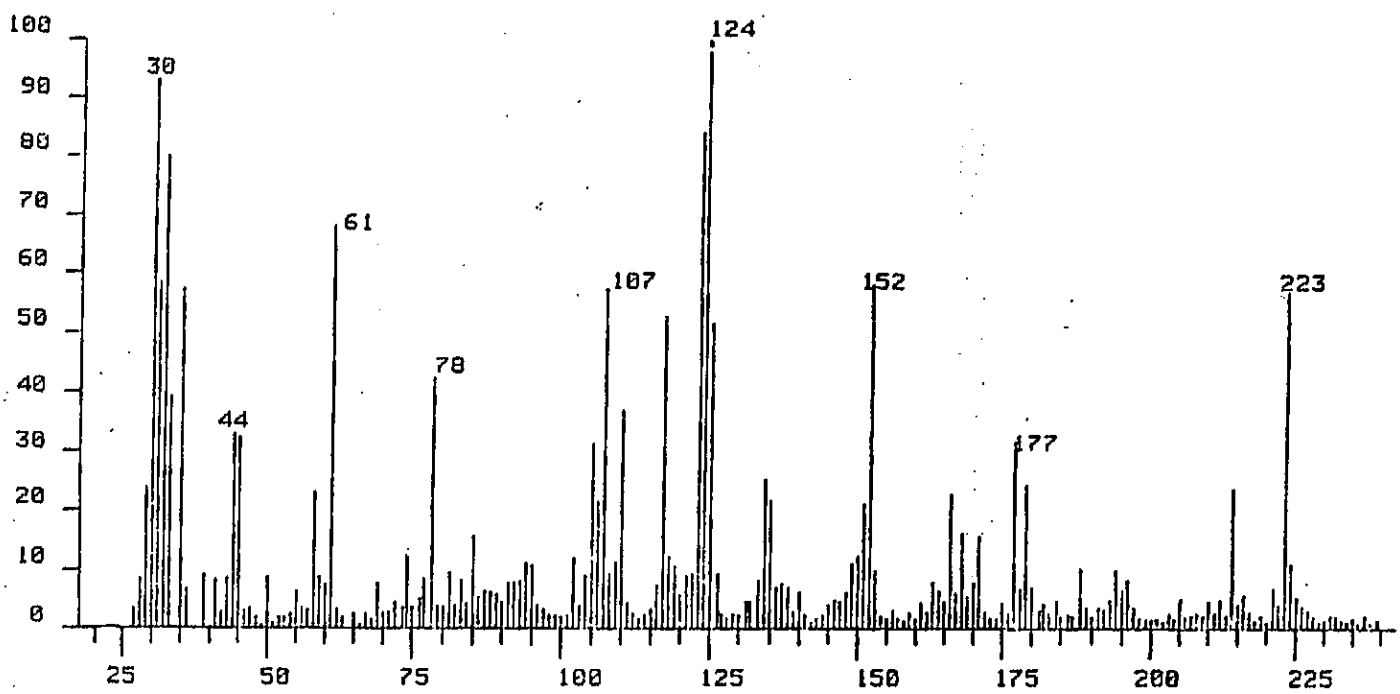


PAGE

PEAK NO.	MEASURED MASS	% INT. NREF	
60	391	7.3	
62	389	3.6	
64	387	5.7	
96	355	4.0	
97	354	10.6	
98	353	41.1	
99	352	4.5	
100	351	6.8	
101	350	5.6	*
102	349	12.1	*
114	337	30.4	
128	323	48.0	
140	311	30.2	
142	309	34.8	
144	307	33.1	
154	297	21.3	
155	296	41.2	*
156	295	100.0	*
157	294	23.4	*
158	293	54.8	*
159	292	20.0	*
160	291	27.0	*
163	289	22.1	
174	279	33.0	
177	277	24.1	*
178	276	30.4	*
179	275	84.3	*
181	273	21.7	*
188	267	33.5	
190	265	35.3	*
192	263	25.3	*
193	262	20.9	*
194	261	70.1	*
209	249	28.1	
212	247	41.5	
240	223	73.3	
242	221	20.2	
281	183	20.4	
286	180	22.3	
289	177	22.3	
300	169	55.0	
301	168	44.8	
322	152	32.2	
335	146	27.1	
371	124	51.8	
381	117	24.3	
390	108	23.0	
438	60	76.9	
440	59	17.9	
443	58	22.7	
460	44	25.0	
473	35	61.8	*
474	35	21.4	*f
477	32	73.4	
481	30	33.3	*

SBHY1 C1

ALKALOID FROM HUNTERIA UMBELLATA
JAJ2 SBHU-1 GAM 22 VIII 86

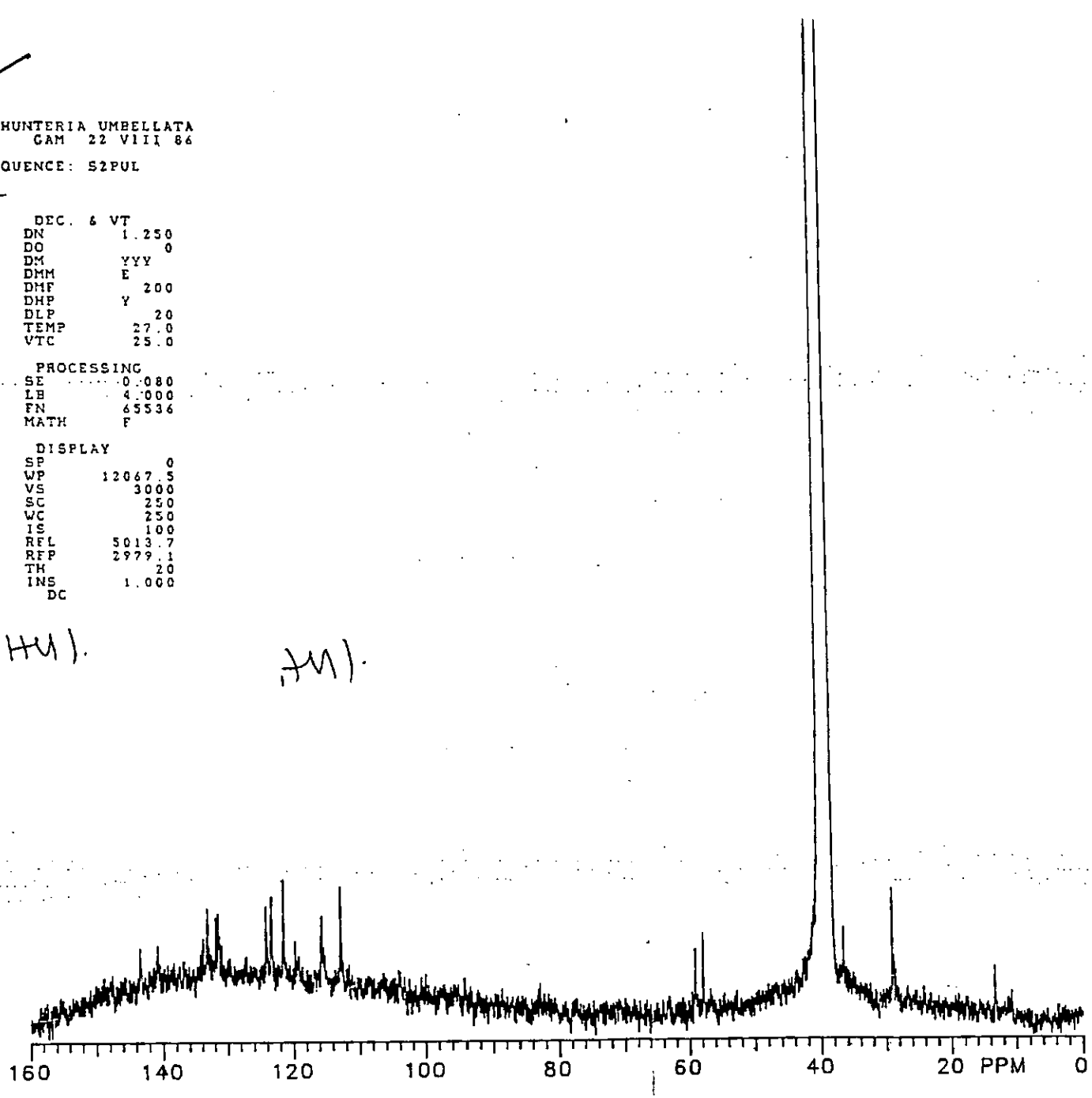
EXP1 PULSE SEQUENCE: S2PUL
DATE 22-08-86
FILE ~~S22C~~

ACQUISITION		DEC. & VT	
TN	13.250	DN	1.250
SW	18867.9	DO	0
AT	0.795	DM	YYY
NP	30016	DMM	E
PU	5.0	DMF	200
F1	0	DHP	Y
D1	0	DLP	20
D2	0	TEMP	27.0
TO	0	VTC	25.0
NT	400000		
CT	400000	PROCESSING	
CTTIME	60.000	SE	0.080
FB	10400	LB	4.000
BS	128	FN	65536
SS	0	MATH	F
IL	N		
IN	N	DISPLAY	
DP	Y	SP	0
		WP	12067.5
		VS	3000
		SC	250
		WC	250
		IS	100
		RFL	5013.7
		RFP	2979.1
		TH	20
		INS	1.000
		DC	

SBHU1.

SBHU1.

233



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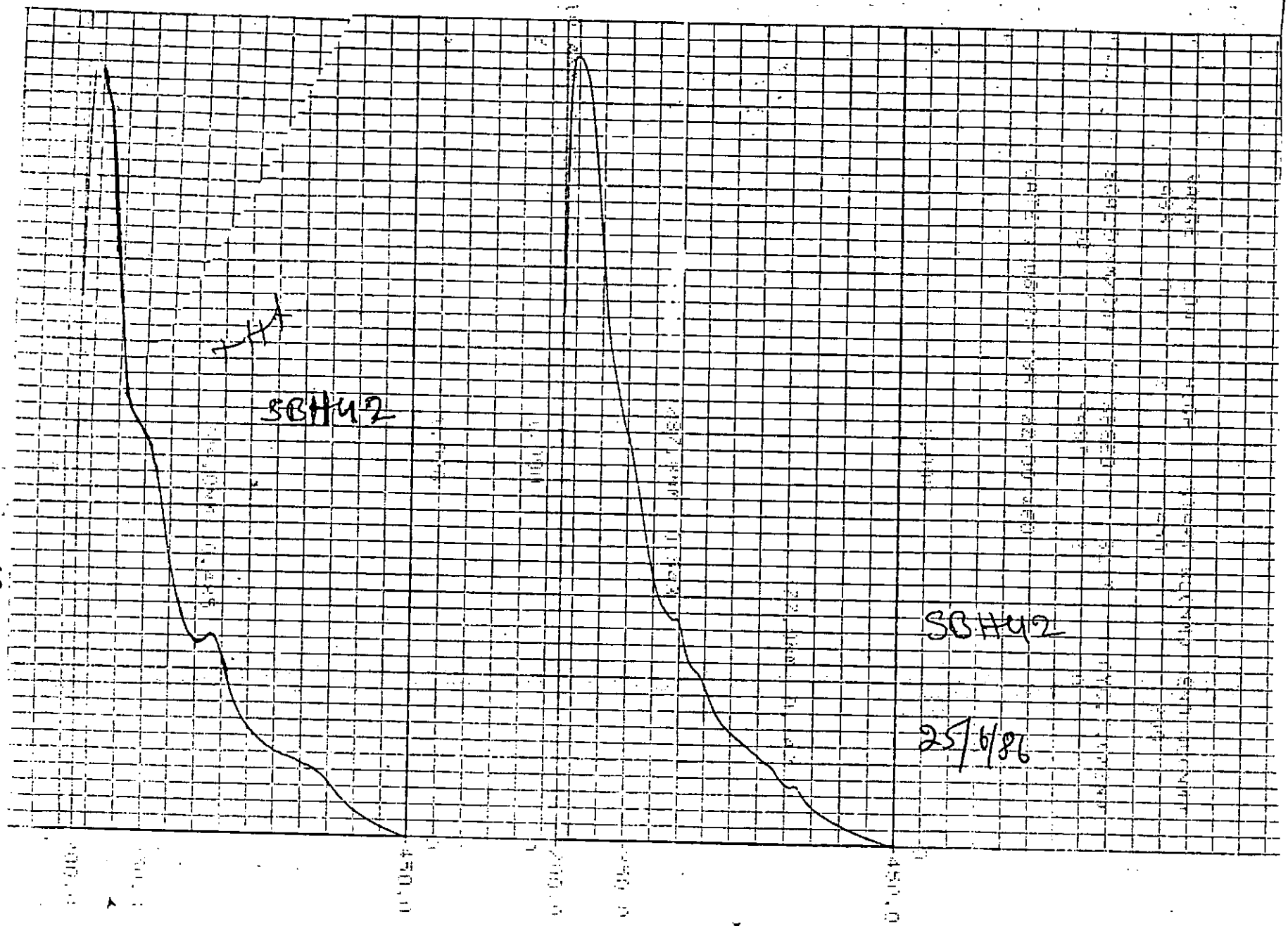

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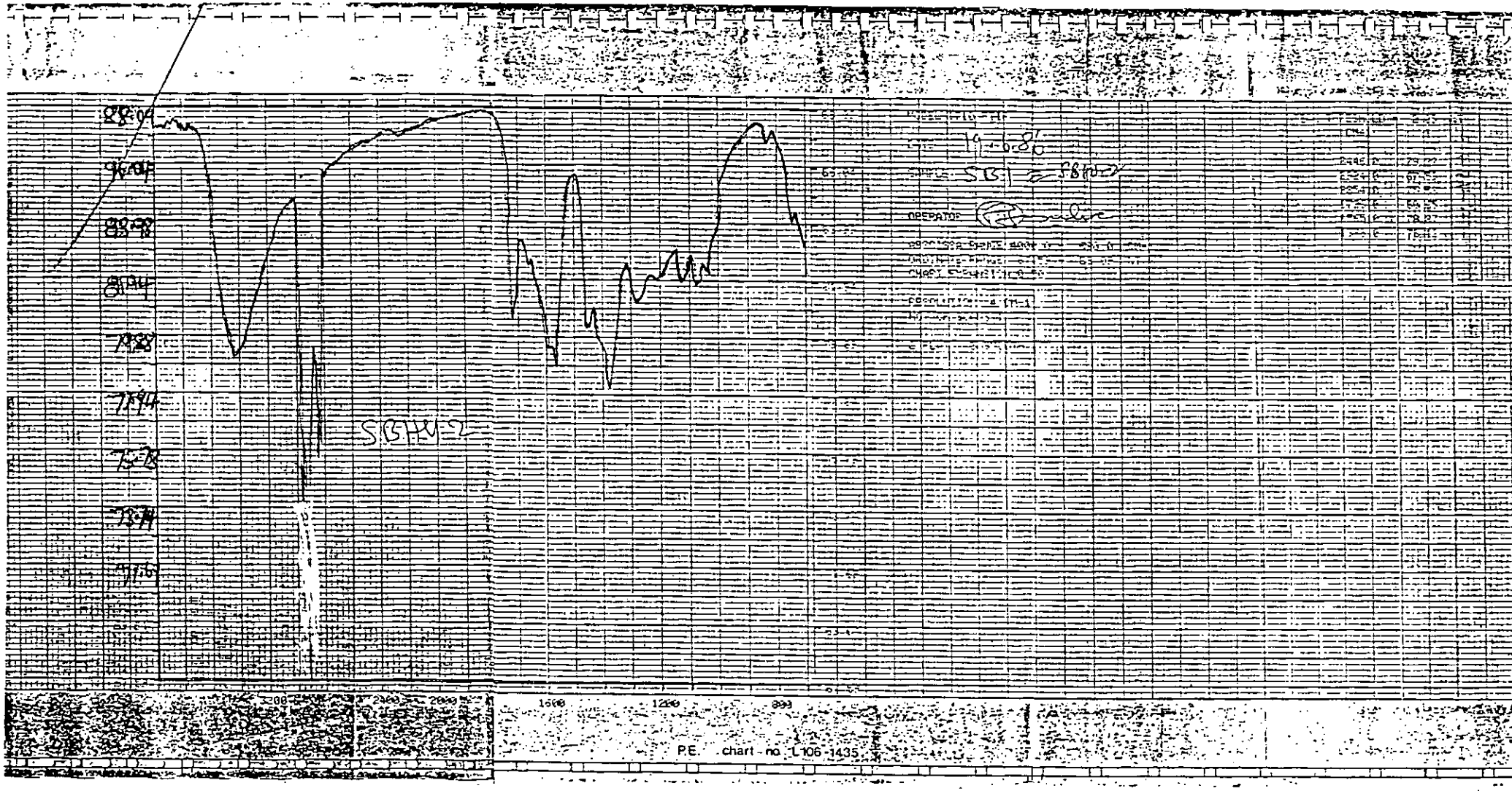
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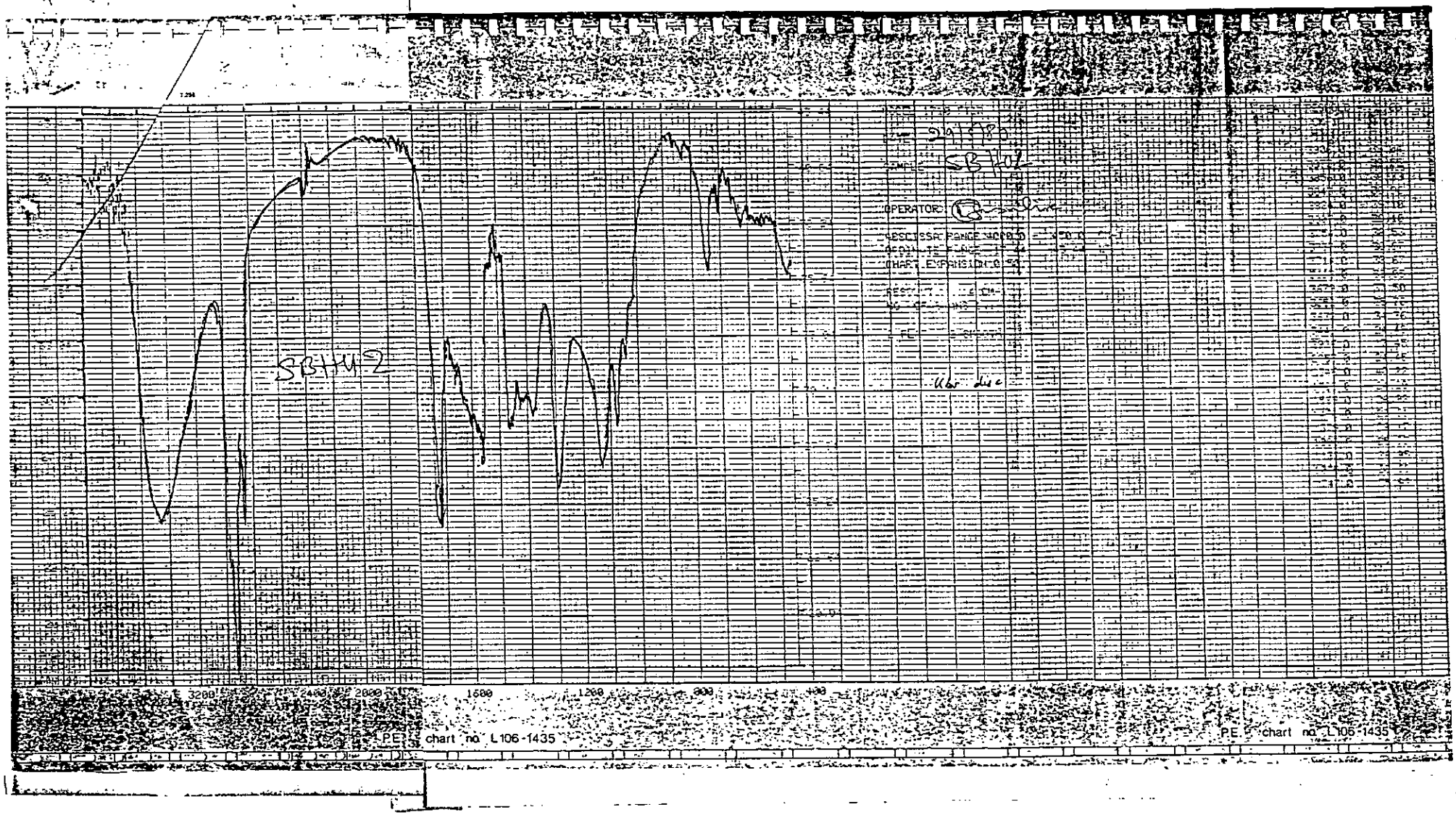
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P.E. chart no. L106-1435
P.E. chart no. L106-1435

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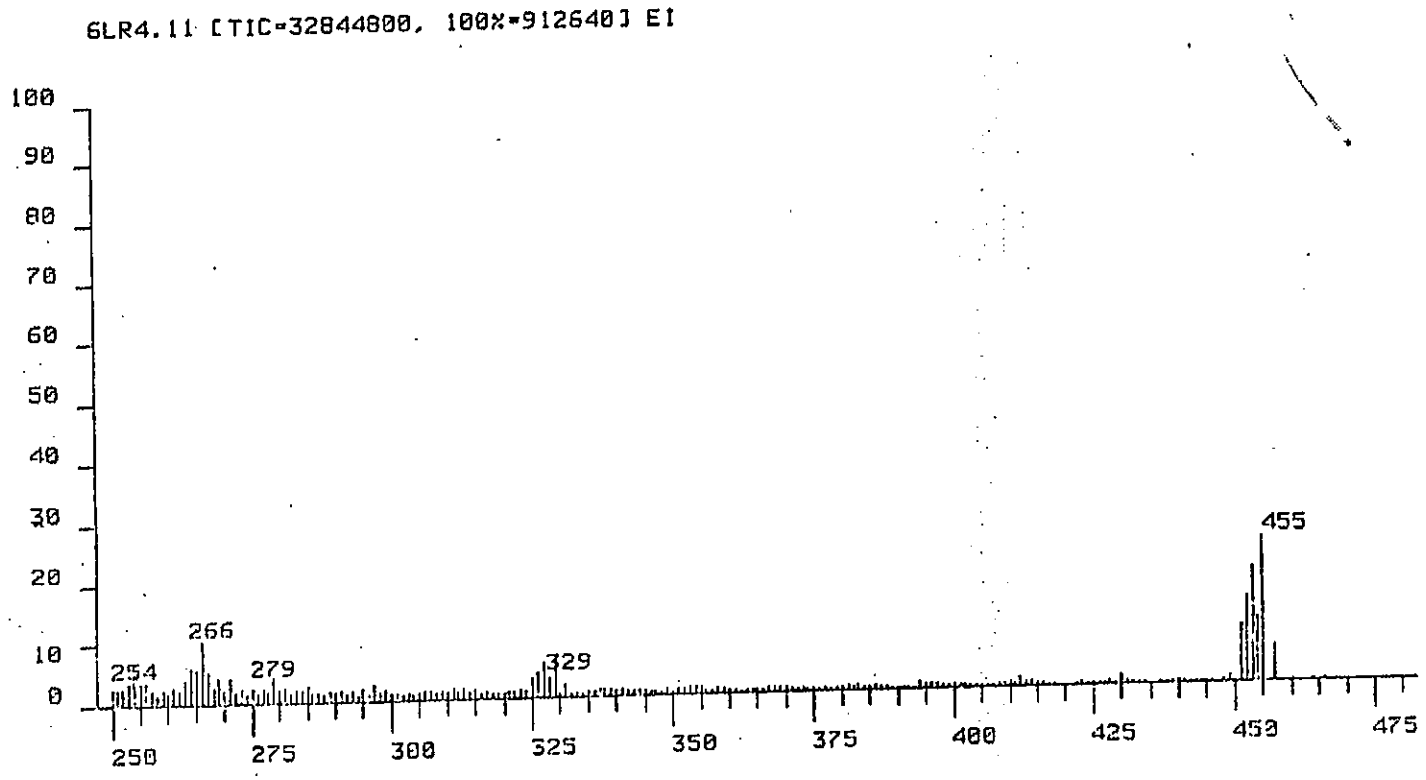
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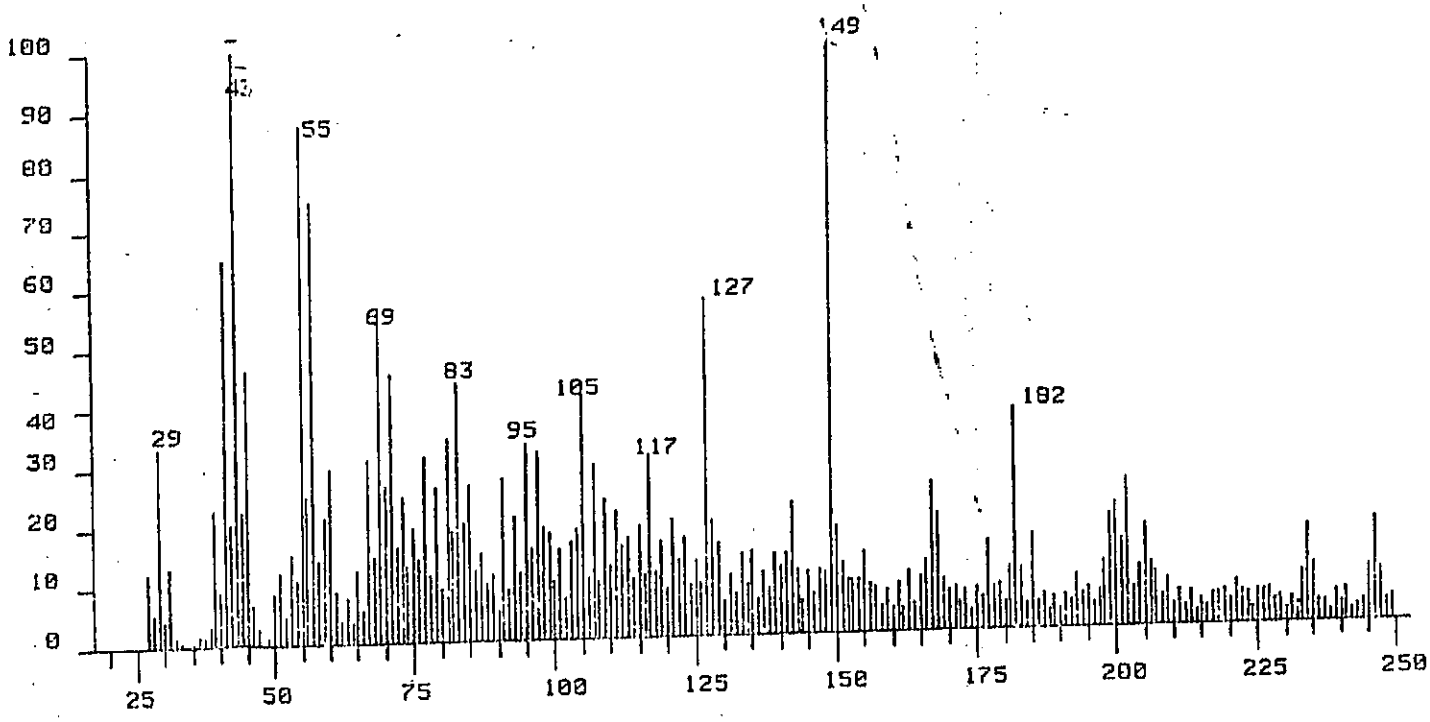
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127
149
182
25
50
75
100
125
150
175
200
225
250

PAGE 1

PEAK NO	MEASURED MASS	% INT. NREF	
5	457	5.9	
6	455	24.1	*
7	454	11.0	*
8	453	19.7	*
9	452	14.2	*
10	451	9.4	*
125	331	2.0	
127	329	6.0	
128	328	3.3	
129	327	5.7	
130	326	4.1	
131	325	3.3	
159	297	2.5	
171	285	2.6	
172	284	2.0	
173	283	2.0	
175	281	2.4	
176	280	2.0	
177	279	4.0	
179	277	2.4	
181	275	2.2	
183	273	2.5	
185	271	4.0	
186	270	2.0	
187	269	4.0	
188	268	2.6	
189	267	5.3	
190	266	10.3	
191	265	5.3	
192	264	5.6	
193	263	3.7	
194	262	2.2	
195	261	2.6	
197	259	2.2	
199	257	2.5	
200	256	3.5	
201	255	3.3	
202	254	6.6	
203	253	3.4	
204	252	2.6	
205	251	2.7	
209	247	9.0	
210	246	17.7	
211	245	9.5	
221	235	9.9	
222	234	16.6	
223	233	8.8	
47	209	8.0	
49	207	9.1	
50	206	11.0	
51	205	17.5	
52	204	10.3	
54	202	75.1	
55	201	15.1	
56	200	21.1	

SBHU2 E

52	204	10.3
54	202	25.1
55	201	15.1
56	200	21.1

PAGE 2

PEAK NO	MEASURED MASS	% INT. NREF
257	199	19.6
258	198	11.3
263	193	8.8
271	185	14.4
274	183	10.4
275	182	37.4
277	181	10.7
282	177	15.2
292	169	8.9
293	168	20.1
294	167	25.6
296	166	11.9
298	165	9.3
301	163	10.4
303	161	8.4
317	150	18.2
319	149	100.0
327	142	22.7
340	139	15.9
344	127	45.6 *
349	123	16.9
351	121	20.0
353	119	16.3
355	117	31.0
357	115	19.3
359	113	17.2
360	112	15.8
361	111	21.8
364	109	23.7
367	107	29.5
369	105	42.6
370	104	19.0
372	103	16.7
375	101	15.5
379	99	18.4
380	98	19.3
382	97	32.0
384	96	15.7
386	95	33.4
390	93	21.1
394	91	27.7
404	85	24.7
406	84	19.3
409	83	43.3
412	82	17.5
414	81	34.3
417	79	26.4
422	77	31.2
427	75	18.7
431	73	24.3
433	72	15.7
435	71	45.6
437	70	26.7
439	69	56.1
443	67	31.0

PAGE 3

PEAK NO	MEASURED MASS	% INT. NREF
455	60	29.5
457	59	21.5
461	57	74.9
463	56	24.9
465	55	87.8
479	45	44.1
481	44	21.4
483	43	95.3
485	42	19.0
487	41	61.0
492	39	21.4
504	29	17.6 *

5BHH2.

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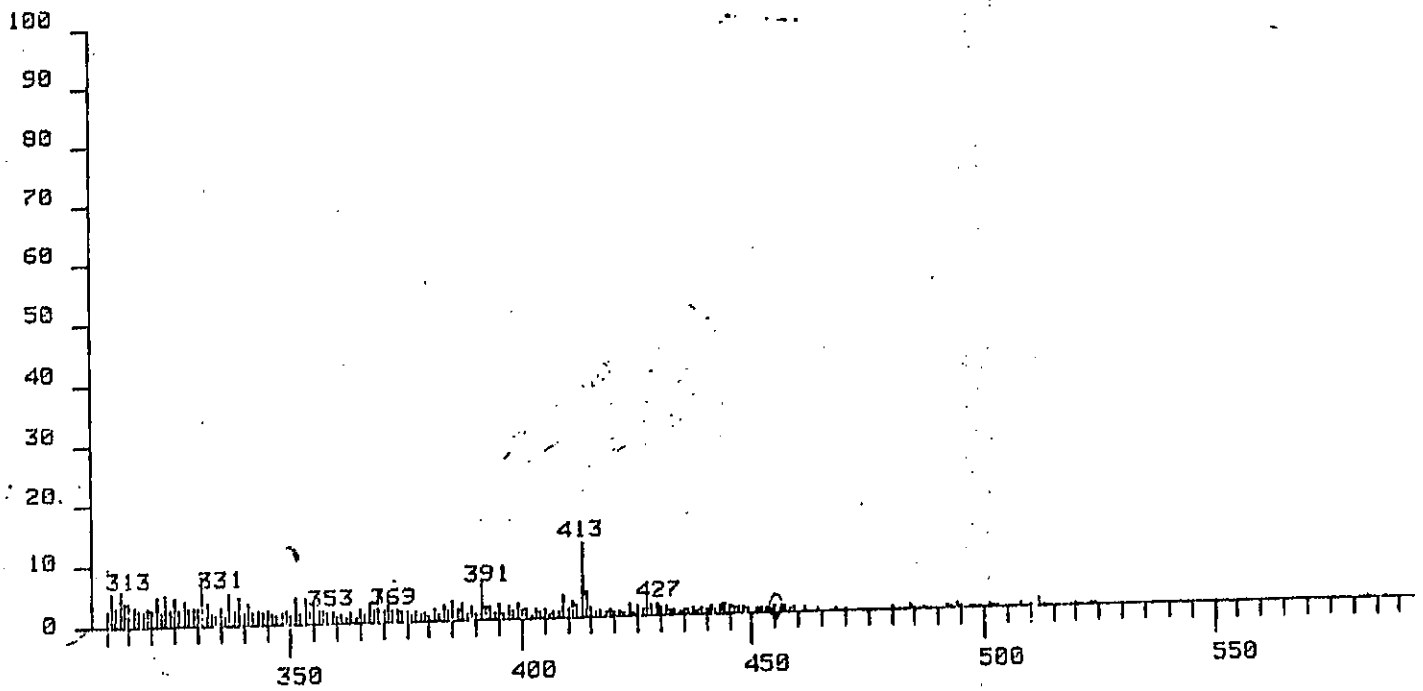
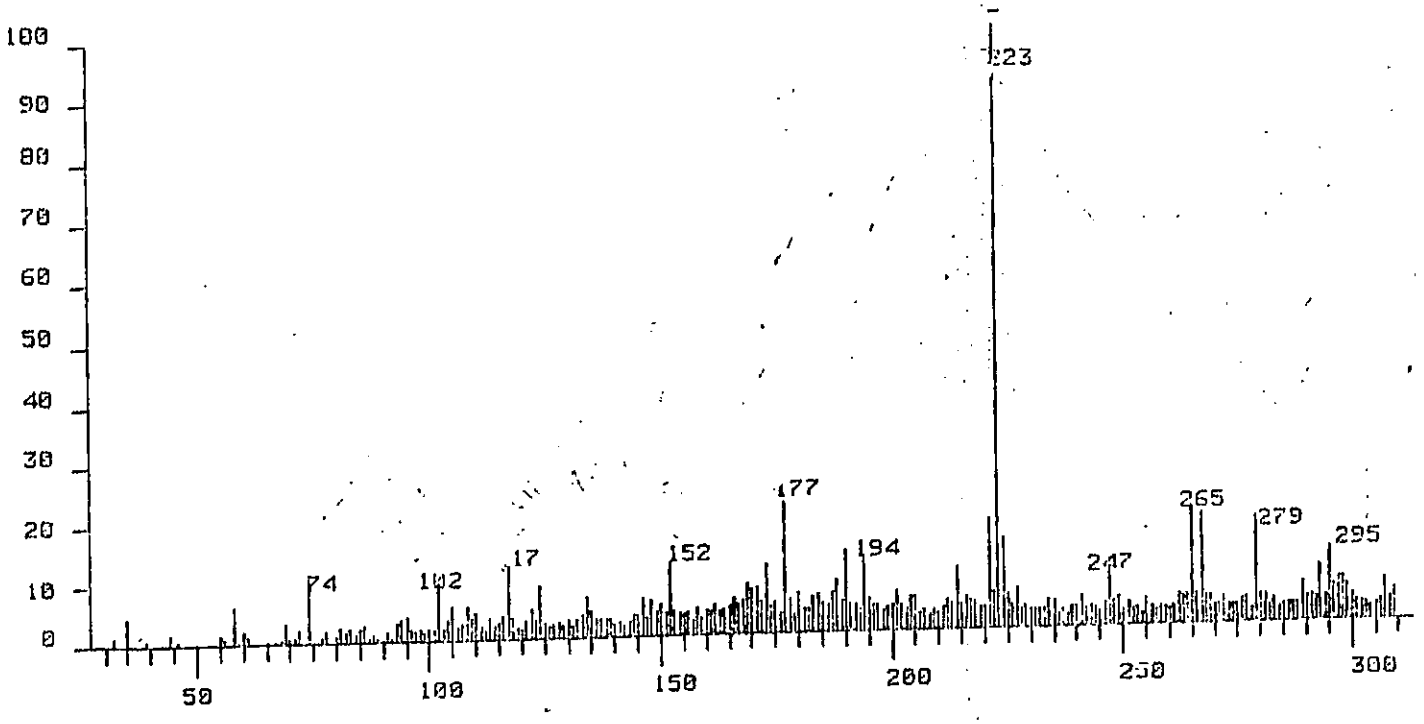




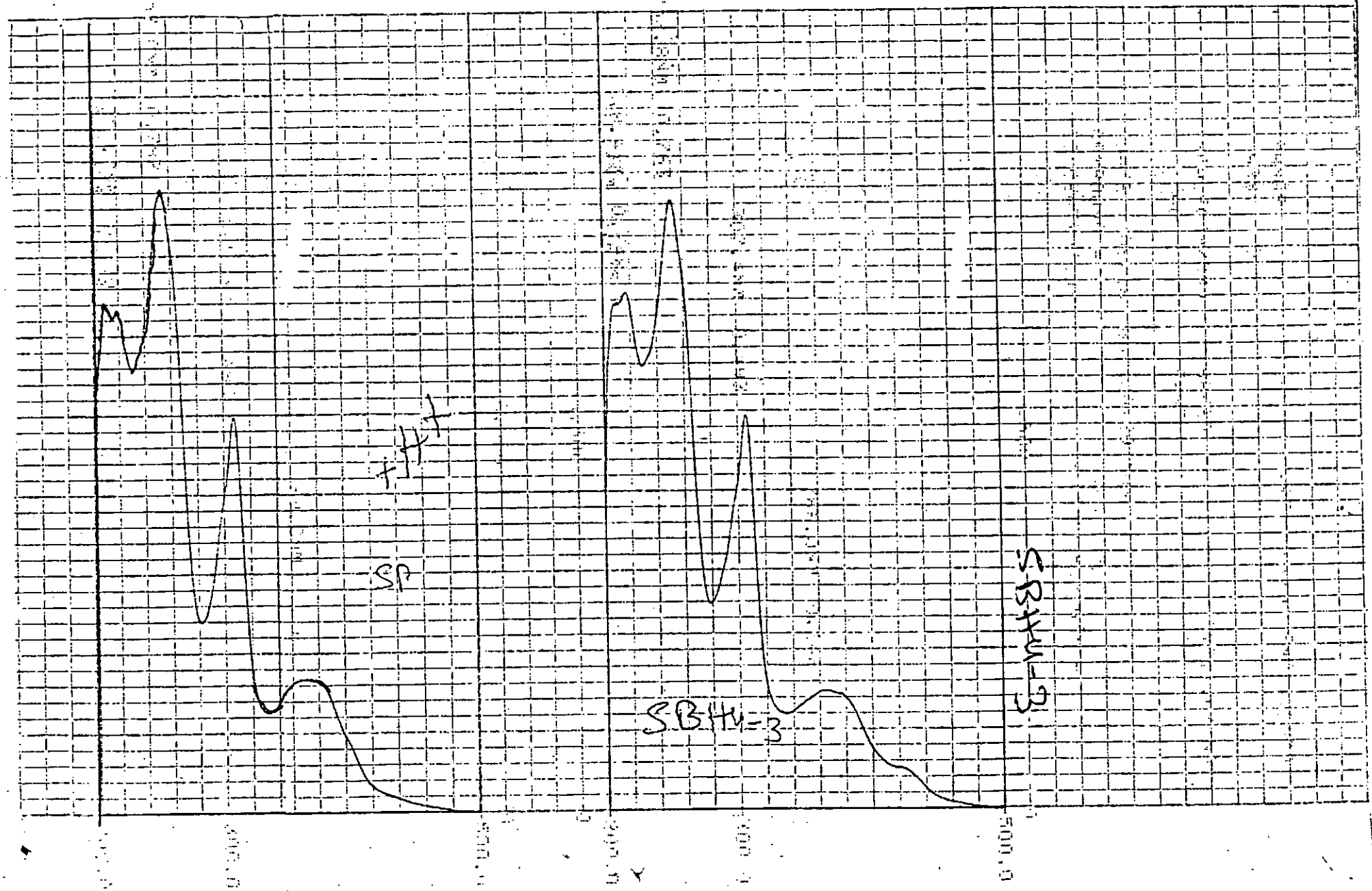
PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF
92	413	12.3
114	391	7.0
136	369	5.0
168	337	5.3
174	331	7.9
182	323	5.2
192	313	5.6
194	311	5.4
196	309	5.1
198	307	7.0
206	299	6.1
207	298	7.2
208	297	7.2
209	296	5.9
210	295	12.4
212	293	9.2
216	289	6.6
226	279	17.8
238	267	18.5
239	266	5.2
240	265	19.1
241	264	5.3
243	262	5.2
258	247	10.8
265	241	5.2
279	227	6.5
281	225	5.8
282	224	15.3
283	223	100.0
285	222	6.2
286	221	18.5
291	216	5.4
293	214	10.2
302	205	5.6
303	204	5.7
306	201	6.6
312	195	5.5
313	194	12.5
317	190	13.4
318	189	5.1
319	188	8.5
320	187	6.7
323	184	6.4
324	183	6.1
327	180	6.7
329	178	5.7
330	177	21.8
332	175	5.3
334	173	11.5
335	172	5.5
336	171	7.7
337	170	7.4
338	169	8.2
339	168	5.8
340	167	5.1

SBHH ? Cl

PAGE 2

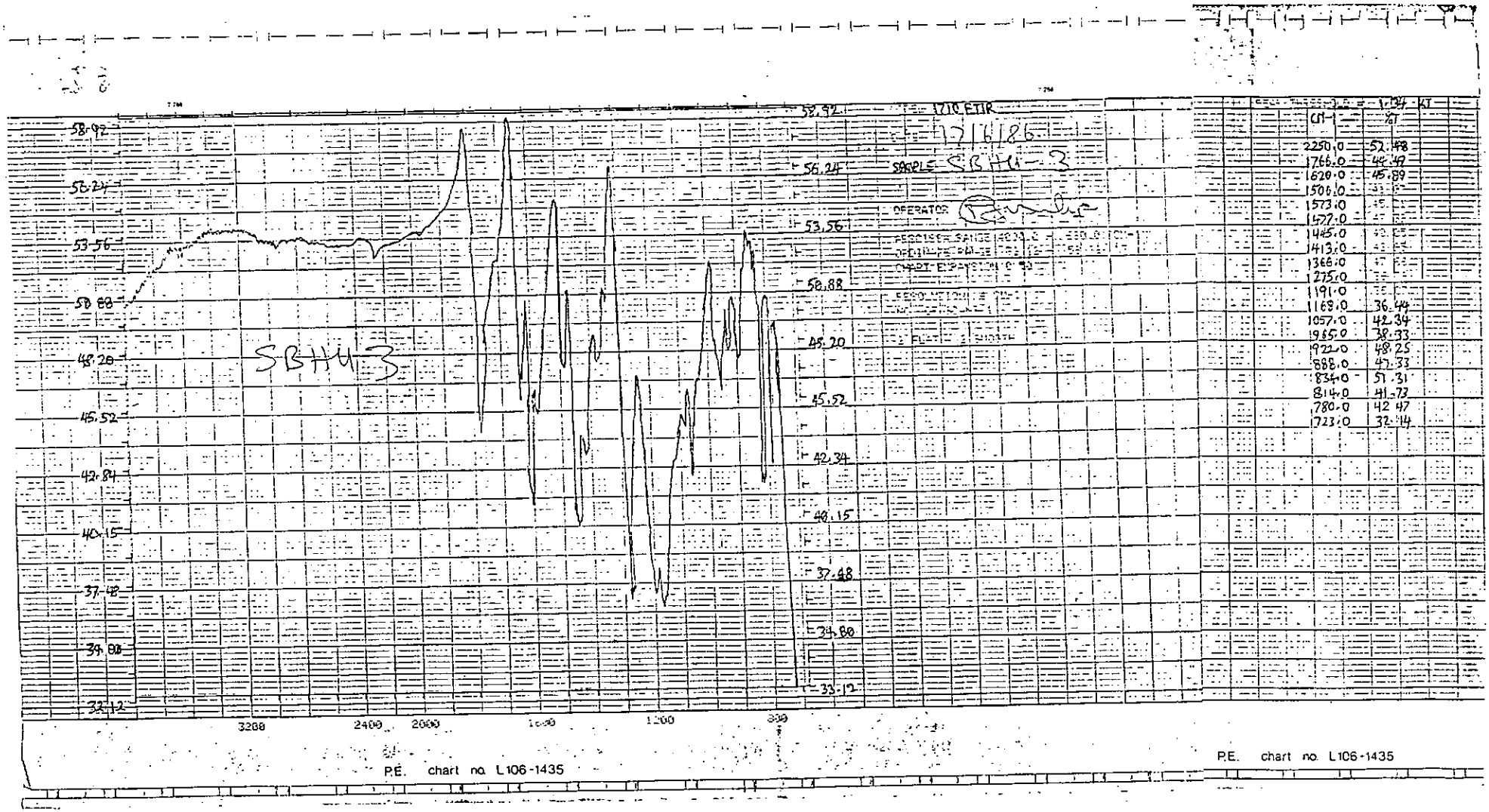
PEAK NO.	MEASURED MASS	% INT. NREF
341	166	6.1
345	162	5.1
355	152	12.1
357	150	5.4
359	148	5.9
361	146	6.2
373	134	7.0
383	124	8.7
385	122	5.1
390	117	12.2
399	108	5.6
402	105	5.9
405	102	9.7
430	74	10.8
441	58	6.2



SMD. CR. CHART 200-91522

7.3

2 of 2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SBH4-3
SAMPLE SBH4-3
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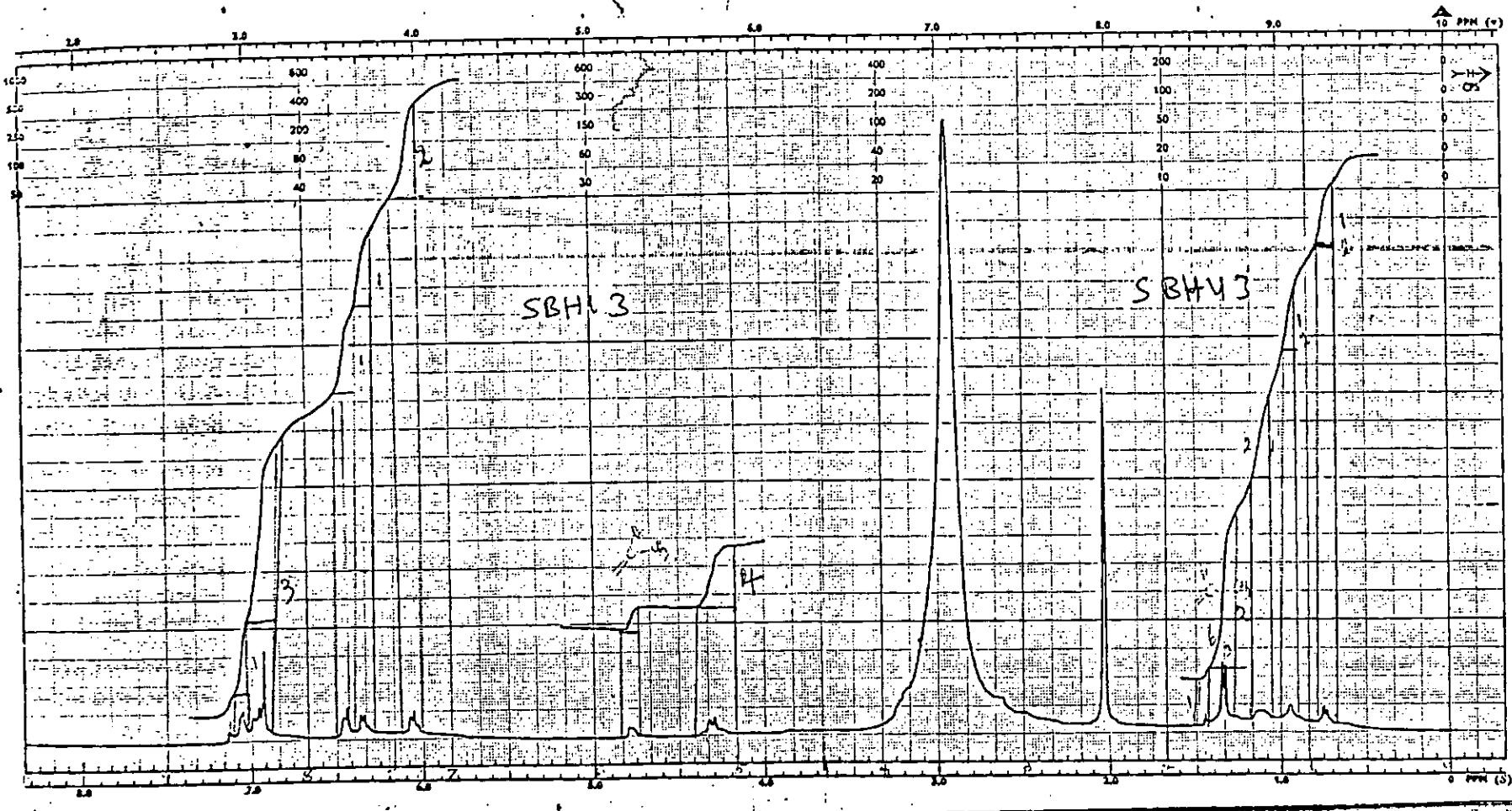
P.E. chart no. L106-1435
P.E. chart no. L106-1435



SPECTRUM NO. 786A

SAMPLE SBH13

SOLVENT DMSO

LOCK

OBSERVE

DECOUPLE

OPERATOR Heather Fox DATE 11/16/06

CHART SC300

varian

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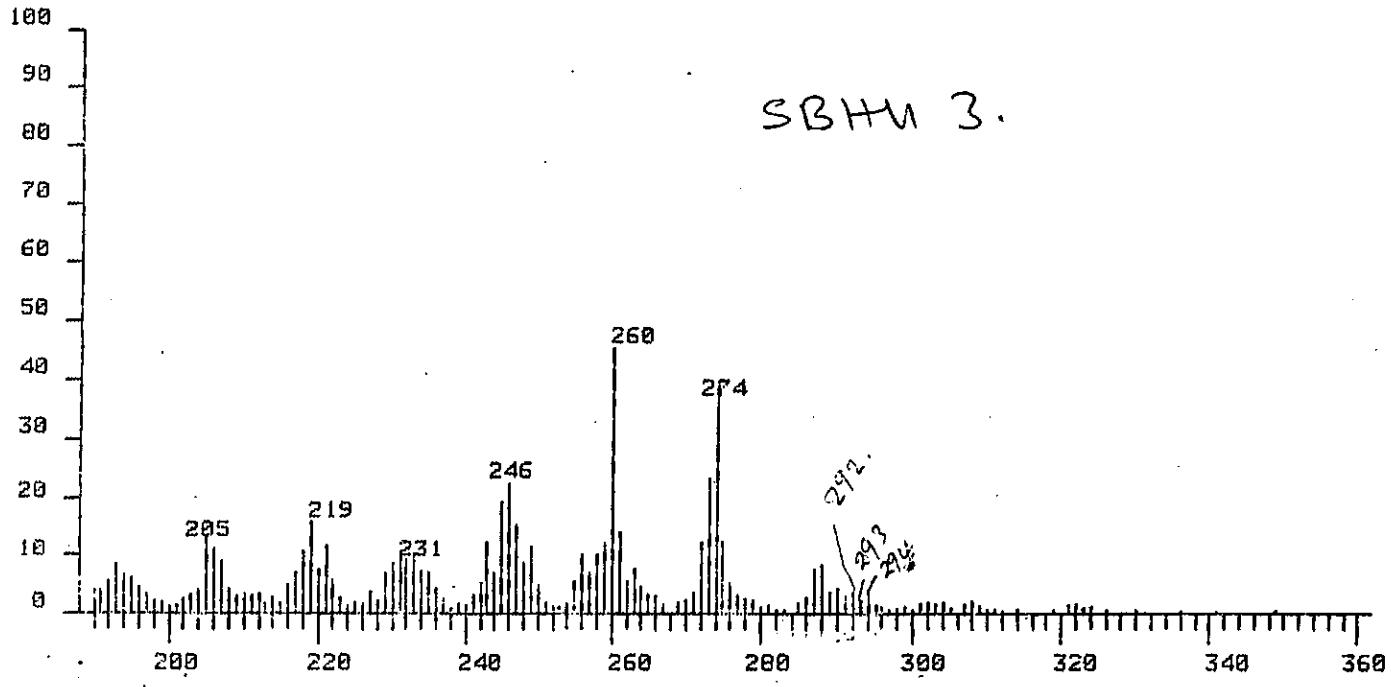
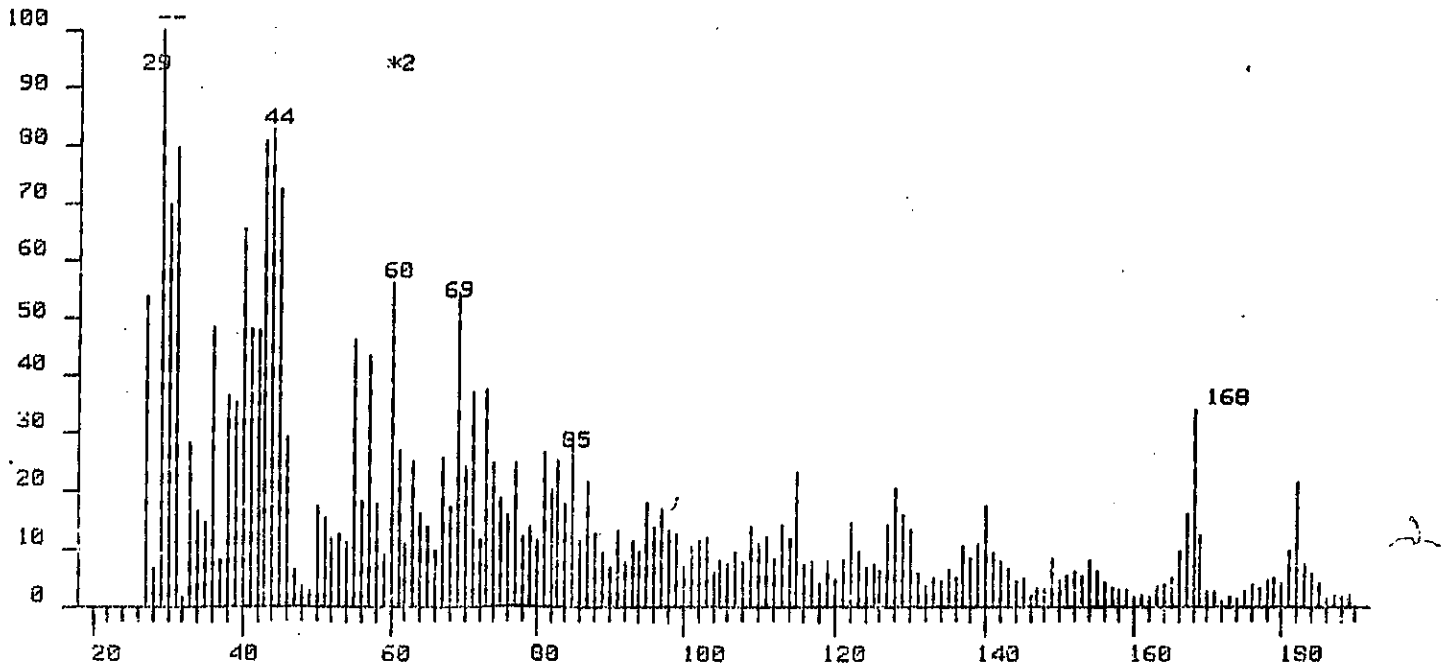
SBHM 3.





246

PEAK NO.	MEASURED MASS	% INT. NREF
47	275	6.2
48	274	19.9
49	273	11.8
50	272	6.2
60	261	7.0
61	260	22.8
62	259	6.0
63	258	5.0
65	256	5.1
72	249	5.7
73	248	4.3
74	247	7.7
75	246	11.3
76	245	9.7
79	243	6.2
89	233	5.0
90	232	4.6
91	231	5.4
92	230	4.3
101	221	5.7
103	219	7.8
104	218	5.4
115	207	4.4
117	206	5.6
118	205	6.7
141	182	10.8
142	181	4.9
154	169	6.1
155	168	17.0
156	167	9.1
157	166	4.8
182	141	4.8
183	140	8.8
184	139	5.4
185	138	4.3
187	137	5.3
198	130	6.7
200	129	7.9
202	128	10.2
204	127	7.0
209	123	4.9
211	122	7.4
212	121.5	-
223	115	11.6
225	114	5.8
226	113	7.0
227	112	4.2
228	111	6.0
230	110	5.5
232	109	6.8
236	107	4.7
242	103	6.1
244	102	5.7
246	101	5.3
248	99	6.3

SBH43 E1

PAGE 2

PEAK NO.	MEASURED MASS	% INT NREF	
249	98	5	
250	97	8.5	
251	96	6.9	
253	95	9.0	
255	94	4.8	
256	93	5.7	
258	91	6.6	
261	89	4.8	
263	88	6.4	
264	87	10.7	
265	86	5.8	
266	85	14.1	
267	84	8.9	
269	83	12.4	
271	82	10.1	
273	81	13.3	
274	80	5.2	
276	79	6.9	
277	78	6.1	
278	77	12.3	
279	76	7.9	
280	75	9.3	
281	74	12.3	
282	73	18.7	
283	72	5.8	
284	71	18.4	
286	70	12.0	
288	69	27.2	
289	68	8.6	
290	67	12.8	
291	66	4.9	
292	65	6.8	
293	64	5.6	*
295	63	12.5	
296	62	5.5	
297	61	13.5	
298	60	28.1	
299	59	9.2	
300	58	17.9	
301	57	43.7	
302	56	18.4	
303	55	46.3	
304	54	1	
305	53	12.7	
307	52	11.8	
308	51	15.0	
309	50	17.1	
312	47	6.2	
313	46	29.3	
314	45	72.7	
315	44	83.0	
316	43	80.9	
317	42	48.2	
318	41	48.4	
319	40	45.8	

PAGE 3

PEAK NO	MEASURED MASS	% INT. NREF	
320	39	35.5	
321	38	34.3	
323	37	4.2	*
324	37	4.2	*
325	36	48.7	
326	35	14.6	
327	34	16.7	
328	33	28.2	
334	31	79.0	
340	30	68.0	
345	29	100.0	
352	28	5.2	*
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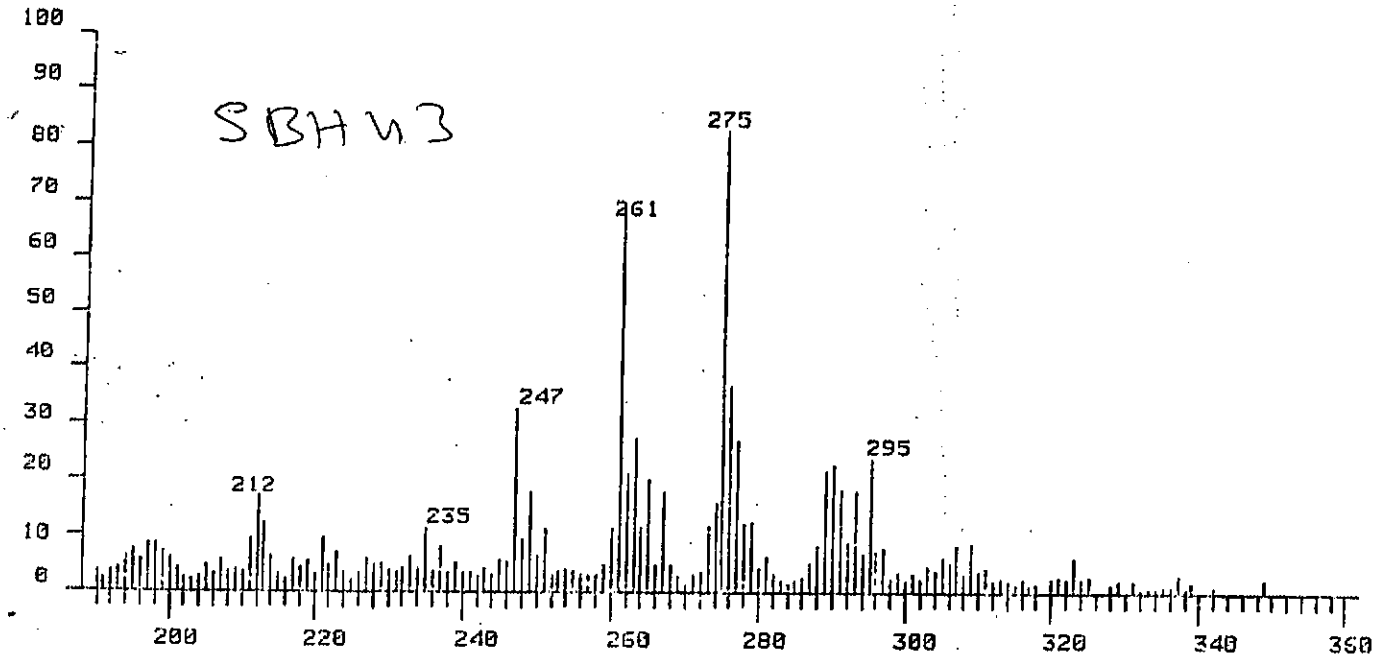
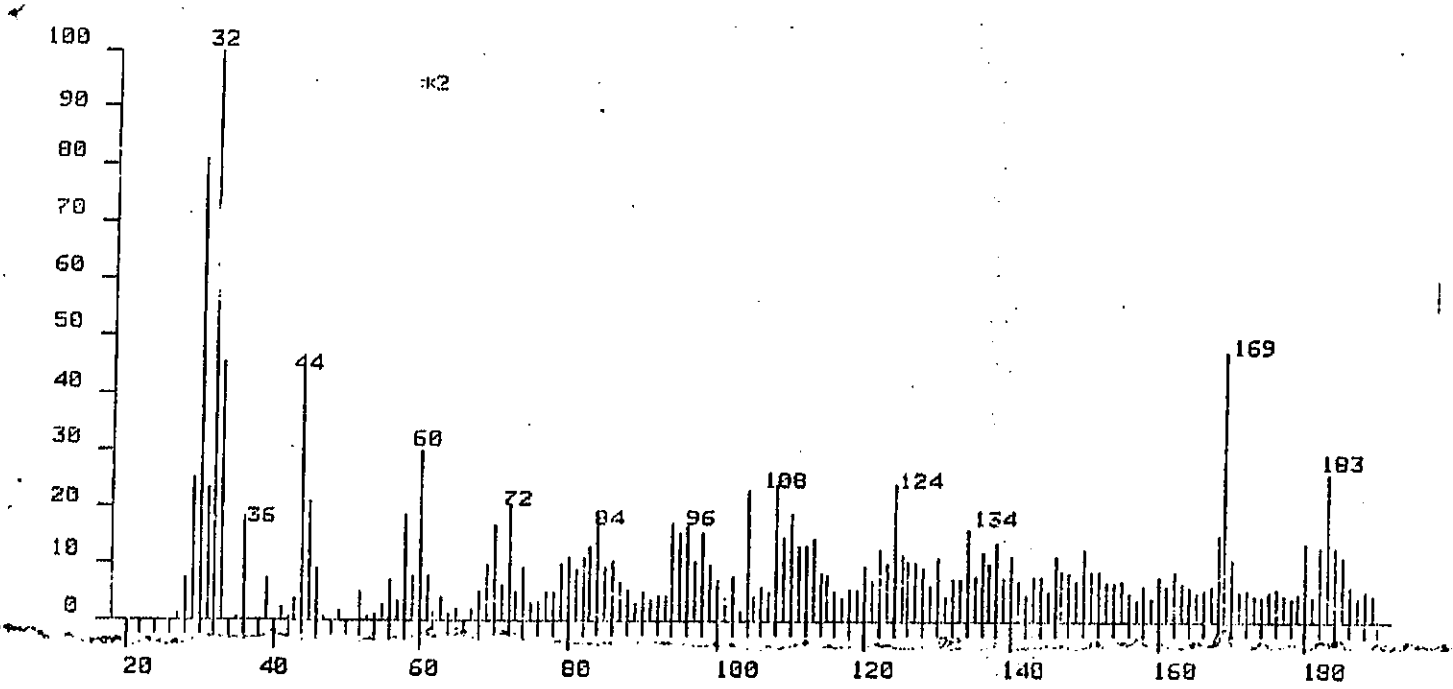




PAGE 1

PEAK NO	MEASURED MASS	% INT. NREF
11	351	1.7
12	350	1.1
13	349	3.7
21	339	1.2
22	337	2.1
24	335	1.1
32	327	1.3
34	325	2.3
35	324	1.7
36	323	3.6
37	322	1.1
38	321	2.1
50	309	6.4
51	308	4.4
52	307	9.3
54	305	3.6
62	297	9.4
63	296	7.2
64	295	29.8
65	294	11.2
66	293	39.4
67	292	5.4
68	291	8.8
69	290	4.3
70	289	7.9
80	279	6.0
82	277	7.8
83	276	5.7
84	275	15.7
85	274	4.8
86	273	12.4
91	268	5.0
92	267	23.5
93	266	5.7
94	265	17.2
95	264	3.2
96	263	8.5
97	262	6.8
98	261	20.0
99	260	3.5
104	255	3.0
108	251	3.3
110	249	8.2
111	248	4.8
112	247	16.6
113	246	4.3
122	237	3.2
124	235	4.6
126	233	3.1
132	227	3.2
136	223	3.3
138	221	5.0
146	213	11.5
147	212	18.3
148	211	4.1

SBH43 C1

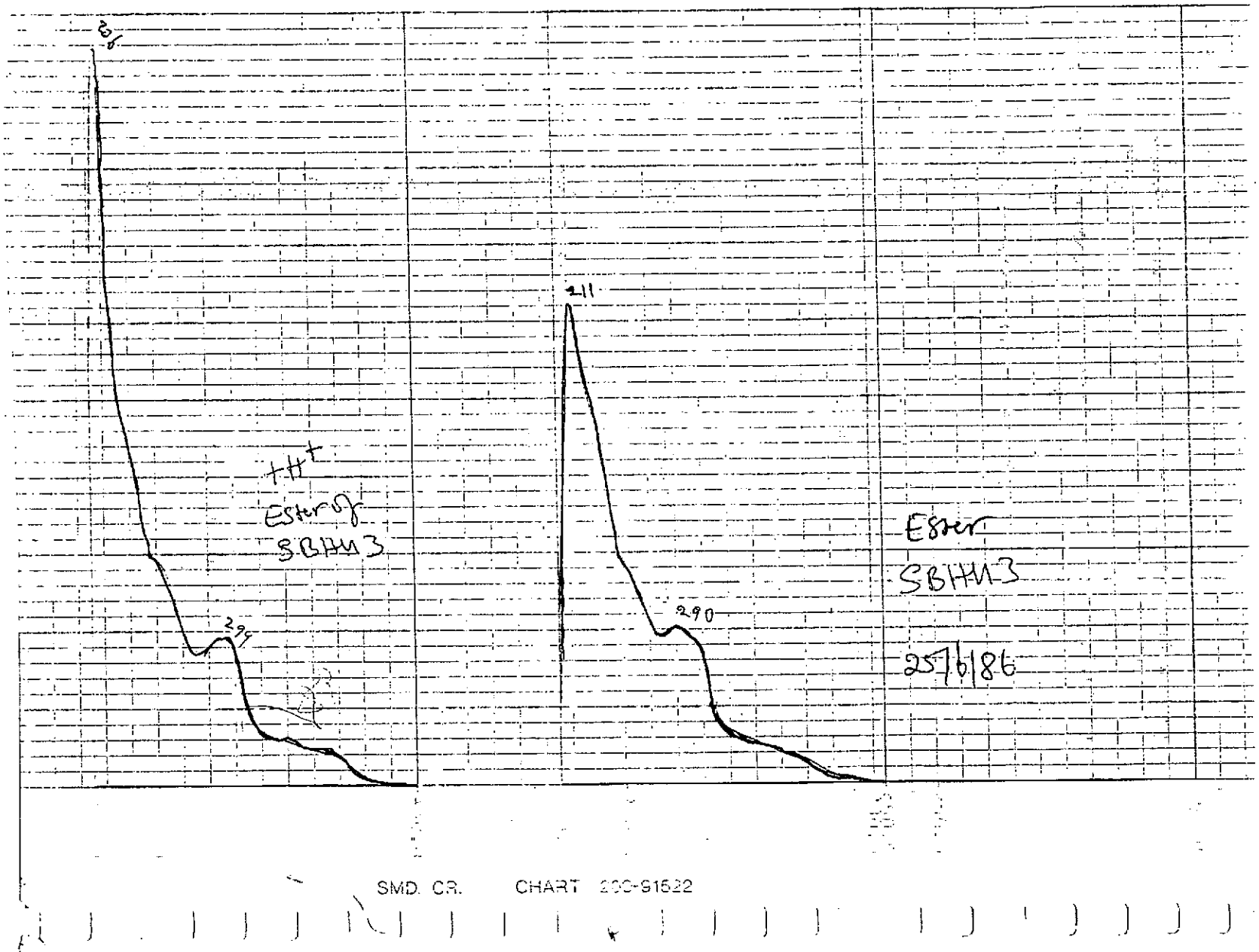
250

PEAK NO.	MEASURED MASS	% INT. NREF
152	207	3.3
153	206	3.2
159	200	4.5
161	198	4.9
162	197	3.8
163	196	4.1
164	195	3.2
165	194	3.4
173	186	3.5
174	185	11.3
175	184	5.1
176	183	14.7
177	182	9.9
178	181	4.1
179	180	16.6
188	171	3.0
189	170	7.8
190	169	23.2
191	168	9.7
192	167	3.5
193	166	3.5
195	164	3.2
196	163	3.4
197	162	3.3
199	160	3.8
203	156	3.0
205	154	4.0
206	153	4.7
207	152	3.9
208	151	3.4
209	150	7.7
213	146	4.3
214	145	3.2
215	144	3.5
216	143	3.?
218	141	3.1
219	140	5.2
221	138	7.7
223	136	4.7
225	134	5.0
229	130	4.4
230	129	3.9
232	127	4.0
233	126	4.1
234	125	3.4
235	124	11.8
236	123	3.4
237	122	3.9
239	120	4.8
241	118	3.1
242	117	3.1
243	116	3.7
244	115	5.4
245	114	4.7
246	113	3.8

PAGE 3

SBHM 3 Cl.

PEAK NO.	MEASURED MASS	% INT. NREF
247	112	4.5
248	111	4.9
249	110	8.5
250	109	4.2
251	108	7.7
257	102	4.9
260	99	3.5
261	98	5.0
262	97	4.0
263	96	4.7
264	95	5.2
265	94	4.6
269	90	3.6
272	87	3.6
273	86	4.0
274	85	4.0
275	84	5.2
278	81	4.4
279	80	3.2
280	79	5.1
281	78	3.0
285	74	5.0
287	72	6.1
289	70	4.9
290	69	4.4
298	61	8.8
299	60	11.3
301	58	9.2
303	56	4.4
304	55	5.1
308	50	7.1
311	47	6.1
312	46	8.6
313	45	20.1
314	43	4.1
316	41	3.6
318	39	5.8
320	36	10.6
323	33	53.0
324	32	75.3
325	31	57.0
327	30	100.0
328	29	36.3
329	28	12.3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SBHH3
257186
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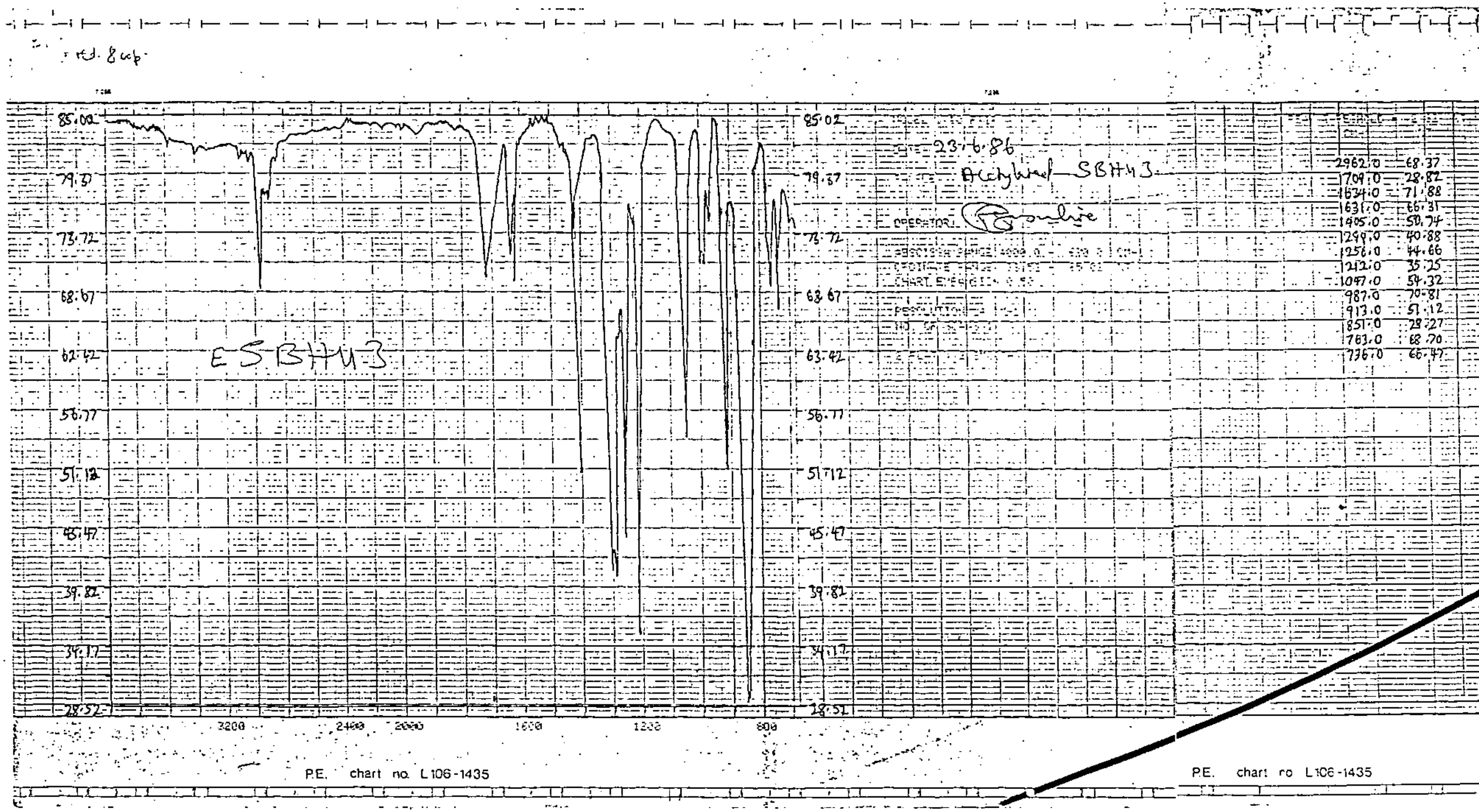
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1405.0 50.74
1299.0 40.88
1256.0 44.66
1212.0 35.25
1047.0 54.32
987.0 70.81
913.0 51.12
851.0 28.27
763.0 68.70
736.0 66.47
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1600
1200
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P.E. chart no. L106-1435

253

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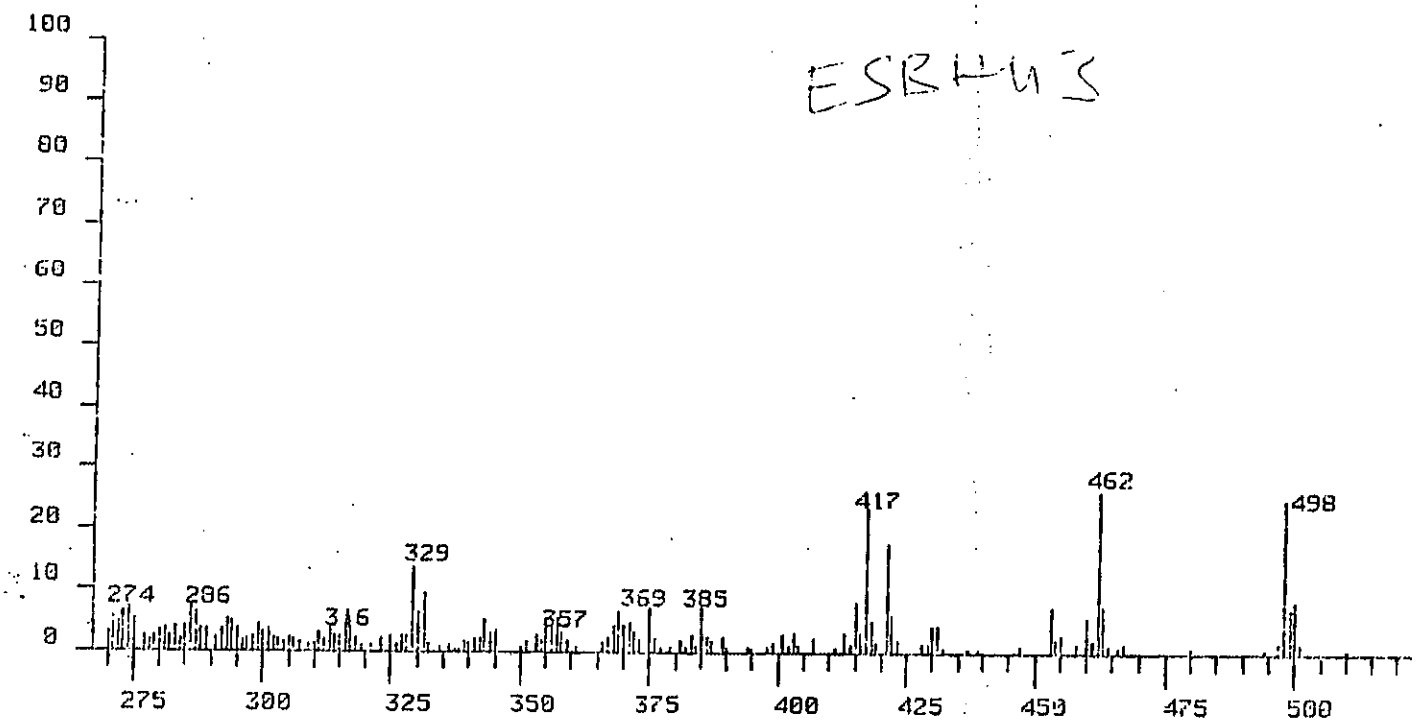
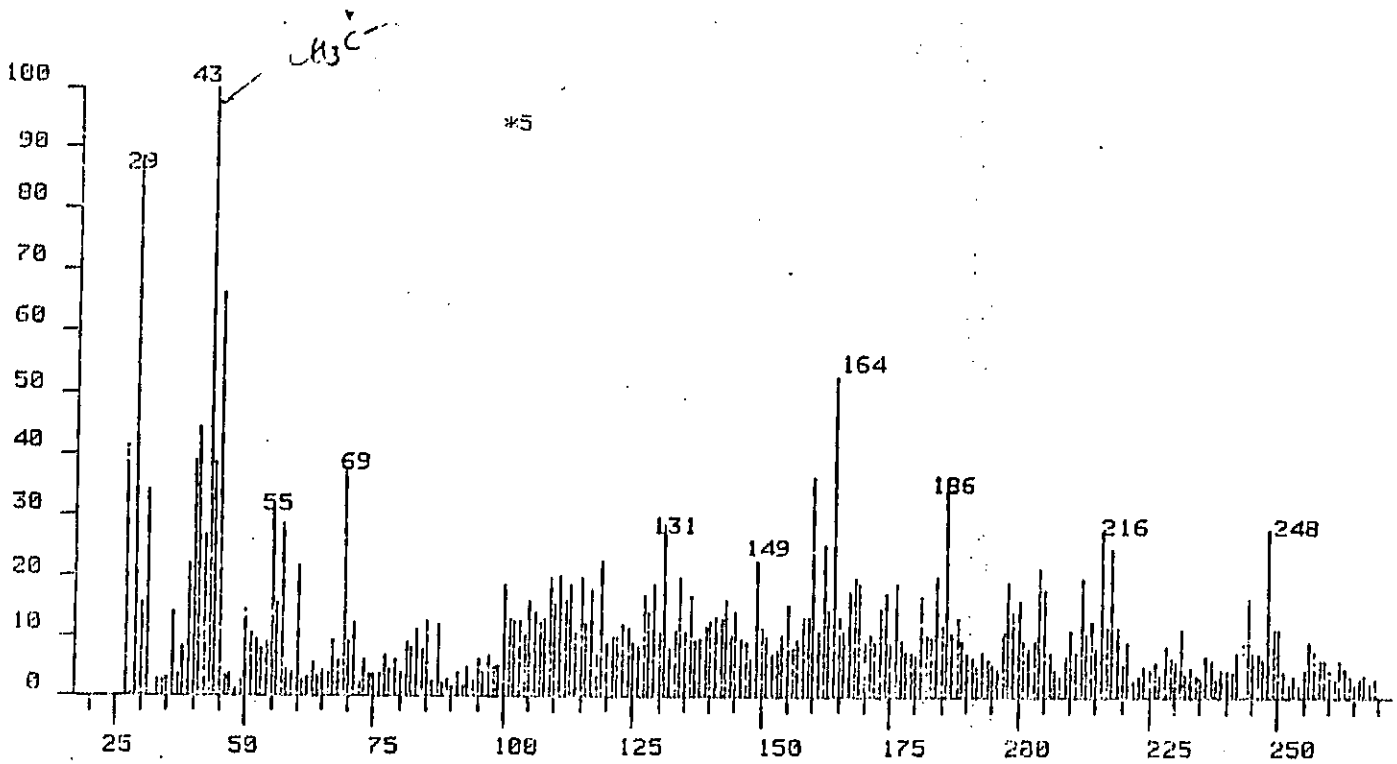




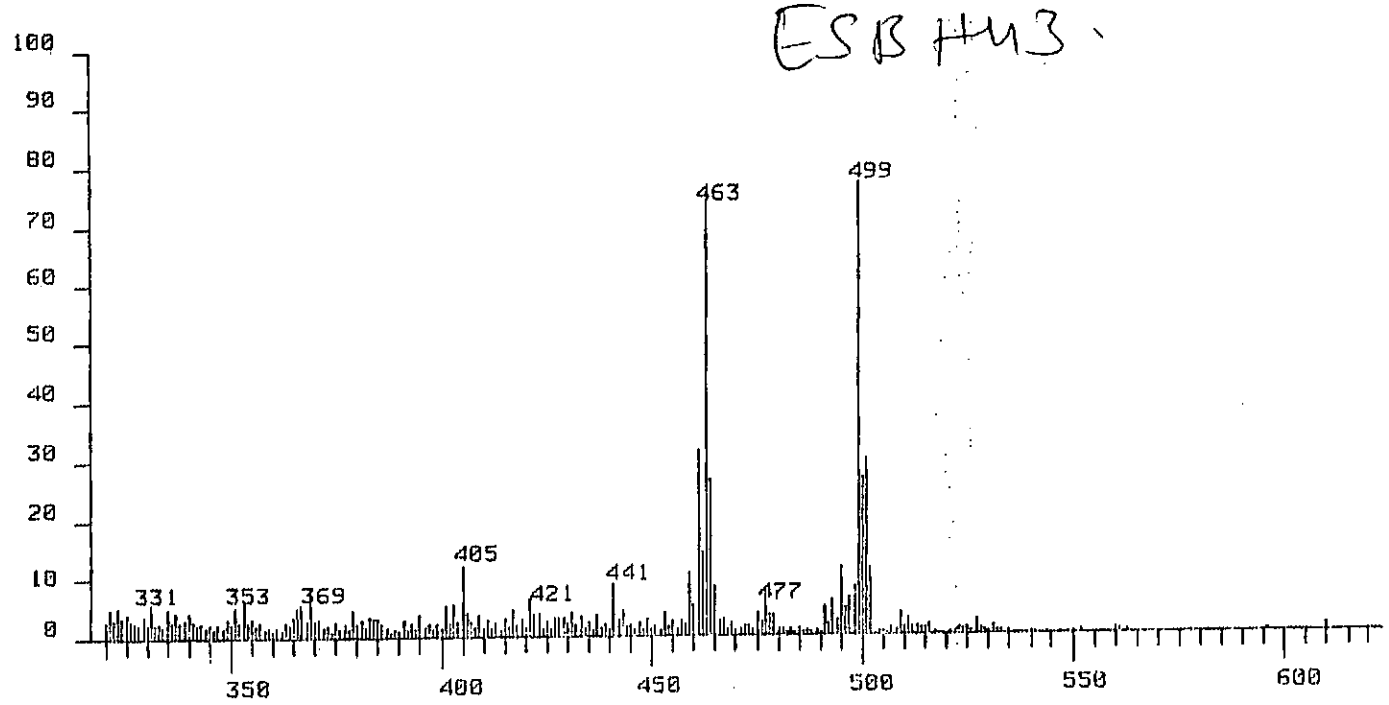
PEAK NO.	MEASURED MASS	% INT. NREF	
3	500	1.7	
5	498	5.0	
14	462	5.3	
31	421	3.5	
34	417	4.8	
36	415	1.6	
92	331	1.9	
94	329	2.8	
160	257	1.6	
161	256	1.9	
167	250	2.3	
168	249	2.3	
169	248	5.6	
173	244	3.3	
174	243	1.8	
175	242	1.5	
231	186	7.1	
253	164	10.6	
257	160	7.3	
323	97	6.9	
325	95	6.3	
333	87	12.2	
335	85	6.6	*
337	84	8.0	
338	83	11.3	
339	82	8.4	
340	81	9.2	
342	79	6.4	
344	77	6.8	
348	73	6.5	
350	71	12.4	
351	70	9.5	
352	69	12.5	*
353	69	24.8	*
355	67	9.6	
362	60	21.7	
365	57	28.6	
366	56	15.3	
367	55	31.2	
368	54	9.2	
369	53	7.9	
370	52	9.3	
371	51	10.7	
372	50	14.4	
377	45	66.3	ethers
378	44	38.7	
379	43	100.0	
380	42	27.0	
381	41	44.3	
383	40	33.1	*
384	39	22.1	
388	36	14.0	
393	31	34.1	ethers
394	30	15.5	
395	29	88.4	

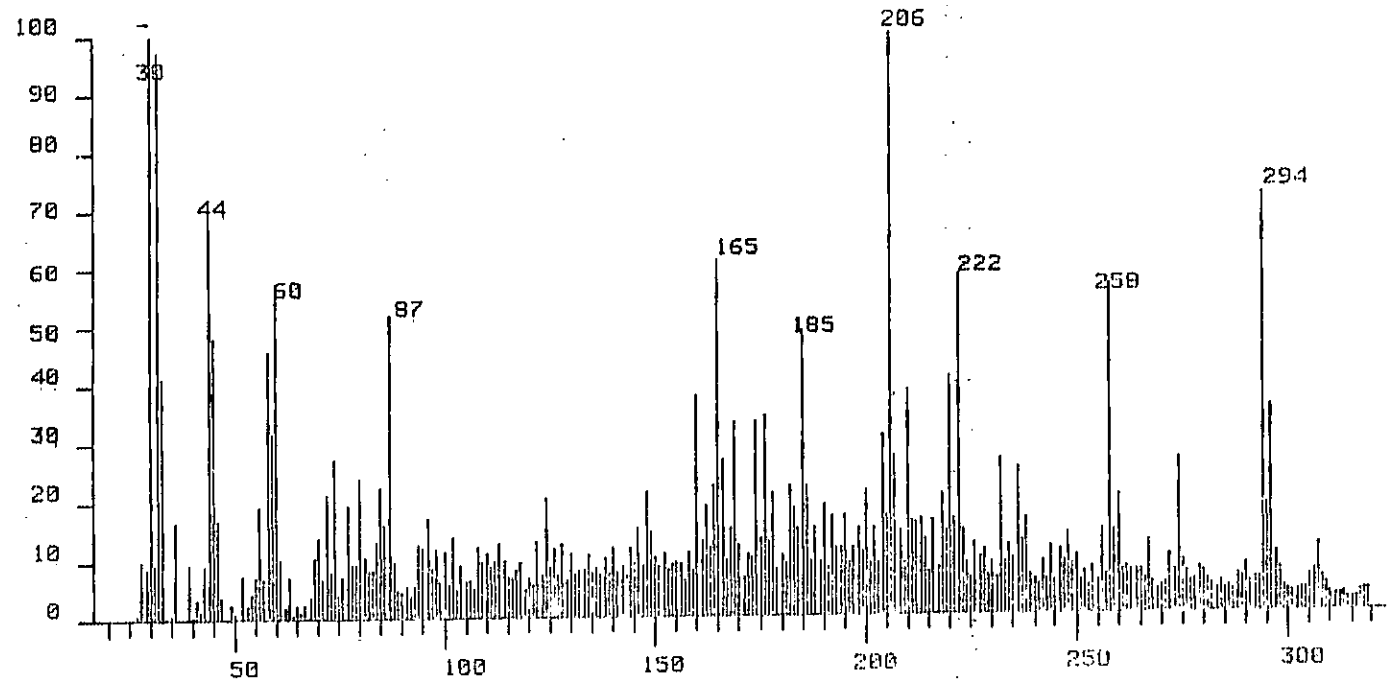
ESBHU3

ESBHU3 EI.

27LR1.20 [TIC=50998272, 100%=10586241] +VE CI, REAGENT:AMMONIA

ESB #43.



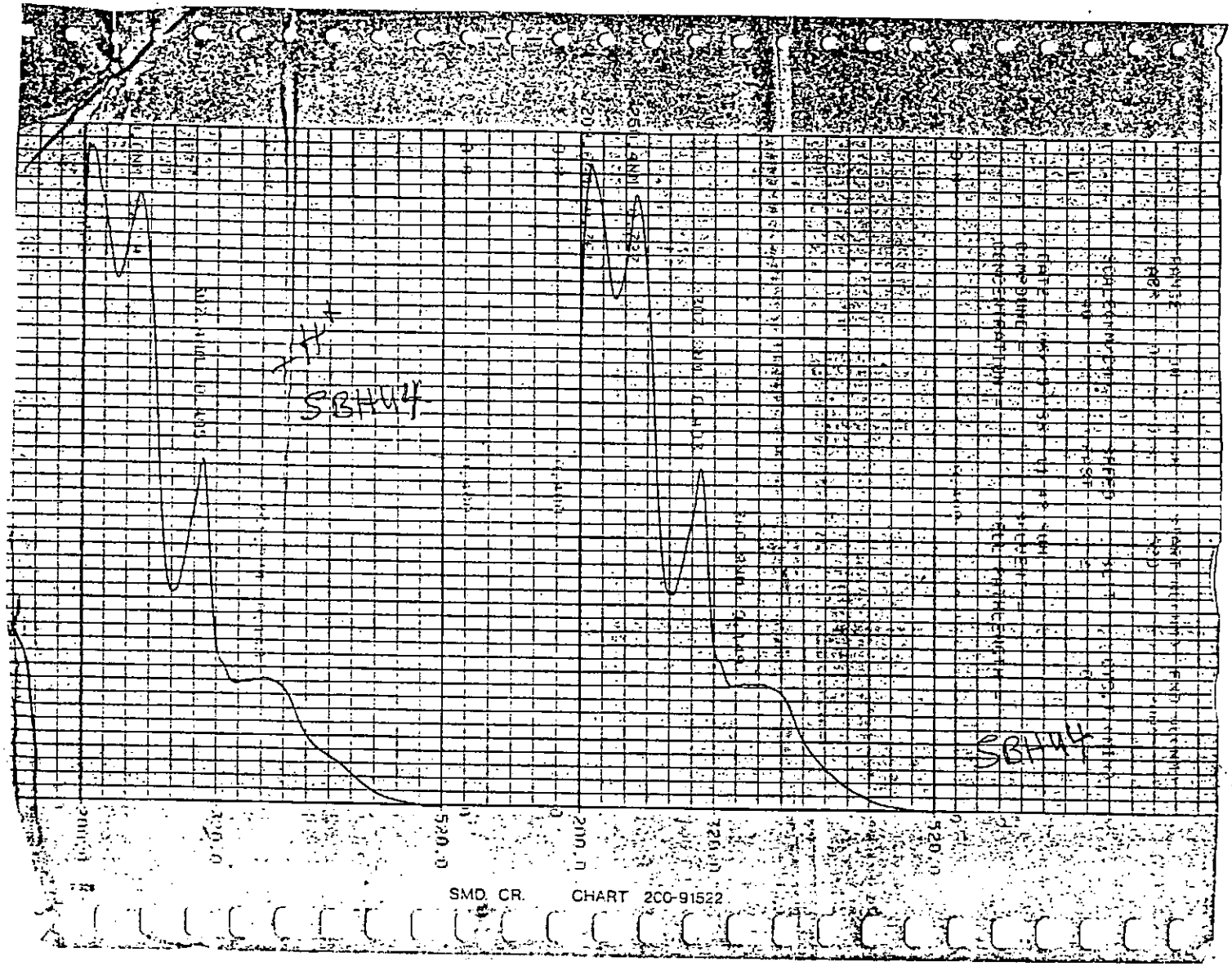


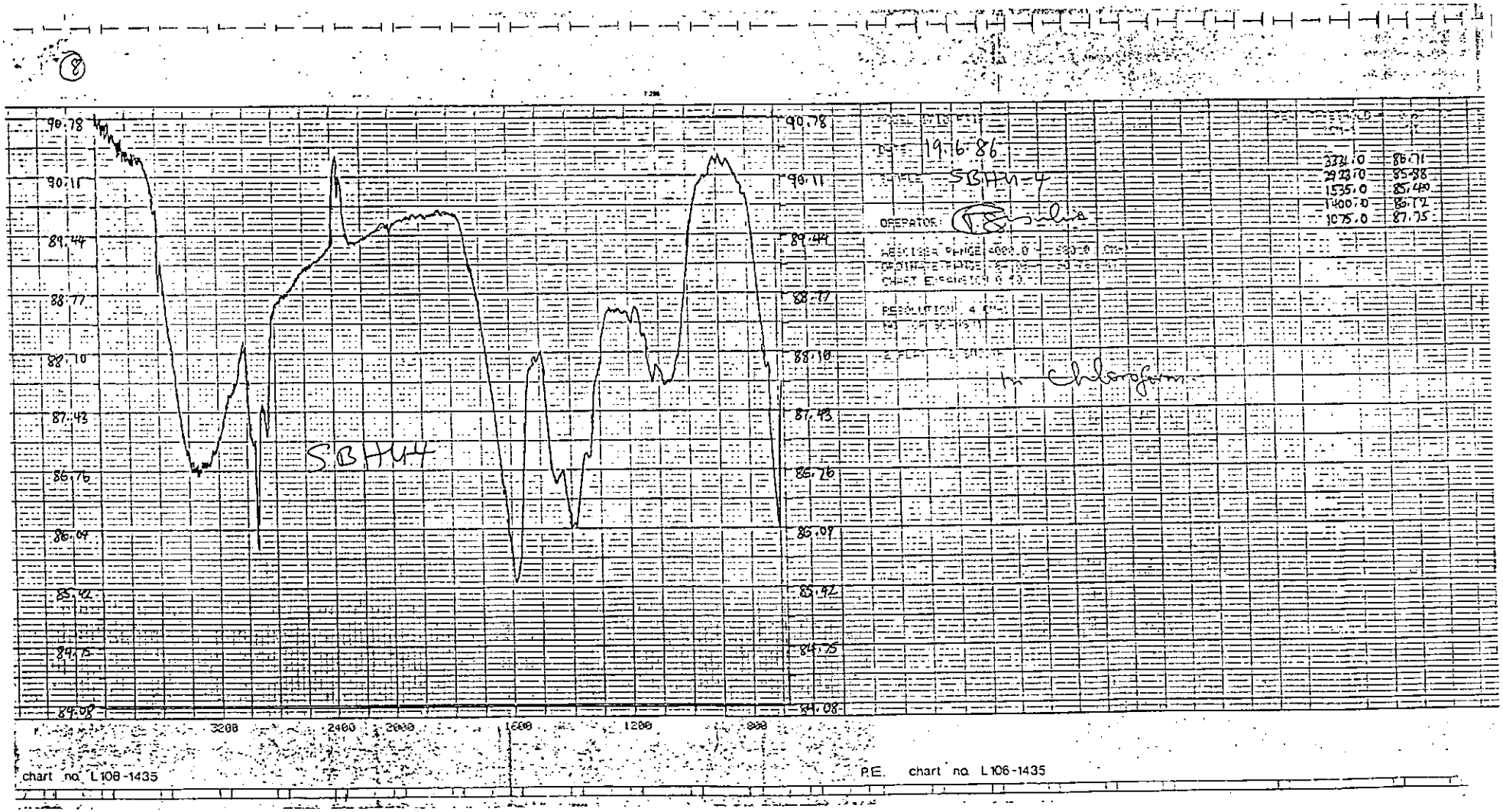
PAGE 1

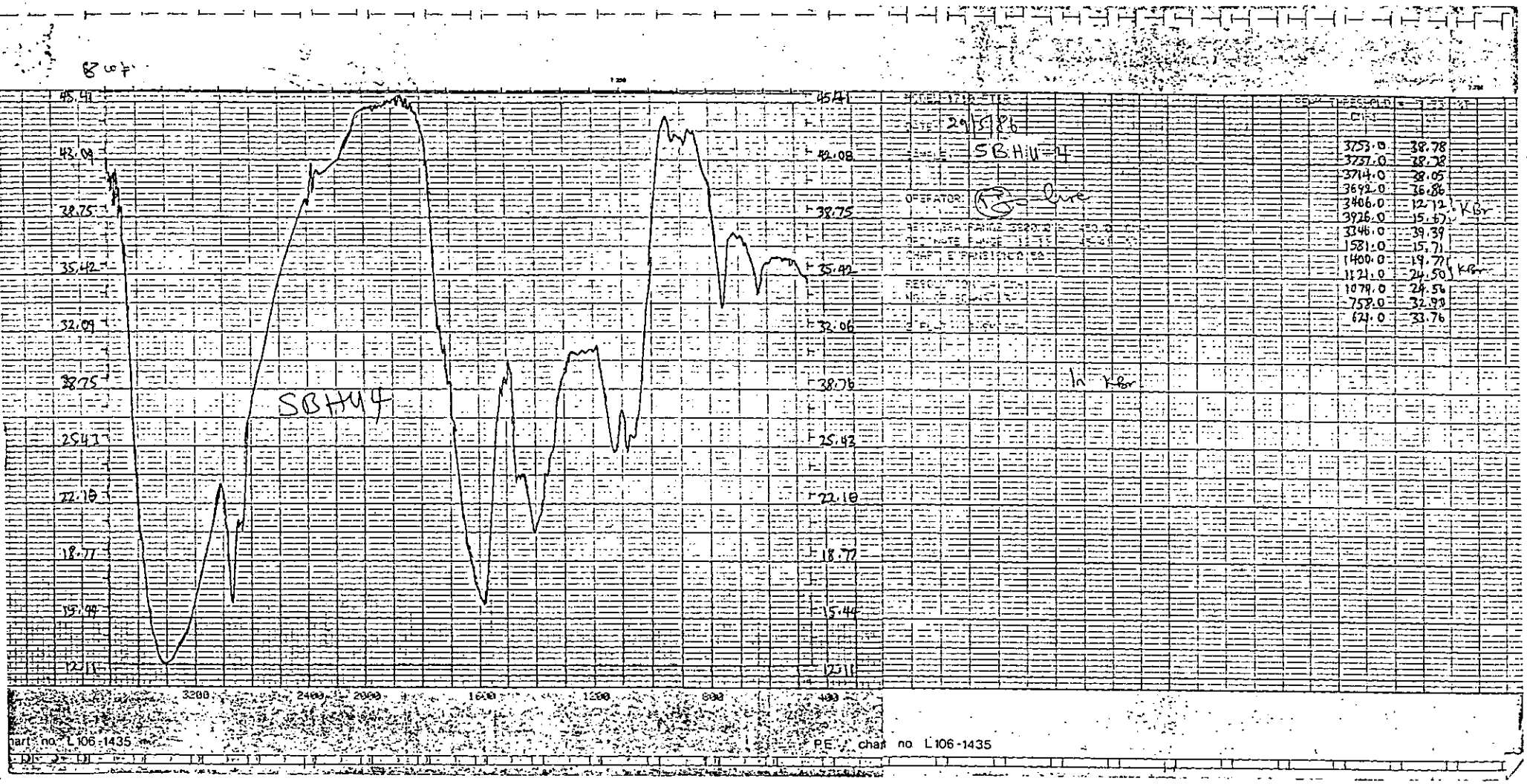
PEAK NO.	MEASURED MASS	% INT. NREF	
64	509	3.7	
70	502	11.4	*
71	501	30.0	*
72	500	27.0	*
73	499	77.2	*
74	498	8.4	*
77	495	11.5	*
95	477	8.0	*
107	465	8.6	*
108	464	26.7	*
109	463	75.6	*
110	462	14.4	*
111	461	31.7	*
113	459	10.6	*
131	441	8.8	
167	405	11.9	
265	307	11.8	
276	296	35.5	
278	294	71.9	
298	274	26.7	
314	258	56.7	
337	236	25.1	
341	232	26.7	
351	222	58.4	
353	220	40.9	
363	210	38.5	
366	207	27.3	
367	206	100.0	*
370	204	31.0	
389	185	48.9	
399	176	34.5	
403	174	33.4	
409	169	33.3	
412	166	26.8	
413	165	61.3	
418	160	37.7	
491	87	52.2	
498	80	24.2	
504	74	27.2	
518	60	57.5	
519	59	31.8	
520	58	45.9	
532	45	48.1	
533	44	70.8	
545	33	41.1	
546	32	97.2	
548	30	94.0	*

ES

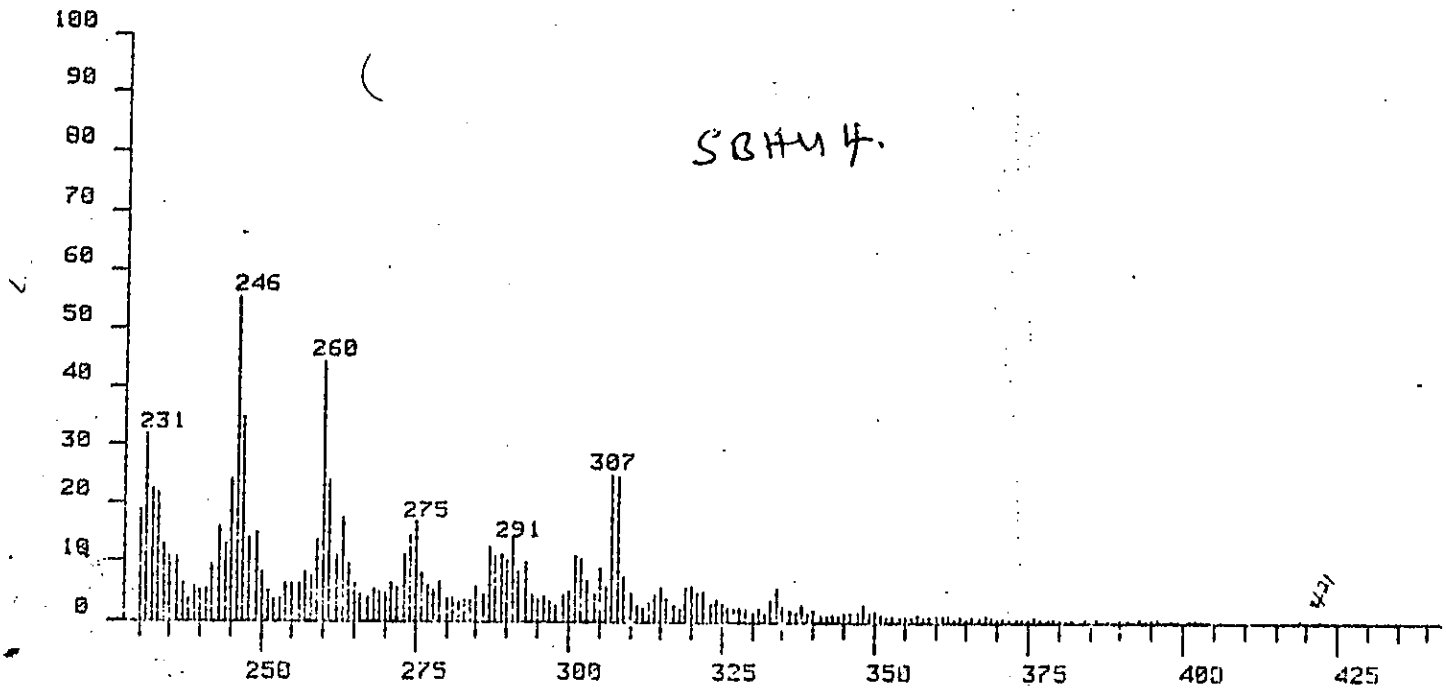
ESBHY3C1.


SBH44
SBH44
SMD. CR. CHART 200-91522


(8)
90.78
90.11
89.44
88.77
88.10
87.43
86.76
86.09
85.42
84.75
84.08
SBHH4
19-6-86
SBHH-4
OPERATOR
in Chloroform
3200
2400
2000
1600
1200
800
chart no. L108-1435
P.E. chart no. L106-1435


SBH#4
45.41
43.09
38.75
35.42
32.09
38.75
25.43
22.10
18.77
15.44
12.11
3200
2400
2000
1600
1200
800
400
P.E. chart no L106-1435
chart no L106-1435
SBH#4
In Kbr
3753.0 38.78
3737.0 38.28
3714.0 38.05
3692.0 36.86
3406.0 12.12
3926.0 15.67
3346.0 39.39
1581.0 15.71
1400.0 19.77
1121.0 24.50
1079.0 24.56
758.0 32.99
671.0 33.76
Kbr
Kbr

17LR12.46 [TIC=52492288, 100%=1025600] EI



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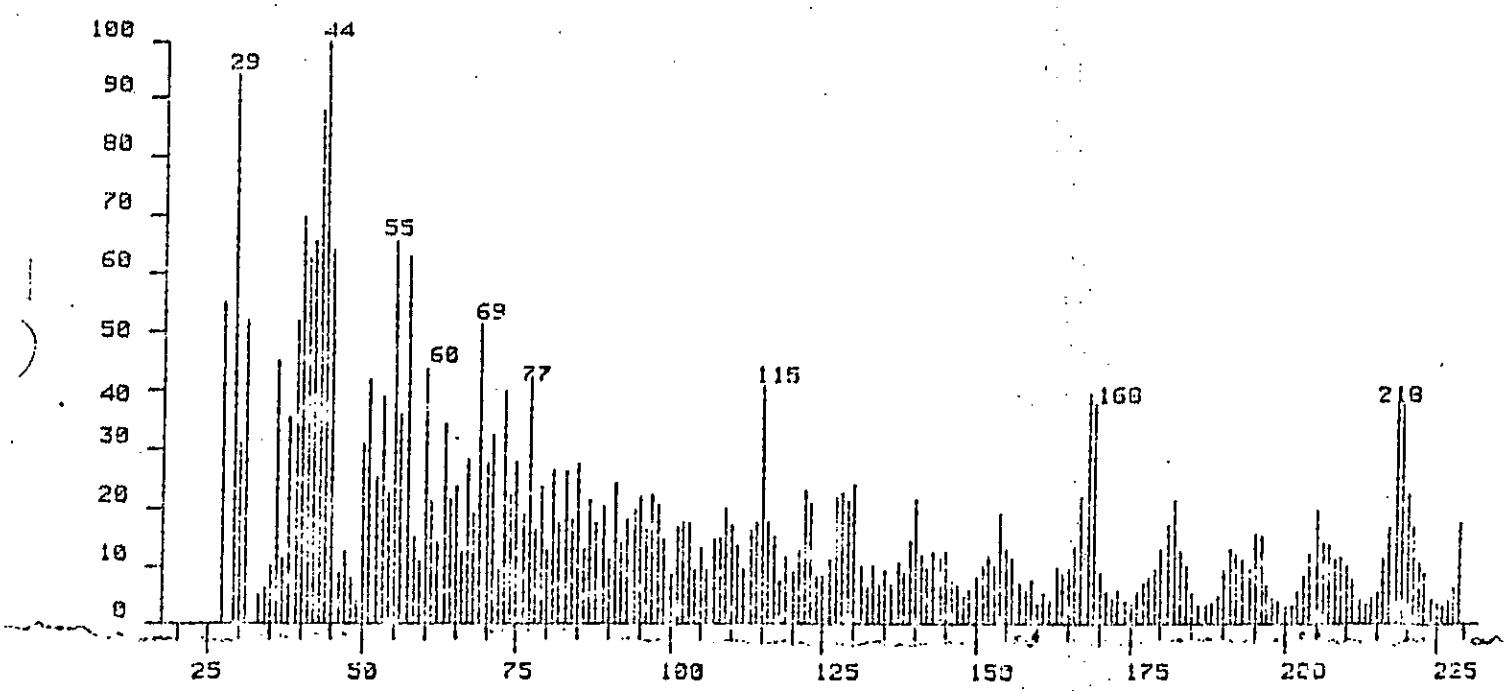


PAGE 1

PEAK NO	MEASURED MASS	% INT. NREF	
72	334	5.6	
73	333	3.7	
82	324	3.8	
84	322	5.2	
85	321	5.0	
98	308	24.6	
99	307	24.8	*
115	291	14.2	
131	275	17.0	
132	274	14.1	
143	263	17.5	
145	261	23.8	*
146	260	44.4	*
147	259	13.5	*
158	249	14.8	*
159	248	14.0	*
160	247	34.7	*
161	246	55.6	*
162	245	24.1	*
163	244	12.?	*
164	243	15.?	*
173	234	17.?	*
174	233	21.8	*
175	232	22.3	*
176	231	31.?	*
177	230	19.0	*
178	229	18.1	*
186	221	16.?	*
187	220	22.6	*
188	219	37.6	*
189	218	39.8	*
190	217	16.?	*
201	207	13.6	
203	204	14.3	*
204	205	19.?	*
215	196	14.?	
217	195	15.6	
222	191	13.?	
232	183	12.8	
233	182	21.6	
234	181	17.?	
236	180	12.5	
252	149	37.0	
254	168	38.4	
256	167	22.2	
258	166	13.0	
361	115	41.0	*
425	85	27.5	
429	83	26.3	
433	81	24.5	
442	77	41.?	
447	75	27.7	
450	73	39.6	
453	71	32.4	
455	70	27.6	

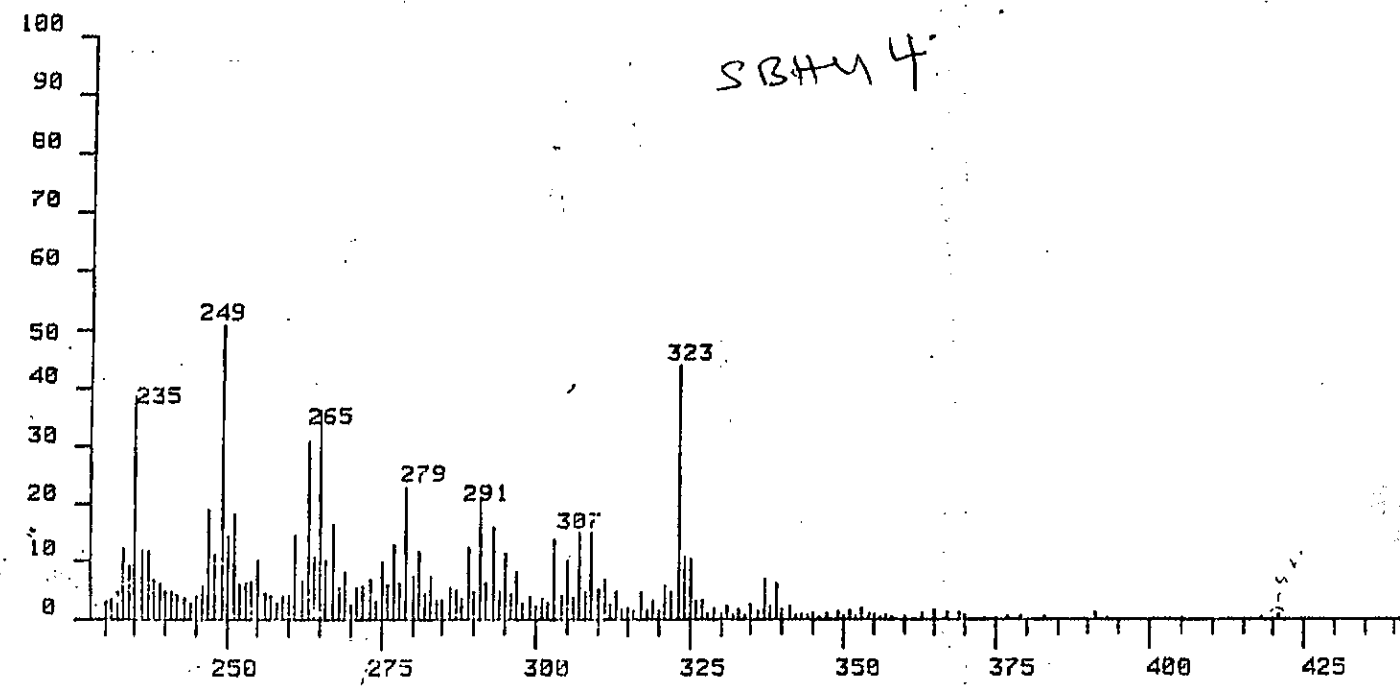
SBH44 E1

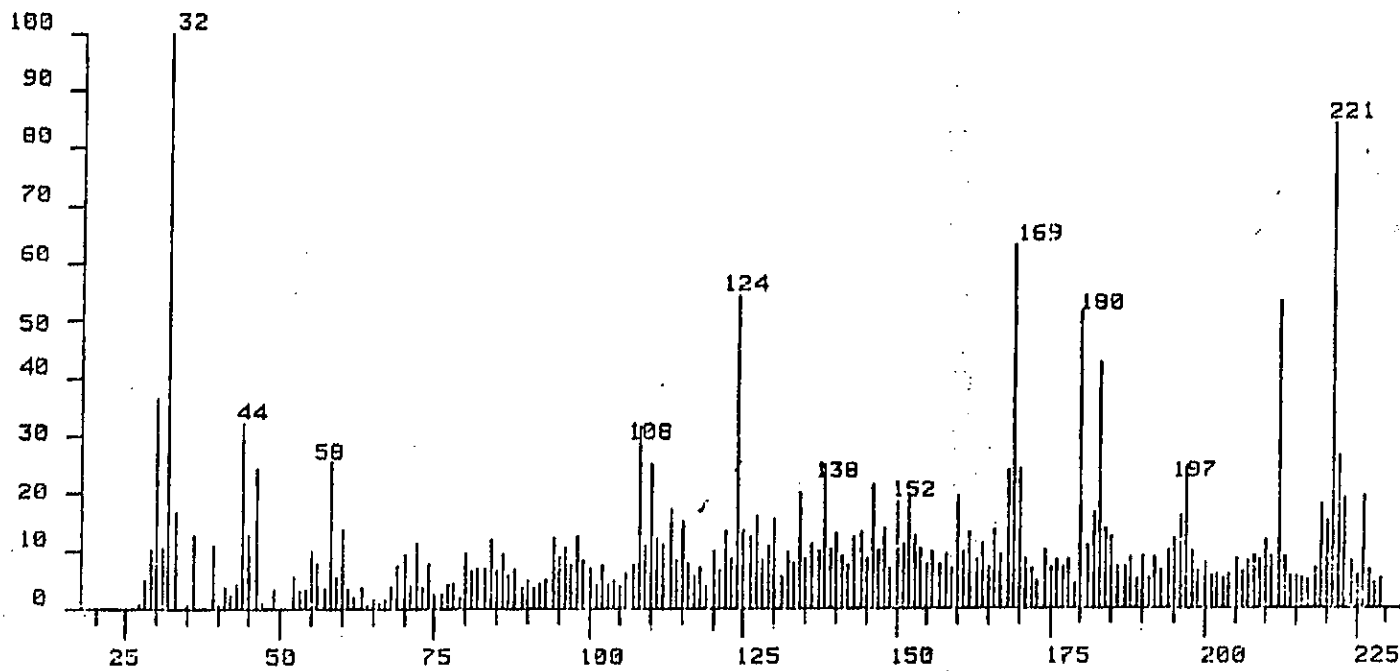
PAGE 2

PEAK NO.	MEASURED MASS	% INT. REF
457	69	51.4
460	67	28.3
467	63	34.3
470	60	44.2
476	57	63.0
478	56	35.9
480	55	65.7
483	53	39.0
484	52	25.3
485	51	41.9
486	50	30.9
493	45	64.0
494	44	100.0
495	43	88.1
496	42	65.6
497	41	62.5
498	40	69.9
499	39	52.0
503	36	45.2
509	31	52.2
510	30	31.0
511	29	94.3
513	27	55.3

17LR12.36 [TIC=18651136, 100%=5599521] +VE CI, REAGENT:AMMONIA

SBH4



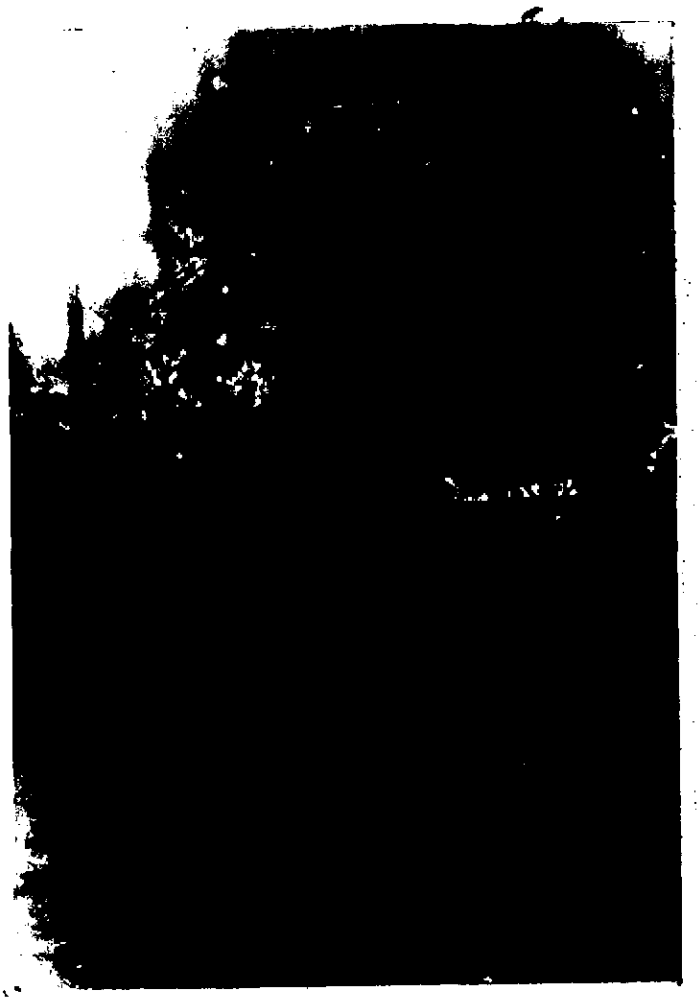


PAGE 1

PEAK NO.	MEASURED MASS	% INT. NREF
34	339	6.3
36	337	7.0
46	327	3.5
48	325	10.2
49	324	10.5
50	323	44.1
51	322	4.9
52	321	5.9
80	293	15.9
82	291	21.5
94	279	23.4
106	267	16.4
108	265	36.4
110	263	31.1
122	251	18.4
124	249	50.8
126	247	19.0
138	235	38.7
147	226	19.2
150	223	18.8
151	222	26.5
152	221	84.2
154	219	17.7
161	212	53.3
176	197	24.5
177	196	16.0
190	183	42.7
191	182	16.6
193	180	51.5
203	170	74.4
204	169	63.2
205	168	24.1
213	160	19.6
221	152	19.7
223	150	18.4
227	146	21.5
235	138	74.8
239	134	20.1
243	130	15.6
246	127	16.0
249	124	54.4
260	113	17.2
263	110	25.3
265	108	31.7
315	58	25.9
324	46	24.4
326	44	31.9
336	33	16.7
337	32	100.0
339	30	36.7

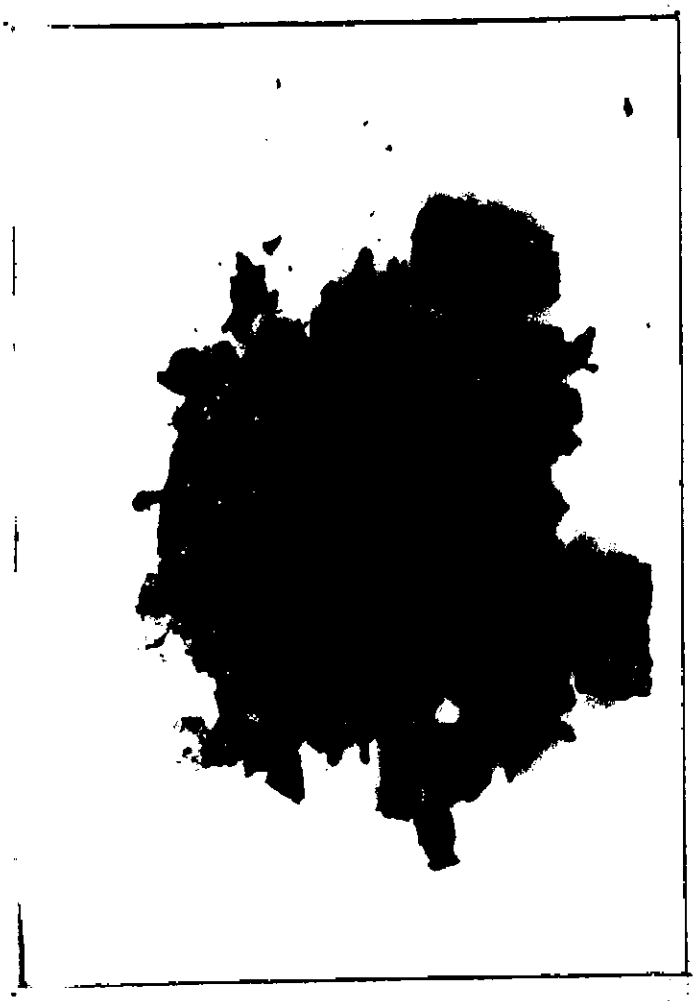
SBH4-4

C1

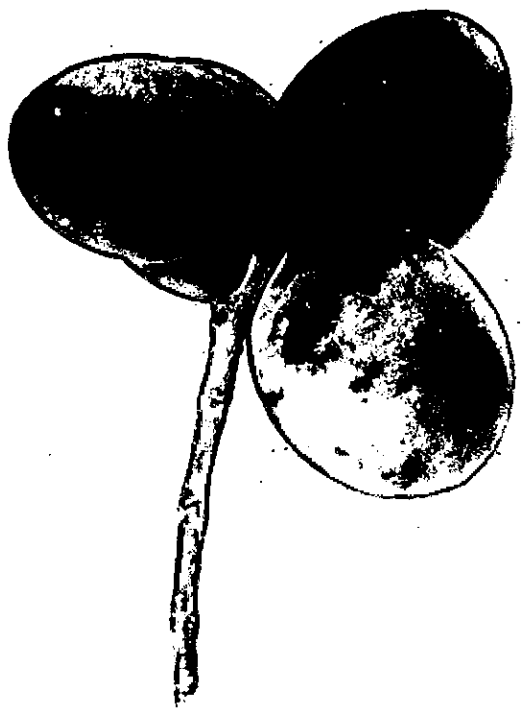


THE PLANT HUNTERIA UMBELLATA

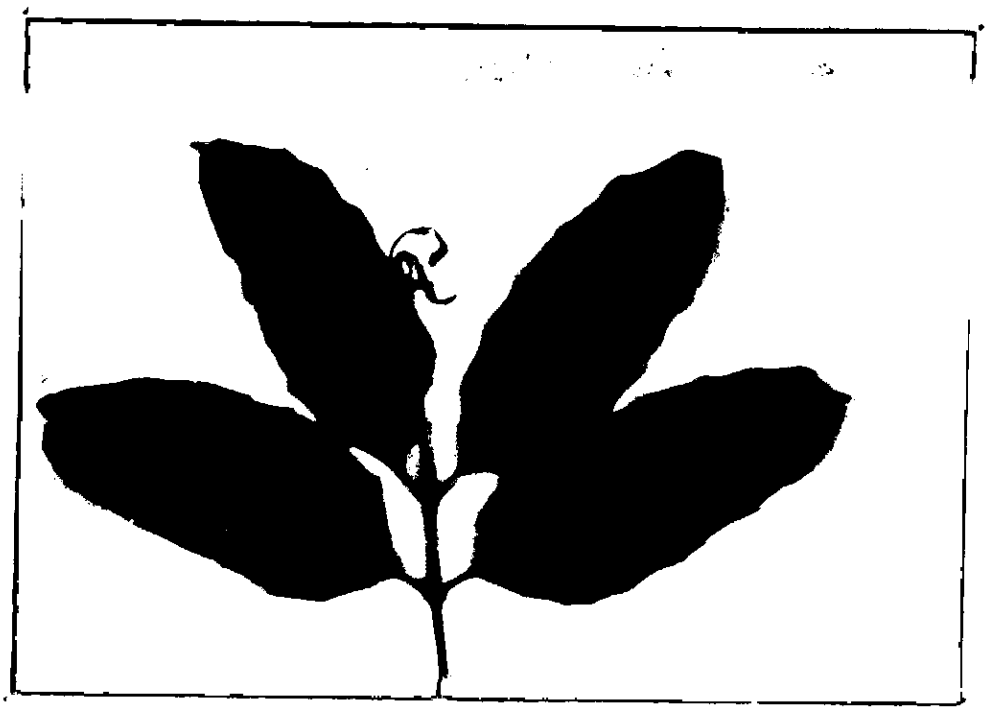
MRS. F. O. OGUNSULIRE REMOVING BARK FROM THE PLANT HUNTERIA UMBELLATA



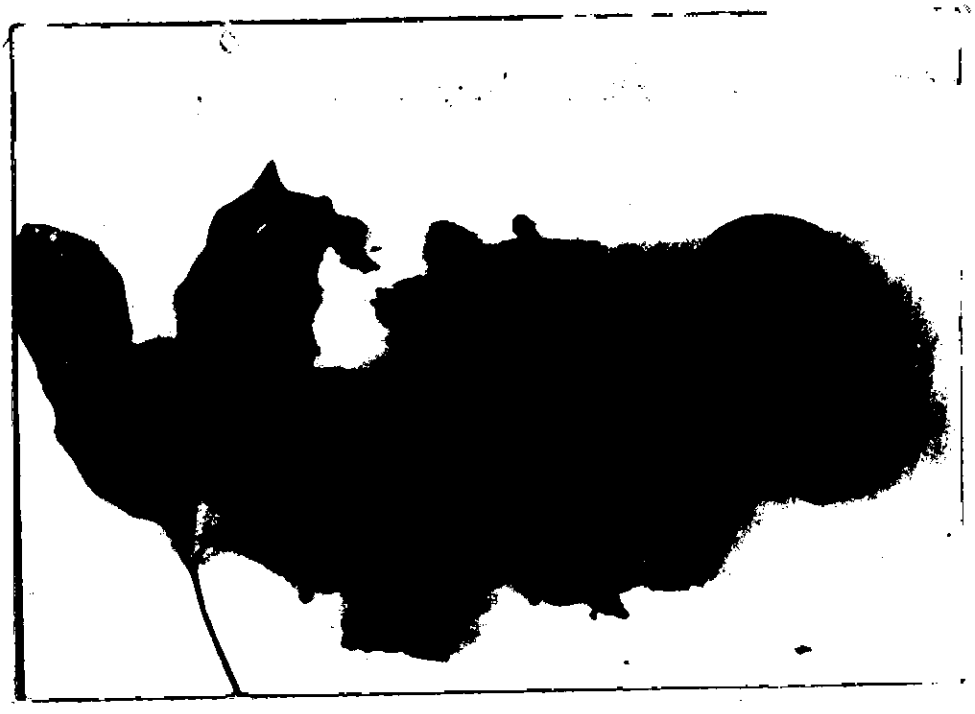
BARK CHIPS OF HUNTERIA UMBELLATA



A BUNCH OF SEED PODS OF HUNTERIA UMBELLATA



LEAVES OF HUNTERIA UMBELLATA



LEAVES, BARK AND SEED POD OF HUNTERIA UMBELLATA