CHEMICAL COMPOSITION

OF

Rumex abyssinicus Jacq.

A thesis

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In Partial Fulfillment

of the requirements of the Degree

Master of Science in Chemistry

By

Alemu Bezabih

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ABSTRACT

Chemical Composition of

Rumex abyssinicus

by

Alemu Bezabih

Research Advisor: Dr.Berhanu Abegaz

Rumex abyssinicus, family polygonaceae is a plant used traditionally for colouring textiles and food. It has several medicinal application such as purgative, anti-diabetic, and taencide. The powdered root of R.abyssinicus was packed on a column and extracted using acetone. The sticky brownish mass (A-1) which was produced on evaporation was further separated by column chromatography and showed to consist of anthraquinone derivatives A-4, A-6 and A-7 which were latter identified as emodin (1,3,8 trihydroxy-6-methylanthraquinone), chrysopyanvl (1,8 dihydroxy-3-methylanthraquinone, and physicone (1,8 dihydroxy-3-methoxy-6-methylanth-raquinone) respectively. The structural ilucidation of emodin, chrysophanol and physicone are based on their ultraviolet, visible, infrared, nuclear magnetic resonance and mass spectras.

INTRODUCTION

Rumex abyssinicus Jacq is an indigeneous wild flowering plant used traditionally for colouring textiles and food. The root of Rumex species has several medicinal applications. It is used as taencide, purgative, antidiabetic, and as a cough remedy.[1,2]

On the genus Rumex, the following biological activities have been reported. Extracts of roots and rhizomes of R. hydrolaphtlum[3] have bacteriostatic activity against Staphylococeus albus, Staphytococus aures, and Bacillus subtile. R. acetosa and R. andreaeanus were found effective against pathogenic fungi[4] inclusing Trichophyton purpureum, T. gypseum and T. granulosum. Antiseptic tannins and polyphenol components which are both bacteriostatic and bacteriocidal to Flexner type and other dysentry bacteria were isolated from R.confertus and R. fenicus[5]. The roots of Rumex species[6] were used as laxatives, but the presence of reduced aglycones in these plants causes undesirable side effect, like vomiting and cramps. The extracts of roots and rhizomes of R. alpinus[7] has 30% the purgative effect of 1,8 dihydroxyanthraquinone. Substances with antioxidant properties for fats, food and dyes were isolated from Rumex species[8]. Extract of R. confertus[9] root is a heart stimulant in frogs and mice and it is also a vasconstrictor to isolated

frogs, rabit and cat organs. Ascobbic acid combined with polyphenols from Rumex[10] species normalized the increased activity of serum ceruloplasmin, decrease blood peroxidase and cytochrome osidase activities and reduce liver glycogen level which were caused by vitamin C and vitamin P dificiencies in pigs.

With regard to the chemical composition of Rumex abyssinicus two compounds of oxalic acid[2] and chrysophanic acid have been reported. However, for the Rumex[11,12] species flavonoids, ascorbic acid, carotenes, proteins, fats, cellulose, free anthraquinone (chrysophanol, rheumemodin and rheochrysidin), anthraglucosides (chrysophanein, rheochrysin, rheumemodin glucoside), tannins and tannoglucosides were identified[13].

2.0 BACKGROUND

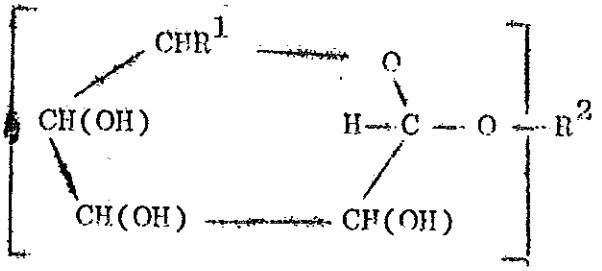
The genus Rumex is found to contain many hydroxyanthraquinones, both unsubstituted and substituted. These compounds occur both in the free state and as anthraglucosides[14].

2.1 ANTHRAGLUCOSIDE

The existance of anthraglucoside in plants, has been known for more than a century and it occurs to a greater or lesser extent in most parts of the plant[15].

2.11 PROPERTIES OF ANTHRAGLUCOSIDES

Anthraglucosides are optically active compounds w which are readily hydrolysed by dilute mineral acids, dilute alkali, or suitable enzyme. The naturally occuring anthraglucosides have the general structure shown in (1). The glycosidic linkage is almost invariable β-with a D-sugar and α with L-sugar.



1

Where R^1 = CH_2OH, H, Me or CO_2H

R^2 = Aryl (anthraquinone derivative)

The glycone moiety of the anthraglucoside can be D-glucose, D-galactose, D-fructose, L-rhamanose, D-xylose, L-arabinose or D-glucuronic acid[16]. D-glucose is the most common in the natural glycosides, where as D-fructose and D-glucuronic acids are rare.

The aglycones[17] of natural glycosides may contain condensed ring system such as hydroxyanthraquinones.

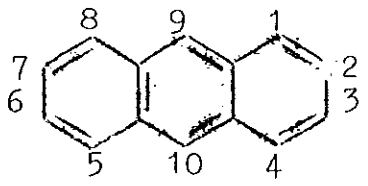
2.2 ANTHRAQUINONES

The synthesis of alizarin from anthracene[18,19] in 1868 and the elucidation of the generic relationship between anthracene, anthraquinone and alizarin, may be said to mark the beginning of a new era in the study of the chemistry of anthraquinone and its derivatives.

The quinone-like character of anthraquinone was first clearly recognized by Graebe and Liebermann[20] who showed that it bore the same structural relationship to anthracene as benzoquinone did to benzene. The generally accepted diketo formula for this compounds was suggested by Fitting[21].

2.21 NOMENCLATURE

The ten position, in anthracene are numbered as indicated below.

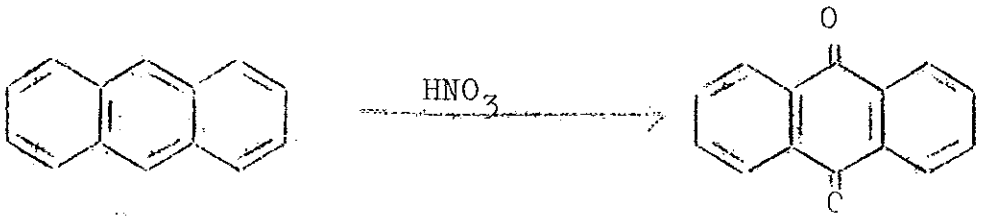


Positions 1,4,5 and 8 are reffered to as the positions; and positions 2,3,6 and 7 as the B. The 9 and 10 positions are some times designated as the meso.

2.22 PREPARATION

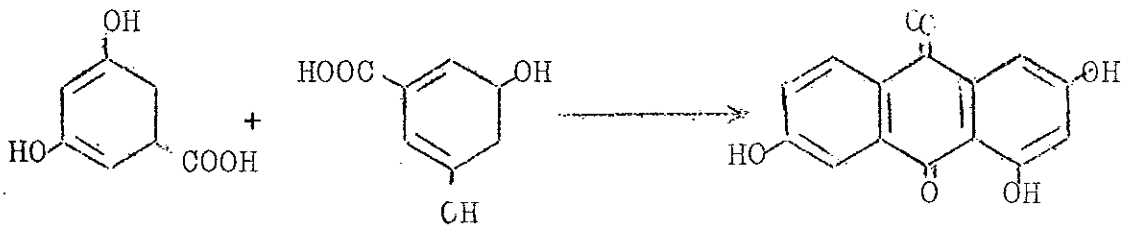
2.221 Oxidation of Anthracene

Anthracene (2) is oxidized by nitric acid to produce anthraquinone (3).



2.222 From benzoic acid

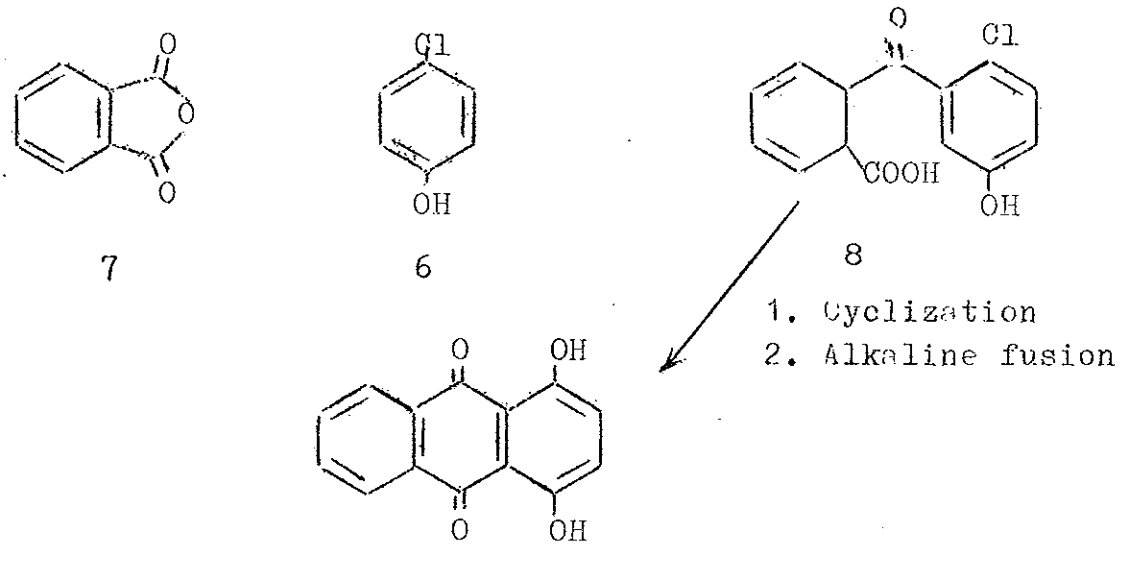
When benzoic acid is heated with a suitable dehydrting agent such as phosphrous pentoxide anthraquinone is obtained[22]. When a hydroxybenzoic acid (4) is used, the condensation proceeds more easily and hydroxyanthraquinone (5) is formed[23].



4 4 5

2.223 From Phthalic anhydride

This method which is useful in the preparation only of derivatives of anthraquinones, especially hydroxyanthraquinones, consists of heating phthalic anhydride and phenol or a phenol derivative with concentrated sulphuric acid and boric acid. Thus P-chlorophenol (6) and phthalic anhydride (7) yield quinizarin (9).

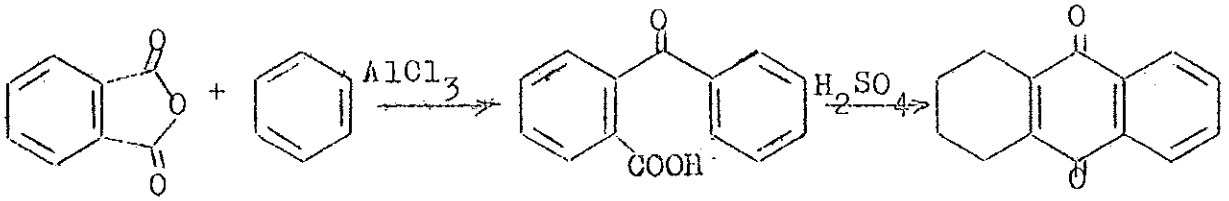


7 6 8

9

2.224 From Phthalic anhydride, benzene in the presence of anhydrous aluminium chloride.

Phthalic acid (10) is first condensed with benzene (11) and aluminium chloride to give O-benzoi-benzoic acid (12) which when heated with sulphuric acid is converted into authraquinone (13).



10 11 12 13

2.23 CHEMICAL PROPERTIES

2.231 Sulphonation

Anthraquinone does not sulphonate easily with ordinary concentrated sulphuric acid, and fuming sulphuric acid is therefore generally employed. This leads to the formation of the - acid together with small amount of disulphonic acids, principally the 2,6 and 2,7 acids[26]. If a small amount of mercuric sulphate is added to the sulponation mixture the acid is obtained almost entirely[27].

Anthraquinone sulphonic acid are fairly easily desulphonated by hydrolysis, although the ease with which the sulphonic group is eliminated varies to a great extent in different substances. Ordinarily the sulphonic acid group in the α position is more easily removed than one in the β position.

2.232 NITRATION

When anthraquinone is nitrated the position is first substituted. No β-nitroanthraquinone is formed in this reaction. The β-nitroanthraquinone can be be prepared by direct nitration and is generally made by nitrating β-aminoanthraquinone and then removing the aminogroup from the resulting 2-amino-3-nitroanthraquinone by diazotisation reaction[28].

2.233 HALOGENATION

Anthraquinone is attacked by halogen, with the greatest difficulty. In gnerall, the halogen derivatives of anthraquinone are obtained by indirect methods, that is, by replacing a substituent already present in anthraquinone molecule. Thus chloroanthraquinone can be prepared from aminoanthraquinone by diazotisation reaction or by treatement

of hydroxyanthraquinone with phosphorous pentachloride, or from anthraquinone suplhonic acid by treatement with chlorine chlorine[29].

2.234 AMIDATION

They are generally prepared either by the reduction of the corresponding nitroanthraquinone with sodium sulphide or by the replacement of substituents groups in the anthraquinone molecule, such as halogen atoms, hydroxyl or sulphonic acid groups.

2.235 HYDROXYLATION

There are four general methods for introducing hydroxyl group into anthraquinone nucleus[19]. They are:

a) Replacement of a sulphonic acid group

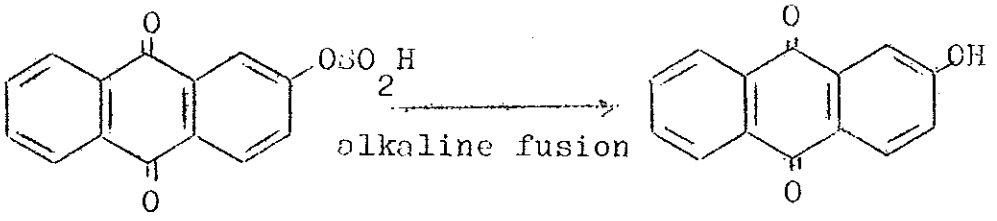
b) Replacement of a halogen atom

d) Replacement of an amino group

d) Direct oxidation

Replacement of Sulphonic Acid group and Halogen atoms.

Hydroxyanthraquinones are prepared by the alkaline fusion of chloro or sulphonic acid derivatives, under oxidative condition.



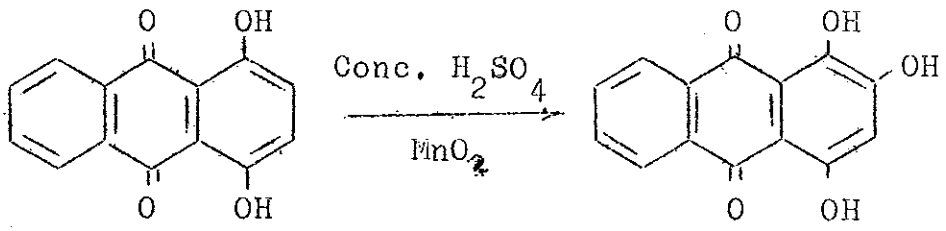
14 15

Replacement of amino group.

Amino group can be replaced with hydroxyl groups, through diazotisation reaction.

Oxidation.

Oxidation of concentrated sulphuric acid solution of hydroxyanthraquinones using manganse dioxide as the oxidant.



16 17

General properties of hydroxyanthraquinone[30].

The hydroxyanthraquinones are of considerable technical importance, like alizarin they are used as dyes and as intermidates in the preparation of other anthraquinone derivatives. They are all yellow, orange or red in colour. Many of these compounds may be vacuum sublimed and this property is utilized in the preparation of hydroxyanthraquinone in a very pure state. As a class, hydroxyanthraquinones dissolve in alkaline and concentrated sulphuric acid giving high coloured solution with characterstic absorption spectra. 2-Hydroxyanthraquinones are usually more acidic than 1-hydroxyanthraquinones. They form salts not only with alkaline metals but also form highly coloured stable salts or cordinated complex with heavy metals such as alluminium, iron, chromium and tin. 1-Hydroxyl groups are acylated much less readily than 2-hydroxyl groups and the resulting derivatives show similar difference in ease of hydrolysis.

Hydroxyanthraquinone having at least one hydroxy in its -position give colouration with alcoholic magnesium acetate, which would be purple for 1-4 dihydroxy derivatives

as in quinizarin, orange red with 1-3 hydroxyanthraquinone (emodin) or 1,8-hydroxyderivative (chrysophanol) and blue violet for 1,2 hydroxy derivative (alizarin)[31].

2.24 SPECTRAL PROPERTIES

2.241 Infrared Spectra

The spectras of anthraquinone derivatives having the same substituents pattern are similar, regardless of the substituents, provided these compounds contained no hydroxyl groups.

In the hydroxyl region anthraquinone with one hydroxyl group in the β-position on the nucleus, or attached to a substituents group, will have one hydroxyl streching band. More than one hydroxyl band will appear only when β-hydroxyl groups are adjacent or are in different environment[32]

α-Hydroxy group attached to the nucleus apparently will show a hydroxyl absorption in a spectrum of a mulled sample. However, with potassium bromide pressed disc and mulls in hexachlorobutadiene, a broad, weak absorption band with center at approximately 2700 cm^{-1} will be observed which correspond to the streching frequency of chelated hydroxyl bands[33].

From the spectral characterstic of the hydroxyl and carbonyl of hydroxy derivative, there is a useful grouping according to the arrangement of the α-hydroxyl group in the hydroxyanthraquinone and its derivatives. The following table shows the carbonyl frequency of anthraquinone grouped according to the arrangement of α-hydroxy group on the nucleus.

Table - 1

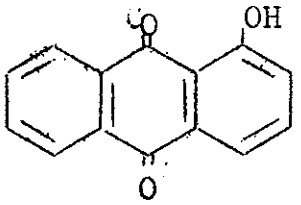
Group No.	Type of Anthraquinone	C = O frequency
1	No. α-OH	1678 - 1653
2	1, α- OH	1675 - 1643
3	1,4; 1,5 $(OH)_2$	1645 - 1608
4	1,8 $(OH)_2$	1678 - 1661
5	1,4,5 $(OH)_3$	1616 - 1592
6	1,4,5,8 $(OH)_4$	1592 - 1575

Depending on the number of carbonyl peaks and their frequency range, it is possible, to place anthraquinones in one of the six groups of table 1.

In anthraquinones with no α-hydroxy groups, the single carbonyl frequency occur between 1678 and 1653m^{-1} and, expect in the case of strongly electrophlic substituent group which raise the frequency a few cm,$^{-1}$ the position

and nature of the substituents groups have no noticable effect on the carbonyl frequency.

The lowered frequency of the -hydroxy group and the accompaning change of intensity and frequency in the carbonyl region is due to " conjugated-chelation "[34] where the strength of hydrogen bonding between the hydroxyl and carbonyl group (18) and (19) is enhanced by resonance with an ionic form (20) having a conjugated system in which the donor-acceptor properties of the chelating center increased.



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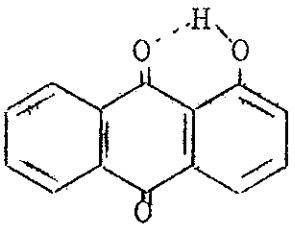

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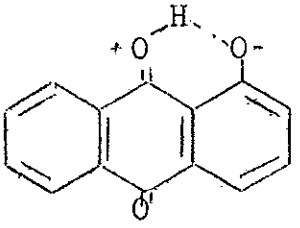


20

A conjugated-chelate system always results in the disappearance or lowering of the hydroxyl streching frequency, and at the same time the carbonyl band is lowered in frequency and increased in intensity owing to the weakend force constant and increased moment of the band.

The average C=C strechinr band in anthraquinones of 1,4 and 1,5 dihydroxy group has a frequency greater than 1575 cm^{-1}, while in the case of the 1,4,5 - trihydroxy group, it is less than 1575 cm^{-1}. For anthraquinones of 1,4,5,8 - tetrahydroxy groups the C=O and C=C bands are in distingushable.

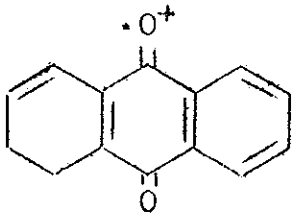
2.242 UV Spectra

The UV spectrum of anthraquinone may be regarded as a cross conjugated acetophenone-benzoquinone system. The transition due to acetophenone and quinone chromophores are designated as (a) and (b), respectively[35]. Thus, anthraquinone in ethanol, have max 243, 252 (a bands) 263,272 (b bands) 325 (a) and 405 (b) mμ. The 405 mu quininoid absorption is susceptible to intensity increased and shift to longer wave lenths by introduction of nuclear hydroxyl groups.

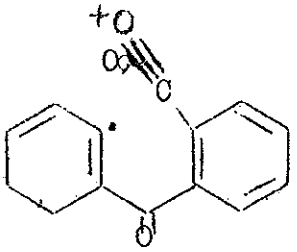
2.243 Mass Spectra

In anthraquinone, the most loosely bound electron, is removed preferentially during formation of the molecular ion from electrons of low energy (10-15 ev) is one of the lone-pair electrons on the oxygen atom. The oxygen positive

ion (O^+) has the capacity of trivalency, as this is developed the electron pair in one of the C-C bonds adjacent to the carbonyl group will become uncoupled to provide an orbital which can give a π-bonding effect with the lone-pair oxygen orbital. Initally, the direction of this bond orbital is not parrallel to the orbital available on the oxygen, so that a complete π-bonding effect will not result. The situation can be represented by as follows:-



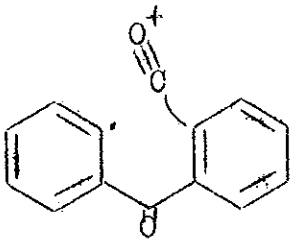
21



22

The positive charge on the oxygen will induce a polarity in the adjacent bonds, the direction being such that its negative end is attached to the carbonyl group. The bond next to the carbonyl group is then " bent " and weakened, but this is compensated by increase in the CO bonding. The electron pair may be regarded as almost entirely attached to the carbon atom of the CO, which

corresponds with usual view of the bonding in neutral CO^{35}. It is illustrated as follow.



23

The carbonyl group is then so weakly attached that the internal energy produced on impact of the ionizing electron can produce the dissociation. The ion remaining is a highly excited fluorenone ion having one bond streched beyond its normal length. By rearranging its electron so that the odd electron is again one of the lone-pair electron, the fluorenone ion can now fragment in a analogous fashin to eliminate further neutral carbon-monoxide to give excited O-diphenylene ion. These process are illustrated by following reaction sequences.

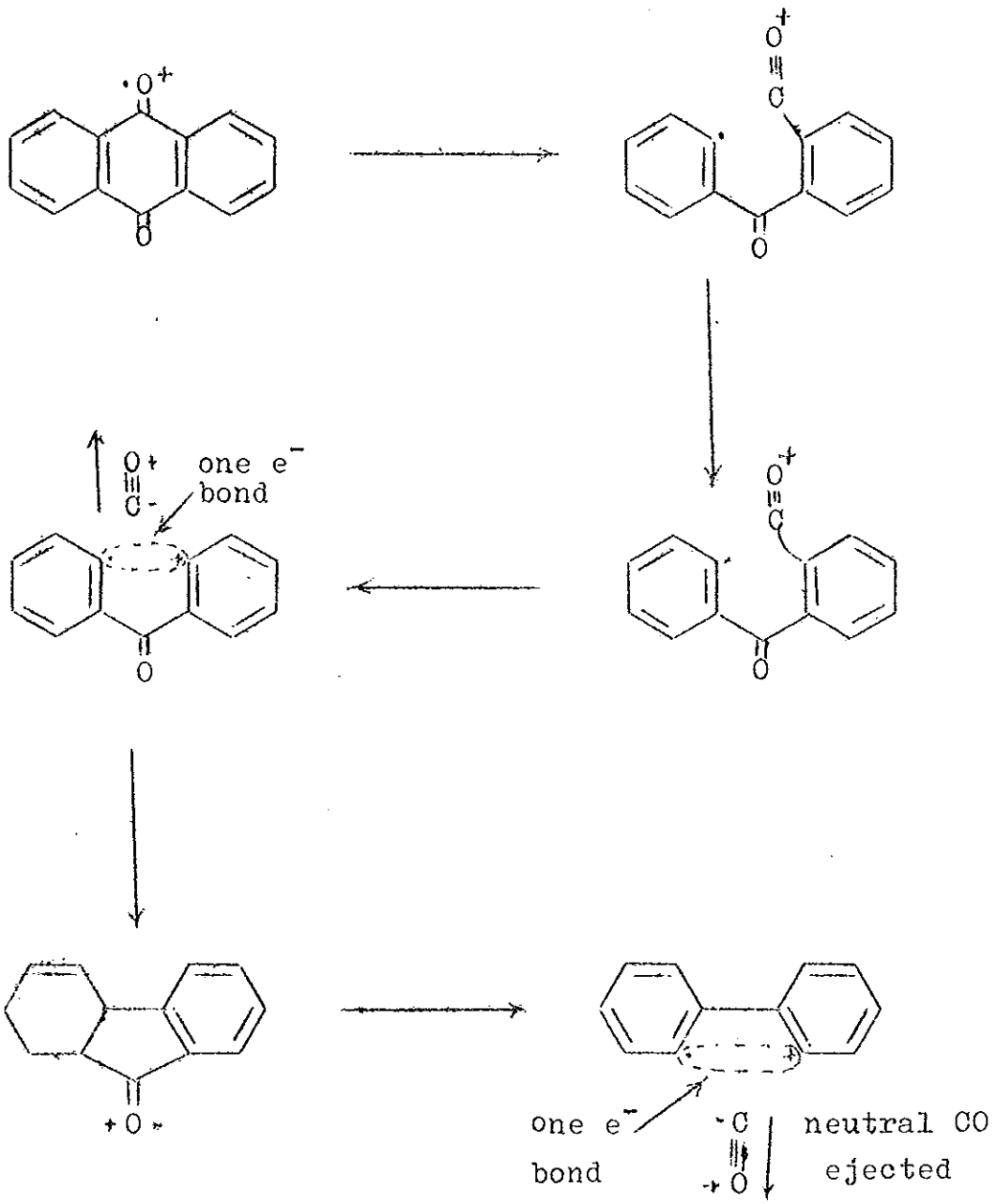
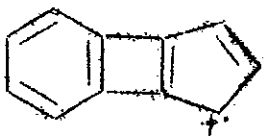


Figure 1

The mass spectra of a number of other substituted anthraquinone[38] have the same strong tendency to lose neutral carbon-monoxide. The hydroxy-anthraquinone are of practical interest. In the case of 2-hydroxy-anthraquinones in addition to two CO molecules which are lost, a further CHO radical is ejected and is fact the three major fragment peaks at masses 196, 168 and 139 in the spectrum of these compounds are due to the loss of these fragments. The ion of mass 139 occurs widely in the spectra of aromatic hydrocarbon and it is thought that its structure might be as shown (24) below



24

This ion can also lose a further electron quite easily with out further fragmentation, which could be seen by the relative large peak at mass 69.5.

Dihydroxy anthraquinone gives peak at mass 156, corresponding to the loss of three CO melecules $(p\text{-}C_3O_3)^{+\cdot}$

This latter ion can lose a fourth CO to give a peak at mass 128. The associated peaks, at mass 126 and 127 are smaller than that at 128. Thus it is seen that there is a difference between the fragmentation of these compounds and the monohydroxy-anthraquinones. In the latter compounds the peaks at 139 is larger than that at 140.

2.25 THE BIOSYNTHESIS OF ANTHRAQUINONES

It is belived that the emodine-type anthraquinones found in higher plants are synthesised via " Polyacetate rule " path way[39].

The primary building block of this biosynthetic pathway was assumed to be a linear poly-β-ketomethylene, formed by repeated head to tail condensation of acetate units[40] (figure 2).

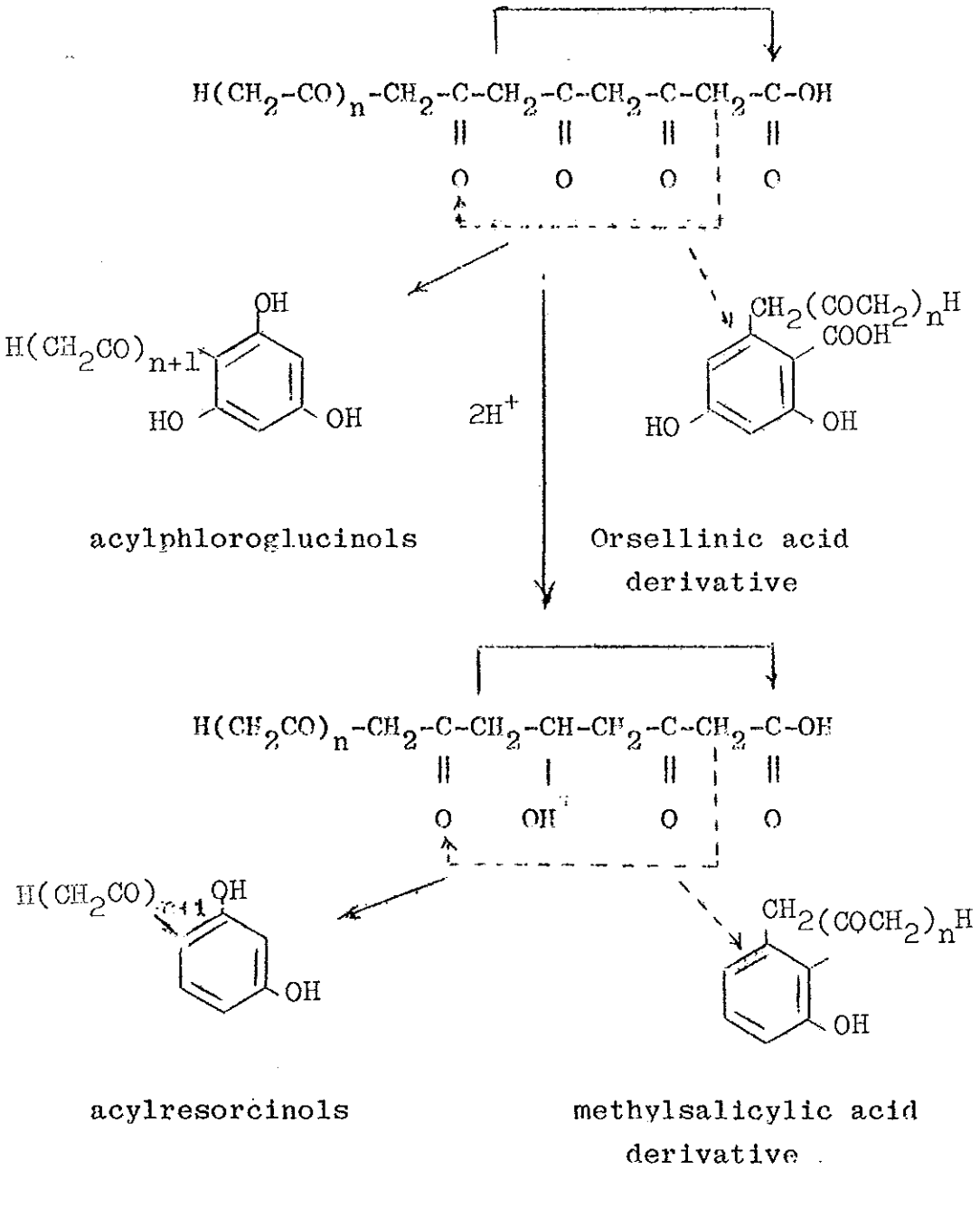
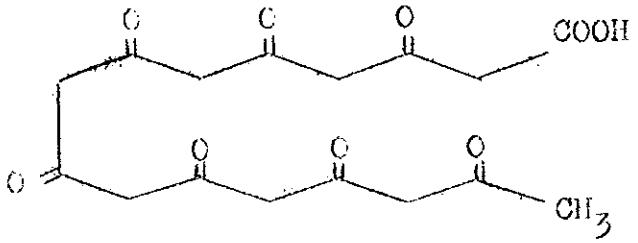


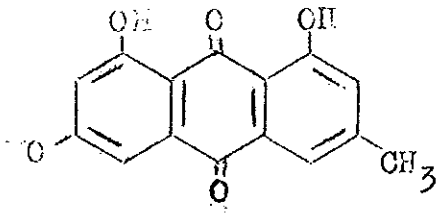
Figure - 2 Illustration of polyactate rule

By secondary transformations, such as reduction, dehydration, condensation, cyclization, rearrangement, and decarboxylation reactions polyacetate molecules of different chain length can lead to a variety of naturally occuring anthraquinones. Especially since additional variants are given by the introduction of methyl or isopenthyl groups at the methylene site, or by replacement of acetic acid in the terminal position by another acid such as linear and branched chain alphatic acid[30].

One of the key reactions of the polyacetate chain is cyclization. Thus an enolate anion may attack the carbonyl carbon of either the keto or carboxyl group at another position of the chain to yield cyclized products as illustrated in figure (2). Both reactions occur with facility in forming six-membered ring and lead to two families of phenols, the acylpholoroglucinol and orsellic acid derivatives respectively. By reduction of the carbonyl not involved in the cyclization and ring-closure by loss of water derivative of methylsalicylic acid or acylresorcinols respectively are generated. Further cyclization gives the respective anthraquinone.

The perfect adherence of certain anthraquinones to the acetate hypothesis is striking indeed. For example emodin a component of the Rumex genera, can be related directly to the poly-β-ketomethylene acid[41].





for other compounds loss or rearrangements of the hydroxyl group and oxidation or removal of the methyl group are required.

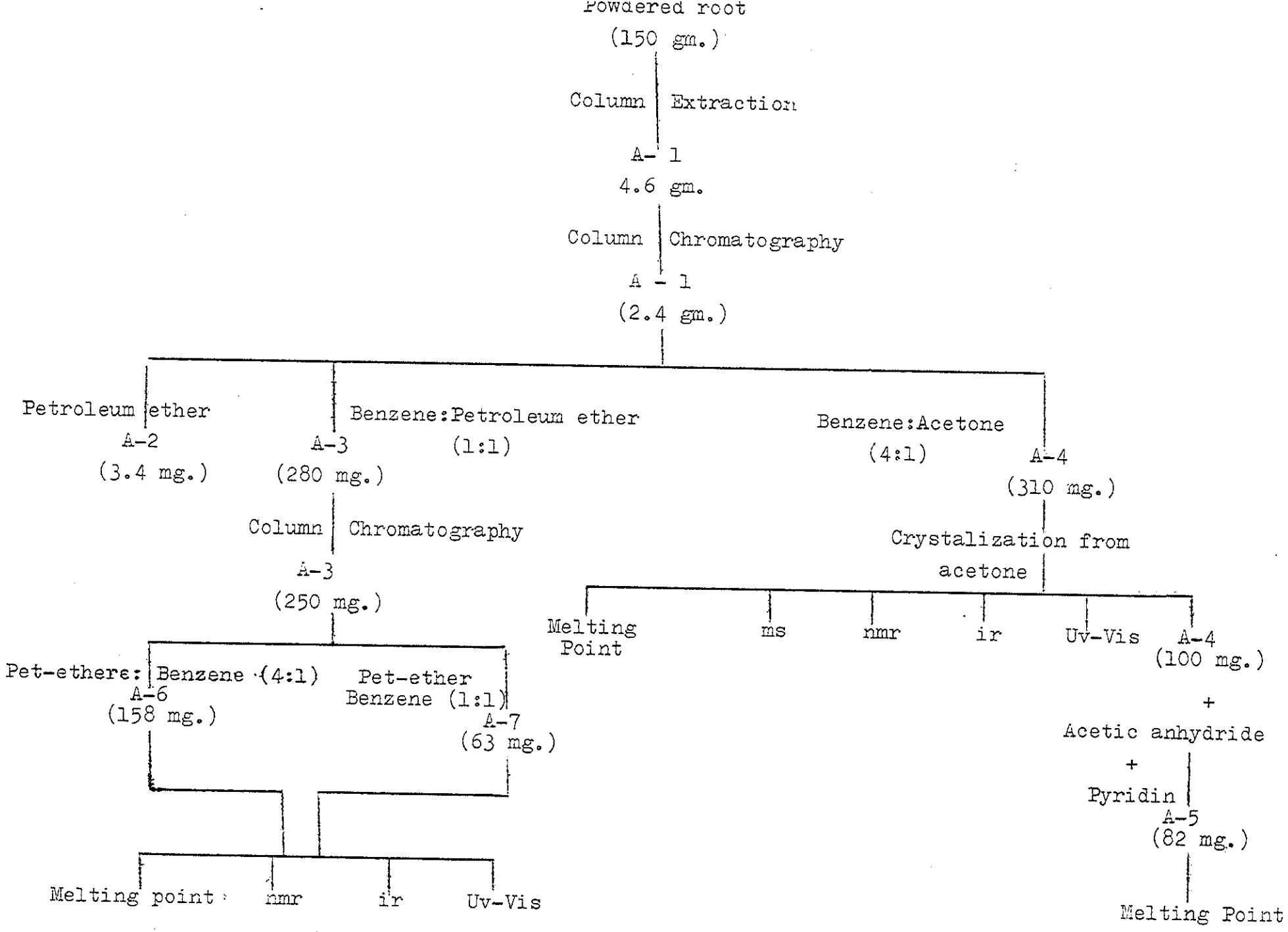
2.26 ISOLATION AND CHARACTERIZATION

Isolation procedure depend on wether free aglycone or the various glycosidic derivatives are desired. For the first, extraction of the plant with rather non polar solvents such as ether, or benzene is effective.

The sugar derivatives, however, are extracted using water, ethanol or water-ethanol mixture. After extraction the solution of glycosides may be concentrated under reduced pressure to obtain crude crystals. These crude crystals may then be purified by repeated crystalisation from acetone water. The glycosides on heating with acetic acid or dilute alcoholic HCl are readily hydrolysed within one hour at $70^{o}C$[30]. After hydrolysis a 1:1 mixture of enthanol-benzene is added and then diluted with 0.5% hydrochloric acid. A layer of benzene separates containing the aglycones. The aglycones obtained by hydrolysis or direct extraction of the plant material may be disolved in benzene and the solution extract with 5% sodium hydroxide. Acidifying the alkaline solution with hydrochloric acid will generate yellow to orange and pigments. The pigments are then extracted from the aqueous layer solution using chloroform. The chloroform extract thus obtained may be evaporated to give the crystals. The crystals then dissolved in benzene and extracted successively with aqueous 5% sodium bi-carbonate and 5% sodium carbonates. A layer of benzene contains the alkaline soluble pigments. The chloroform layer forms the carbonates soluble pigments, while the

aqueous layer forms the bicarbonate soluble pigments. Further isolation could be done by column chromatography.

For identification of anthraquinone derivatives the Bortrager reaction is routinley used. Some of the unknown material is boiled in dilute aqueous sodium hydroxide for few minutes. The alkaline solution is cooled, acidified, and extracted with benzene. When the benzene phase is separated and shaken with dilute alkali, the benzene losses its yellow colour and the alkaline phase becomes red if anthraquinones are present. The test is not specific for anthraquinones, naphtaquinones give positive reaction. Direct spectral observation of a benzene solution may also be made for characterization of anthraquinones derivatives. Anthraquinones show broad absorption at about 437 nm. The colours given with alcoholic magnesium acetate solution are characterstic of different hydroxylation pattern. Meta hydroxyls give an orange, para a purple, and ortho violet.


Powdered root
(150 gm.)
Column Extraction
A- 1
4.6 gm.
Column Chromatography
A - 1
(2.4 gm.)
Petroleum ether
A-2
(3.4 mg.)
Benzene:Petroleum ether
(1:1)
A-3
(280 mg.)
Column Chromatography
A-3
(250 mg.)
Pet-ethere: Benzene (4:1)
A-6
(158 mg.)
Pet-ether
Benzene (1:1)
A-7
(63 mg.)
Melting point
nmr
ir
Uv-Vis
Benzene:Acetone
(4:1)
A-4
(310 mg.)
Crystalization from
acetone
Melting
Point
ms
nmr
ir
Uv-Vis
A-4
(100 mg.)
+
Acetic anhydride
+
Pyridin
A-5
(82 mg.)
Melting Point

3.2 Characterization of Compound A-4

3.21 Thin layer chromatography

It gave single spot (R_f 0.45, benzene: acetone, 4:1) on tlc.

3.22 Colour tests

It turns red violet with sodium hydroxide and orange-red with methanolic magnesium acetate and negative to zirconium nitrate.

3.23 Melting point

It melts with decomposition at 254-6^{o}C.

3.24 Mass spectrum (m^{+}/e 270) figure 3

Mass	270	139	115	128	213	77	168	69	242
Relative intensity	100	21	11.6	11.5	10.5	10	10	9.5	8.9

3.25 Proton magnetic resonance spectrum (figure 4)

The nmr spectrum in D_6-DMSO showed (ppm valves are given in δ) at:- 2.35 (s,3H), 6.53 (d,1H), 7.02 (d,2H), 7.33 (d,1H)

3.26 Infrared spectrum (figure 5)

The ir spectrum showed strong absorption peaks at 3392, 1662, 1622, 1477 cm^{-1}

3.27 uv-vis spectrum (figure 6 & 7)

The uv-vis spectrum showed a broad absorption at 437 nm and sharp peaks at 289,266,252 and 222 nm.

3.28 ^{13}C nucleur magnetic resonance (figure 8)
The experimental and calculated ^{13}C chemical shift are given in table (4)

The one major pigment A-4 was obtained substantially pure and readily identified.

It turns red-violet with sodium hydroxide (anthraquinone test), orange-red with alcoholic solution of magnesium acetate[18] which indicates that two hydroxyl groups are in position 1,3 or 1,8 of the nucleus. It was negative to zirconium nitrate indicating the absence of two adjacent hydroxyl groups[42]. The uv spectrum showed an intense benzenoid absorption band at 222 nm and strong quininoid electonic transition (FT) absorption at 254 nm and 289 nm. Furthermore the ir absorption band 1662 cm^{-1} and 1622 cm^{-1} are that of carbonyl and chelated carbonyl.

It can be deduced from the mass spectrum (M^+/e 270) the molecular formula is $C_{15}H_{10}O_5$. Anthraquinones with molecular weight 270 are listed in table 3. The peaks at mass 255 (m^+-15) and the mass 253 (m^+-17) are attributed to the loss of a methyl group and a hydroxyl groun from the parent ion respectively. The peaks at

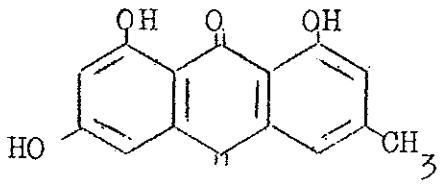
242 (m^+-28), 214 (m^+-56) and 186 (m^+-84) corrosponds to the loss of one, two and three CO moleculas respectively. In addition to the CO molecules lost a further CHO radical is ejected and in fact the fragments at mass 241 (m^+29) and 213 (m^+-CO-29) are due to the loss of this fragment. This pattern of fragmentation suggests that A-4 is a hydroxyanthraquinone derivative[31,32].

The visible spectrum of A-4 has an absorption maximum at 437 nm which indicates the presence of two hydroxyl groups[49,50]. These must be in the position 1,8 since a 1,4 or 1,5 arrangments would produce " congugate chelation "[32] in both carbonyls, the carbonyl-absorption at 1662 cm^{-1} and 1622 cm^{-1} in the infra-red spectrum of A-4 (figure 5) show infact that only one is thus chelated.

The nmr spectrum showed (ppm valves are given in δ) singlet at 2.35 (3 protons, CH_3-ar) and four aromatic protons at 6.52 (IH), 7.02 (2H) and 7.33 (IH) in which the four aromatic protons exhibit long range coupling indicates that there are two aromatic protons in each ring that are in meta position in both cases. Since position 1,8 is occupied by two hydroxyl groups justified

above, then the aromatic hydrogens must be at position 2,4,5 and 7. The substitution pattern is thus 1,3,6 and 8.

From the molecular formula $C_{15}H_{10}O_5$ the two other substituents must by a hydroxyl group and a methyl group. Since 1,8 positions are occupied by two hydroxyl groups, then the methyl group and the hydroxyl group must be at position 3 and 6. The structure is thus given:-



25

Hydroxyanthraquinones of molecular weight 270

Fundametal structure of anthraquinone.

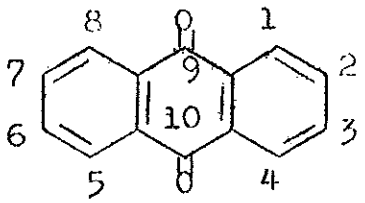


Table - 3

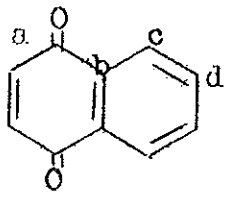
	Anthraquinone	Melting Point°C	Triacetate Melting point°C
1.	1,2-dihydroxy-6 hydroxy-mthylanthraquinone[36]	330	
2.	1,3-dihydroxy-2 hydroxymethylanthraquinone[33]	330	
3.	1,4-dihydroxy-2 dydroxy-methylanthraquinone[36]		
4.	1,5-dihydroxy-3 hydroxy-methylanthraquinone[37]		
5.	1,8-dihydroxy-2 hydroxy-methylanthraquinone		
6.	1,7-dihydroxy-6- hydroxy-methylanthraquinone[33,34]	224°	
7.	2,8-dihydroxy-1- hydroxy-methylanthraquinone[33]		
8.	1,2-dihydroxy-3- methoxyanthraquinone[37]	242°	
9.	1,4-dihydroxy-2- methoxy-anthraquinone[33,34]		
10.	1,3-dihydroxy-2- methoxy-anthraquinone[34]	220°C	
11.	2,5-dihydroxy-1- methoxy-anthraquinone[34]		
12.	2,5-dihydroxy-1- methoxy-anthraquinone[33]		

Anthraquinone	Melting Point°C	Triacetate Melting Point°C
13. 1,2,3-trihydroxy-5-methylanthraquinone[33]	235-240	217-18
14. 1,2,3-trihydroxy-6-methyanthraquinone[33]	275	-
15. 1,2,3-trihydroxy-7-methylanthraquinone[33]	311-313	188-90
16. 1,2,3-trihydroxy-8-methyanthraquinone[33]	297	208-10
17. 1,2,3-trihydroxy-3-methylanthraquinone[37]		
18. 1,2,5-trihydroxy-3-methylanthraquinone[33,34]		
19. 1,2,5-trihydroxy-6-methylanthraquinone[33]	282	256-8
20. 1,2,5-trihydroxy-8-methylanthraquinone[33]	300	204-5
21. 1,2,6-trihydroxy-7-methylanthraquinone[33]	218-320	204-5
22. 1,2,6-trihydroxy-8-methylanthraquinone[33]	330	
23. 1,2,7-trihydroxy-6-methylanthraquinone[33]	330	232-3
24. 1,2,8-trihydroxy-6-methylanthraquinone[33]	216-217	
25. 1,2,8-trihydroxy-7-methylanthraquinone[33]	287-8	185-6

A_o, A_m and A_p = additive parameters of the chemical shift of carbon atoms at ortho, meta and para substitutions respectively.

Table-4

Substituent effect on anthraquinone on the ^{13}C chemical shift (ppm)[37]



where a = 184.7
b = 131.8
c = 126.2
d = 133.7

Substituents	on the same carbon	ortho	meta	para
$-CH_3$	9.3	0.8	0.0	-2.9
-OH	26.9	-12.7	1.4	-7.3

The assignment of carbons of compound A-4 for ^{13}C nmr spectra is given by structure (25)

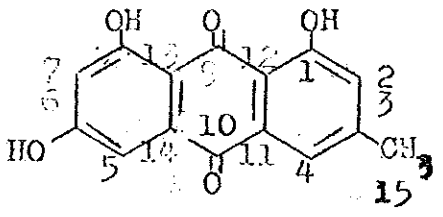


Table (5)

The experimental and calculated ^{13}C chemical shift of compound A-4.

Assignment Carbons	Experimental (ppm)	Calculated (ppm)
C1	161.3	153.0
C2	107.8	109.0
C3	165.4	162.4
C4	108.7	106.0
C5	120.3	119.3
C6	148.0	144.7
C7	123.9	121.9
C8	164.3	155.0
C9	189.4	
C10	180.9	
C11	132.5	130.6
C12	108.7	112.0
C13	113.0	116.0
C14	132.5	130.6
C15	21.4	

The down field deviation on the order of 8ppm for C1 and C^{8} are due to the small 1,3 interaction (δ-syn axial effect) of the carbonyl group with the hydrogen of the hydroxyl groups. The slight difference for C12 and C13, possibly due to the δ-effect. The overall experimental and calculated ^{13}C chemical shift are in a resonable agreement for the structure of compound A-4.

The ^{13}C nmr of compound A-4 was compared with ^{13}C nmr of authentic emodin[55] and was found to be identical.

The melting point of A-4 and the melting point of the triacetate derivatives were identical to the melting point of emodin and its triacetate derivatives[51]. The uv and nmr of A-4 was identical with the uv[52] and nmr[53] spectrums of emodin. Hence the compound A-4 was identified as emodin on the basis of mp, mp of triacetate, nmr, uv, ir and ^{13}C nmr.

3.3 Characterization of Compound A-6

3.31 Thin layer chromatography

It gave single spot (Rf 0.92, hexane: ether, 4:1) on tlc.

3.32 Colour tests

It turned red-violet with sodium hydroxide and orange-red will methanolic magnesium acetate.

It was negative to zirconium nitrate.

3.33 It melted with decomposition at 187-9°C

3.34 Proton magnetic resonance spectrum (figure 11)

The nmr spectrum in $CDCl_3$ showed (ppm) at 2.40 (3H), 6.97 (IH), 7.20 (IH), 7.5 (2H), 7.61 (IH) 11.74 (I,OH) and 11.84 (I,OH).

3.35 Infrared spectrum (figure 12)

The ir spectrum showed strong absorption peaks at 3450, 1685, 1620, 1480, 1460, 1380, 1280, 1220 cm^{-1}

3.36 uv-vis spectrum (figure 13 & 14)

Broad absorption at 430 nm, sharp peaks at 287, 277, 256, 226 nm.

3.37 Mass spectrum (M^+/e 254) figure 10

Mass	254	57	127	76	65	114
Relative intensity	100	76	71.4	67	57	48

It turned red-violet with sodium hydroxide (anthraquinone test) and gave orange-red with methanolic magnesium acetate which indicates that two hydroxyl are in position 1,3 or 1,8 of the nucleus. It was negative to zirconium nitrate indicating the absence of hydroxyl group ortho to one another. The uv-spectrum of A-6 showed an intense benzenoid absorption band at 226 nm and a strong quininoid electonic transition bands at 256 and 287 nm. The ir absorption bands at 1685 cm^{-1} and 1620 cm^{-1} are that of carbonyl and chelated carbonyl. From the mass spectrum (m^+/e, 254) the molecular formula is $C_{15}H_{10}O_4$. A peak at mass 239 (m^+-15) is atributed to the loss of a methyl group from the parent ion, one mass at 237 (m^+-17) to loss of a hydroxyl group. In addition to two CO molecules which are lost a further CHO radical is ejected and in fact the three peaks at masses 226, 198 and 169 in the spectrum (figure 10) of this compound are due to the loss of these fragments. This pattern of fragmentation is a characteristic of dihydroxyanthraquinone. Therefore from the characteristic

colour reaction, uv, ir and ms datas A-6 must be a hydroxyanthraquinone derivative.

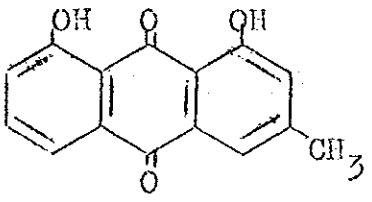
The visible spectrum of A-6 has an absorption maximum at 430 nm which indicates the presence of two hydroxyl groups. These must be in the 1,8 position since a 1,4 or 1,5 arrangments would produce " congagate chelation " in both carbonyl groups, the carbonyl abs absorption 1685 cm^{-1} and 1620 cm^{-1} in the infra-red spectrum of A-6 (figure 11) show in fact only one is chelated.

The proton nmr spectrum of A-6 (figure 10) showed the presence of five aromatic protons (δ, ppm) at 6.97 (IH), 7.20(IH), 7.50 (*H) and 7.61 (IH). At 2.4 (CH_3- Ar) is that of alkyl hydrogens attached to the aromatic ring while at 11.61 (I OH) and 11.74 (I OH) are due to two hydroxyl groups. The positions 1 and 8 have been assigned to the two hydroxyl substituents on the basis of the visible and the infrared spectrums of A-6. Then the substitution pattern for the five aromatic hydrogens and the methyl group are at positions 2,3,4,5, 6 and 7. Each ring would then acommodate only three substituents. Therefore the three hydrogens are on one

ring while the two hydrogens and methyl group are on the other ring. Since it was not possible to measure the coupling constant of the aromatic hydrogens from the nmr spectrum (figure 8), it was found difficult to identify the coupling pattern. However, the possible position for the methyl group would be at 2,3 or 4 position of the anthraquinone.

The dihydroxyanthraquinone derivatives having methyl group at 2 and 4 positions were eliminated on the basis of their melting point and the melting point of their diacetate derivatives[51].. The melting point as well as the melting point of the acetate derivative of 1,8 dihydroxy-3-mthylanthraquinone (chrysophanol) was identical to that of compound A-6.

The structure A-6 is thus given by



Comparison of the uv, proton nmr, and ir spectras data of chrysophanol with compound A-6 were found to be identical. Therefore compound A-6 is identified as chrysophanol.

3.4 Characterization of Compound A-7

3.41 Thin layer chromatography
It gave single spot (R_f 0.81, Hexane: ether, 4:1) on tlc

3.42 Colour test
It turns red-violet with sodium hydroxide and orange red with methanolic magnesium acetate. It was negative zirconium nitrate.

3.43 Melting point
It melted with decomposition at 205-7°C. The melting point of acetate derivative was 182-184°C.

3.44 Proton magnetic resonance (figure 15)
The nmr in $CDCl_3$ showed (δ ppm) at 2.48 (3H,S), 3.97 (3H, S), 6.7 (IH), 7.10 (IH), 7.40 (IH), 7.63 (IH), 12.17 (I-OH), and 12.37 (I, OH).

3.45 Infra-red spectrum (figure 16)
The ir spectrum showed strong absorption peaks at 2950, 1690, 1620, 1580, 1500, 1380 cm^{-1}.

3.46 uv-vis spectrum (figure 17 & 18)
Broad absorption at 432 nm and sharp peaks at 286, 256, 255 nm.

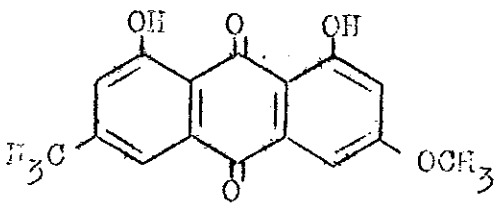
3.47 Mass spectrum (m^+/e 284) figure 14

mass	284	254	255	128	226	241
Relative intensity	100	20	15	10	9	6

Using the same approach as A-4 and A-6, A-7 was found to be anthraquinone derivative whose hydroxyl groups are in position 1 and 8. The infra-red spectrum (figure 16) indicates only one carbonyl is chelated. It mass spectrum (figure 14) showed a parent ion peak at m^+/e 284 and the molecular formula that represent this parent ion is given by $C_{16}H_{12}O_5$. The peaks at mass 269 (m^+-15) and 267 (m^+-17) are due to the loss of a methyl and hydroxyl groups from the parent ion respectively. The peaks at mass 256 (m^+-28) and 228 (m^+-56) are due to the loss of one CO and two CO molecules, where as the peaks at mass 255 (m-29) and mass 253 (m^+-31) could be accounted for the loss of HCO and a methoxy radicals. The pattern of fragmentation suggests that A-7 is a hydroxyanthraquinone derivative[37].

The nmr spectrum of A-7 (figure 15) at 2.48 (3H, s) and at 3.97 (3H, s) shows the presence of an alkyl and a methoxy attached to the aromatic ring respectively. The

aromatic portion showed four protons that exhibit long range coupling. These hydrogens are at position 2,4,5 and 7 of the nucleus since position 1,8 are occupied by two hydroxyl groups. The substitution pattern is thus at position 3 and 6. From the molecular formula and nmr datas the two other substituents must be a methyl and methoxy groups at position 3 and 6. Hence the structure is given by:-



The melting point of A-7 and its acetate derivative was identical with the melting point of physicone[51] and its diacetate derivative respectively. The nmr spectrum of A-7 was found to be identical to the nmr spectrum of physicone[56]. The mass spectrum of A-7 was compared with the mass spectrum of authentic physicone and found to have 40% fit due to minor impurities of A-6. The compound A-7 is identified as physicone on the basis of its MP, mp of its diacetate derivative, nmr, ir and ms spectrums.

4.0 EXPERIMENTAL

Melting points were determined on Thomas Hoover Capillary Melting point apparatus and are uncorrected. Infera-red spectra were determined in KBr pellets using Perkin-Elmer Model 727E grating spectrophotometer. Ultraviolet vissible absorption spectra were measured in ethanol on Pye Unicam Model SP 8-100 spectrophotometer. Nuclear magnetic resonance spectra were obtained in D_6-DMSO on a Varian T-60A spectrometer and are reported as ppm value relative to tetramethyl silane (TMS) as internal reference. Mass spectra were determined on a Finnigan Model 3200F GC/MS spectrometer.

Analytical thin layer chromatography (tlc) was seen on Eastman chromatogram sheet code 6060 silica gel. The products were detected by ammonia vapour and by their ultraviolet fluoresence. Column chromatography were performed on a silica gel 60-120 (mesh (Merck).

4.1 Cold extraction of R. Abyssinicus

150 grams of powdered Rumex abyssinicus was packed on a column. This was eluted with 1.3 liters of distilled acetone. 4.6 grams of stiky brownish

material A-1 was produced on evaporation. The experiment was repeated to get more of A-1.

4.2 Column chromatography of A-1

2.4 grams of A-1 was disolved in acetone and 15 grams of silica gel was added. Then the solvent evaporated by rota evaporator. This was applied to 2.0 cm diameter column packed with 80 grams of silica gel. The column was first eluted by 880 ml of petroleum ether (5_0 -60°C). One yellow band (A-2) was completely removed. It was insufficient quantity (3-4 mg) for further investigation.

The column was eluted with 1200 ml petroleum ether-benzene (1:1). A yellow band was removed whose fraction was monitered by analytical thin layer chromatography (tlc) using benzene-acetone (4:1) solvent system. Evaporation of the solvent gave 280 mg yellow crystalline material (A-3). It was crystallized from chloroform. The column was eluted with 1.5 liters of petroleum ether, benzene and acetone (1:1:1). A light red band was removed whose fraction was checked on tlc for single spot only. On vacuum evaporation gave 310 mg orange-red crystal A-4. It was crystalized from acetone.

4.3 Column chromatography of A-3

250 mg of A-3 disolved in 10 ml of chloroform was applied to 1.8 cm diameter column packed with 25 grams of silica gel. The column was eluted first by petroleum ether and then by gradient elution with petroleum ether and benzene solvent system. Every 50 ml fraction was checked by tlc (hexane: ether, 4:1) and single spots of the same R_f were combined and concentrated under vacuum gave 110 mg yellow substance A-6, which was chrystalized from chloroform. Further elution gave a fraction whose tlc showed two yellow spots (R_{f_1} = 0.92, R_{f_2} = 0.81, ether: Hexane, 1:4) and evaporation under vacuum gave 90 mg yellow crystaline substance A-3. Latter fractions on elution showed single yellow spot R_f 0.81 on tlc and evaporation under vacuum gave 40 mg light yellow crystal A-7. It was crystalized from chloroform. The fraction A-3 90 mg was subjected for repeated column chromatography as before and gave 48 mg of A-6 and 23 mg of A-7.

4.4 Acetylation of A-4

A mixture (100 mg) of A-4 acetic anhydride (10 ml) and pyridin (2 ml) was made to react at room

temperature with stirring using a magnetic stirrer for four hours. It was pured in to a crushed ice where by a yellow solid mass A-5 separated. It was filtered, washed well, and finally crystalized from acetone yielding the acetyl derivative. The melting point of the acetyl derivatives is 194-196^{o}C.

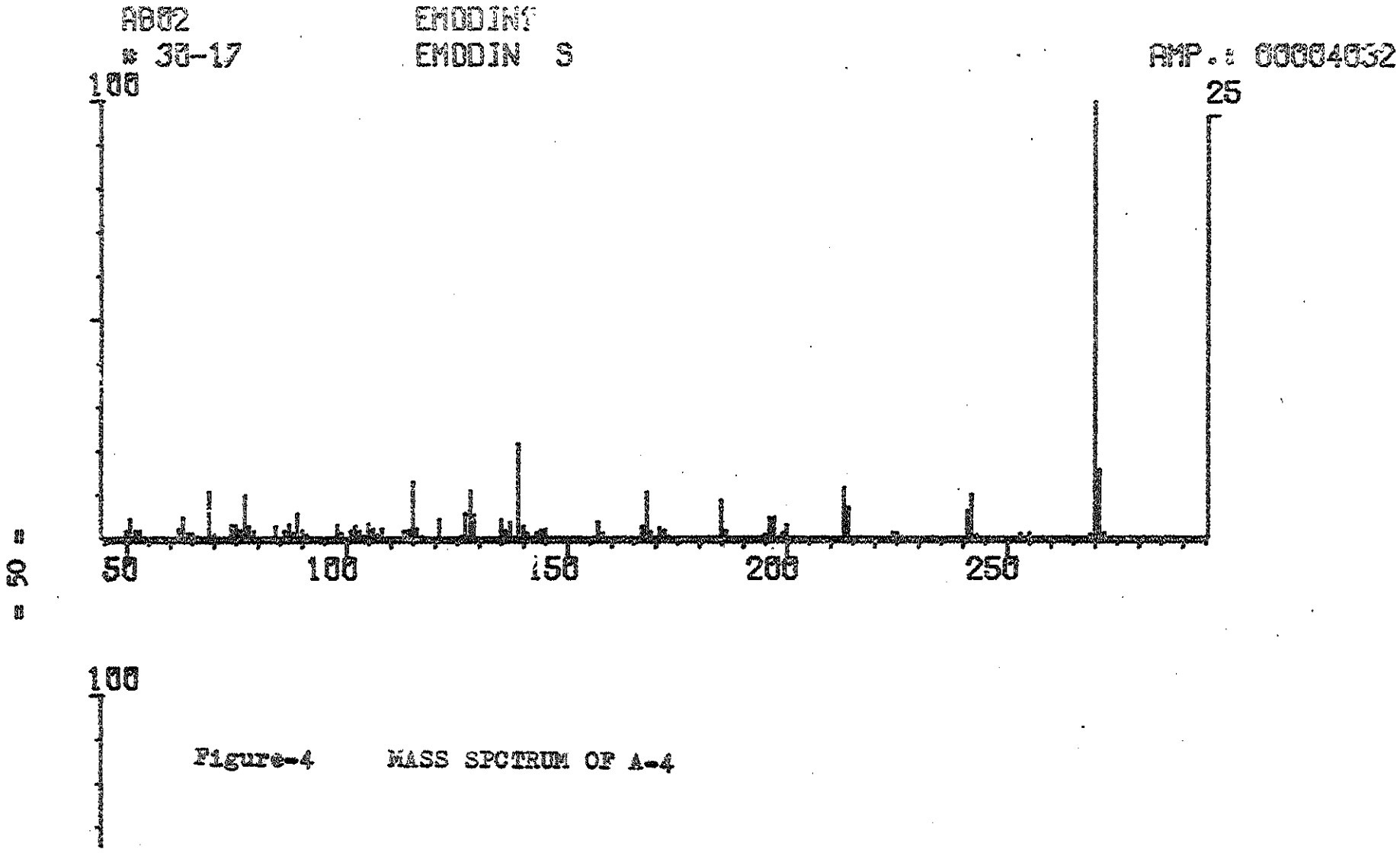


Figure-4 MASS SPCTRUM OF A-4

S-omDTx
Wavelength (nm)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

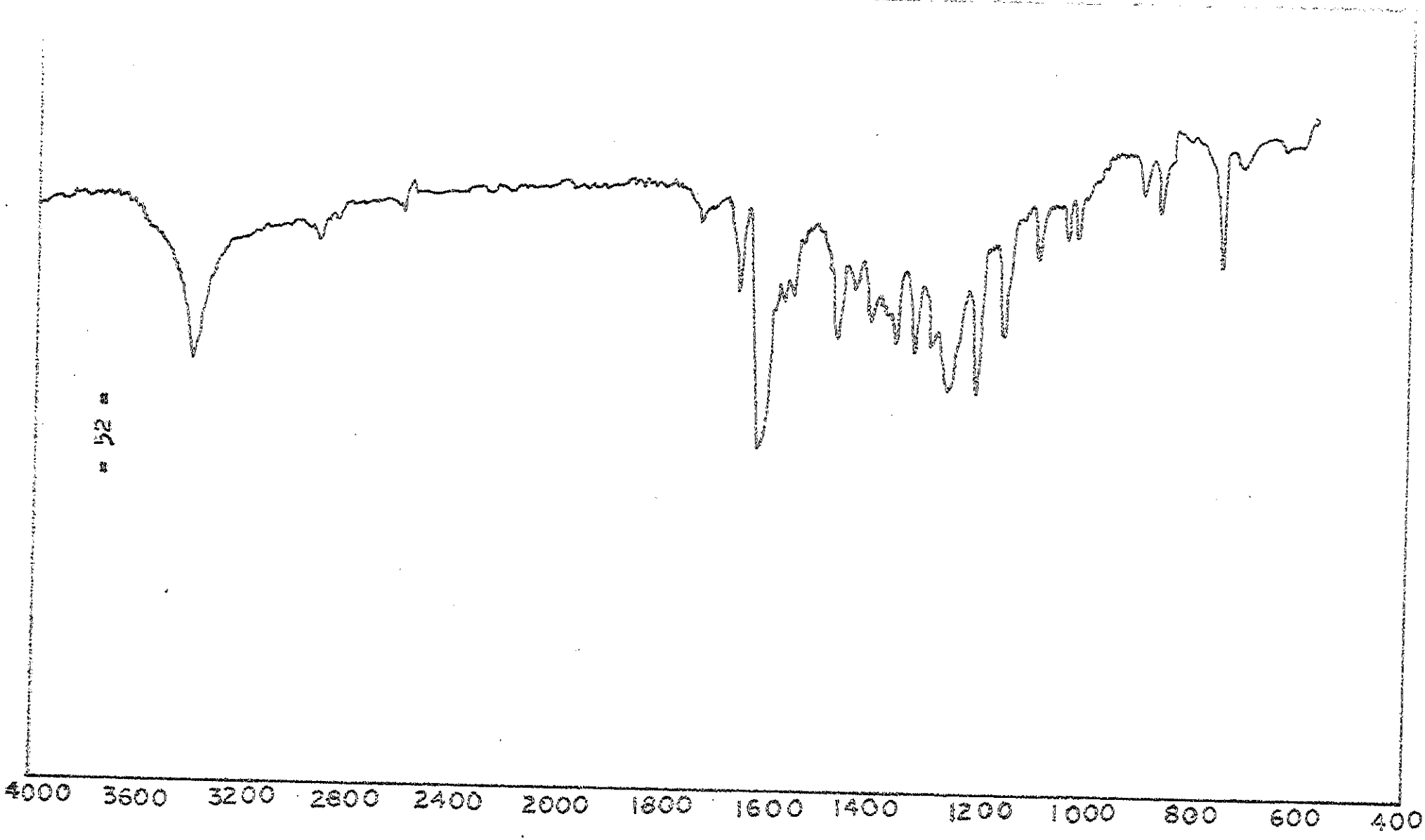


Figure-6 IR SPECTRUM OF A-4

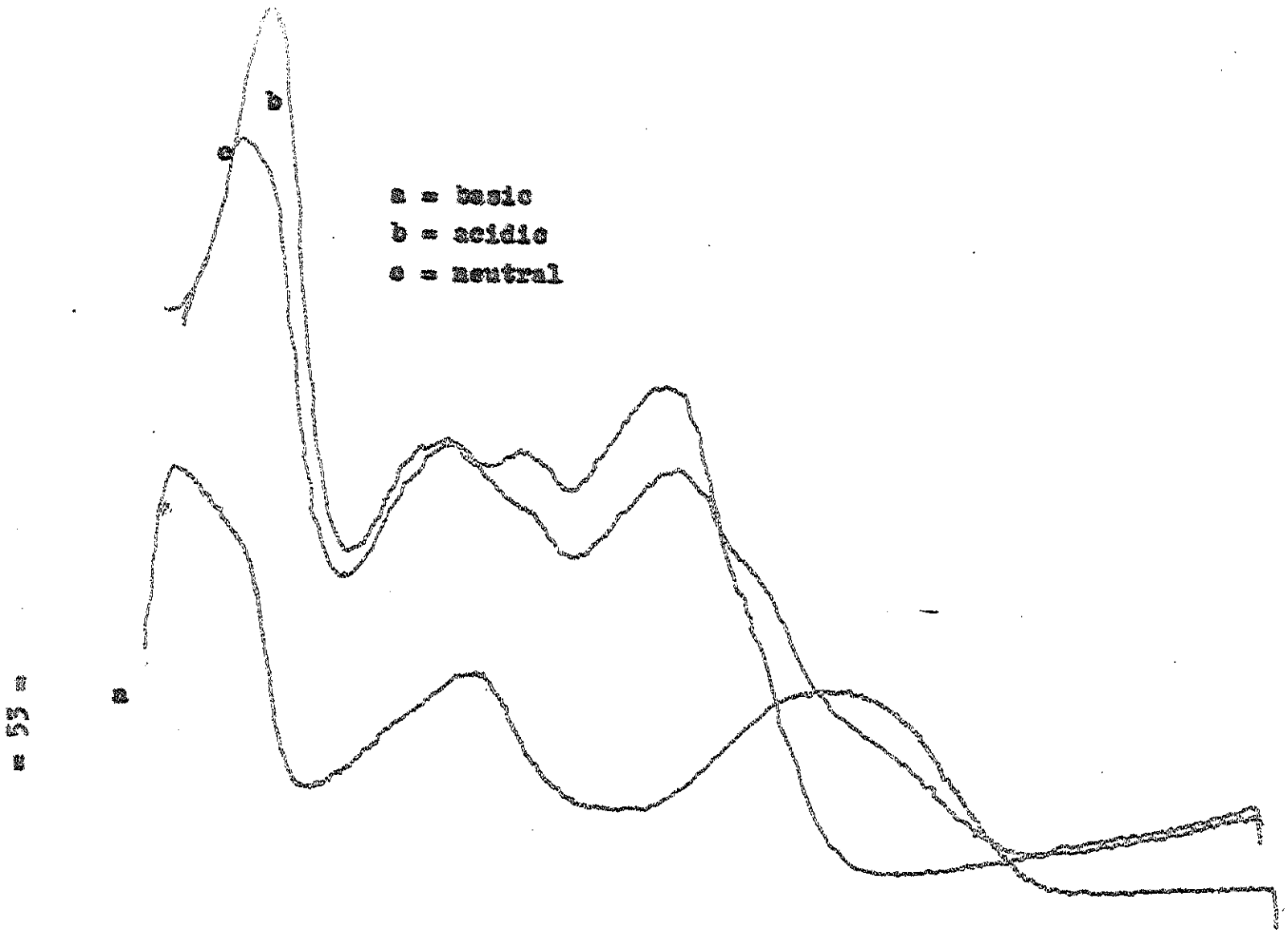


Figure-7 U V SPECTRUM OF A-4

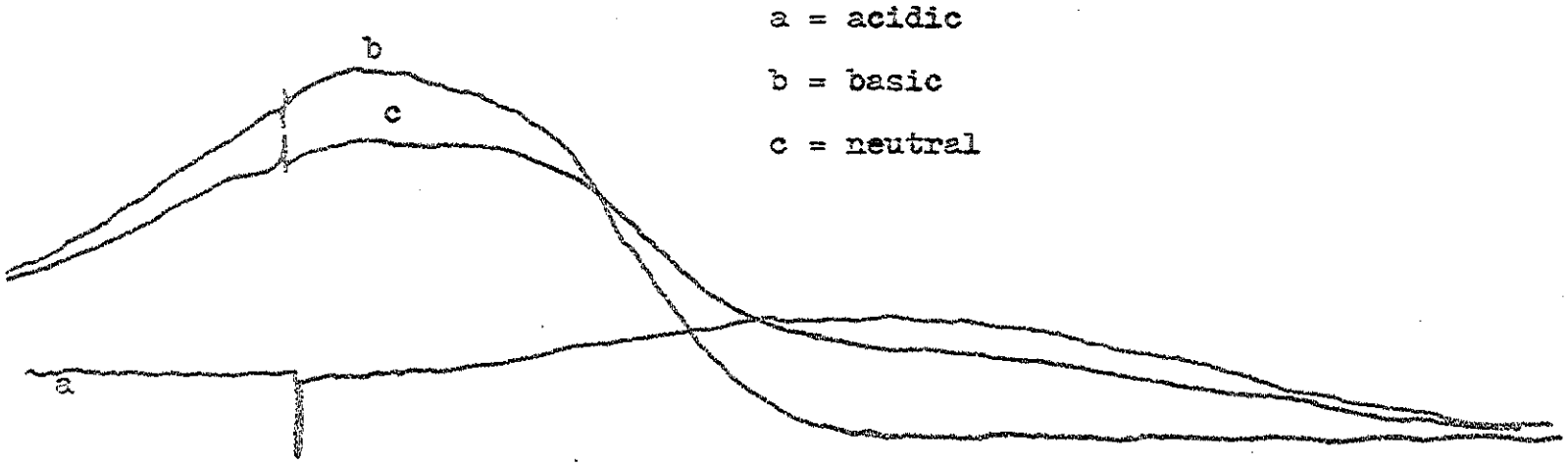


Figure-8 VISIBLE SPECTRUM OF A-4

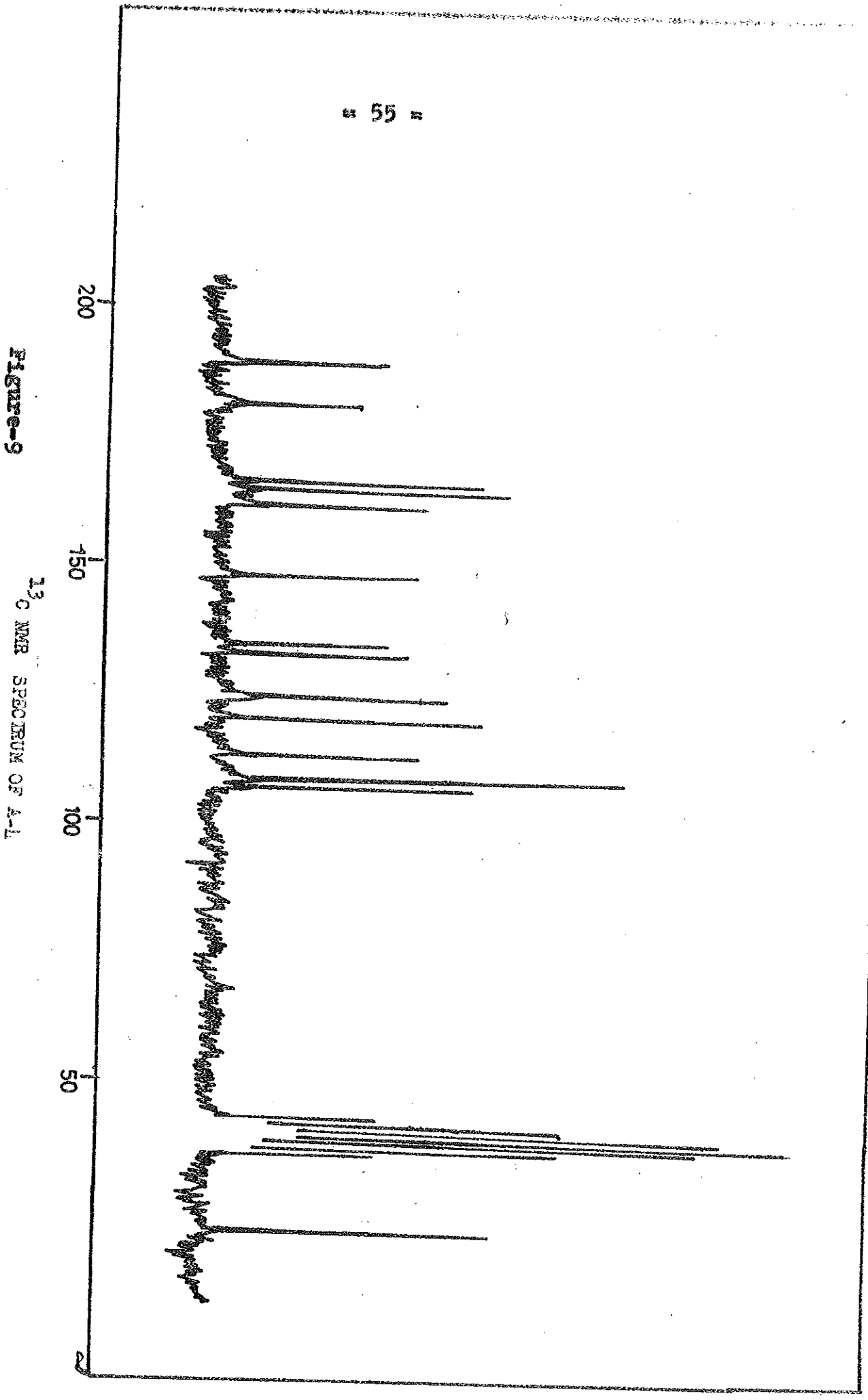


Figure-9

^{13}C NMR SPECTRUM OF 4-1

FIGURE-10 MASS SPECTRUM OF A-6

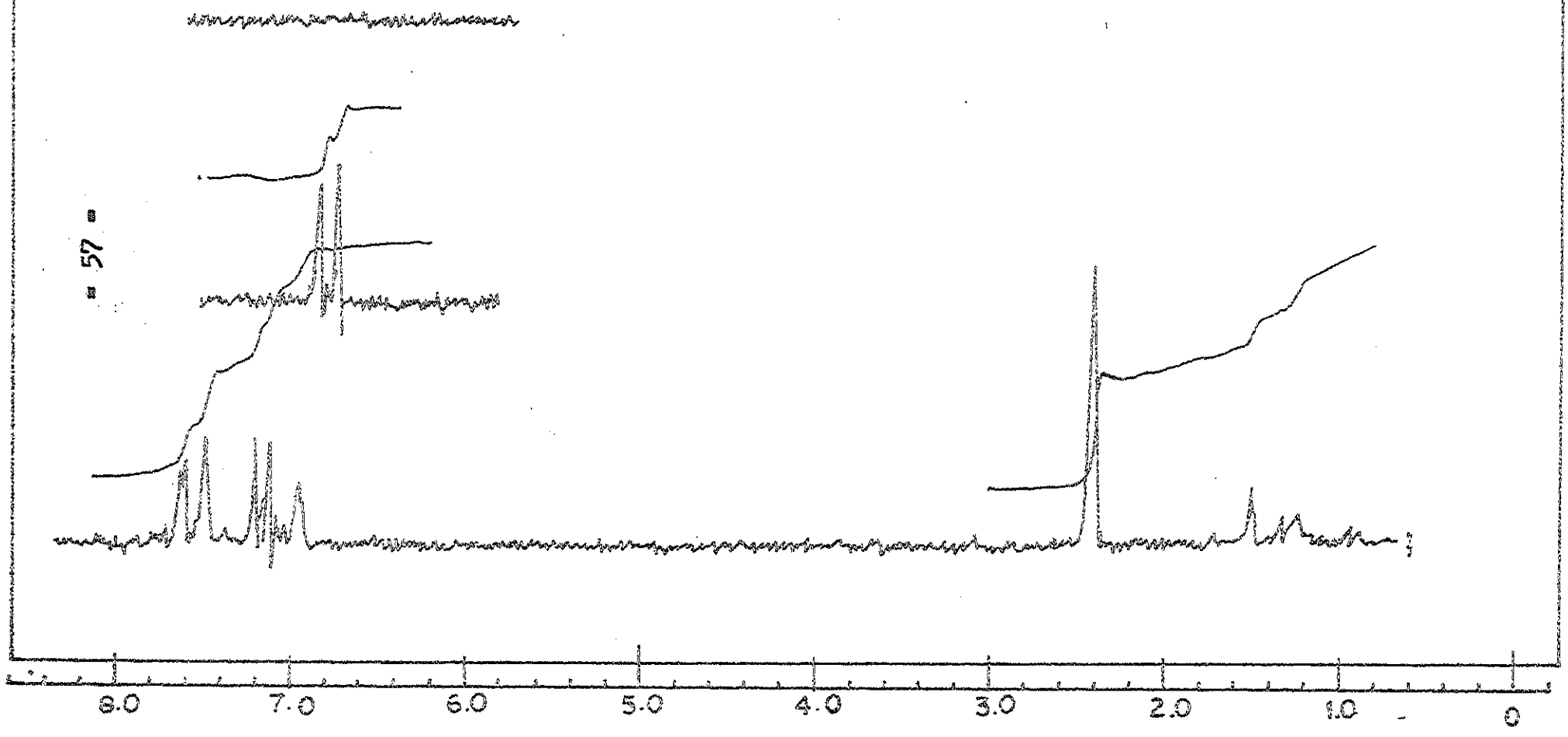


Figure - 11 N M R SPECTRUM OF A-6

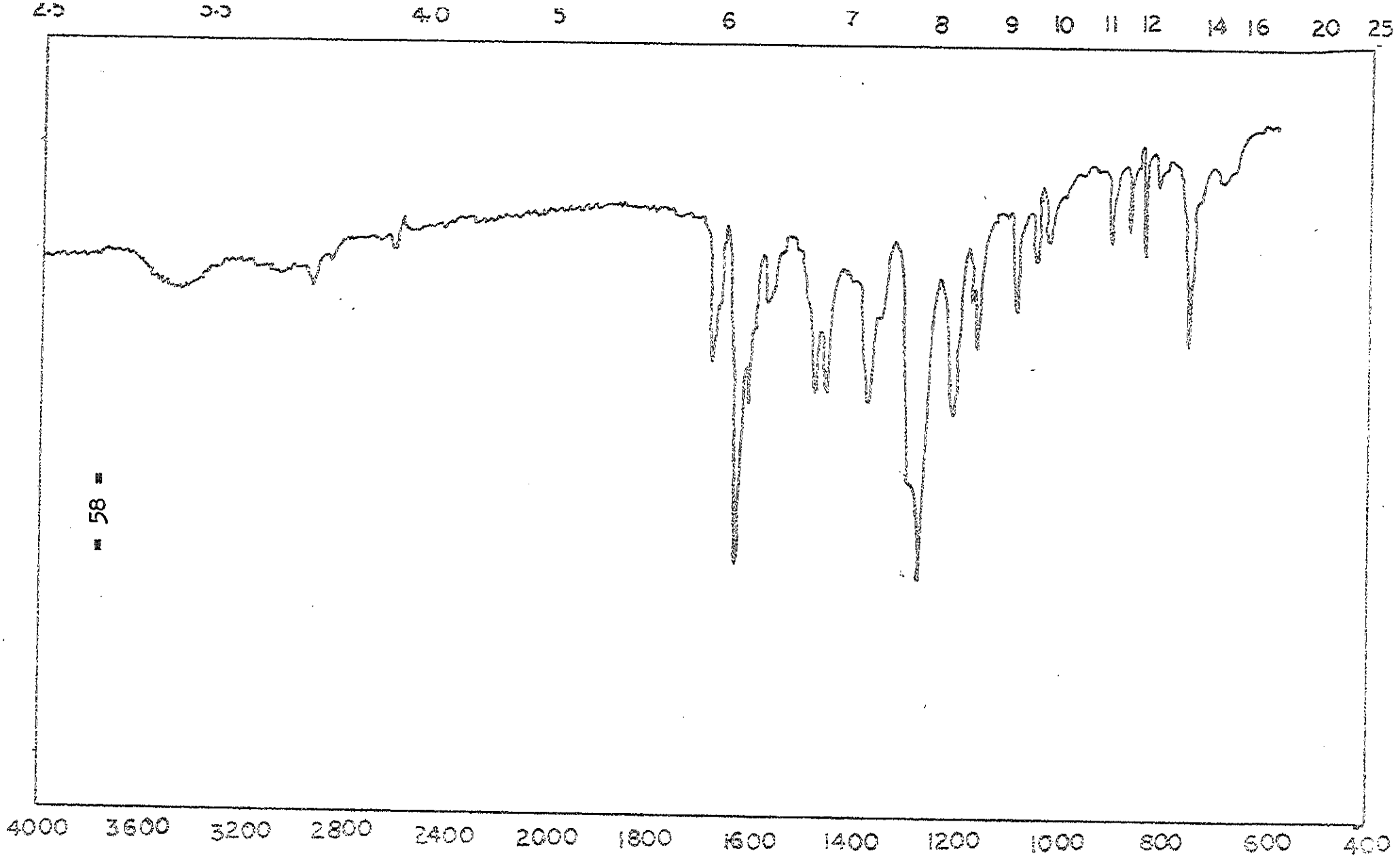


Figure -12 IR SPECTRUM OF A-6

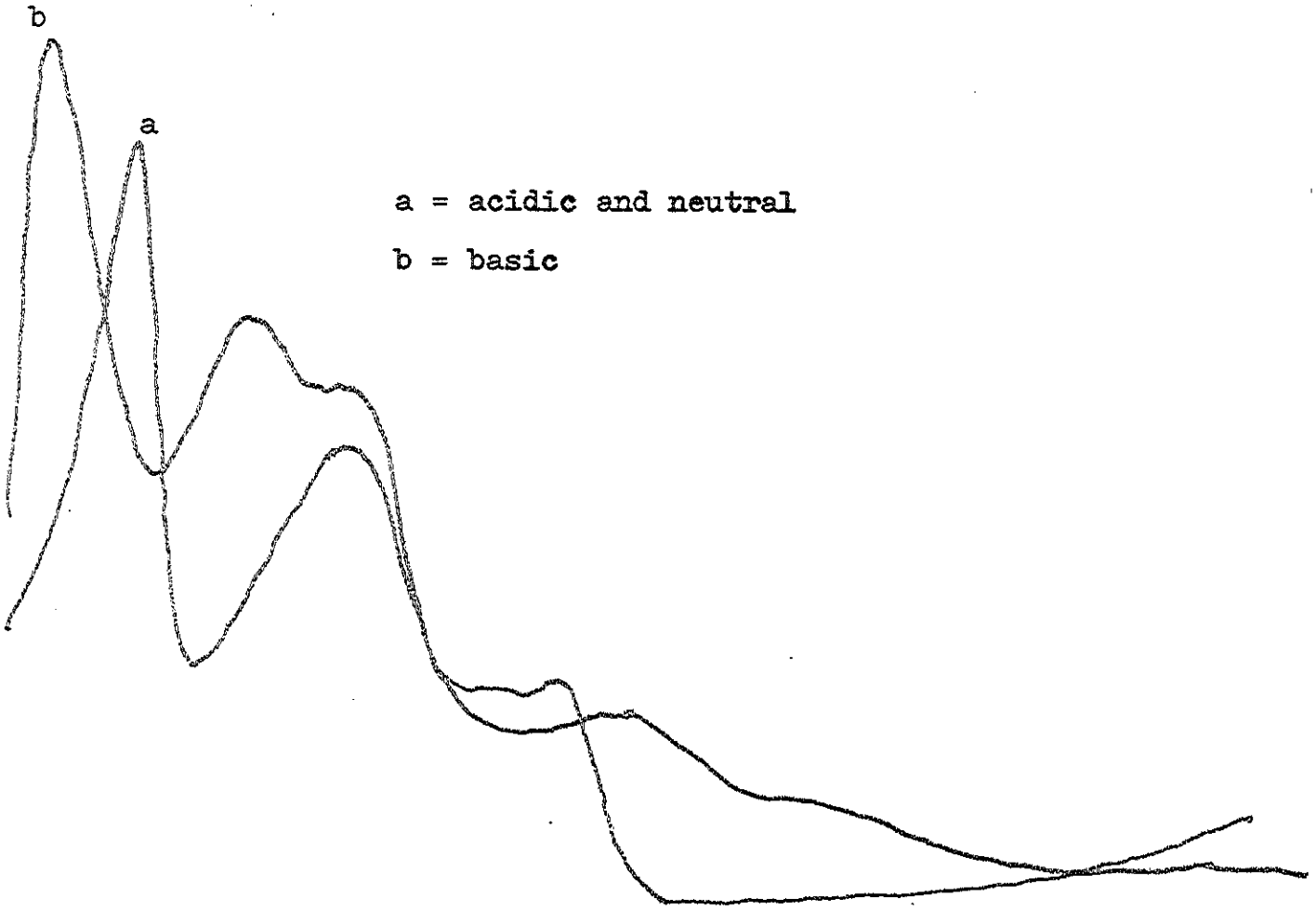


Figure - 13 U V SPECTRUM OF A-6

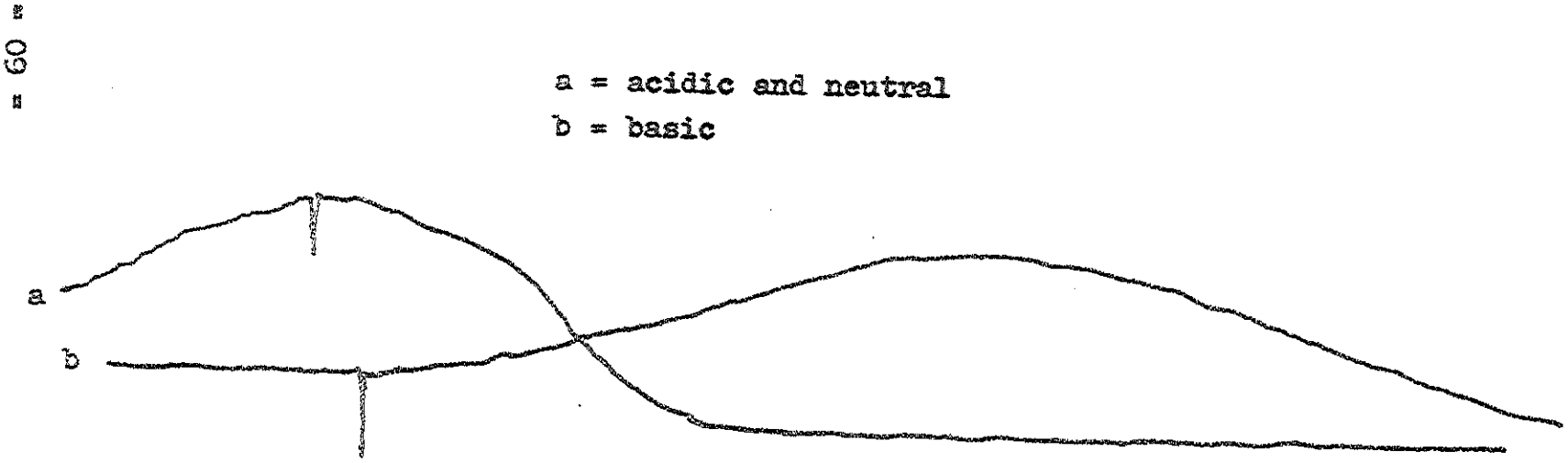


Figure -14 VISIBLE SPECTRUM OF A-6

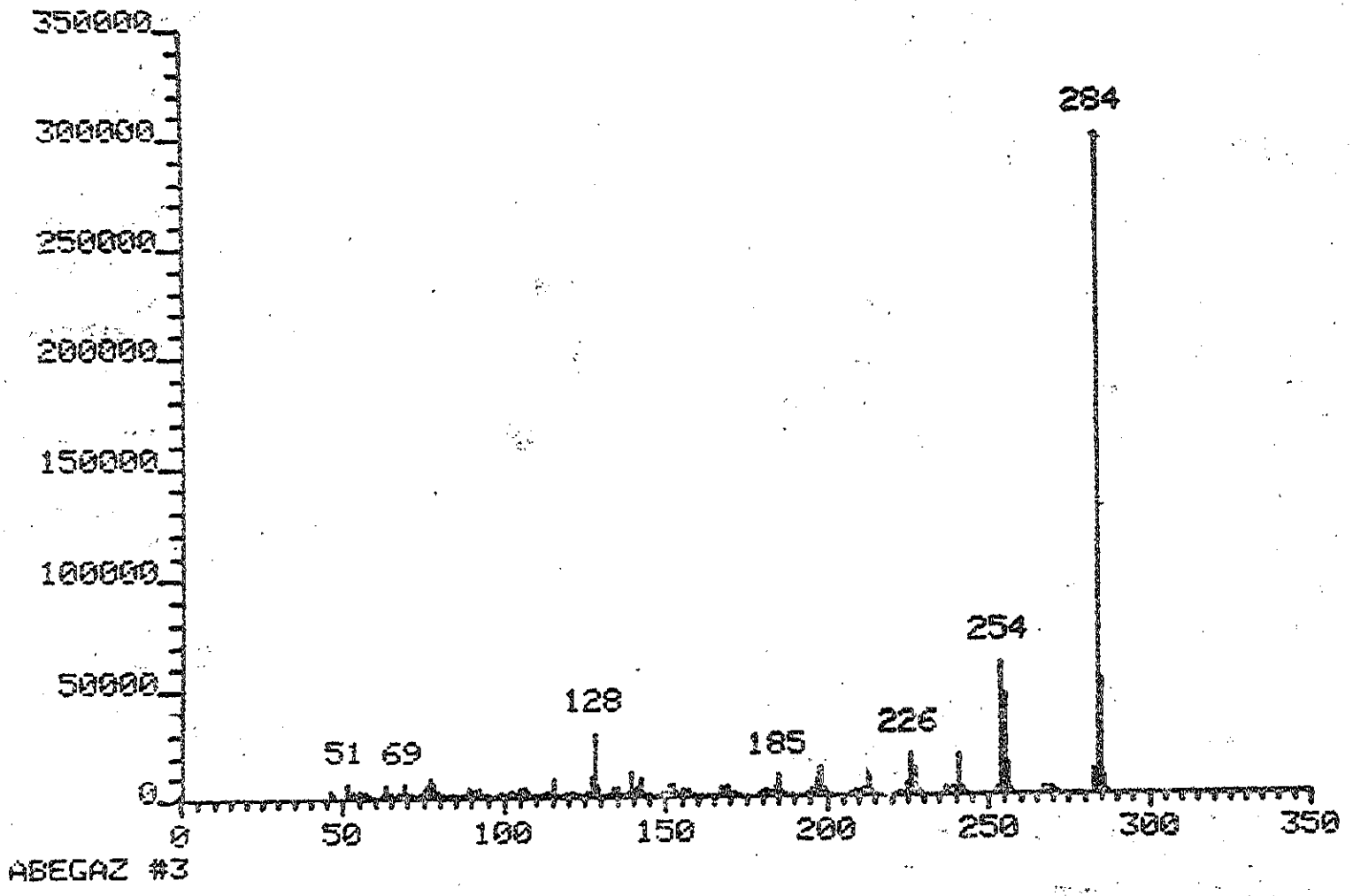


Figure-15 MASS SPECTRUM OF A-7

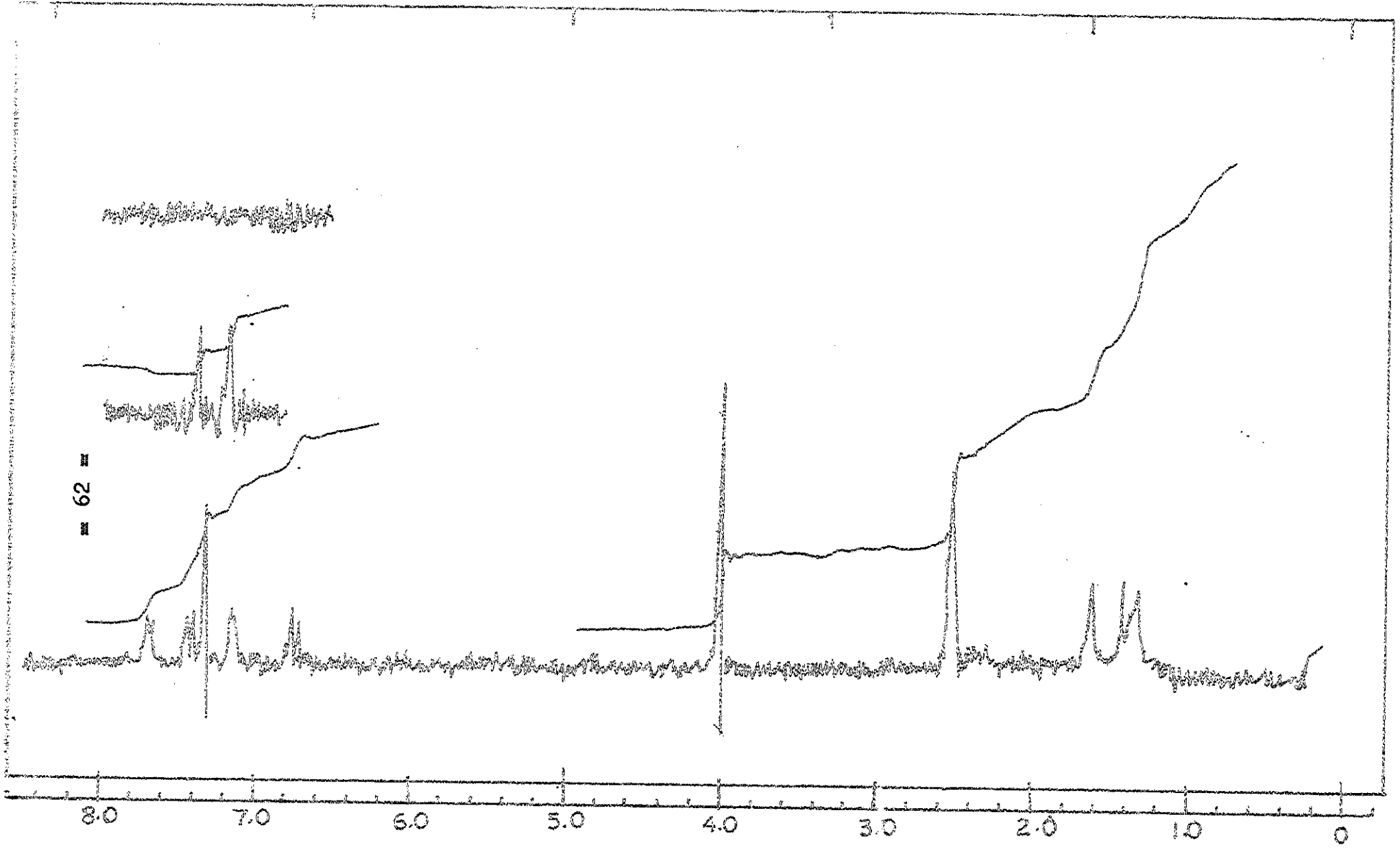


Figure-16 N M R SPECTRUM OF A-7

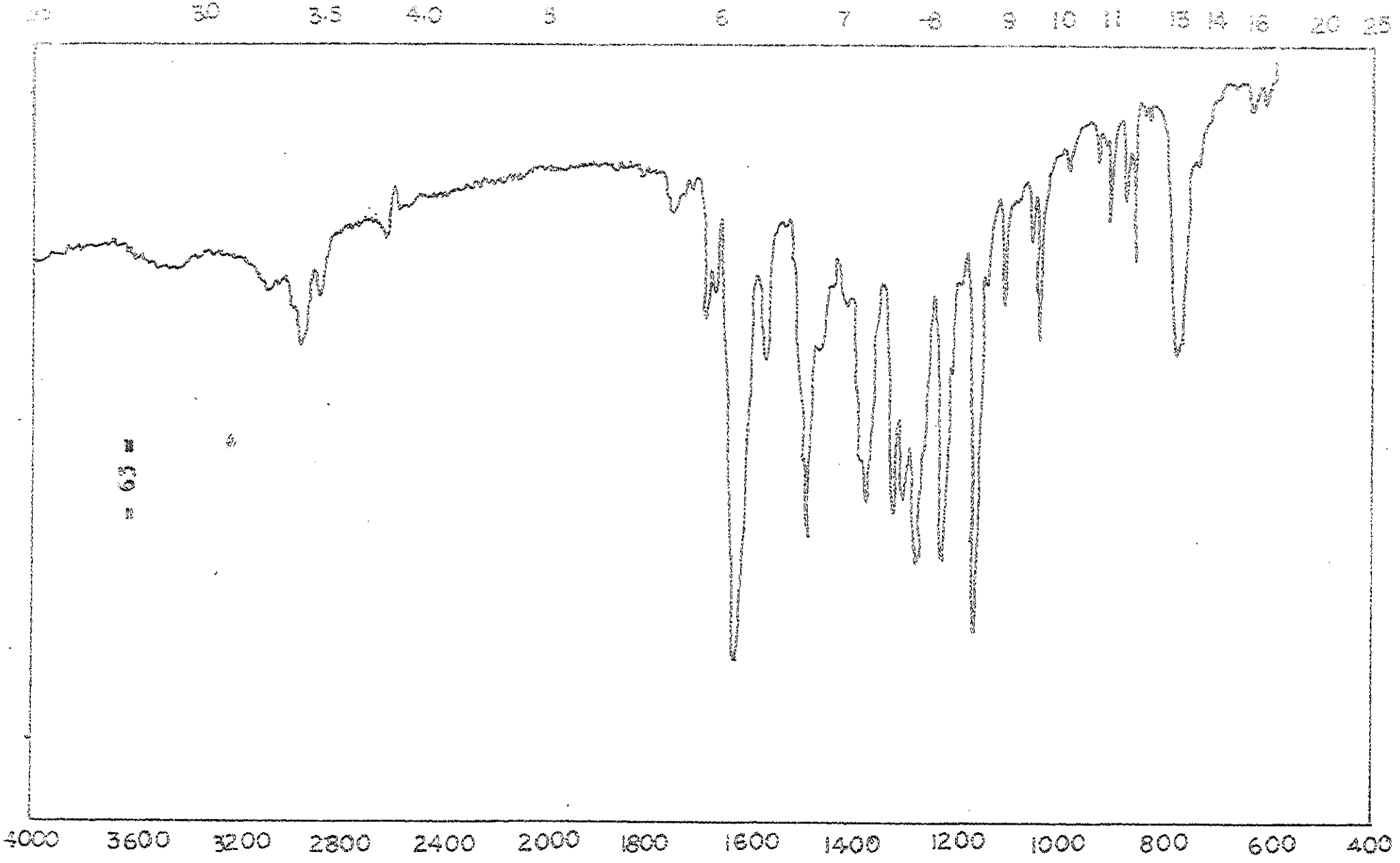


Figure-17 IR SPECTRUM OF A-7

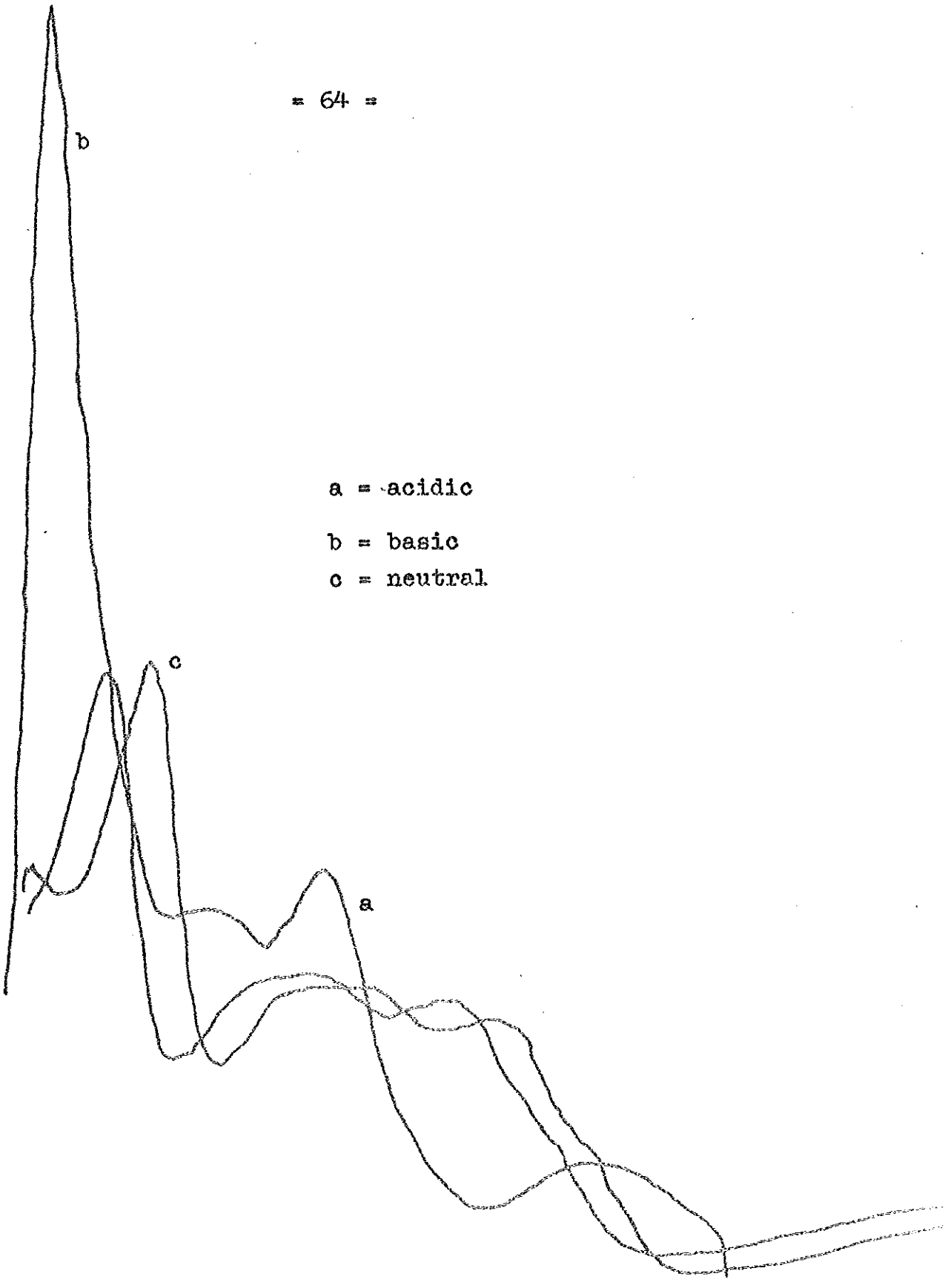


Figure- 18 U V SPECTRUM OF LA-7

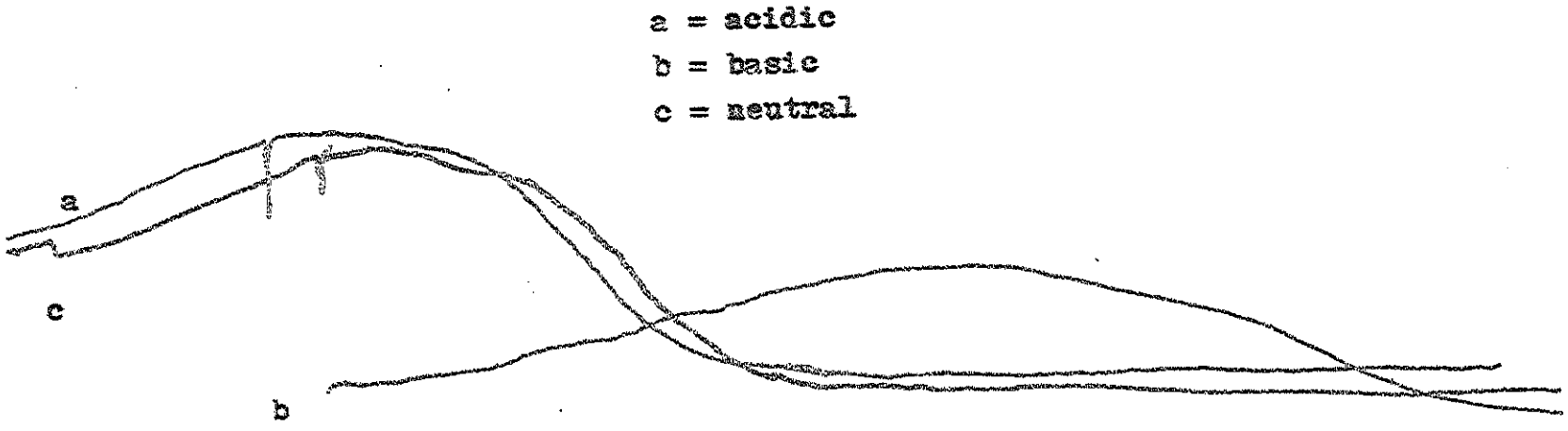


Figure-19 VISIBLE SPECTRUM OF A-7

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