Title:- The Preliminary Phytochemical Investigation of the Leaves of Croton Macrostachyus. (Euphorbiacae family).

by MIDAMBO, B. KAGAI (MISS)

A project submitted in partial fulfilment of the requirements for the award of the Bachelor of Pharmacy degree of the University of Nairobi, Kenya.

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DEDICATION

To the entire MIDAMBO family headed by Mr. Erastus Midambo whose hard work and foresight has seen many to success.

Acknowledgements:

My sincere gratitude is extended to my supervisor Dr. Addae-Mensah of the Pharmaceutical chemistry section, Department of Pharmacy whose advice, guidance and continous encouragement throughout this project was very much well taken and appreciated. Through his help and influence it was possible to get the NMR and MS spectra run.

The assistance from the technical staff of the Department of Pharmacy, cannot be taken for granted and more particularly Mr. Richard Gibbson Mwalughu whose help was highly appreciated.

Last, but not least, my appreciation goes to my fellow working colleagues Miss Constance Wandera and Miss Grace Karanja who livened up the laboratory atmosphere.

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ABSTRACT

During the preliminary investigation of the petroleum ether extract of the leaves of Croton macrostachyus, two compounds were isolated:-

i). Probably 3-cyclohexyl eicosane.

ii). A compound which is probably a triterpenoid.

An account of the traditional medicinal uses of croton species in Africa is given as well as the research work done on croton species in the period 1978 and 1979.

Research work already done on Croton macrostachyus prior to 1985 is also recorded.

INTRODUCTION:

Croton Macrostachyus is a tree 20 to 40ft tall with grey bark in the family Euphorbiacea with yellow white flowers and slightly three lobed fruits half an inch in diameter. The tree is wide spread in tropical Africa growing in Savannah forest [1,2] Croton macrostachyus is found in the forested areas of East Africa and it is known as follows in the local dialects. Musundzu (Kakamega); Mutundu (Kamba and Kikuyu); Mutuntu (Meru) and Muwulugu (Hehe) (3). The plant has been used as antihelmintic for tapeworm and as a purgative. The ash from burnt leaves is licked for coughs. The juice from a fresh leaf is applied on fresh wounds to hasten blood clotting. Juice from boiled roots is drunk for malaria and venereal diseases. The bark peeled from stems and roots, boiled in water, is used to bath newly born babies as a remedy against skin rashes. The seed and resins are however considered poisonous [3]. The Chagga use the leave-juice with fresh green leaf of Embelia Kilimandscharica as an antihelmintic. The plant is considered an abbysinian taenifuge. The bark which is said to contain crotin is used in East Africa as a

purgative (2).

Other species in the genus croton have been shown to contain active principles having medicinal or toxic properties and have also been used in traditional medicine in Africa.

The seed of Croton Elliottianus is a purgative in man with 0.1g to 0.2g. producing a mild effect and o.4g. a drastic effect. The fixed oil produced by the seed also has the purgative effect but is less irritant than the seed. The Masai use it as a purgative after mixing the bark with curdled milk. Small doses in man are diuretic and so is the oil. Both seed and oil are mildly antihelmintic but have no cholagogue action with the systemic action of haemolysis and haemorrhagic spots in the tissue, the action of oil in these respects being feebler than the seed. [2].

Croton gratissimus has been used as a remedy for fevers by the Transvaal Sotho. The charred and powdered bark is used to treat bleeding gums by brushing them with the powder. They also use the leaf as one of the ingredients for 'smoking' rheumatic patients. The Zulu use the plant as cathartic and an eruptive irritant.

Zulu use the plant as Cathartic and an eruptive irritant. The bark is applied for its irritant action on the chest wall, in any painful respiratory condition and for intercostal neuralgia. It is also used for dropsy, indigestion and pleurisy. The Zulu also use the powdered bark as one of the ingredients of a remedy inserted into the uterus for disorders of that organ. The bark is said to contain the toxalbumin crotin. The Kgatla make an eye lotion for animals from a cold infusion of the leaf and use the root as a charm medicine. The leaf, stem and the fruit yield an aromatic oil (calamus like) [2].

The Transvaal and East African people have used the bitter bark of Croton gubouga as a malarial remedy. The seed is also used. The powdered bark is usually made into a pill and is said to produce benefit, but opium may be an ingredient. The same bark has been used as a fish poison in Gazaland and East Transvaal The seed and bark cause intense burning sensation in throat and mouth. Salivation, slight nausea and slight purgation is also seen and these effects are thought

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to be due to the presence of an acrid principle. The bark has a slight but not unpleasant odour and the dust from it produces sneezing with a burning sensation in the throat and on the tongue. The Luvale administer an infusion of the root to thin babies to make them fat [2]. Another croton species used for influenza and malaria treatment is Croton meny-hartii. [2,3].

In East Africa, Croton megalocarpus's pounded bark is soaked in water overnight and the extract drunk as a remedy for intestinal worms and for the treatment of whooping cough. The plant species is said to contain a toxalbumin. The Masai administer a decoction of the bark with blood as a tonic. Tests for antibiotic activity are negative. [2,3]. Another plant species used as a vermifuge and purgative by the Nyamwezi is Croton Mubango. [3].

Croton Pseudopulchellus's roots are used as a decoction for relief of asthma by the Nyamwezi. The leaves are boiled and applied to chest for colds. Twigs and leaves are used with the twigs of Teclea nobilis in making a vapour bath for the treatment of syphilitic

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sores, at the same time the powdered root of crossopteryx febrifuga being applied locally. The root and leaf are thought to contain the toxalbumin crotin. An infusion of the leaves is given to cattle as a remedy for anthrax. The leaves are also burnt in among crops as an insecticide [2].

The powdered bark of Croton Sylvaticus is a Swati remedy for gall sickness in cattle. The bark yields a tanning matter used in Gazaland as a fish poison. The root is a remedy for pleurisy and indigestion and is said to be avoided by birds as they are fatally poisonous to them. A decoctus of leaves is used as a wash for body swelling caused by Kwashiorkor or Tuberculosis. The roots are also pounded to make poultices for swellings. A decoction from the bark of the roots is taken orally as a remedy for tuberculosis. An infusion of the leaves is also taken as a purgative [2,3].

Croton Scheffleri is used as remedy for miscarriages while croton Zambesicus is used by the Masai with Villosa as a strengthening medicine. [3].

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The leaves of Croton dichogamus are dried and burnt for inhalation by in fumigation of a patient with fever. It is an excellent remedy for chest ailments. The leaves are chewed or dried and smoked as cigarette by the Sukuma. It also acts as a remedy for stomach diseases;chopped roots are added to soup made from goat's meat and taken as tonic [3].

The strongly scented roots of croton jatrophoides are used for colds and stomachache (2).

From the outgoing remarks it is important to screen plants that are used as traditional remedies by the various groups of people to find out more about the pharmacology and chemistry of the active principles. The present project is part of an on going programme of investigating the East African crotons which have hitherto received very little phytochemical and /or pharmacological attention.

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CHAPTER ONE

RESEARCH WORK DONE ON CROTON GENERA(EUPHORBIACEAE FAMILY) IN THE PERIOD 1978-1979.

Before this period, various reserchers had done some phytochemical screening of plants native to N.E. Brazil for the presence or absence of alkaloids, steroids, triterpenoids, hydroxy anthraquinone derivatives, flavonoids, saponin compounds and antibiotics. Some of the croton species that were subjected to this screening included Croton rhamnifolius (leaf), croton compostris and croton refusa (leaves)[4]. The species worked on in the period under review are discussed below.

Croton sublyratus and Croton columnaris.

The trend in research at this time was a deliberate search into anti ulcer, anticancer/co-carcinogenic components in the plants. Mishima et al (5) studied the furanoditerpene, plaunal (I) and its acetate were prepared by extraction of Croton sublyratus and croton columnaris and additional chemical treatment. Plaunol (I) and its acetate had antipeptic ulcer activity (data given in rats).

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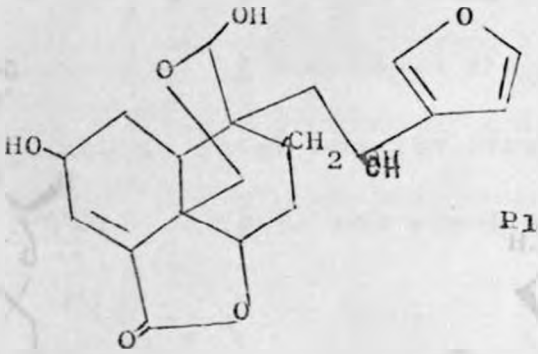

Plaunol(I)

Pharmacological screening directed towards finding antipeptic ulcer substances of plant originled to findings that the acetone extract of crude drug of Croton sublyratus by Eiichi Kitizawa et al [7]. Two new diterpene lactones named plaunol A (III) and plaunol(IV) showed significant inhibitory activities against reserpine induced ulcer in mouse and shay-ulcer in rat. From anti-reserpine active fraction, 18-hydroxy geranyl geraniol (V) was isolated and from anti-shay active fraction the new diterpene lactones designated plaunol A and plaunol B were isolated by silica gel column chromatography. The structures of plaunol A and geranyl geraniol were determined by X-ray analysis while that of plaunol B was determined by chemical and spectral data.

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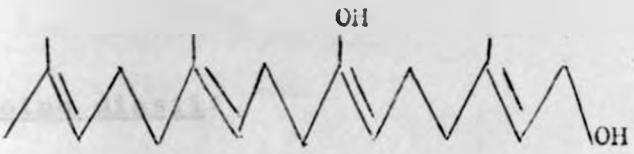
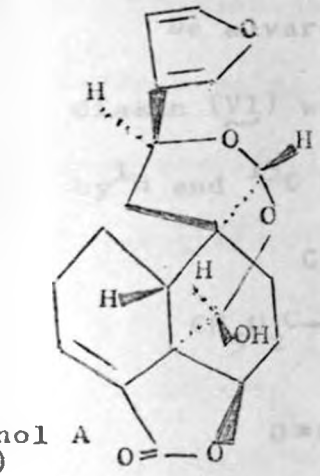


Geranyl geraniol(V)



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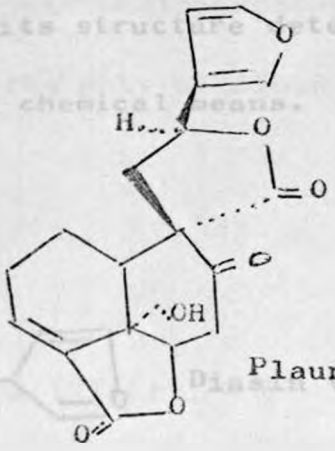

Plaunol B (IV)

Croton Flavens.

The relationship between co-carcinogens of the diterpene ester type from this plant and oesophageal cancer in Curacao was then investigated by Weber et al [6]. Three highly irritant and tumour promoting croton factors F_1-F_3 and the corresponding 3cryptic croton factors F_1-F_3 were isolated and characterised as new esters of 16-hydroxy and 4-deoxy, 16-hydroxy phorbol. A suggestion was put forward that tumour promoters of the phorbol ester type, ingested through the wide spread and frequent use of Croton flavens, may be causally related to the high rate of oesophageal cancer in Curacao.

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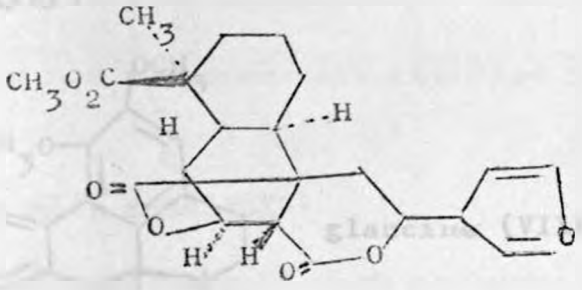
Croton diasii:

De alvarega et al worked on the ground wood and diasin (VI) was isolated and its structure determined by ^{1}H and ^{13}C NMR analysis and chemical means.



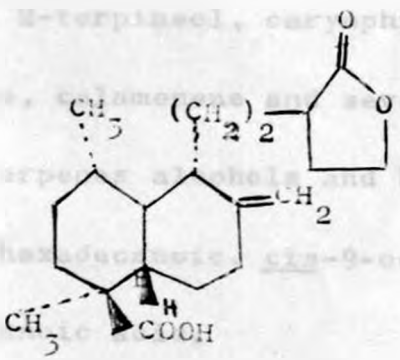
Diasin (VI)

Croton californicus:

The furanoid diterpene (-) hardwickiic acid and the long chain l-triacontanol ($CH_3(CH_2)_{28}CH_2OH$) were first reported in this species' fruit [9].

Croton niveus.

Rojas E.T. and Rodriguer H.L. isolated Nivenolide (VII) a diterpene lactone from this plant and the structure determined by chemical and spectral means [10].



Nivenolide (VII)

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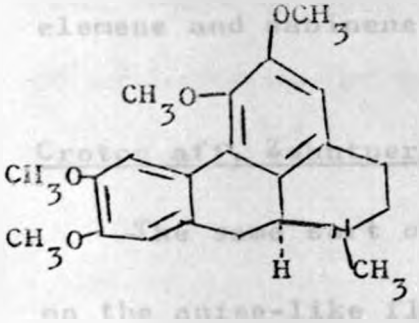
Croton draconoides:

Alkaloids of this croton species were studied by Bettole et al and found to contain thaliporphine and glaucine (VIII). Taspine was the only alkaloid in the latex [11].



glaucine (VIII).

Croton Sonderianus:

Within this same period (1978-79), research on essential and fatty oils of various crotons have also been reported. The essential and fatty oils of Croton sonderianus (stem and leaves) were studied by Craveiro et al (12) who reported the presence of α-pinene, β-pinene, myrcene, 2-terpineol, caryophyllene, humulene, α-elemene, γ-elemene, calamenene and several unidentified sesquiterpenes alcohols and hydrocarbons. The fatty acids yielded hexadecanoic, cis-9-octadenoic and 9-methyl-heptadecanoic acid.

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Croton argyrophylloides (leaves)

The volatile constituents of this croton were investigated and found to contain α- pinene, sabinene 1,8 cineole, β-elemene, β-caryophyllene, 2-humulene, γ-elemene and an unidentified seisquiterpene alcohol and a seisquiterpene hydrocarbon. For the first time β-elemene and sabinene were reported in this plant [13].

Croton aff. Zehntneri:

The same sort of work as above was also done here on the anise-like flavour of this plant and showed that estragote, the major constituent of essential oils in thisspecies occurs together with minor quantities of camphor, trans-anethole, isoborneol, caryophylene, γ-elemene, safrole, methyl isoeugenol heptadecane and eicosane. This was the first report of these compounds in the genus. 15 predominant volatile constituents of the oil wereidentified by Gas-chromatography-mass spectroscopy.

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The sort of work exemplified here, shows the high potential in the study of the various constituents of the croton species in question - croton macrostachyus - which is one of the most widely used croton species in Kenya. Some work has already been done but on judging from amount of work done, there is still greater chances of exploitation of the field.

CHAPTER TWO.

WORK PREVIOUSLY DONE ON CROTON MACROSTACHYUS.

In the course of a continuing search for tumour inhibitors of plant origin. alcoholic extracts of the fruits of Croton macrostachyus showed significant inhibitory activity in Lewis Lung carcinoma in mice (LL). Kupchan et al in 1968 [15] worked on the isolation and structural elucidation of crotepoxide (IX) a novel tumour inhibitory cyclohexane diepoxide derivative from this plant.

Fractionation of the ethanol extract guided by assay against LL revealed that an active principle, was concentrated successively in methanol layer of a 10% aqueous methanol/skellysolve B partition and in the 1-butanol layer of 1-butanol/water partition. Further fractionation involving silicic acid chromatography yielded the crotepoxide ($C_{18}H_8O_8$).

- M.P. 150-151°C.
- $[\alpha]_D^{25}$ = +74 (C1.70 $CHCl_3$);
- λ_{Max}^{MeOH} 274 mμ (ε=1050 and 281mμ (ε = 860).

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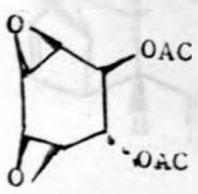
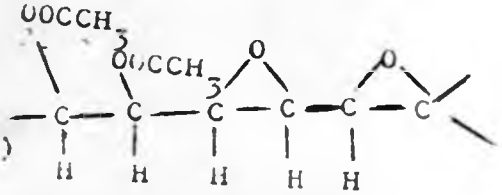
$\nu_{max}^{CHCl_3}$ 3.35, 5.71,5.78,6.24,6.31,6.89,7.29,7.89 8.20,9.00,10.24 and 11.24 μ.

- NMR signals (in $CDCl_3$) at δ 2.28 (5H,m,aromatic); 4.27 (1H,d,Jxy = 9.5HZ > CHOAC); 5.42 and 5.75 (2H, doublets J = 12.0HZ CH_2OCOPh); 6.32 (1H,d, J_{BC} = 2.5HZ); 6.56(1H,d,d, J_{BC} = 2.5 and J_{AB} = 4.0H$_z$); 6.90(1H,d,d, J_{AB} = 4.0 and J_{AY} = 1.5HZ) 7.88 (3H,S, acetate) and 7.95 (3H,S,acetate).

Crotepoxide belongs to a small group of naturally occuring highly oxygenated cyclohexane derivatives, other members of which are terreic acid (X), Epoxydone (XI), senepoxide (XII) seneol (XIII) and Shikimic acid (XIV). However crotepoxide has the diepoxide functionality. This function has been shown earlier to confer tumour inhibitory activity on other classes of synthetic compounds. Investigations are underway to determine the significance of various structural features in relationship to the tumour inhibitory activity of crotepoxide.



Crotepoxide(IX)



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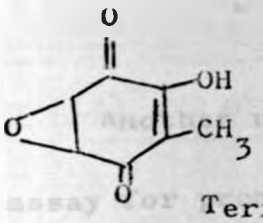
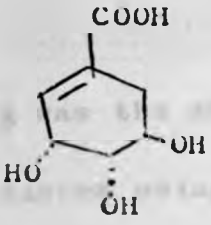
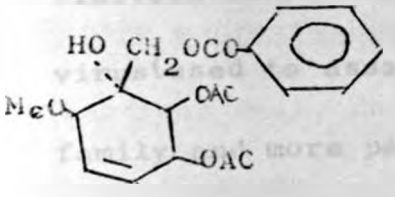


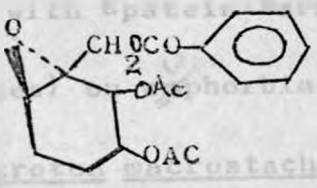
Terreic Acid (X)



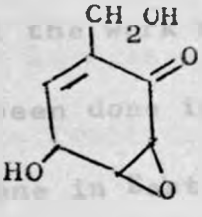
Shikimic acid (XIV)



Seneol (XIII)

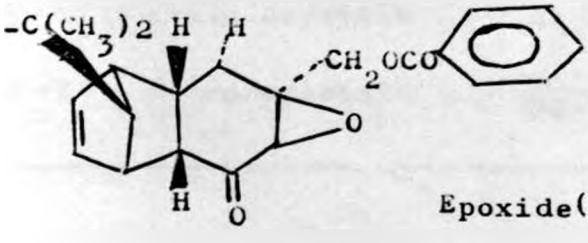


Senepoxide (XII)



Epoxydone (XI)

Odak et al [16] then embarked on the total synthesis of the dl crotepoxide (usually isolated from fruits of croton macrostachyus and from the leaves and stem of piper futadzura) by effecting it from epoxide (XI) in 9 steps.



Epoxide(XV)

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Another undertaking was the short term in vitro assay for promotor substances using human lympho-blastoid cells latently infected with Epstein-Barr virus(used to assay such substances) on euphorbiaceae family and more particularly in croton macrostachyus and croton megalocarpus.

Most of the work reported in the period under survey has been done in Brazil, America, Japan and virtually none in East Africa where the plant occurs in plenty. More so, in the research done some parts of the plant had not been studied like the twigs and leaves. Therefore, bearing in mind the variation in components (quantitative and qualitative) in a plant with variation in geographic and morphological differences the research project was and is a course worth taking.

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CHAPTER THREE - THE PRESENT INVESTIGATION

RESULTS AND DISCUSSION.

The petroleum ether extract of the leaves of Croton macrostachyus was separated into seven main fractions BK-1, BK-2, BK-3,BK-4,BK-5, BK-6, BK-7 on a chromatographic column- silica gel; Benzene: chloroform (I:I) as eluting solvent and eluting further with more solvents with increasing polarity. The characteristics of the various fractions are shown in table 1.

Table 1:

Fraction	Colour of Crystals	Melting Point ^{o}C	Yield (g)
Bk-1	white crystals	70-71	0.0670
BK-2	white crystals	74-76	0.2136
BK-3	white crystals	70-74	0.0623
BK-4	cream crystals	68-72	0.0304
BK-5	light green	76-78	0.2628
BK-6	cream crystals	75-77	0.0150
BK-7	Brown crystals	72-74	0.0855

No further work was done on fractions BK-1,BK-3, BK-4, BK-5, BK-6, BK-7.

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CHARACTERISATION OF BK-2.

Fraction BK-2 was recrystallised from methanol to give the white crystals with m.p. 74-76°C (uncorrected). An attempt to deduce the structure of BK-2 was done from ^{1}H NMR, mass spectroscopy (MS) and IR(KBR). The IR spectrum (fig. 1) showed significant peaks at 1445-1485 cm^{-1} corresponding to C-H bending alkane - CH_2; 2960-2850 CM^{-1} corresponding to C-H stretching alkane. It is clear from the IR spectrum that there is no oxygen functionality such as OH or C=O in the compound.

The NMR spectrum (fig 2) showed only one large single peak at δ=1.25 (singlet). In view of difficulties with the instrument, no intergration could be done on this peak. The only deduction from the spectrum is that there are many methylene protons ($-CH_2-$). No other signals could be seen from the above data, it can be concluded tentatively that the compound is a hydrocarbon.

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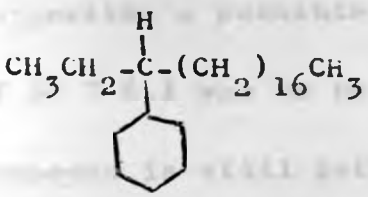
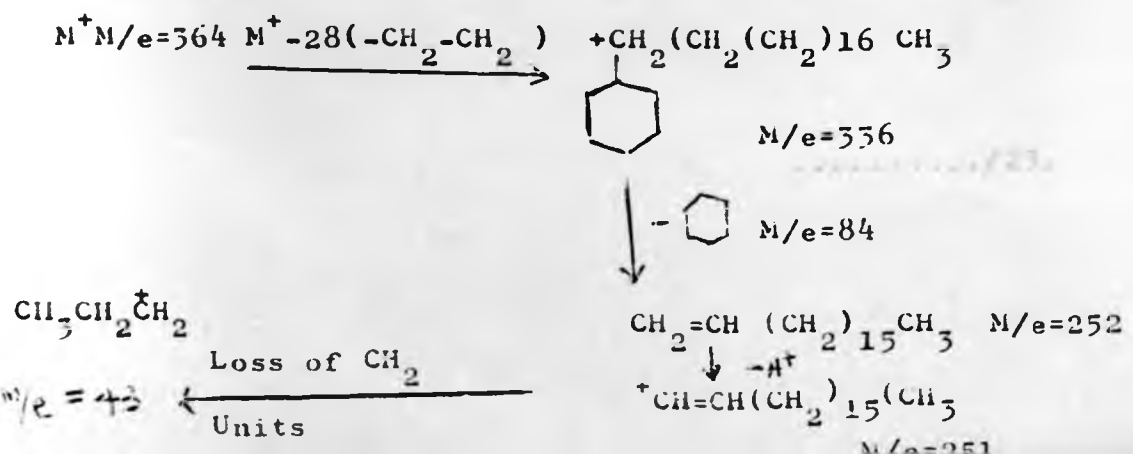
The mass spectrum (fig. 3) supported this possibility. It gave a molecular ion (M^+) peak at 364, another peak at 336 due probably to loss of $-CH_2-CH_2-$ and then a typical hydrocarbon fragmentation pattern between M/e=258 and M/e=43. From the above tentative conclusion, the M^+ peak value and consultation of literature [18] the following possible compounds were considered; 7-cyclohexyl eicosane $C_{26}H_{52}$; 5-cyclohexyl eicosane ($C_{26}H_{52}$). 9-cyclohexyl eicosane ($C_{26}H_{52}$); 2 cyclohexyl eicosane; 4-cyclohexyl eicosane ($C_{26}H_{52}$); 1,4-dimethyl -3-n-Octadenyl cyclohexane and 3 cyclohexyleicosane ($C_{26}H_{52}$) (XVI). On the basis of the fragmentation it is suggested that structure is the 3-cyclo hexyl derivative.



3-cyclohexyl eicosane (XVI)

The following fragmentation pattern is proposed:-



EXAMINATION OF THE MOTHER LIQUORS:

BK-2.1 - Recrystallised from acetone to give cream crystals (10mg) and the R_f values in several solvent systems determined (fig.4 and table 2). No standards were available for comparison. This could have been desirable in structure characterisation of BK-2.1.

BK-3-6.1

This is the combination of the BK-3.1, BK4.1, BK-5.1 and BK6.1 mother liquors as from TLC they seemed to be composed of similar components. After the recrystalisation from methanol, it gave cream white crystals with M.P 110-112°C (uncorrected). The compound gave a deep purple colour with anisaldehyde on TLC, suggesting a possible triterpenoid. Characterisation of BK 3-6.1 was to be attempted. HNMR,MS data on this compound is still being awaited to enable us to suggest a possible structure.

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Table 2.

Solvent System		BK3-6.1 (variuos spots obtained 1, 2, 3, & 4) 1'	2'	3'	4'
Pure chloroform:	(0.60)	0.07	0.14	0.19	0.49
Chloroform benzene	(3:1)	0.04	0.07	0.12	0.39
Chloroform Benzene	(1:1)	0.22	0.29	0.45	0.82
Toluene:	(4:1)	-	0.56	0.80	0.90
Chloroform Ether	(1:1)	-	0.35	0.47	0.67
Toluene	(1:1)	-	0.75	0.53	0.48

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CHAPTER FOUR - EXPERIMENTAL:

^1H NMR was done on a Perkin-Elmer instrument at 60mHz in $CDCl_3$ with TMS as the internal reference. Analytical TLC was carried out with silica gel 60 GF_{254} as the adsorbent. The plates were prepared by the conventional methods. Column chromatography was on silica gel 60(0.063-0.200mm=70-230 mesh ASTM)

IR spectrum was recorded on a Perkin-Elmer IR spectrophotometer 727B. Information on the instrument used for MS was not available. Melting point was determined using Gallenkamp m.p. apparatus as well as Koffler hot-stage m.p. apparatus.

Anisaldehyde spray reagent (1% v/v anisaldehyde, 1% v/v concentrated sulphuric acid in glacial acetic acid) was used for visualisation of TLC spots. The reagent was used when freshly prepared.

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Extraction:

The dried leaves of Croton Macrostachyus were ground to powder in a mill. The plant material was collected from Karatina about 200km. North of Nairobi about 2,000m above sea level in July/August 1985. 600g of this powder was extracted in a soxhlet extractor with petroleum ether (boiling point range 60-80°C) for 48 hours. The extract was concentrated in a rotary evaporator to 150ml and put in a refrigerator for three days after which a green brown solid deposited. This was then filtered, the solid washed with methanol of the colouring material & methanol soluble components to give a cream white solid (4.2g). To find out the most suitable solvent system for the seperation of the various compoents present in the solid on column chromatography, the solid was examined by thin layer chromatography(TLC) on microscope slides using various solvent systems and silica gel GF 254 as adsorbent and anisaldehyde reagent for detection

The following solvent systems were found most suitable and were then used in the column chromatography

in the given order of increasing polarity.

1. Benzene: Chloroform (5:1)
2. Benzene: Chloroform (1:1)
3. Chloroform
4. Chloroform: methanol (1:1)
5. Methanol
6. Ethyl Acetate.

Separation (column chromatography).

A column 45cm long was used with silica gel GF 254 (0.063-0200mm 720-230mesh ASTM).

The crude extract was dissolved in minimum amount of chloroform and added to the top of the column to form a uniform layer of the solution. The eluting solvents were added in the given order and the column left to drip at the rate of 20 to 30 drops per minute and 30ml fractions collected. TLC was done to monitor the separation of the various components from the crude extract using silica gel and chloroform: benzene (1:1) as eluting solvent.

...../27.

Fractions that seemed to contain similar components were combined into 7 major fractions BK-1, BK-2, BK-3, BK-4, BK-5, BK-6, and BK-7. The fraction were evaporated to dryness on the rotary evaporator and recrystallised from methanol.

EXAMINATION OF THE SEPARATED SOLIDS

Hydrocarbon BK-2 (Probably 3-cyclohexyl eicosane)

m.p.-74-76°C IR(KBR) showed peaks at Umax 2960-2850 cm^{-1} (C-H stretch); 1485-1445 cm^{-1} (CH bending) ^1H NMR ($CDCl_3$ TMS as internal reference) δ 1.25(s), MS M^+ at m/z 364. Other prominent peaks are indicated in the discussion.

Data from the above spectroscopic and physical methods was analysed and used in deducing the compound BK-2. No further work was done on the other fractions.

EXAMINATION OF THE MOTHER LIQUORS:

The mother liquors were spotted on TLC and developed in $CHCl_3$: Benzene (1:1) and the spots compared.

...../28.

BK 2.1 The mother liquor was evaporated to dryness on the rotary evaporator and then recrystallised in Acetone to give whitish crystals (10mg), whose TLC in various solvents was performed and the R_f values calculated.

BK3-6.1 This is the combination of mother liquors BK 3 to BK6, evaporated and recrystallised in methanol to give cream white crystals (0.0284g) m.p 110-112°C (uncorrected). The TLC of the compound was in the solvent systems shown in table 2 and similarly the R_f values calculated and tabulated in table 2. Samples for ^1H NMR and MS analysis were supplied, but at the time of submitting this report, results of ^1H NMR and MS were still not available. No work was done on BK-1 and BK-7 mother liquors.

.....29.

FIGURE 1 — I.R. SPECTRUM FOR [illegible]

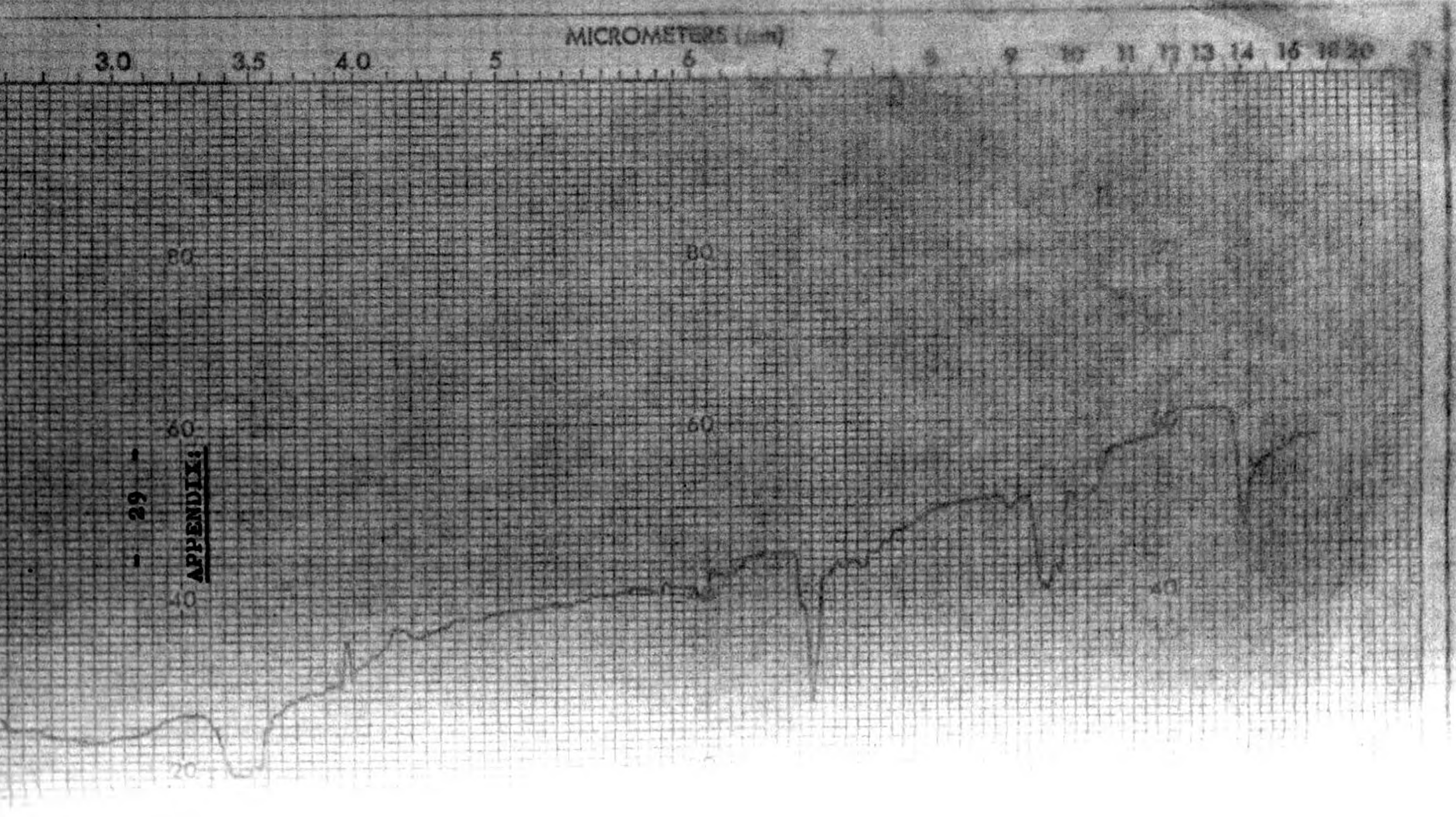


FIGURE 1 — IR SPECTRUM FOR BK-2

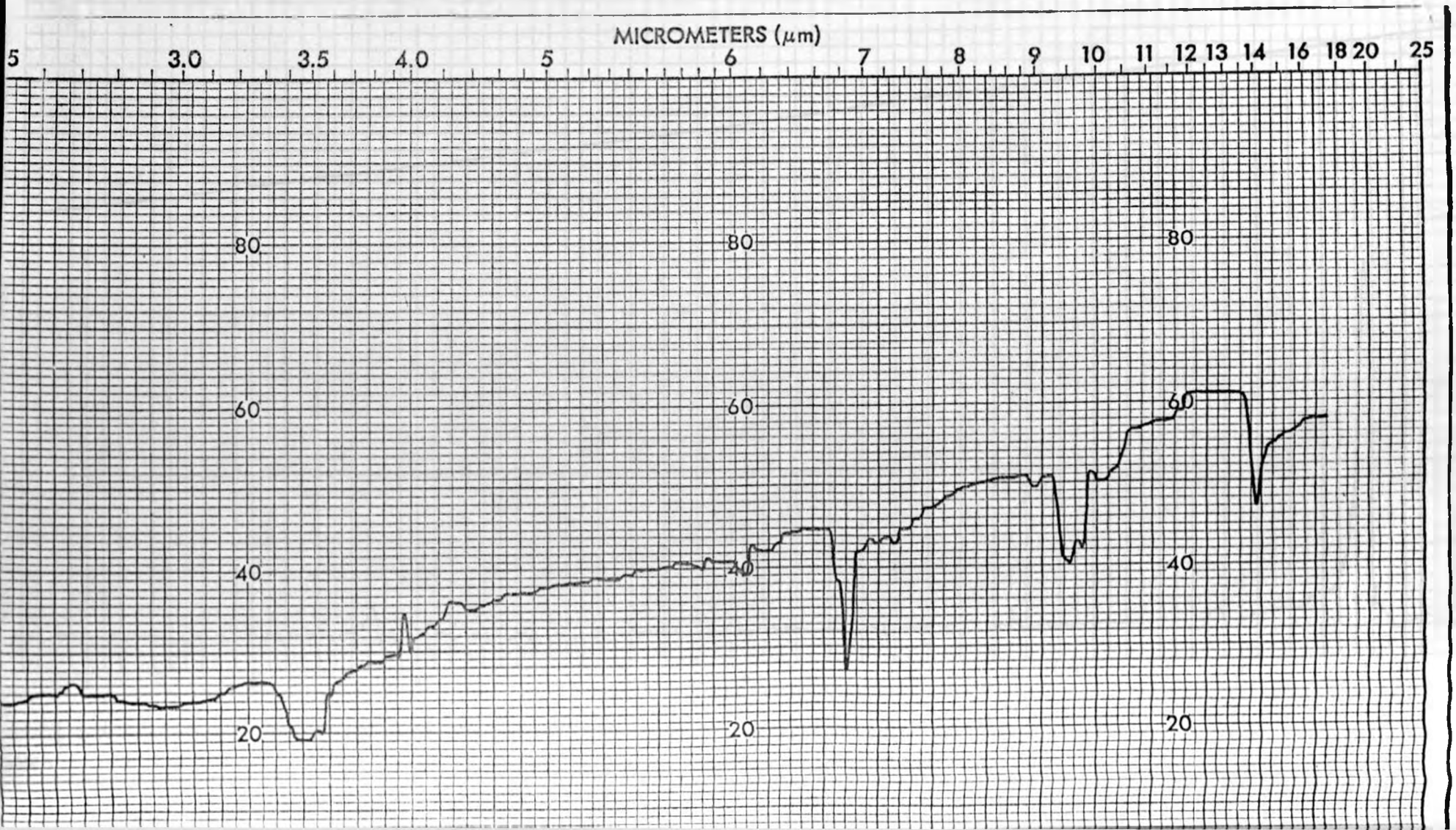


FIGURE 2 – THE ^1H NMR SPECTRUM FOR BK-2

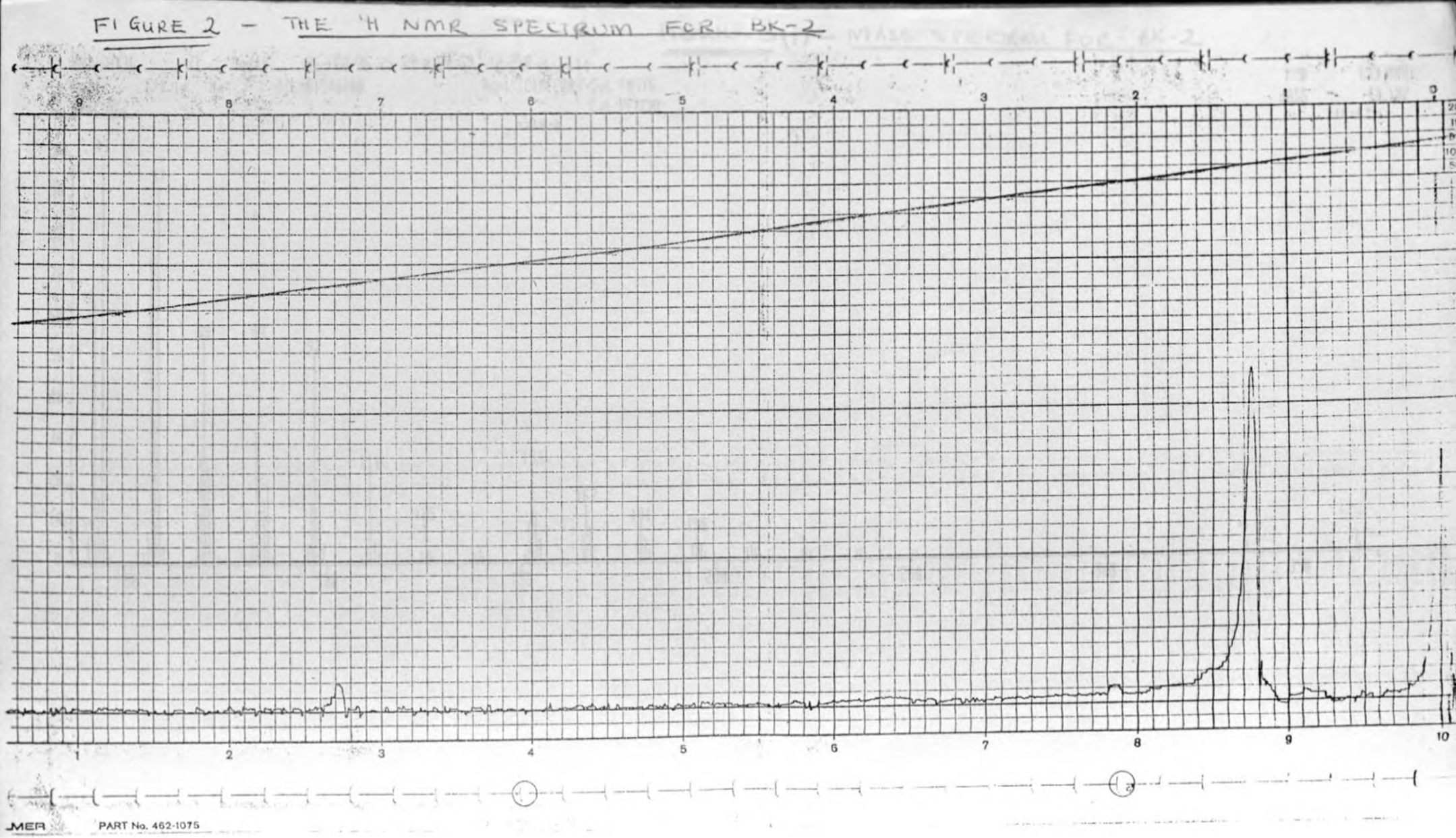
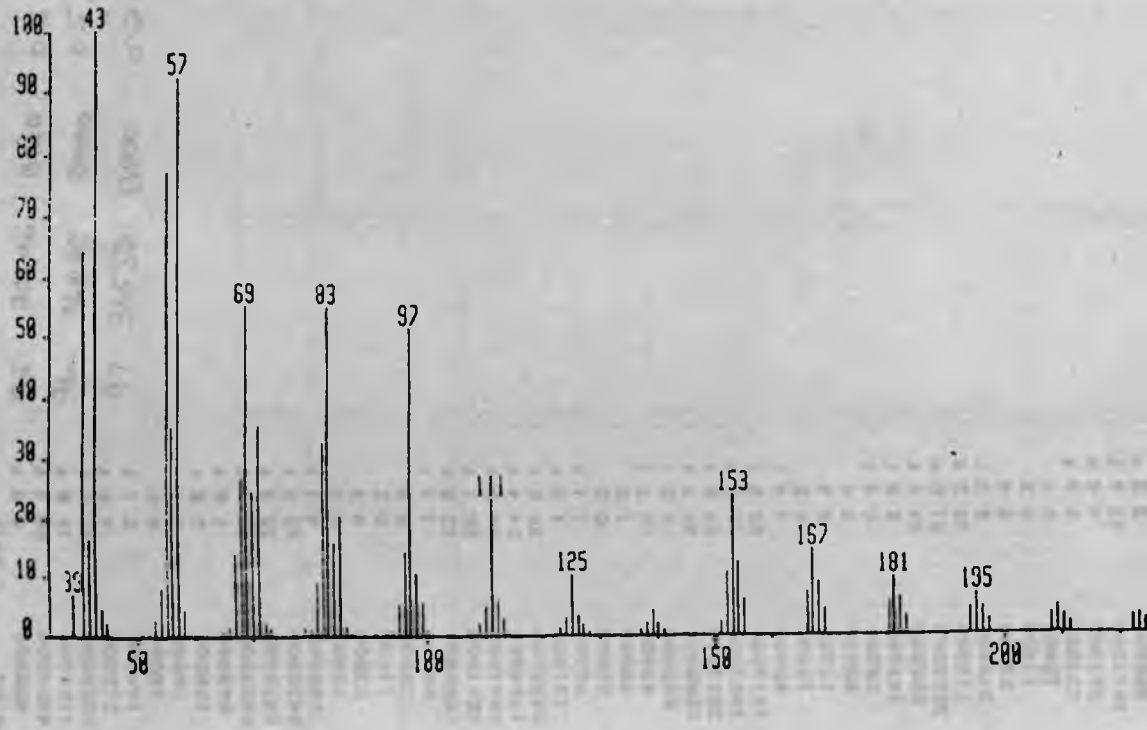


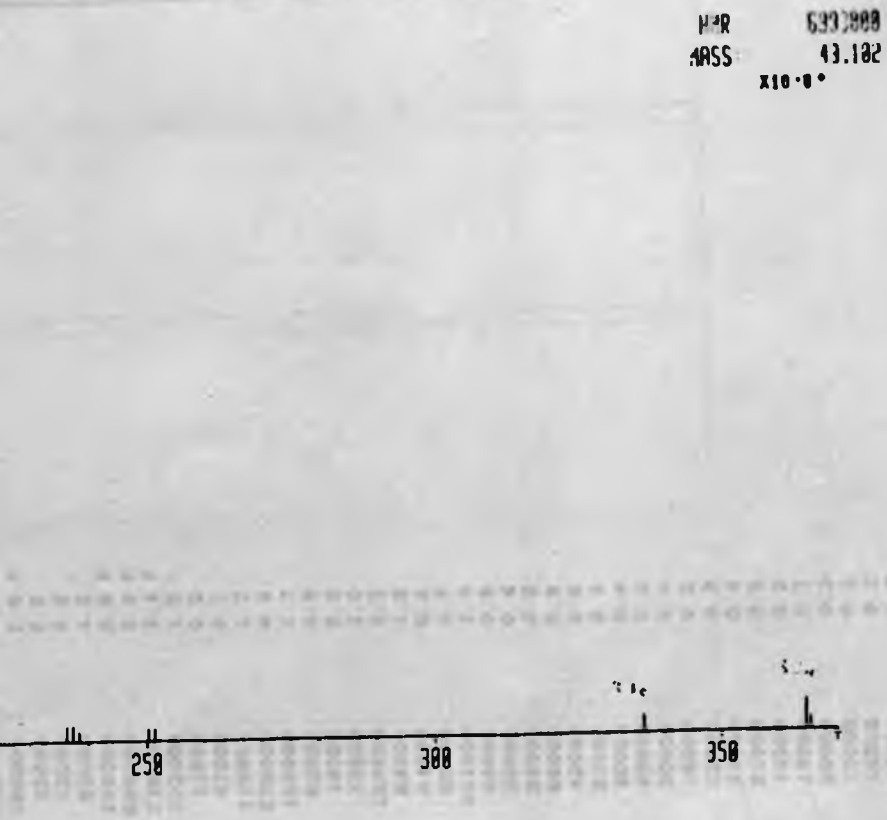
FIGURE 3(i)

AH26OH13G x1 Sgd=3 26-FEB-86 15 03:8 88 59 12-250 EI+
Bpm= I=1.1v Hw= TIC=61546880 Acnt ICIPECORU Sys FMSTS
Text BK2 Cal PFKCAL

*x10+0



- MASS SPECTRUM FOR BK-2

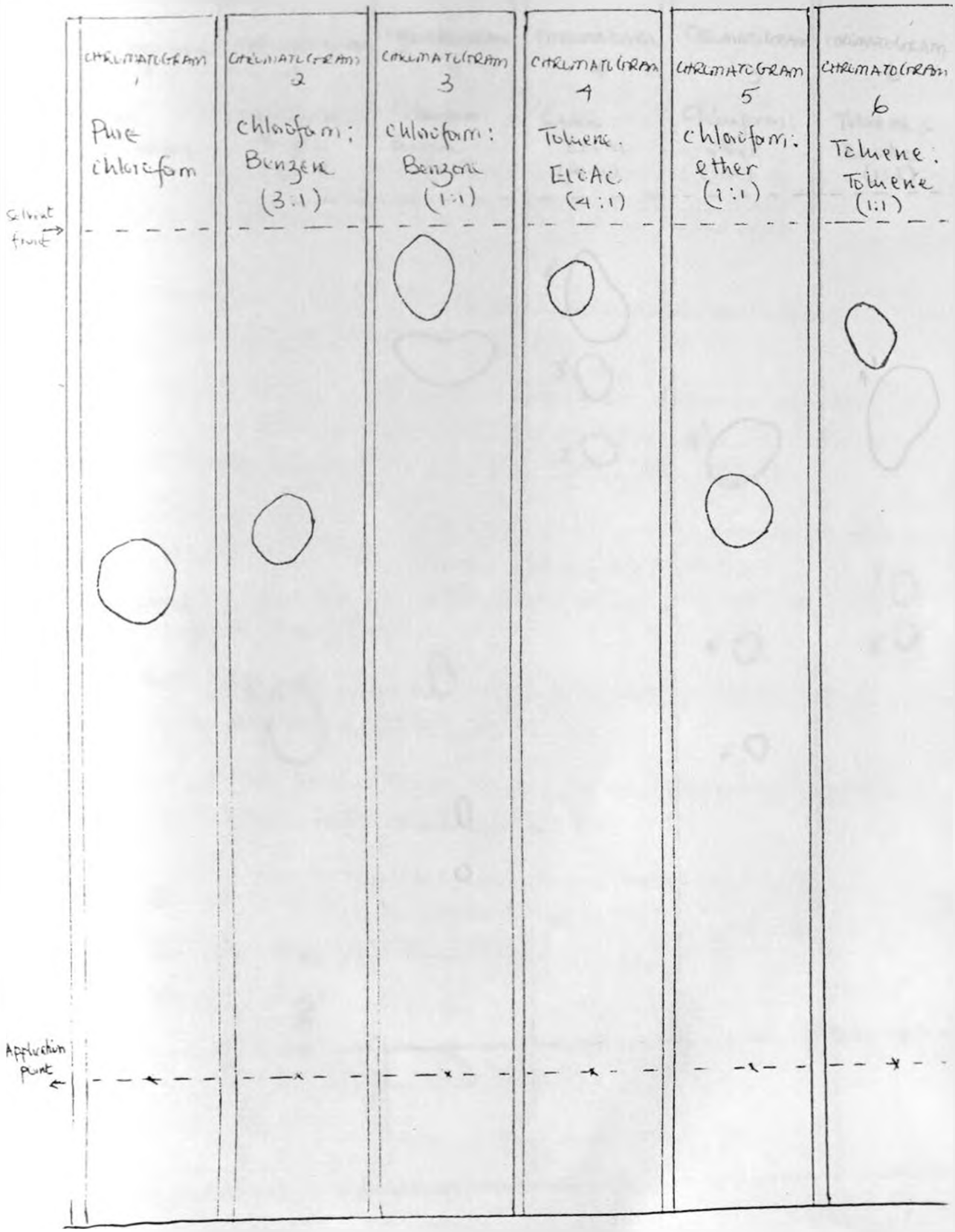


=0 I=1.1v Mm=0 TIC=61546000 Acnl ICIPECBRO Sys PHSIS
t:BK2 Cal:PFKCA

at No	Mass	Abs. Ht	% Base				
1	38.99	485000	6.9 F	95	336.46	19000	0.3
2	41.07	4484000	64.1 F				
3	42.11	1162000	16.6 F	96	364.48	34000	0.5
4	43.10	6993000	100.0 F				
5	44.09	312000	4.5 F	97	365.50	13000	0.2.
6	45.07	144000	2.1 F				
7	50.98	15000	0.2				
8	52.12	10000	0.1				
9	53.03	178000	2.5 F				
10	54.13	543000	7.8 F				
11	55.13	5403000	77.3 F				
12	56.14	2472000	35.3 F				
13	57.14	6436000	92.0 F				
14	58.15	285000	4.1 F				
15	59.03	22000	0.3				
16	60.00	23000	0.3				
17	61.05	11000	0.2				
18	65.03	38000	0.5				
19	66.04	88000	1.3 F				
20	67.12	968000	13.8 F				
21	68.12	1867000	26.7 F				
22	69.12	3841000	54.9 F				
23	70.15	1704000	24.4 F				
24	71.15	2493000	35.6 F				
25	72.16	131000	1.9 F				
26	73.14	79000	1.1 F				
27	77.06	22000	0.3				
28	79.06	70000	1.0				
29	80.10	47000	0.7 F				
30	81.15	597000	8.5 F				
31	82.17	2288000	32.7 F				
32	83.17	3831000	54.8 F				
33	84.17	1091000	15.6 F				
34	85.19	1413000	20.2 F				
35	86.21	95000	1.4 F				
36	87.10	17000	0.2				
37	91.11	17000	0.2				
38	93.10	25000	0.4				
39	94.12	28000	0.4 F				
40	95.20	347000	5.0 F				
41	96.20	995000	14.2 F				
42	97.20	3569000	51.0 F				
43	98.21	717000	10.3 F				
44	99.22	372000	5.3 F				
45	100.11	29000	0.4				
46	107.14	11000	0.2				
47	108.12	10000	0.1				
48	109.12	137000	2.0 F				
49	110.21	323000	4.6 F				
50	111.21	1611000	23.0 F				
51	112.22	386000	5.5 F				
52	113.23	180000	2.6 F				
53	114.17	15000	0.2				
54	122.15	13000	0.2				
55	123.15	82000	1.2				
56	124.15	196000	2.8 F				
57	125.24	695000	9.9 F				
58	126.25	216000	3.1 F				
59	127.17	102000	1.5				
60	128.17	11000	0.2				
61	137.17	47000	0.7				
62	138.16	119000	1.7				
63	139.18	278000	4.0				
64	140.18	122000	1.7				
65	141.19	62000	0.9				
66	151.17	14000	0.2				
67	152.19	73000	1.0				
68	153.20	164000	2.3				
69	154.21	84000	1.2				
70	155.20	41000	0.6				
71	166.22	50000	0.7				
72	167.22	101000	1.4				
73	168.22	60000	0.9				
74	169.25	30000	0.4				
75	180.23	36000	0.5				
76	181.24	66000	0.9				
77	182.26	42000	0.6				
78	183.25	21000	0.3				
79	194.22	30000	0.4				
80	195.25	45000	0.6				
81	196.25	30000	0.4				
82	197.28	15000	0.2				
83	208.27	22000	0.3				
84	209.29	31000	0.4				
85	210.30	21000	0.3				
86	211.32	11000	0.2				
87	222.30	18000	0.3				
88	223.29	20000	0.3				
89	224.30	15000	0.2				
90	236.31	16000	0.2				
91	237.34	17000	0.2				
92	238.36	10000	0.1				
93	250.32	18000	0.2				

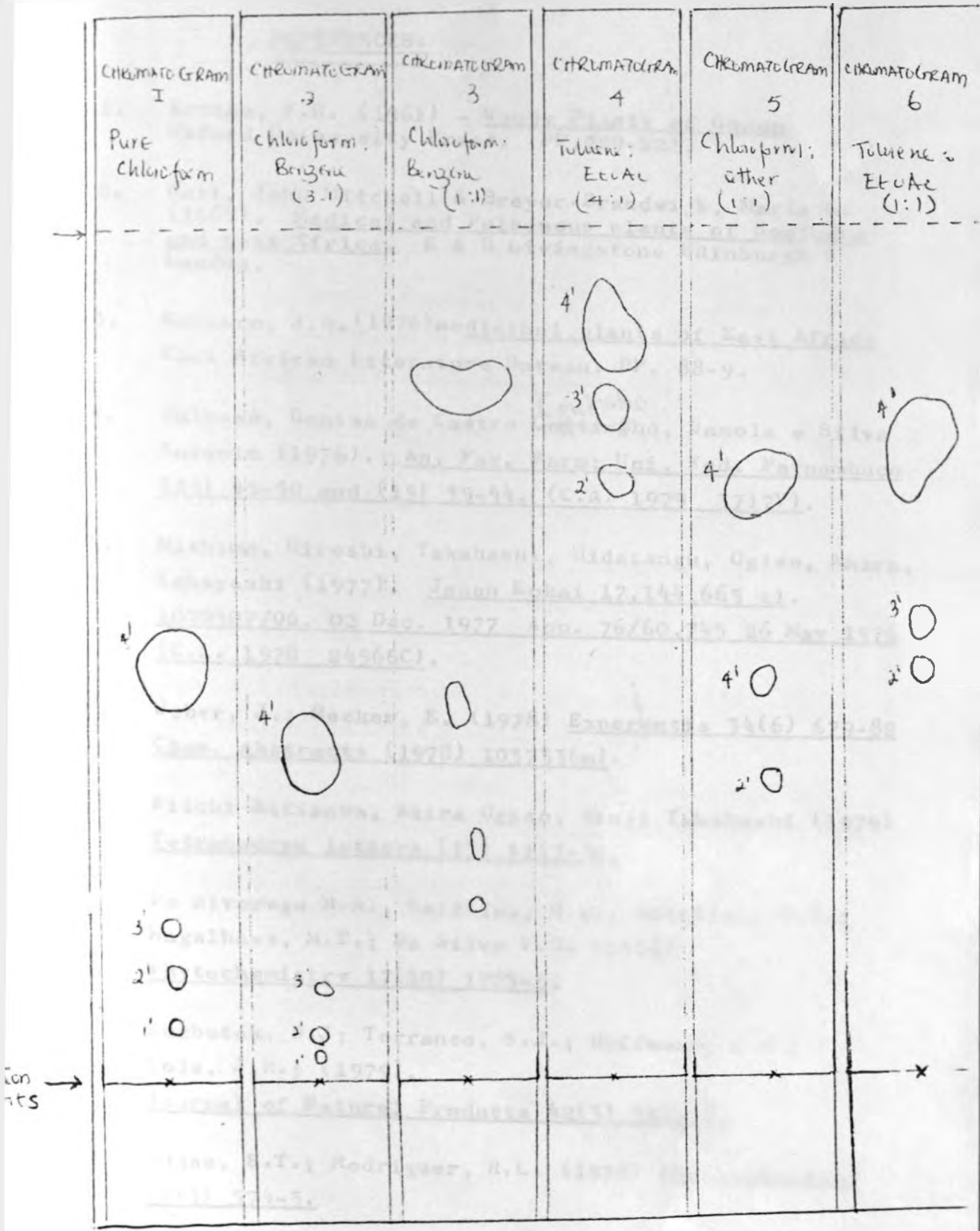
FIGURE 3(ii) - Mass Spectrum data for BK-2

FIGURE 4: CHROMATOGRAMS OF AK 2 METHOL LIQUOR SOLIDS IN VARIOUS SOLVENT SYSTEMS



NB THE SPOTS WERE PINK

FIGURE 5 : CHROMATOGRAMS OF BK 3-6 MOTHER LIQUOR SOLIDS IN VARIOUS SOLVENT SYSTEMS



Key - colour of Spots

1' - Grey
2' - Green
3' - pink
4 - purple

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