CHEMICAL COMPOSITION OF CROTON MACROSTACHYS

A Thesis

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In Partial Fulfillment

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Master of Science in Chemistry

By

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To Ato Agonafir Makonnen, Elilta,

Elias and Makonnen Agonafir who sacrifised

all that my education required

TABLE OF CONTENTS

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ABSTRACT

Chemical Composition of Croton Macrostachys

Root-Bark

By

Dirshaye Menberu

Research Advisor: Dr. Berhanu Abegaz

The root-bark powder of Croton macrostachys Hochstex. A. Rich. (Euphorbeaceae) (ጠጠኘ), used in Ethiopia for curing spreading wounds, was extracted with diethyl ether. The crude extract, on fractionation by column chromatography (Silica gel) yielded a major solid compound A (mp 210-212°) crystallizable from acetone-methanol (9:1) into beautiful needle aggregates, the structure of which was identified, using IR, PMR and MS data, to be that of lupeol, a known antitumor agent. The tumor inhibitory compound Crotepoxide which was previously isolated from the fruits could not be detected in the root-bark extract. Preliminary investigation also showed the presence of trace solids B and C having the same R_f on TLC. B was water isoluble white solid while C was water soluble and showed strong blue fluorescence under UV-lamp. The presence of quaternary alkaloid was also confirmed in the root-bark.

LIST OF TABLES

LIST OF FIGURES

CHEMICAL COMPOSITION

OF

CROTON MACROSTACHYS

I. INTRODUCTION

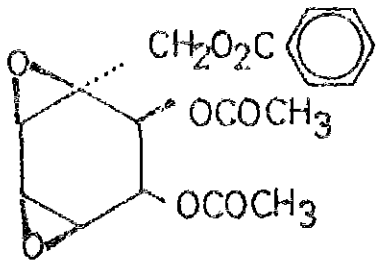
Croton macrostachys Hochst ex. A. Rich (Euphorbiaceae) is a tree that grows in the lowlands as well as in the highlands of Ethiopia.

Description[1]: The tree is usually 6 meters to 12 meters high, sometimes attaining 24 meters. Its bark is grey, when slashed it is brown, even, occasionally with dark brown to black spots and has a strong spicy odour as of pepper. Its flowers are yellow-white in colour, sweet-scented and normally dioecious. This tree is widely spread in regions of 600 meters to 2000 meters elevation. Occasionally it reaches timber size in wetter forests.

The bark of this tree was widely used in Ethiopian medical folklore for the treatment of spreading wounds, besides its common use as an anti-fungal, anthelmintic, taenicidal and purgative agent. The method of dosage preparation and administration of the bark as an effective treatment for "Nekersa" i.e., spreading wounds, was submitted in writing (Amharic) by the grandchildren of Deressa Amente (1884-1975). The later had used this plant in treating patients and before his death had emphasized to his children and grandchildren the importance of this plant and suggested to them that they should endeavour to have scientists and clinicians study it. Another traditional herbalist[2]

enriched the information (in Jan. 1981) by explaining that it is specifically the root-bark of the female tree that is very effective for the purpose.

The plant is also identified by the synonyms Croton accuminatum R. Br. and Rottlera schimperi Hochst. ex.A. Rich, however no chemical investigation has been reported under these two names. Literature survey on C. macrostachys revealed that fruits collected from Ethiopia were studied by Kupchan[3] and crotepoxide (1), the novel tumor inhibitory cyclohexane diepoxide derivative, was isolated. It was found to be effective against both Walker 256(IM) carcinosarcoma and Lewis lung carcinoma in animals. The structure[4] of crotepoxide, $C_{18}H_{18}O_8$, MP. 150-151, ( ) D^{25} + 74 (c. 1.7 in $CHCl_3$) λ_{max}^{MeOH} 274 and 281nm (ε=1,050 and and 860), was shown by X-ray studies to be as follows:

1. 

The infrared spectrum of crotepoxide in chloroform gave the following absorption peaks: 2985, 1751, 1730, 1602, 1585, 1451, 1372, 1271, 1220, 1111, 1042, 977, and 899 cm^{-1}. The PMR signals of the compound in deuteriochloroform were:

: 7.72 (5 H, m, aromatic), 5.73 (1 H, d, J_{XY} = 9.5cps CHOAc), .58 and 4.25 (2 H, doublets J = 12.0cps, CH_2OCOPh), 3.68

(1 H, d, J_{AB}=4.0 cps), 3.10 (1H, d.d, J_{AB}=4 and J_{AY}=1.5 cps), 2.18 (3H, s, acetate) and 2.05 (3H, s, acetate). On crotepoxide, synthetic methods[5a,b,c] have been developed and even its isolation from Piper futokadzura[6] has been reported, although no further work has since been carried out on C. macrostachys, except for an alkaloid screening test that indicated the presence of tertiary and quaternary alkaloids in the stem bark[7]. Therefore the aim of this project was to perform chemical investigation on the root bark, to see if crotepoxide is present here also, as the traditional plantlore places greater emphasis on the root bark than on the fruits or any other part of the plant.

II. Chemical Composition and Biological Activities of the Genus Croton.

This genus is well known in many countries for its medicinal activities and a lot of work has been reported in the chemical literature. The results have also been summarized in chemical and/or phyto-chemical reviews[8a,b]. The important classes of compounds mentioned are the terpenoids, alkaloids, flavonoids, saponins and tannins; as well as many other miscellaneous compounds.

A. Terpenoids: Of the class, the diterpenoids are the most commonly isolated compounds of the genus. Monoterpenoids, sesquiterpenoids, steroids and triterpenoids of only a few species have been reported as shown in Table 1.

Table 1. Terpenoids Isolated From Croton Genus

Class	Name	In (Name of Species)	Part of Plant
Monoterpenoids	α-Pinene; Sabinene;		
and	1,8- Cineole; β-elemene		
Sesquiterpenoids	2 - Humulene	C. argyrophylloides[9a]	Leaves
	Camphor; isoborneol	C. Zehntneri[9b]	
	Caryophyllene; γ-elemene	both species	
Diterpenoids	Phorbol	C. rhamnifolius[10]	
"	Phorbol esters	C. tiglium[11a, b]	Fruits
"	Phorbol esters	C. flavens[12]	roots
"	Plaunol	C. columnans[13]	whole plant
"	Plaunol A, B--	C. sublyratus[14]	root & stems
"	Crotofolin E	C. corylifolius lam[15]	
" Clerodane	Corylifuran and Crotofolin A.	C. corylifolius[16]	leaves & twigs
"	Diasin	C. diasii[17]	trunk wood
"	Draconin	C. draco[18]	
	Crotocaudin	C. caudatus[9a]	
" nor-clerodane	Iso-Crotocaudin	" [9b]	stem-bark
" nor- "	Crotonin	C. Lucidus[20a,b]	leaves & twigs

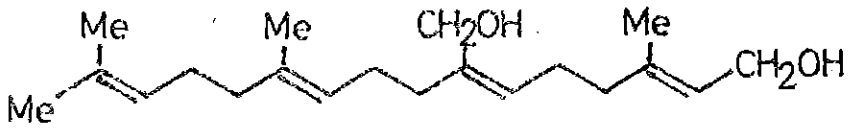
Table 1. Terpenoids Isolated From Croton Genus (Contd.)

Class	Name	In (Name of Species)	Part of Plant
Dit. nor-clerodane	Dehydro-crotonin	C. Cajucara[21]	
Diterpenoid, nor-furanoid	Hardwickicacid and methyl barbascoate	C. Californius[22a]	whole plant
	Hardwickic acid and 11-dehydro hardwickic acid	C. oblongifolius[22b]	
" acid	Argyrophilic acid	C. argyrophylloides[23]	
" " Pimaroid,	Oblongifoliol and Five other iso-pimaradiene derivatives	C. Oblongifolius[24-27]	stem bark
"	Cascarillin	C. Cascarilla[8b]	
"	Nevenolide	C. neveus[28]	leaves
Steroid	β-sitosterol	C. caudatus[29]	
		C. Oblongifolius[27]	stem bark
		C. Sparsiflorus[30]	
C_{13}-compound of steriod origin	Vomifoliol	C. Linearis[31a]	
Triterpenoids		C. trinitatus and C. lobatus[31b]	
	β-amyrin; taraxerol; taraxerone, taraxeryl acetate	C. caudatus[19a, 29]	
	Acetyl aleuritolic acid	C. oblongifolius[32]	

The biological activities of many croton species can be attributed mainly to their terpenoid composition, especially the diterpenoids in their various forms. This will be shown later on in Table 4. Three compounds given below are examples of active diterpenoid derivatives with varied structures.

Plaunol - diterpenoid alcohol
from C. columnans[13]
an anti-digestive tract ulcer

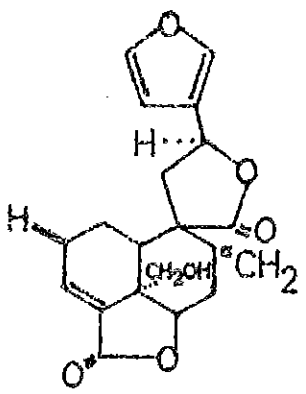
2.



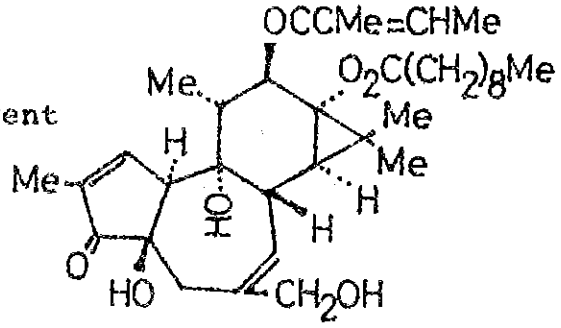
Plaunol B, - diterpenoid lactones
from C. sublyatus
anti-peptic ulcer

Phorbol 12-tiglate, 13-decanoate - diester
from C. tiglum
an antileukemic agent

3.

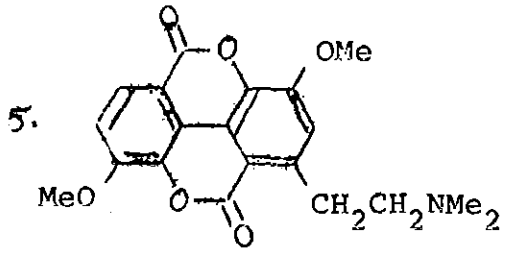


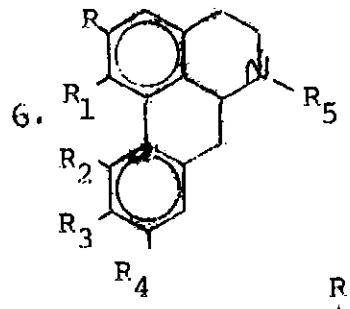
4.

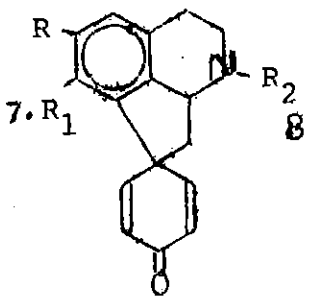


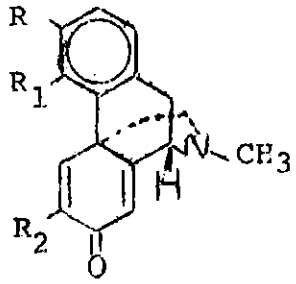
B. Alkaloids: An anti-inflammatory alkaloid, taspine, (5) has been isolated recently from C. lechleri[33] and from the late of C. draconoides[34]: It has an unusual structure.

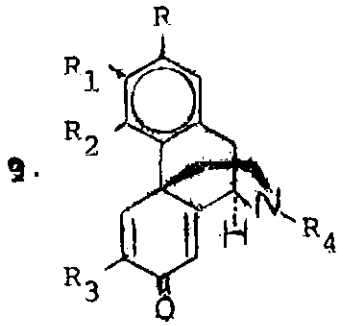
It has been suggested that it may be derived from aporphine precursors since aporphine alkaloids, thaliporphine and glaucine, are found in the leaves and twigs of C. draconoides. All the alkaloids isolated from the genus so far have been classified in structure as aporphine (6), proaporphine (7), morphinandienone (8,9) bases and reduced forms of these[8b].











Many of the known alkaloids isolated from the genus are given in Table 2 and the biologically active alkaloids are reported again in Table 4.

Table 2. Alkaloids isolated from the Genus Croton

Structural type	Name	In (species name)
Aporphine	Taspine	C. draconoides,[34] C. lechleri[33]
	Thaliporphine, glaucine	C. draconoides[34]
	Wilsonirine, hernovine, N-methyl hernovine, O^{10}- methyl hernovine,	C. wilsonii[8a]
	Sparsiflorine	C. sparsiflorus[35]
Morphinandienone	Sinoacutine, norsinoacutine	C. linearies, C. plumier[8a]
	Flavinantine, flavinine salutaridine	C. balsamifera, and C. flavens[8a]
dihydroaporhine	Crotsparinine, n-methyl-Crotsparinine	C. sparsiflorus[35]
Proaporphine	Crotonosine	C.linearis, C. discolor[8a]
	Pronuciferine	C. linearis[8a]
	N-methyl crotonosine	C. plumieri[8a]
	Linearisine jacularine discolorine, jaculadine	C. linearis, C. discolor[8a]
	Crotsparine, N-methyl-Crotsparine; N, O-dimethyl-Crotsparine	C. sparsiflorus[35]

C. Flavonoids: Table 3 gives some of the recently isolated compounds of this class.

Table 3: Flavonoids isolated from the genus croton

Name of Flavonoid	in (species name)
Hexamethoxy xanthone	C. californicus[36] (whole plant)
Vitexin, saponaretin, orientin Iso-orientin, vicenin -1	C. Zambesicus[37]
Quercitin, procyanidin	C. Oblongifolius[36]
Rutin	C. Sparsiflorus[39]

D. Saponins: Of 26 species tested for saponins, 9 gave positive results[8b]

E. Tannins: These have been detected in or isolated from seven of sixteen species tested[8b].

F. Miscellaneous isolated or detected compounds: Dotrioctanol[28] crotin-1[40], fatty acids[41,8b], 1-triacontanol[22], isoeugenol, eugenol, P-cymol, angelic acid, citral, vanillin, catechin, 4-hydroxy-hygric acid, crotonoside, sucrose and several common aminoacids[8b] have been isolated or detected.

Table 4 gives a summary of the medicinally important (useful and dangerous) compounds so far identified in the genus.

Table 4. Biologically active compounds isolated from the genus croton

Class of compound(s)	Name of compound	In (species name)	Biological activity
Cyclohexane diepoxide deriv.	Crotepoxide	C. macrostachys[3]	Tumor inhibitor
Essential oils	Estragol trans-anethole, methyl-isoeugenol safrole, camphor isoborneol, caryophyllene, γ-elemene	C. zehntneri[9b]	flavor
Protein	Crotin - 1	C. tiglium[40]	hemolytic
Diterpenoid(s) alcohol	Plaunol	C. columnans[13]	anti ulcer
" lactones	plaunol A,B,C,--	C. sublyratus[14]	
" acid	argyrophilic acid	C. argyrophylloides	antimicrobial
" ester	phorbol 12 - tiglate-13-decanoate	C. tigluim[11a]	antileukemic
" "	another phorbol derviative ester	C. tiglium oil[11b]	inflammatory
" "	Another phorbol derivative ester	C. flavens[12]	esophageal cancer
Alkaloid	Taspine	C. draconoides & C. Lechleri[33, 34]	anti-inflammatory
	Pronuciferine	C. linearis[8a]	
	Crotonosine	C. Linearis and C. discolor[8a]	local anesthetic
	N-Methyl crotsparine	C. sparsiflorus[35]	hypotensive

G. Crotepoxide: Returning to crotepoxide, the known component of C. macrostachys, relevant data have been recorded earlier and isolation method is given below in order to facilitate its separation and identification from the bark extract if it is indeed present.

Method of isolation of Crotepoxide: An extract can be obtained from the plant material by extraction with diethyl ether, and the extract concentrated by distillation under reduced pressure. Treatment of the crude extract with n-hexane precipitates crotepoxide and similar compounds. The crotepoxide can be separated by filtration, dissolved in benzene, applied on silica gel and eluted with benzene to wash off the non-polar impurities. Elution with benzene: ethyl acetate (1:1) washes the crotepoxide off the column. This can be concentrated and the impure crotepoxide obtained can be recrystallized from ethyl acetate - n-hexane to give colorless prisms (MP 153^{O}).

This is only one of the many methods of isolation which was used to obtain crotepoxide from Piper futo-kadzura. It has also been isolated from the ethanol extract of C. macrostachys[3], the details of which are not easy to perform here. The given method can be modified depending on the type of impurities existing with crotepoxide in the plant parts.

III. Results and Discussion

A. Extraction of C. macrostachys root-bark powder: Root-bark powder of C. macrostachys packed in a glass column was eluted with diethylether. The solvent was removed by distillation under reduced pressure and a semi-solid product, yellowish in color was obtained.

B. Fractionation of the semi-solid product: The semi-solid product was applied to a silica-gel column and eluted successively with the solvents: hexane (500 ml), hexane-chloroform 9:2 (400 ml), 3:1 (400 ml) and 3:2 (550 ml), hexane-chloroform-ethylacetate 5:4:1 (250 ml), 5:3:2 (300 ml), 1:1:2 (200 ml), and 1:1: increasing amounts of ethyl acetate (300 ml), and ethyl-acetate alone (200 ml). Fifty ml fractions were collected and a total of 63 fractions were eluted. On concentration of each under reduced pressure, the first 23 gave yellowish liquids and semi-solids, 24 to 29 gave powdery solids, 30-34 gave yellowish liquids, 35 to 41 gave yellowish semi-solids and to 63 gave semi-solids and liquids (yellow in color).

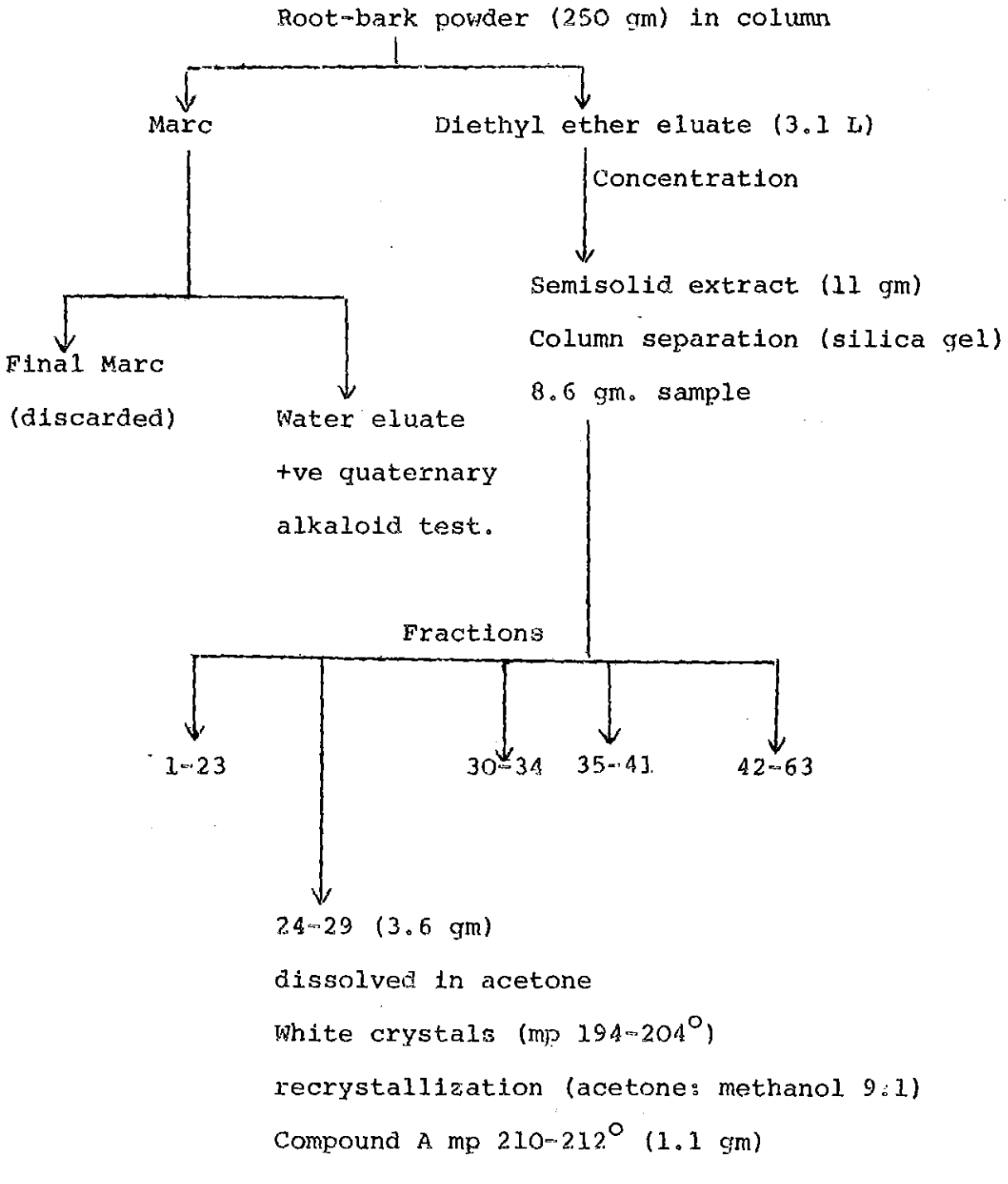
C. Purification of Compound A

The solid from combined fractions 24-29 was completely dissolved in hot acetone, filtered hot and left for a few days. A crystalline solid (mp 194-204^{o}) separated. This was recrystallized repeatedly

until long, needle-like crystals of compound A (mp 210-212°) were achieved (Figure 1).

Figure 1

Extraction and Fractionation of C. Macrostachys

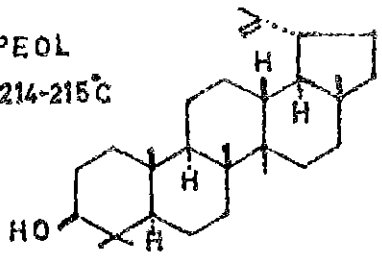


D. Characterization of Compound A

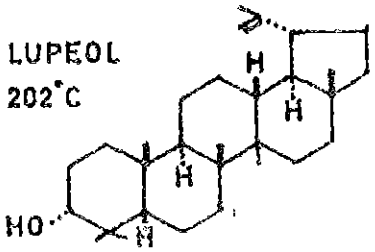
The PMR data of crude A indicated a triterpeniod structure. The mass and infrared spectra (pp[27, 28]) of crystalline A (mp 210-212°) showed a molecular ion peak of 426 and a hydroxyl function (3325 cm^{-1}) respectively. The PMR spectrum of the crystalline A was also taken (p[29]). The presence of 1 hydroxyl function was confirmed by acetylating the crude A (mp 200-207°) for 2 weeks at room temperature followed by 1 hr. of heating (boiling water bath). The compound obtained, A-1, showed a molecular ion peak of 468 (p[30]) and the IR (p[31]) showed no more peak of the hydroxyl function. Compound A gave a positive Leibermann-Burchard test and decolorized a solution of bromine in carbon-tetrachloride. All the triterpenoids of mw 426 were reviewed and those having mp range of 194-217° were selected (structures 10 to 21).

STRUCTURE OF TRITEPRENOIDS[44]

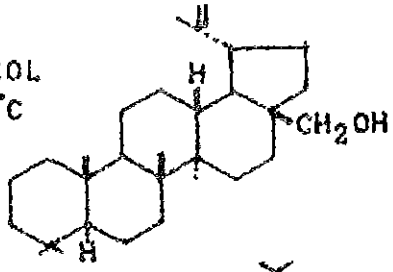
10. LUPEOL
mp 214-215°C



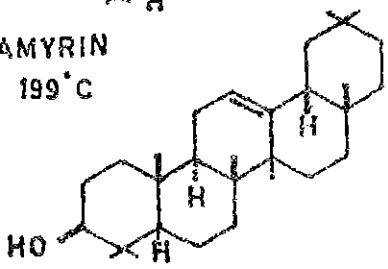
11. EPILUPEOL
mp 202°C



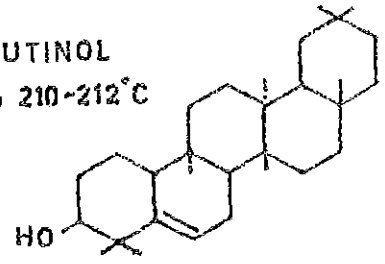
12. JASMINOL
mp 209°C



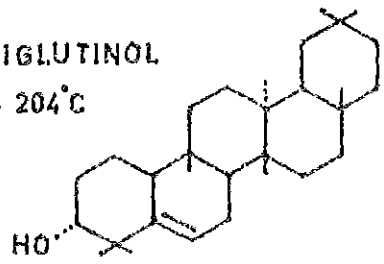
13. β-AMYRIN
mp 199°C



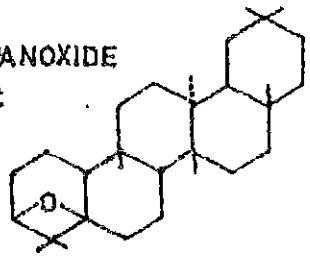
14. GLUTINOL
mp 210-212°C



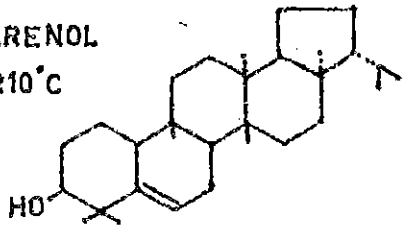
15. EPIGLUTINOL
mp 204°C



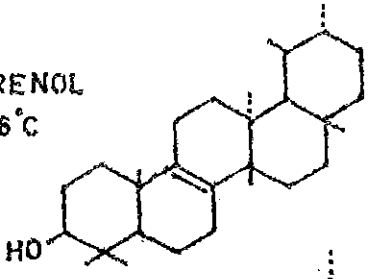
16. DENDROPANOXIDE
mp 207°C



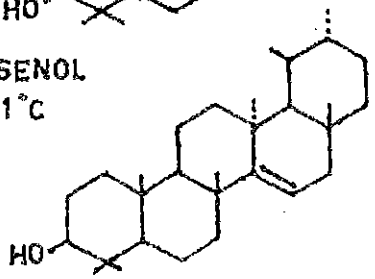
17. SIMIARENOL
mp 210°C



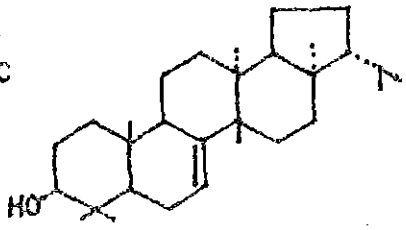
18. BAUERENOL
mp 206°C



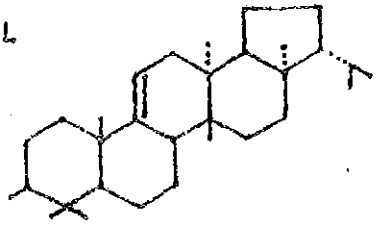
19. ISOURSENOL
mp 201°C



20. MOTIOL
mp 217°C



21. FERNENOL
mp 194°C



Structure 16 was eliminated immediately because it does not possess any unsaturation.

Comparison of the PMR, MS and IR spectra of A with the available literature data indicated very close correspondence with those of structure 10[42a,b, 43a,b].

In the PMR spectrum of A, the vinylic protons were observed at δ =4.6. The IR of A indicated a peak at 3080 cm^{-1} (= C$\diagdown^{H}$ Stretching band) and a strong absorption band at 1640 cm^{-1}, both confirming the presence of a terminal olefin characteristic of structures 10, 11 and 12. Furthermore, the lupane skeleton was confirmed by the major peaks of the MS (218, 207 and 189) besides the m/e peak at 426. This eliminated all the other structures (13-15 & 17-21).

In addition to the high melting point of A, the presence of β- OH peak in its IR data (1040 cm^{-1} due to the stretching vibration of the C-O bond) and $[\alpha]_D^{20.5}$ +26.7 (lit. + 27) favoured the structure 10 over 11. Structure 12 was finally eliminated by the following observations:

a) Acetylation of compound A was difficult indicating that the hydroxyl group was not primary.

b) The oxidation product of A, A-2, was found to be a ketone and not an aldehyde as was shown by the IR of A-2 (absorption at 1715 cm^{-1} (p.[33]).

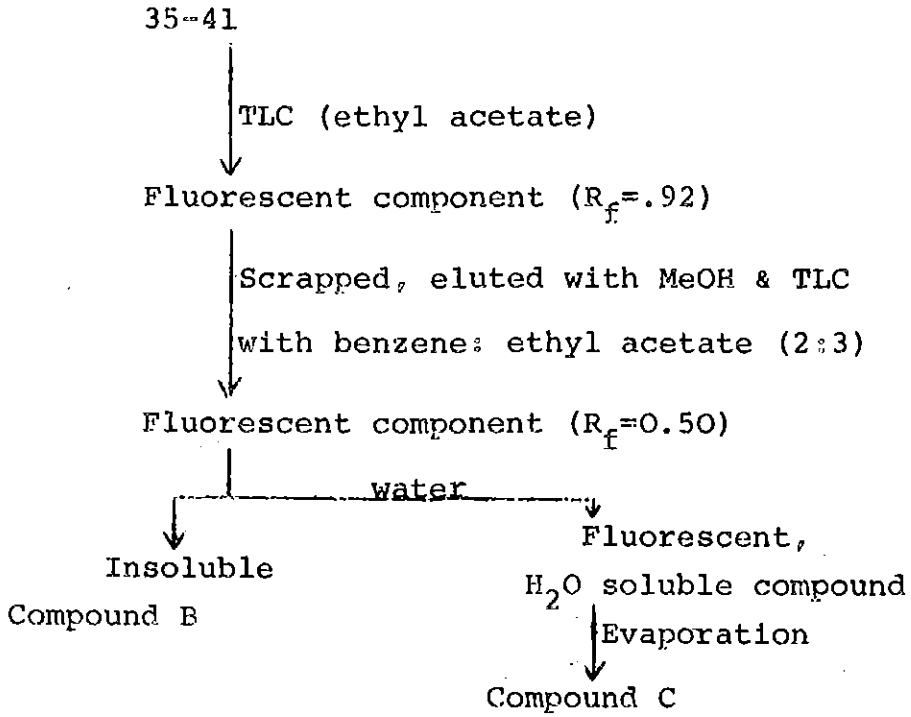
E. Preliminary Investigation of Other Components

1. Fluorescent Component: Analytical TLC of semi-solid fractions 35-41 using hexane-chloroform (1:1) revealed a fluorescent band (R_f:0.17). The best solvent system for this component was found to be benzene-ethyl-acetate (2:3) in which its R_f increased to 0.50

This fluorescent component was then separated by preparative TLC. Mass spectral analysis of this component showed an m/e peak of 470. However this was found to be a mixture because, when water was added to it, the component separated into a colorless solid (B) and a fluorescent aqueous solution. On evaporating the water from the aqueous solution a trace compound C was observed (Figure 2).

Figure 2

Fractionation of Fraction 35-41



2. Quaternary alkaloid: The water eluate of the Marc, left on the column after dietylether treatment, gave an orange brown solution. When tested with either Dragendorff or Mayer's reagents this solution gave an appreciable quantity of precipitate indicating the presence of quaternary alkoloid(s).

Chloroform failed to extract the alkaloid(s) from an aqueous solution made alkaline to litmus with a saturated solution of sodium carbonate.

IV. Conclusion

The major solid component of the diethyl ether extract of the root-bark (as well as the stem-bark) powder of C. macrostachys was found to be lupeol (10). (The stem-bark was the first sample taken up in this project but it was abandoned because of its low yield in total extract, 1.8%, when compared with that of the root-bark 4.4%). The presence of lupeol, as a major product is reported herein for the first time. So far, no species of this genus other than C. macrostachys has been reported to contain even trace amounts of this compound.

The procedure that was developed for the isolation of crotepoxide from Piper futokadzura was used in this project. However no trace of the epoxide could be detected. It is therefore concluded that this compound is not present in detectable amounts in the root-bark of C. macrostachys.

Whether the presence of crotepoxide in the fruits and that of lupeol in the bark of the same plant indicates any biogenetic relationship between these two compounds is a question that is open for speculation.

Facts of medicinal interest about lupeol are given in the literature. Lupeol is said to be effective in treating rheumatism and urinary tract infections.[45] Miles and Kokpol[42b] isolated and identified lupeol from the chloroform extract of the roots and leaves of

Sarracenia flava as one constitutent in this plant responsible for antitumor activity against lymphocytic leukemia p-388. In an earlier work, lupeol (reported: MP 210-212°, $[\alpha]_D^{20}$ + 25°) was identified as one component of the chloroform extract of the stem-bark of Alnus Oregona (Betulaceae)[46] responsible for antitumor activity against the walker 256 (5WA 16) tumor system. This shows that lupeol is a known antitumor agent. Therefore the activity of the root-bark powder of C. macrostachys against "Meshero Nekersa" may be due to its lupeol content. Eventhough the meaning of the Amharic term "Nekersa" is often confused, it is sometimes used to describe certain types of cancer. However, even if the disease is bacterial in origin, again lupeol may be of some therapeutic value, because at least lupeol acetate[47], isolated from Mikania monagasensis, was shown to be one of the compounds found active against staphylococcus aureus and Candida albicans. On the other hand, this part of the plant contains many other compounds whose nature have not yet been identified; for example, the quaternary alkaloid(s) may possess similar medicinal activity. Therefore, it is impossible at this stage, to give conclusive results on the active principles of the root-bark of C. macrostachys that are responsible for curing spreading wounds.

V. Experimental

PMR spectra were determined on solutions prepared in deuteriochloroform with TMS as internal standard, using a Varian T-60 A spectrometer. IR of the solids in KBr pellets were determined using a Perkin Elmer model 727 B Grating spectrometer. Mass spectra of the solids dissolved in acetone were recorded on a Finnigan model GC/MS 3200F Spectrometer. Adsorbent used for column Chromatography was silica gel $60F_{254}$ (Merck) 70-230 mesh. Analytical and preparative thin-layer chromatography were carried out on 0.25 mm thick Polygram Sil G/UV_{254} of 0.20 mm thick TLC alluminum sheets silica gel $60F_{254}$ (Merck) and 2mm thick silica gel PF_{254} BM) respectively. Melting points were determined with Thomas Hoover Capillary Melting Point Apparatus.

The first plant sample used for this project was the stem-bark bought from Markato. Then the root-bark used finally was obtained from the roots collected from Shoa-Sebeta (2,200 m elevation) on Feb. 1st 1981. The root was washed with water to make it free from mud, slashed and the bark crushed well into small pieces, spread on cheese-cloth and sun dried for two days. This was ground in a blender and the powder was sieved in an 80-mesh seive. This was again separated from fine powder using a 50 - mesh sieve. The coarse powder that was left on the 50-mesh sieve, referred to as the root-bark powder, was used

for extraction.

Extraction of the root-bark powder of C. macrostachys

A long glass column (34 cm by 5 cm diameter) was plugged with cotton at the bottom and a filter paper (5 cm diam.) was placed over the cotton. Five layers of 50 gm. each of root-bark powder (i.e. 250 gm) separated by filter paper were packed dry in the column. This was eluted slowly by diethyl ether and a total volume of 3 liters eluate was collected over 24 hrs. Concentration of this under reduced pressure gave 11 gms (4.4%) of a yellowish semi-solid product.

Fractionation of the Diethyl ether extract

The semi-solid product (8.6 gm) was thoroughly mixed with 10 gm of dry silica gel until a powdery mixture was obtained. Silica gel (90 gm) was packed with hexane into a glass column (2 cm diam.) The powdery mixture was transferred onto the top of the column and elution with hexane continued. Fractions of 50 ml each were collected. The first fraction gave a brownish yellow concentrate which when treated with methanol gave a brittle solid (.052 gm). The following 22 fractions (9 with hexane, 8 with hexane:-chloroform (9:2) and 5 with the same solvent system (3:1)) gave yellow oils on concentration. The 24th fraction gave a yellowish-white solid. Two further fractions with the above solvent (3:1) and

3 more fractions with same solvent system (3:2) when combined with fraction 24 gave 3.65 gm solid. Eight more fractions with the above solvent (3.2) gave yellow oils and semi-solids. After 37 fractions had been collected, the solvent was changed to hexane: chloroform: ethyl acetate (5:4:1) and 5 fractions were collected. The next 6 were obtained by using the above solvent (5:3:2). Then 4 fractions were collected by the same solvent (1:1:2) which was gradually made more polar by increasing the proportion of ethyl acetate as fractions 53 to 59 were collected. The last 4 fractions were eluted with ethyl acetate alone.

Isolation of Compound A from fraction 24-29

The solid obtained from fractions 24-29 (3:65 gm) was first stirred with hexane (10ml) and then filtered. The residue was then stirred with methanol and filtered. The resulting solid was dissolved in 20 ml hot acetone, filtered hot and left for a week. The hexane and methanol washings also were left open. White crystalline solids were obtained in all three (though only trace quantities were found in the washings). The crystalline solid obtained from the acetone solution was filtered and then repeatedly recrystallised from acetone methanol (9:1). Finally long needle-like crystals of compound A were obtained and the melting point improved from 194 - 204^{o} to 210 - 212^{o}. Compound A was observed to be highly soluble in the oily

products produced in the fractions just before and after fractions 24-29. Finally characterized to be lupeol, compound A gave the following data:

MP: 213 - 214° (Lit. 214 - 5°), $[\alpha]_D^{20.5}$ + 26.7 C,0.025 in $CHCl_3$) (Lit. $[\alpha]_D^{25}$ + 27)

PMR: ($CDCl_3$, HZ): 47, 52, 59, 64, 59, 49, 103

(Lit) 46, 50.5, 57.5, 62, 57-59, 47.5, 102

IR: (KBr) cm^{-1}: 3325, 3080, 1640, 1040, 980, 890

(Lit.) in C Cl_4 & CS_2): 3630, 3073, 1640, 1040, 883

MS: m/e, 426 with major fragments at 218, 207 and 189 (same as literature data [42b])

Acetylation of compound A: Compound A (0.200 gm) was taken in a flask fitted with a reflux set up. Acetic anhydride (5 ml) and pyridine (5ml) were added and this was kept for over two weeks at room temperature, but the solid did not disappear during this time. The reaction mixture then was heated in a boiling-waterbath for 1 hr. and a homogenous solution was obtained. This solution was poured over 50 gms of ice in a beaker and stirred until hydrolysis of acetic anhydride was complete. The resulting solid suspension was filtered and compound A-1 (1.8gm) was obtained. It was recrystallized from acetone and the acetylation proved by ms (468). Its mp was determined (213-14°), (Lit. mp 217-218°) when re-crystallized from ethanol). The spectral data are:

PMR: ($CDCl_3$, HZ) 46, 51.5, (57.5), 63, 57.5, 48, 103

(Lit) 49.5 - 52.5, 62.5, 56.5, 47.5, 102,

IR (KBr) Cm^{-1}: 3085, 1745, 1640, 1245, 1025, 1015, 975

(Lit.) in CCl_4 & CS_2), 3071, 1640, 1243, 1026, 1015, 978

MS: m/e 468

Oxidation of compound A with Jones Reagent: Compound A (0.200 g) was dissolved in acetone (30 ml) and Jones reagent (2 ml) added and stirred for 2 hrs at room temperature. The greenish precipitate was filtered and the filtrate diluted with water (50 ml). The resulting solution was extracted 5 times with 40 ml portions of diethyl-ether. The combined ether extract was twice washed with 15 ml of water and the ethereal solution was dried over anhydrous Sodium sulphate and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in benzene and applied to a column of silica gel (10 g) packed in benzene. Elution was carried out with benzene. The first fraction, eluted with 50 ml benzene, was concentrated and the solid obtained was crystallized from methanol to give A-2 (mp 154-156°), (Lit. mp 166-168°, $[\alpha]_D^{20}$ + 60.6° (C, 0.5 in $CHCl_3$)[48]. Compound A-2 needed further purification but since the quantity was small, the crude was used to obtain the following spectral data:

PMR (CD_3Cl_3:, HZ) 57, 61, 64, 64, 57, 102

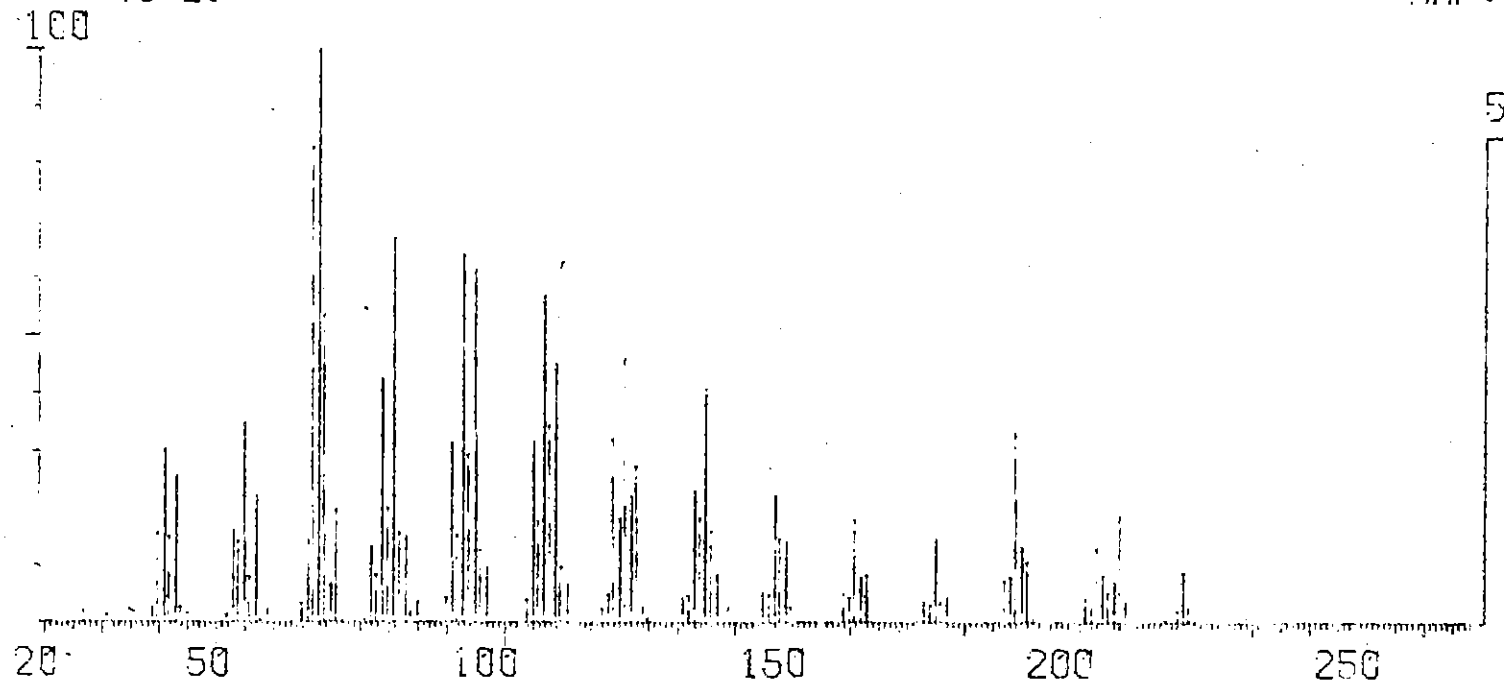
Lit. for lupanone[42.a] 56.5-58.5, 62.5, 65, 65, 56.5-58.5, 46.5

IR (KBr) 1641 3070

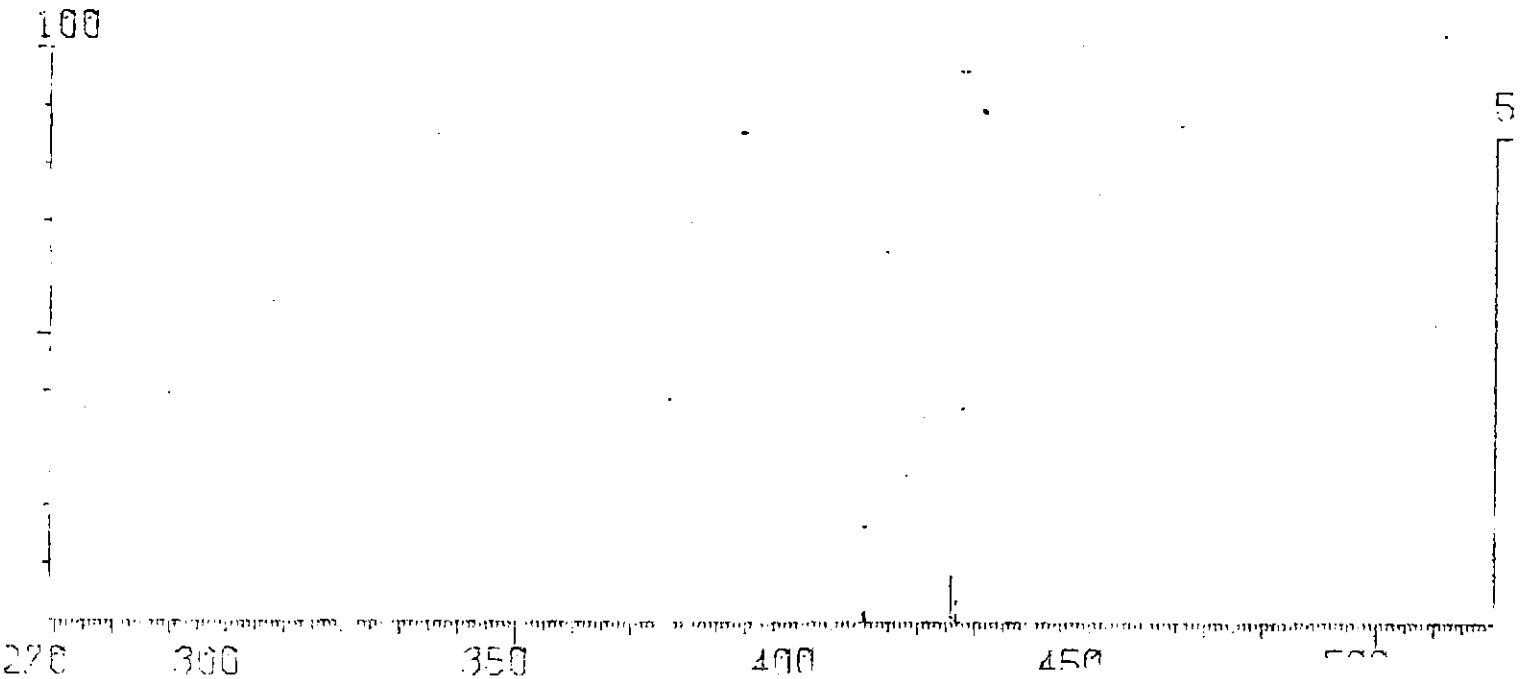
Lit (in CCl_4 or $CHCl_3$) 1641 (C=C stetching) 3070 (C-H stretching)

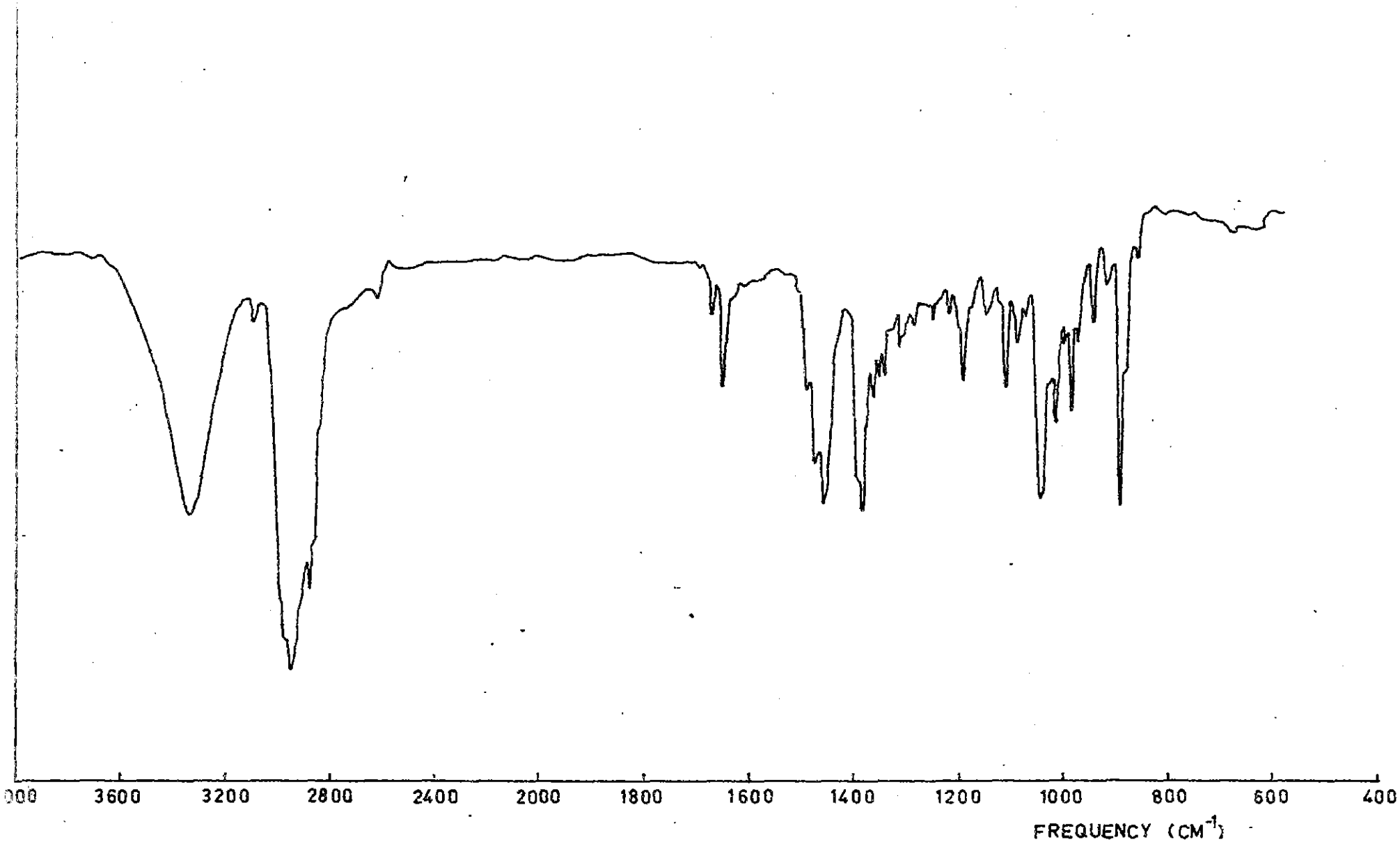
DM-16 LUPEOL DERIVATIVE

40-20 AMP.: 00101184

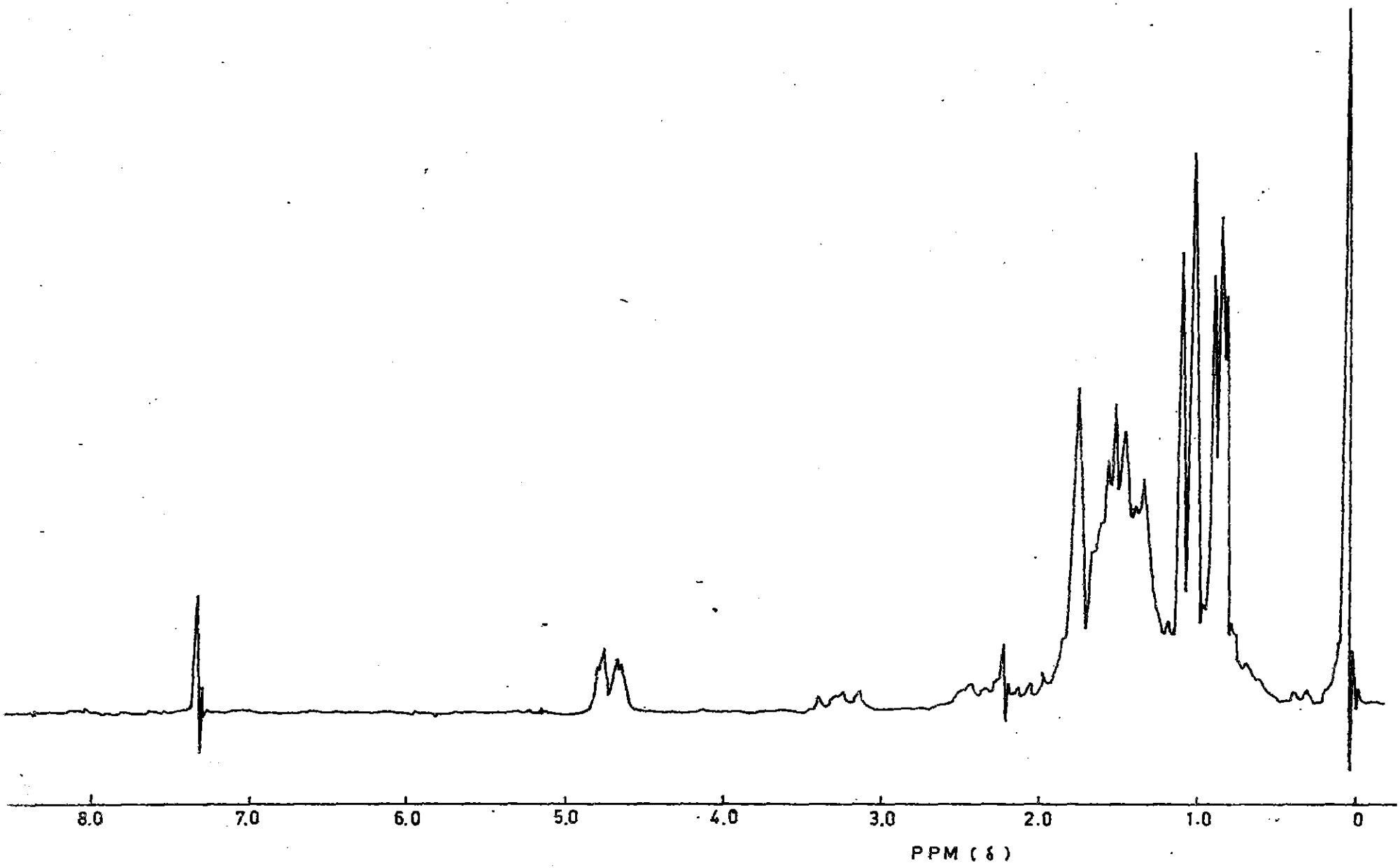


27 Spectra





INFRARED SPECTRUM OF A CRYSTALS (KBr)



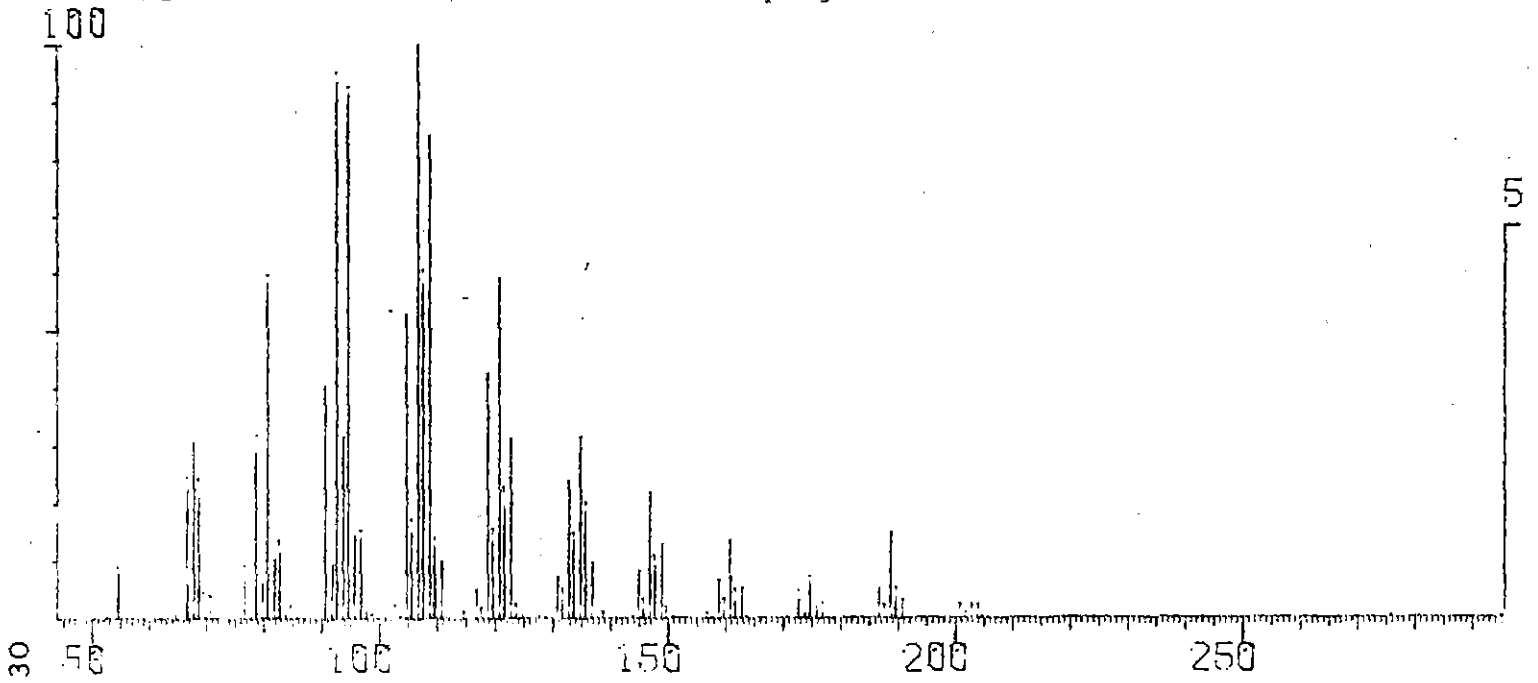
Pmr SPECTRUM OF COMPOUND A CRYSTALS IN CD Cl_3

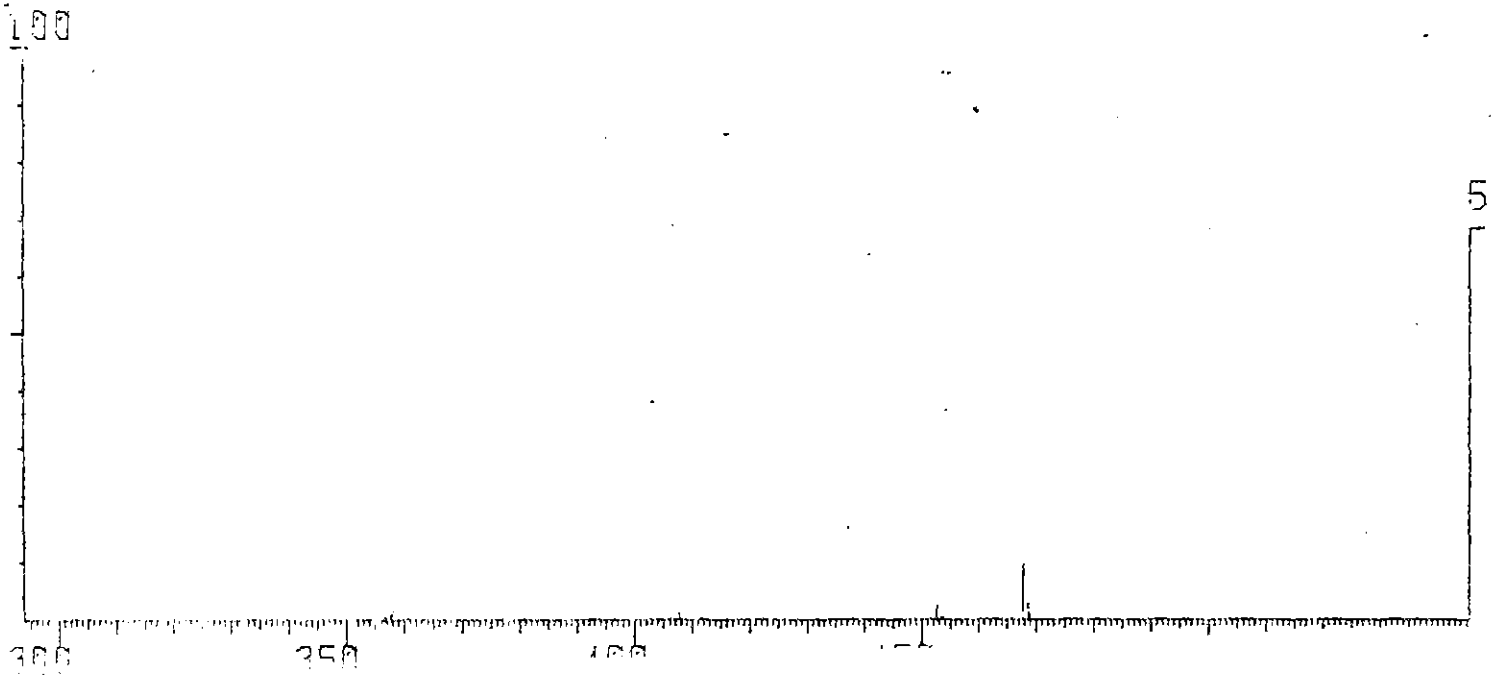
DM15

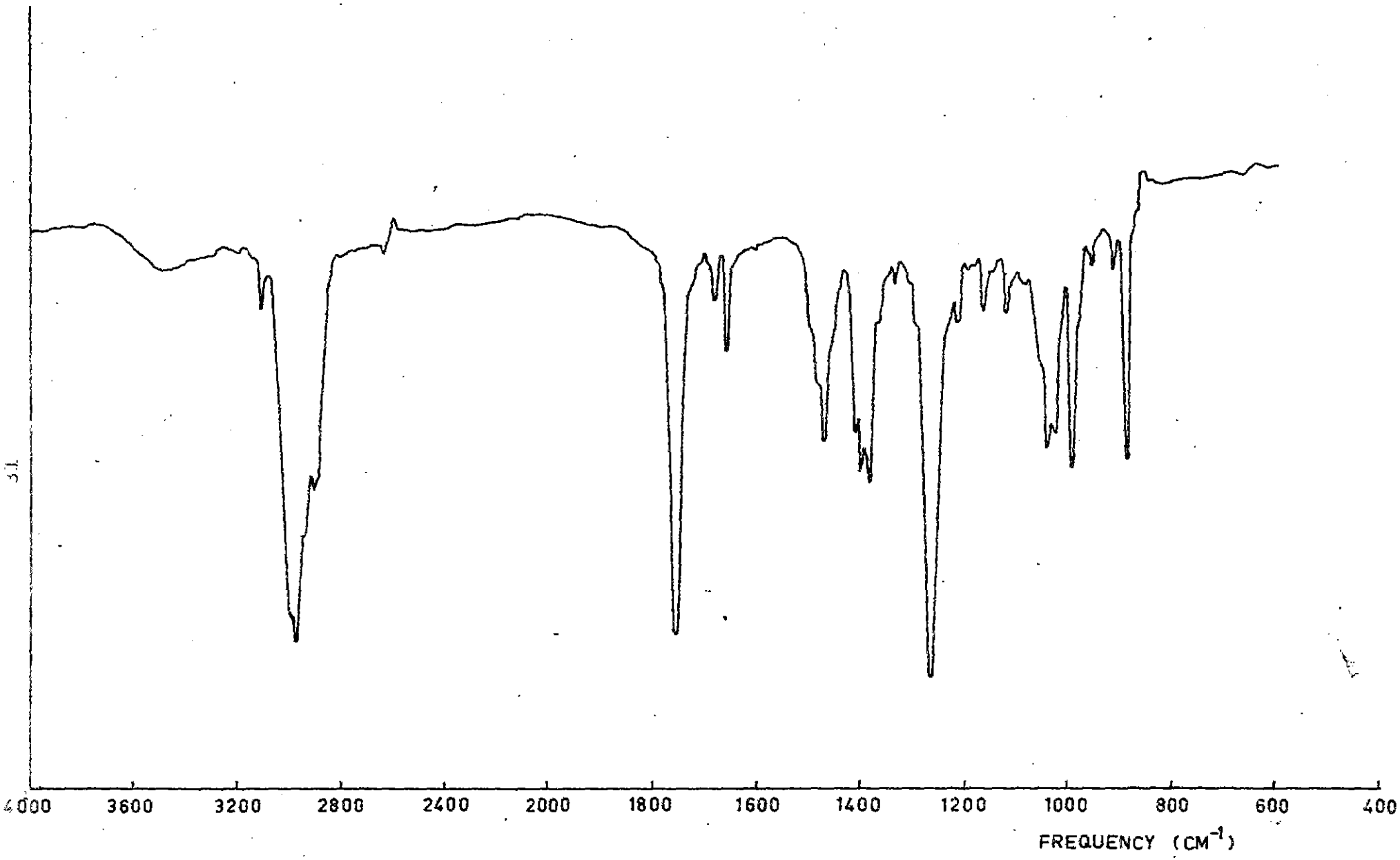
48--23

Lupenyl acetate

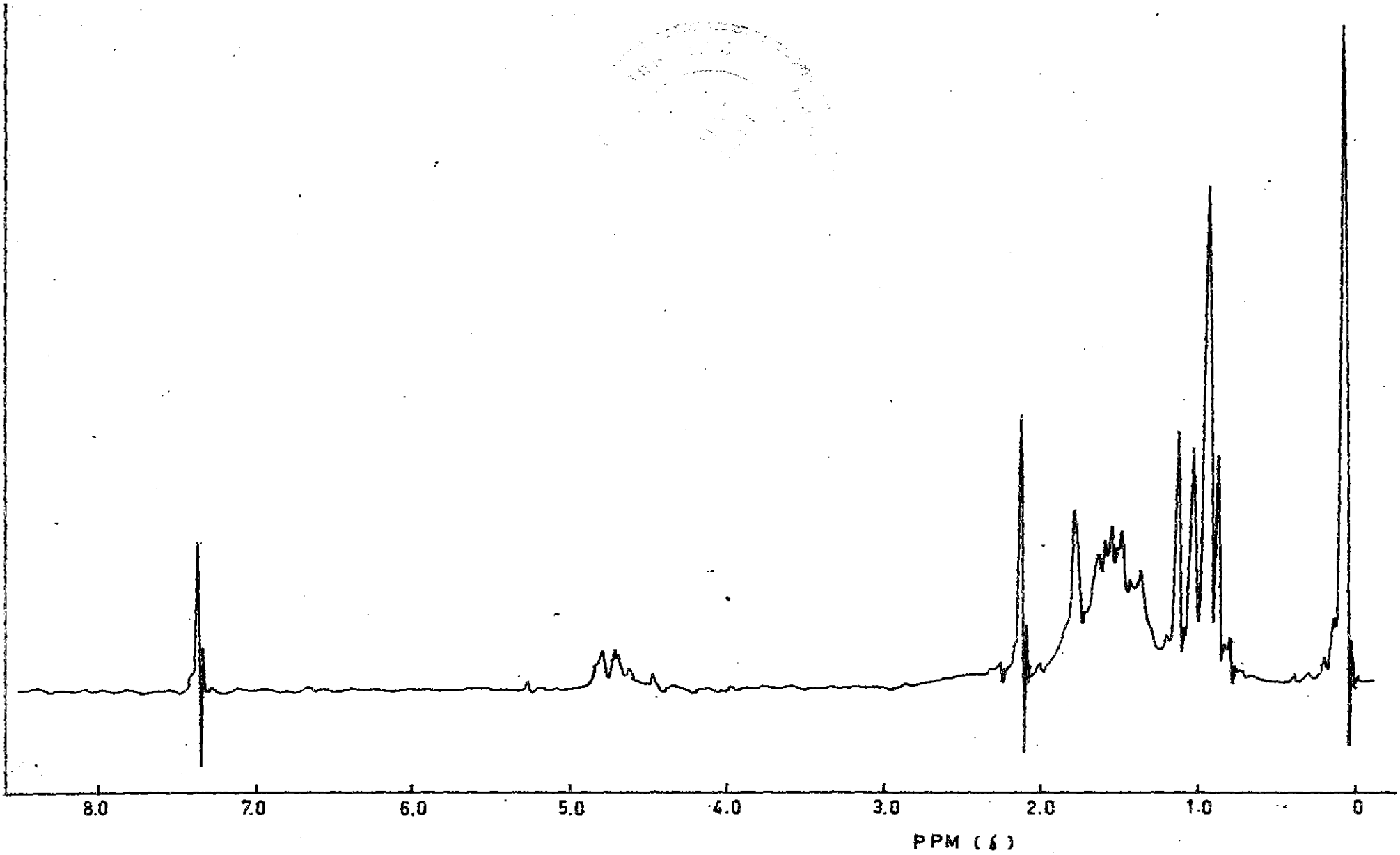
AMP.: 00008212



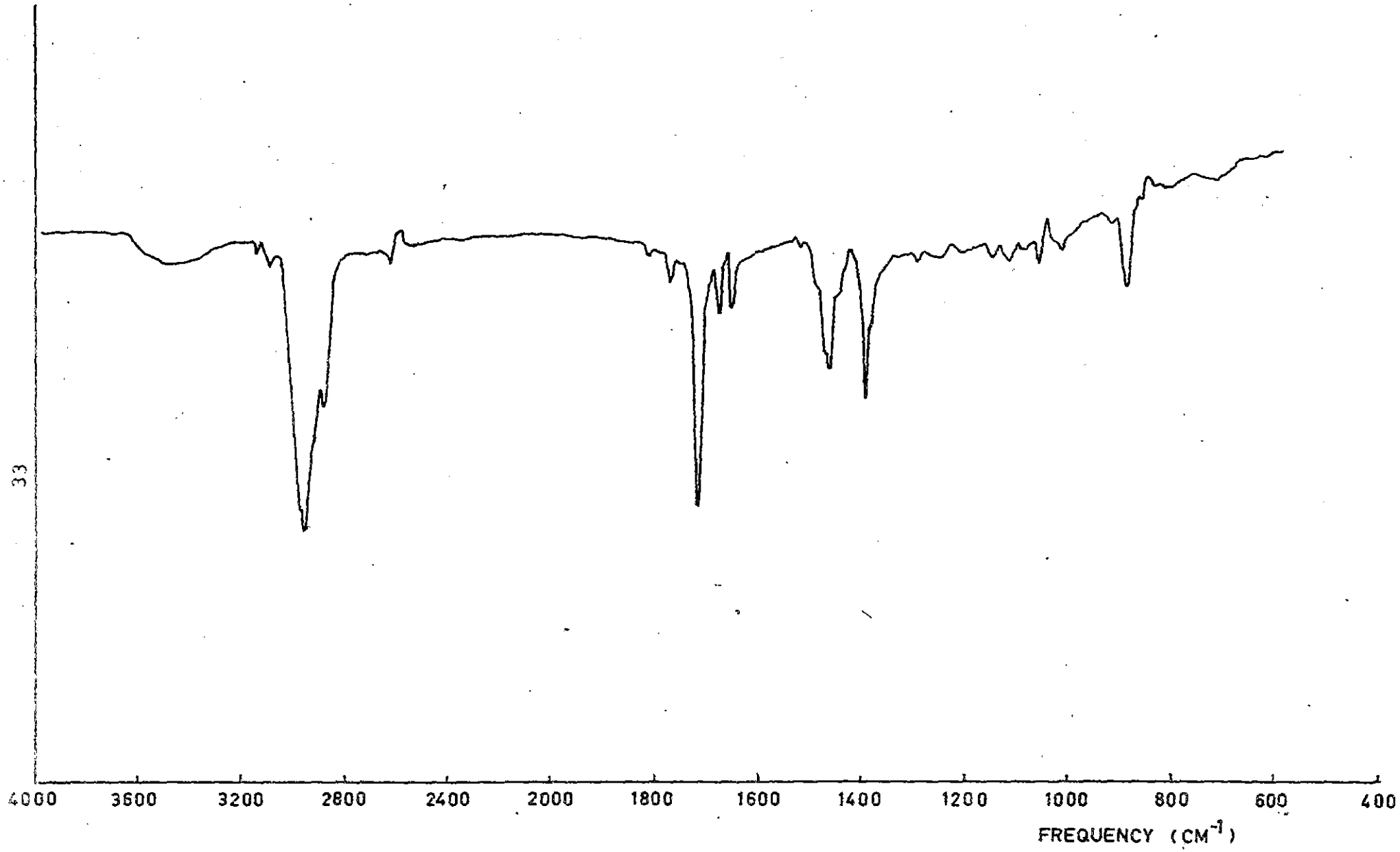




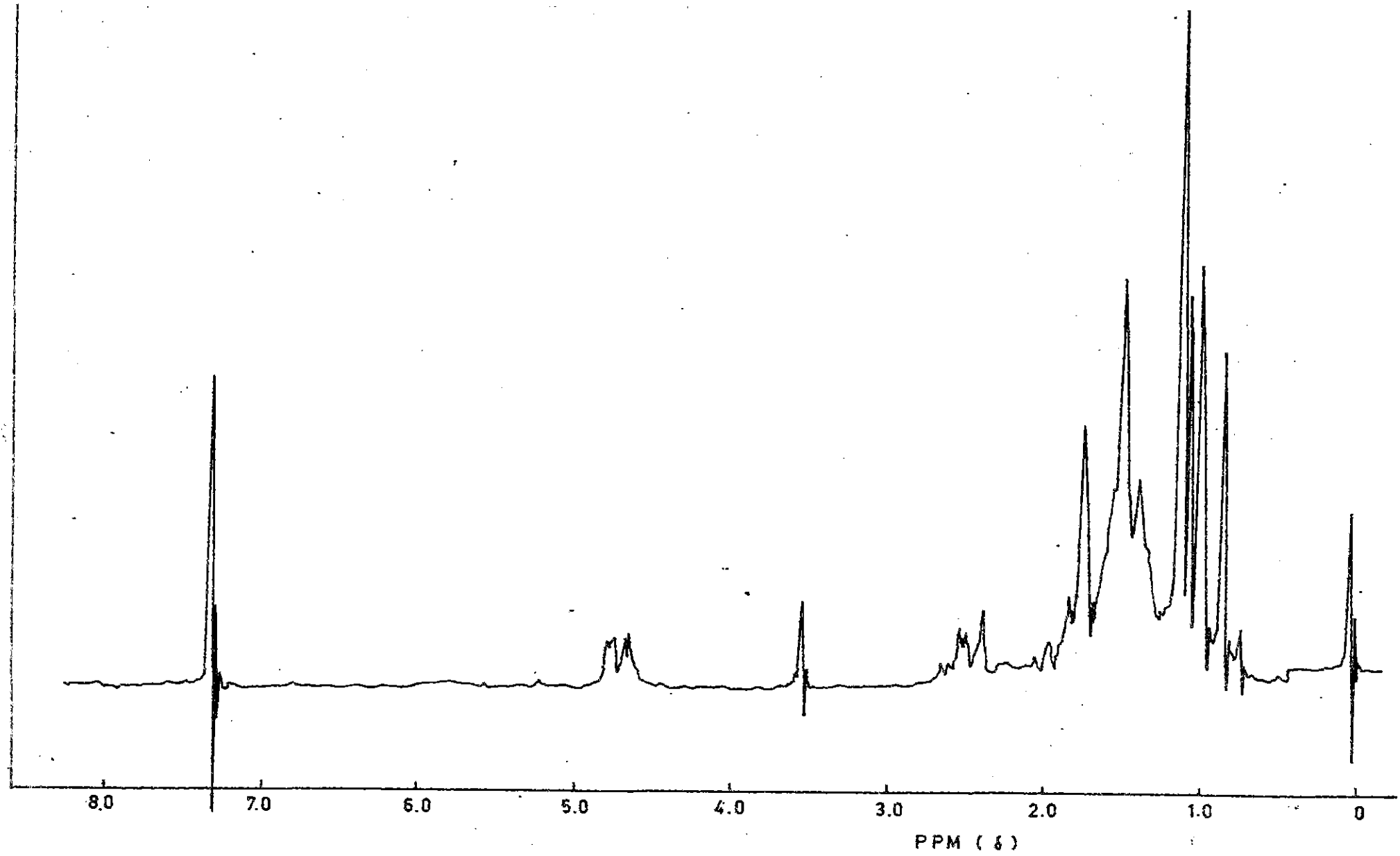
INFRARED SPECTRUM OF A1 CRYSTALS (KBr)



pmr SPECTRUM OF COMPOUND A1 CRYSTALS IN CD Cl_3



INFRARED SPECTRUM OF A2 CRYSTALS (K Br)



Pmr SPECTRUM OF COMPOUND A2 CRYSTALS IN CD Cl_3

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