PHYTOCHEMICAL STUDIES ON SOME *ALOE* SPECIES OF THE *SAPONARIAE* GROUP.

BY

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This thesis is my original work and has not been presented for a degree in any University.

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The thesis is dedicated to my beloved parents.

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ABSTRACT

The taxa Aloe with its more than 350 species are widely distributed in Tropical Africa. They have found wide recognition for their cosmetic and medicinal uses. The taxonomic irregularities and the inadequacy of phytochemical information in many of these plants have attracted our attention.

During our phytochemical survey of the Saponariae group of Aloe, using chromatographic techniques more than twenty distinct compounds were detected. Eight of these were isolated from the subterranean stems of Aloe graminicola. The compounds isolated were found to be mainly anthraquinones. One of these was isolated for the first time in nature and the name **aloegramonol (1)** was suggested. The other isolated compounds included; **chrysophanol (2)**, **asphodelin (3)**, **helminthosporin (4)** **aloesaponol-I (5)**, **aloesaponarin-I (6)**, **aloesaponarin-II (7)** and the phytosterol, **β-sitosterol (8)**.

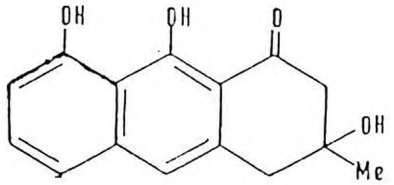
Two other compounds, namely; **aloesaponol-II (9)** and **laccaic acid-D methyl ester (10)** were identified from the subterranean stems of the same plant by HPLC comparison with authentic samples. The HPLC system developed here also enabled us to identify

aloesaponol-I, aloesaponol-II, laccaic acid-D methyl ester and **asphodelin** from the roots of A. graminicola.

Comparative TLC analyses of the root extracts of six Aloe species, namely; A. graminicola, A. lateritia, A. duckeri, A. dumetorum, A. secundiflora and A. nyeriensis (var. kedongensis) enabled us to identify **chrysophanol, asphodelin, aloesaponol-I, aloesaponol-II, aloesaponarin-I, aloesaponarin-II** and **laccaic acid-D methyl ester** from these plants. These compounds were also identified from the subterranean stems of A. graminicola and A. secundiflora and also from the flowers of A. graminicola and A. nyeriensis (var. kedongensis).

Aloesaponol-I, aloesaponol-II, aloesaponarin-I, aloesaponarin-II and **laccaic acid-D methyl ester**, which were detected in all plants analysed in these studies appear to be restricted to this genus. Based on the distribution of compounds an attempt was made to correlate phytochemical information with taxonomic classifications.

Antibacterial test showed the acetone extract of the roots of A. graminicola to be active against E. coli and B. sbutilis at a concentration of 250 µg/ml.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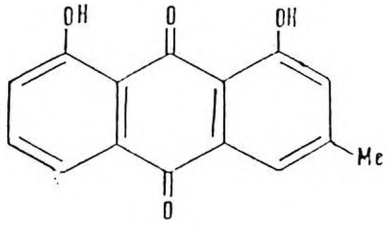
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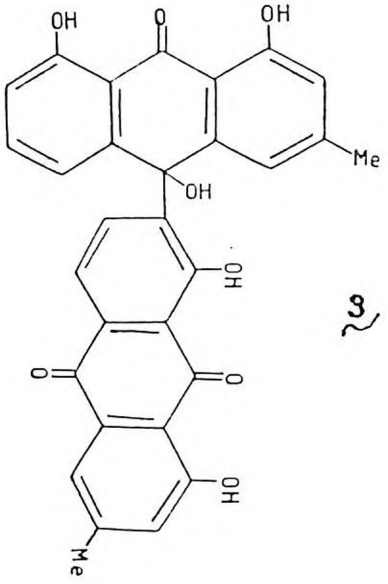
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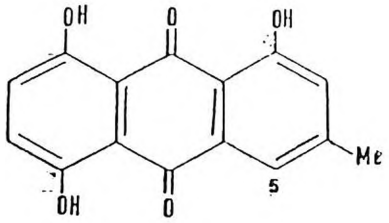
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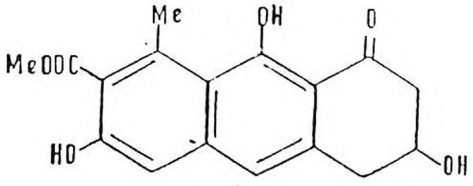
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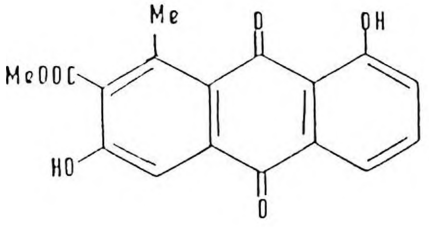

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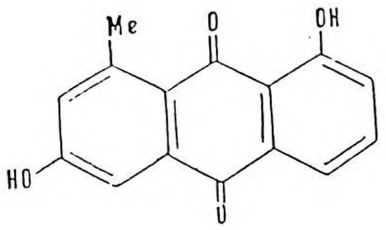

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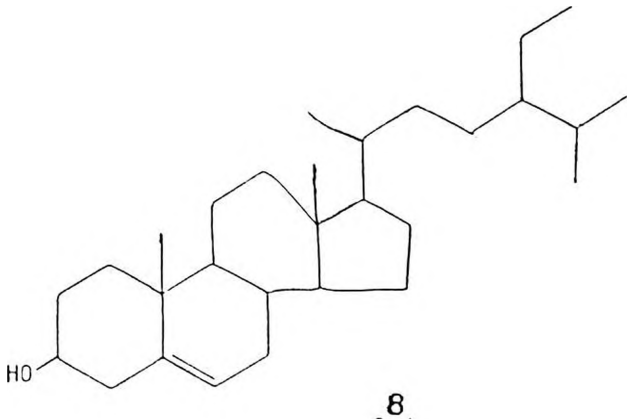

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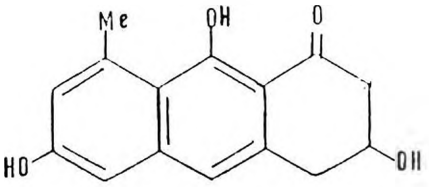

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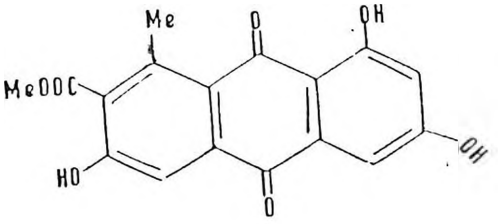

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CHAPTER 1

INTRODUCTION

1.1. BOTANICAL INFORMATION ON *ALOE*.

Aloe plants are widely distributed in tropical and subtropical Africa including Madagascar. The plants vary in size and hight. In all cases leaves are spiked with or without organized white spots. Flowers are orange-red to golden yellow on single or multiple stalk system (Reynolds, 1966).

The taxa *Aloe* has been considered to belong to the heterogenous family, Liliaceae. Recently, two different classifications, which do not include *Aloe* in the family Liliaceae have been proposed. Cronquist (1981) recognised the family Aloeaceae as distinct from Liliaceae, while Dahlgren and Clifford (1982), have placed *Aloe* under the subfamily Aloeideae in the family Asphodelaceae. Later Dahlgren *et al*. (1985) reclassified *Aloe* under the subfamily Asphodeloideae, in the family Asphodelaceae. These make, at least, three different classifications available.

There are more than 350 Aloe species reported and new species are being described regularly. This big taxa has been divided into several groups (Reynolds, 1950, 1966). However some of the divisions were considered to be artificial (Reynolds, 1985a).

The Saponariae group with its 39 species is considered to be the most problematic in taxonomic classification (Reynolds, 1950, 1966). The same author has described this group to be heterogenous within itself and indicated the problem in knowing where one species ends and the next begins.

Because of the taxonomic problems in the Saponariae group, it was suggested that the easiest way to identify a specimen of Aloe belonging to this group, be based on the particular region of collection (Bayer 1975). However, Forster (1989) has pointed out that, such kind of identification were far from being ideal, and suggested the importance of generating chemical informations in solving the taxonomical problems on this group. The same method was also put forward earlier on (Reynolds, 1985a).

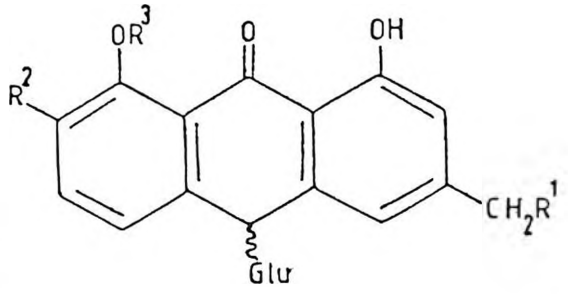
1.2. MEDICINAL INFORMATION ON ALOE.

The traditional uses of Aloe can be traced to the first century A.D., in Greek herbal medicine (Reynolds, 1950). Since then, several Aloe species have been used in traditional medicine for the treatment of a wide range of health complications. The purgative property and its uses for the treatment of wound, burn and any other skin disease were widely accepted (Lewis & Elvin-Lewis, 1977). Some other ailments are listed in Table-1.

TABLE-1. SOME TRADITIONAL USES OF ALOE.

PLANT	USES	REFERENCE
A. excelsa	Remedy for veneral diseases, asthma and abdominal pains.	Mebe (1987)
A. graminicola	Induction of vomiting.	Kokwaro (1976)
A. klifiensis	Reduction of swelling.	"
A. latifolia	Treatment of boil, injuries and sores.	Reynolds (1950)
A. rabaiensis	Induction of vomiting and diarrhoea.	Kokwaro (1976)
A. secundiflora	Treatment of malaria, headache, pneumonia.	"

The purgative effects observed on Aloe, were shown to be due to **barbaloin** (11) and **homonataloin** (12) (Reynolds, 1985a).

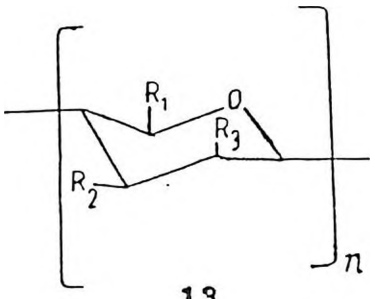


11 R^1 = OH, R^2 = R^3 = H

12 R^1 = H, R^2 = OH, R^3 = Me

Modern application of Aloe, include the treatment of X-ray burns (Lewis & Elvis-Lewis, 1977), the treatment of ulcers (Zawahry et al., 1973) and preparation of shampoos, shaving and skin care creams (Leung, 1977).

Morton (1961) associated the skin healing properties of the leaves of Aloe with its muciliginous nature and later with an antibacterial property (Morton, 1989). Recently, **carrisyn** (13), extracted from A. vera **(Linn.)** was claimed to be the active component which helps skin and gastrointestinal healing. **Carrisyn** was also shown to have some curative effects in AIDS patients (McAnnalley & Bill, 1987).



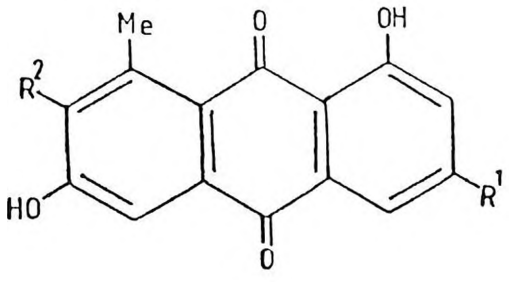
13

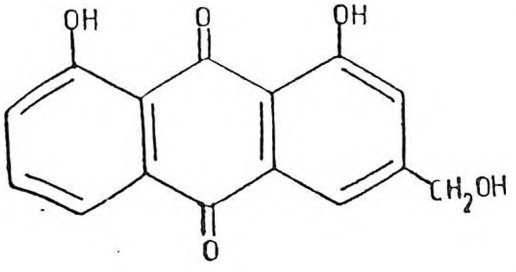
R_1 = CH_2OH, CO_2, CH_2Ac

R_2, R_3 = OH, OAc, CH_2OAc

n = 2-50,000

Apart from these properties of Aloe, a wide range of pharmacological activties, in various species of Aloe have also been reported. Antiviral activities of Aloe vera were reported by Sydiskis et al., (1987) where aloe-emodin (14) and related anthraquinones were noted as active principles. The anthraquinones, aloesaponrin-I (6), deoxyerythrolacin (15) and laccaic acid-D methyl ester (10) isolated from Aloe saponaria showed antimicrobial activities (Yagi et al., 1983).





14

6 R^1 = H, R^2 = COOMe

10 R^1 = OH, R^2 = COOMe

15 R^1 = OH, R^2 = H

1.3. PHYTOCHEMICAL INFORMATION ON ALOE.

1.3.1. COMPOUNDS OF ALOE.

Because of the wide applications of the leaves of Aloe species, in traditional medicine, the phytochemical information available on Aloe are mainly on the leaves. A. saponaria is the only plant with some information on the roots and subterranean stems (Yagi et al., 1974, 1977a,b, 1978a,b, 1983).

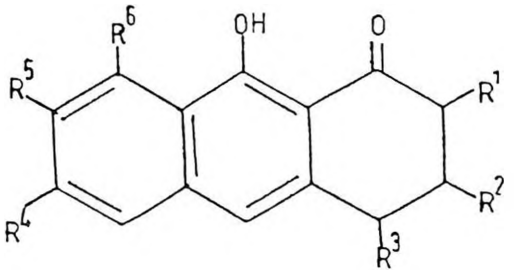
Most of the compounds so far reported from Aloe are anthraquinone derivatives, including anthrones and pre-anthraquinones. Chromones, phenyl pyrones alkaloids, lignins and steroids have also been reported.

1.3.1.1 ANTHRAQUINONE DERIVATIVES.

The taxa Aloe is widely associated with the presence of anthraquinone derivatives. These are some of the most widely distributed naturally occurring phenolics found in lower and higher plants. They are claimed to have arisen by at least two different biogenetic routes (Thomson, 1971).

The anthraquinones found in Aloe appear to have been derived through the polyketide pathway (Yagi et al., 1978b, Grun & Franz, 1981). Compounds found at various stages of this pathway were identified; i.e. pre-anthraquinones, anthrones and anthraquinones.

Some pre-anthraquinones and their derivatives were isolated from the subterranean stems of A. saponaria (Table-2). These compounds have not been reported from any other plant.



CPD	R1	R2	R3	R4	R5	R6
5	H	OH	H	OH	COOMe	Me
9	H	OH	H	OH	H	Me
16	H	H	OH	Me	H	OH
17	OMe	H	OH	Me	H	OH
18	H	OH	H	OGlu	COOMe	Me
19	H	OH	H	OGlu	H	Me
20	H	H	OH	Me	H	OGlu
21	OMe	H	OH	Me	H	OGlu

TABLE-2. PRE-ANTHRAQUINONES OF ALOE SAPONARIA.

COMPOUND	REFERENCE
Aloesaponol-I (5)	Yagi et al. (1974)
Aloesaponol-II (9)	"
Aloesaponol-III (16)	Yagi et al. (1977a)
Aloesaponol-IV (17)	"
Aloesaponol-I-glucoside (18)	Yagi et al. (1977b)
Aloesaponol-II-glucoside (19)	"
Aloesaponol III-glucoside (20)	Yagi et al. (1983)
Aloesaponol IV-glucoside (21)	Yagi et al. (1977b)

Study on the formation of pre-anthraquinones and anthraquinones in Callus tissue of A. saponaria, has showed that pre-anthraquinones have been metabolized into anthraquinones, in the presence of light (Yagi, et al., 1983). Based on this observation the authors have explained why, pre-anthraquinones were confined to the subterranean stems of A. saponaria.

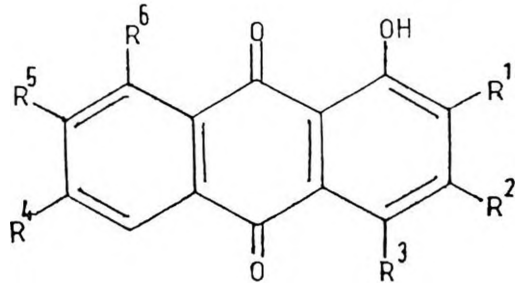
The leaves of Aloe are very rich sources of anthrones, all of which are C-10-glycosides. **Barbaloin (11)** and **homonataloin (12)** which are considered to be characteristic of the genus have been reported from the leaves of over a hundred Aloe species (McCarthy 1969, Reynolds, 1985b).

Unlike anthrone glycosides, which were reported as the major constituents of the leaves of Aloe species, the free anthraquinone concentration in the leaves appear to be low. **Aloe-emodine (14)** is the most widely distributed anthraquinone in the leaves of Aloe (Reynolds, 1985a).

From the subterranean stems of A. saponaria some anthraquinone derivatives (Table-3) have been isolated.

TABLE-3. ANTHRAQUINONES OF ALOE SAPONARIA.

COMPOUND	REFERENCE
Aloesaponarin-I (6)	Yagi et al. (1974)
Aloesaponarin-II (7)	"
Chrysophanol (2)	Yagi et al. (1977a)
Deoxyerythrolaccin (15)	"
Helminthosporin (4)	"
Isoxanthorin (22)	"
Laccaic acid-D methyl ester (10)	Yagi et al. (1974)

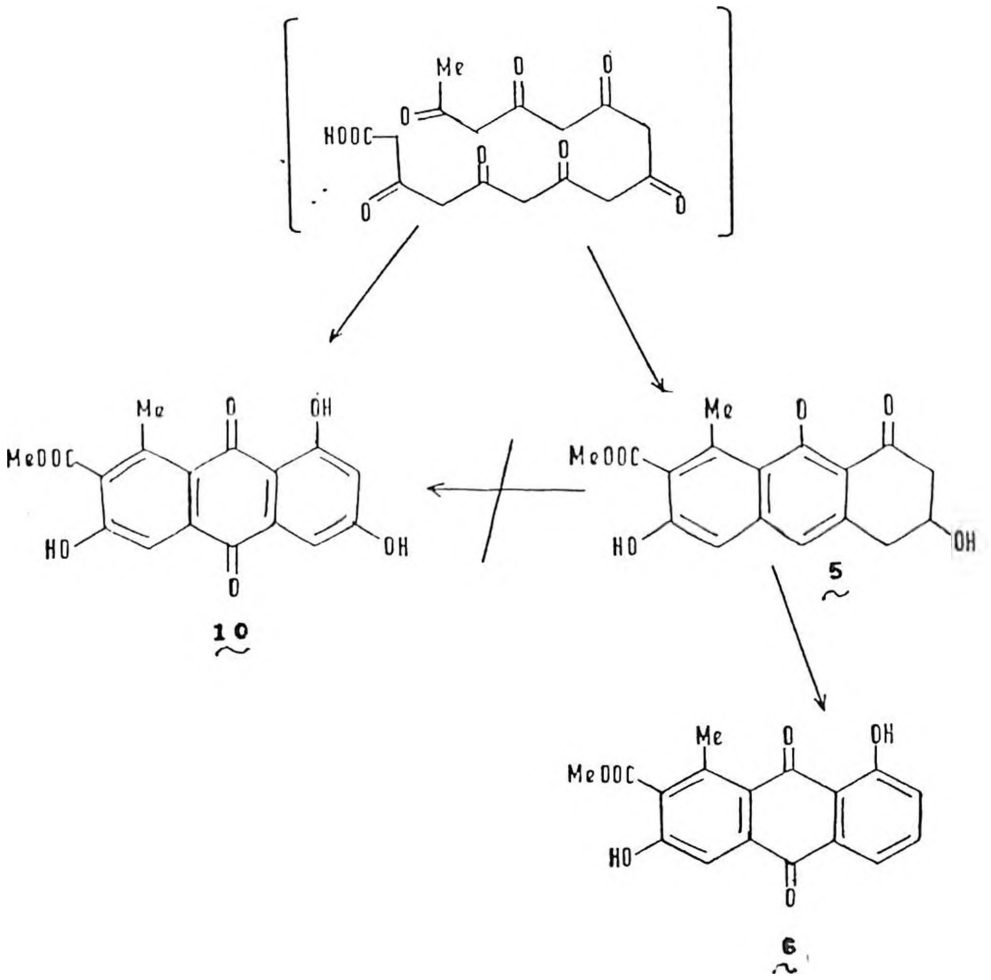


CPD	R^1	R^2	R^3	R^4	R^5	R^6
2	H	H	H	Me	H	OH
4	H	H	OH	Me	H	OH
6	H	H	H	OH	COOMe	Me
7	H	H	H	OH	H	Me
10	H	OH	H	OH	COOMe	Me
15	H	OH	H	OH	H	Me
22	OMe	H	OH	Me	H	OH

Among the anthraquinones isolated from A. saponaria, **chrysophanol (2)** is widely distributed in nature and it was also reported from the leaves of some Aloe species (Thomson, 1971). **Deoxyerythrolaccin (15)** was earlier reported from Bulea monsperma **Kuntze** and Zizyphus maruitiana **Lam.** (Reynolds, 1985a), while, **helminthosporin (4)** was first reported as a fungal metabolite (Reynolds 1985a). **Aloesaponarin-I (6), aloesaponarin-II (7), laccaic acid-D methyl ester (10)** and **isoxanthorin (22)** have not been reported from any other source, apart from A. saponaria.

Biosynthetic studies with the roots of A. saponaria have shown **aloesaponarin-I (6)** to have been derived from the pre-anthraquinone, **aloesaponol-I (5)**, while **laccaic acid-D methyl ester (10)** was shown to have been derived by a parallel route (Scheme-1), (Yagi et al., 1978b).

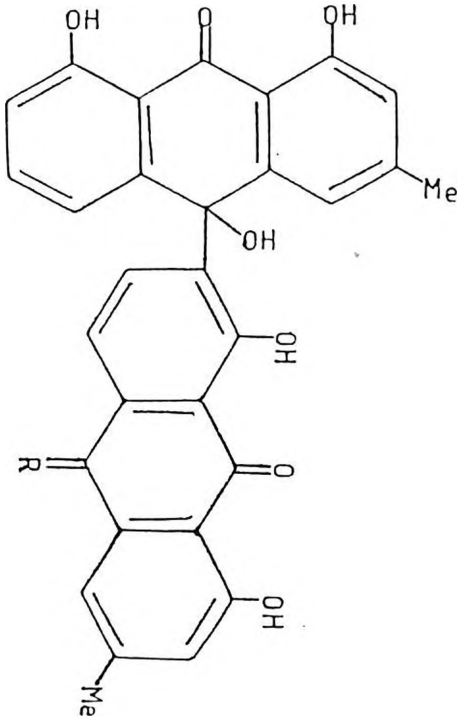
Scheme-1. Biogenetic relationships between aloesaponol-I (5), aloesaponarin-I (6) and laccaic acid-D methyl ester (10).



The bi-anthracene derivatives isolated from A. saponaria (Table-4) were derived from chrysophanol and chrysophanol 9'-anthrone through oxidative coupling. Except for asphodelin (3) which was earlier isolated from Asphodelus microcarpa (Gonzalez et al., 1973), these compounds have not been reported from any other source.

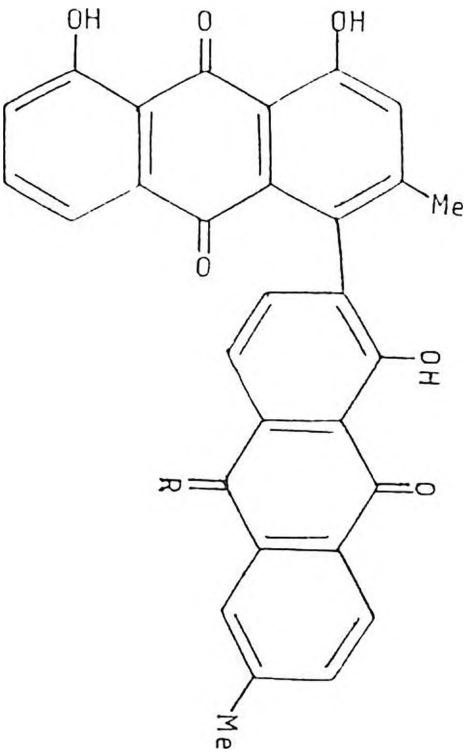
TABLE-4. BIANTHRAQUINONES OF ALOE SAPONARIA.

COMPOUND	REFERENCE
Asphodelin (3)	Yagi et al. (1978a)
Asphopdelin-9'-anthrone (23)	"
COMPOUND A (24)	"
COMPOUND B (25)	"



3 R = O

23 R = H2

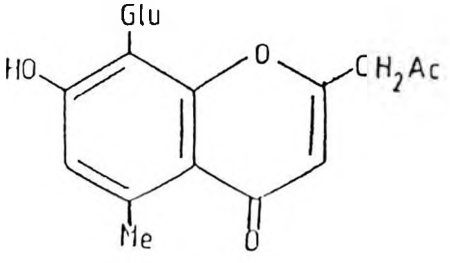


24 R = O

25 R = H2

1.3.1.2. OTHER COMPOUNDS.

Apart from anthraquinone derivatives, the chromone, **aloesin** (26) (=**aloeresine B**) and the phenyl pyrone, **aloenin** (27) were also reported from the leaves of many Aloe species (McCarty et al., 1967). These compounds have also been isolated from the subterranean stems of A. saponaria (Yagi et al., 1977b). Lignins (Graf & Alexa, 1982), alkaloids (Dring et al., 1984) and phytosterols (Waller et al., 1978) were also reported from Aloe.



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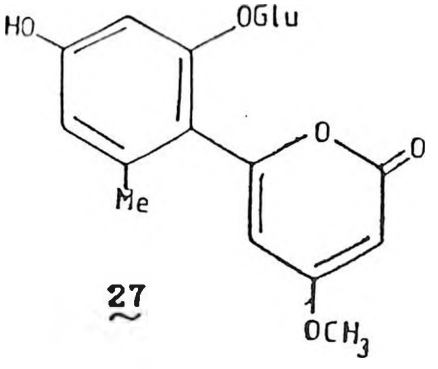

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1.3.2. CORRELATION OF PHYTOCHEMISTRY WITH TAXONOMY.

In Aloe, where classifications at higher as well as at species level are challenging, chemical information have been used in solving taxonomical problems. Dahlgren et al, (1982, 1985), have considered phytochemical information, among other taxonomical characters, when reclassifying Aloe (originally belonging to Liliaceae) under the family Asphodelaceae. Rheede Van Oudtshoorn (1963, 1964) has considered the distribution of some anthraquinones in some genera of the family Liliaceae, when supporting, the previously suggested taxonomic affinity between Aloinae and Asphodelinae.

In an attempt to correlate chemical information with taxonomical grouping proposed by Reynolds (1950), studies on the distribution of some anthraquinone derivatives in various Aloe species have been conducted (Rheede Van Oudtshoorn, 1963, 1964). However, no conclusion was drawn from these studies.

Reynolds (1985b) has analysed about 200 Aloe species and distingushed 88 major chromatographic zones, by TLC. Based on the distribution of these chromatographic zones, the author has pointed out few trends of correlation of chemical information with

taxonomical groupings, given by Reynolds (1950, 1966).

Out of the 88 chromatographic zones detected in the study, only four were chemically identified. The anthrone C-glucosides **barbaloin (11)** and **homonataloin (12)**, which are considered to be typical of the genus, were detected in many species. Out of 200 species analysed, **barbaloin** was detected in 33%, while, **homonataloin** was found in 14% of the samples. These two compounds were not observed to co-occur in the same plant, however, this was not correlated with taxonomic classification. The chromone derivative **aloesin (26)** was detected in 35%, while the phenyl pyrone derivative **aloenin (27)**, occurred in 12% of the samples analysed.

Such comparative phytochemical studies have obviously contributed in solving some taxonomical problems by providing additional evidence to conclusions drawn from botanical observations. However, most of the phytochemical information available on Aloe could not be correlated with the classification available todate. This could partly be due to the fact that the taxonomical classification on Aloe is far from being complete as many new species are continously being discovered while, some are being merged. In addition, most of the information are on few commercial species.

1.3.3. THE SAPONARIAE GROUP.

The Saponariae group which has been considered to be challenging in taxonomic classification, due to its heterogenousity, phytochemical information have been recommended to be used in solving this problem (Forster, 1989). However, the only chemical information so far available on this group is only on one species, namely; A. saponaria (Yagi et al., 1974, 1977a,b, 1978a, 1983). The compounds reported from the roots and subterranean stems of this plant were anthraquinone, pre-anthraquinone and bianthracene derivatives. Most of these have not been reported from other sources and some of the compounds showed antimicrobial activities.

It is worth to note that, apart from A. saponaria, almost all phytochemical information on Aloe are on the leaves. Little is known on other parts of these plants.

1.4. AIMS OF THE PRESENT STUDY.

The taxonomical problems in Aloe, in particular, in the Saponariae group and the absence of enough phytochemical information have attracted our attention. In addition, the absence of enough information on the flowers, roots and subterranean stems of Aloe and the presence of novel anthraquinone derivatives (some of which showed antimicrobial activities) in the roots and subterranean stems of A. saponaria have further attracted our attention. Thus it became necessary to study the flowers, roots and subterranean stems of some Aloe species of the Saponariae group. Some of our major objectives became:

1) To establish the distribution of compounds in the flowers, roots and subterranean stems of some Aloe species belonging to the Saponariae group and compare with some species belonging to other groups.

2) To find if there could be compounds, which could be used as markers in taxonomic classifications.

3) To find if the antimicrobial activities reported from A. saponaria could also be observed in other plant(s).

CHAPTER 2.

RESULTS AND DISCUSSION

2.1. PRELIMINARY TESTS.

The roots, flowers and subterranean stems of six Aloe species were collected. On cross section cutting of the roots and subterranean stems of the plants, deposition of yellow pigments were observed. Preliminary tests on the acetone extracts with KOH indicated the presence of quinones, suggesting the yellow colouration could be due to these pigments. From biogenetic point of view (Yagi et al., 1974, 1977a,b) they were considered to be anthraquinones.

TLC analyses of the acetone extracts of the roots, flowers and subteranean stems of the six species collected, showed the presence of at least twenty major chromatographic zones (Table-5).

TABLE-5. Distribution of compounds in some Aloe species.*

** Art-	AY-34 (ET)*	AY-34 (SST)	AY-34 (FR)	NEWTON-3085 (ET)	NEWTON-3539 (RT)	NEWTON-3188 (RT)	NEWTON-3231 (RT)	NEWTON-3407 (RT)	NEWTON-3087 (RT)	NEWTON-3422 (RT)	NEWTON-3614 (ET)	NEWTON-3620 (RT)	NEWTON-2350 (ET)	NEWTON-2496 (RT)	AY-33 (ET)	AY-33 (SST)	AY-31 (RT)	AY-31 (FR)
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
3	+	+	-	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	-	-	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-
8	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-
10	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
12	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-
16	+	-	-	+	+	+	-	?	?	+	-	-	-	-	+	-	+	-
17	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
18	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
19	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
20	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

* See section 4.2. for identifications of plants.

** Chromatographic zones; Art-1 = Aloe root compound 1

From Aloe graminicola, sixteen chromatographic zones were identified and this plant appeared to be the richest both in variety and concentrations and hence became our target plant for extensive study.

Some of the spots detected in this plant were yellow to orange in colour and gave positive quinonoid tests (KOH) and phenolic tests (**fast blue salt B (F.B.S.)**). The remaining spots were colourless but UV sensitive and also gave positive phenolic tests to **fast blue salt B** spray reagent. Some of the colourless spots developed colours, with time. This indicated, some kind of chemical transformation(s) taking place on the surface of silica gel. On the other hand TLC analyses on silica gel which was impregnated with **oxalic acid**, did not show any colour change for any of the spots. Thus the silica gel used in all column chromatography was impregnated with **oxalic acid.**

2.2. ISOLATION AND STRUCTURE ELUCIDATION OF COMPOUNDS FROM THE SUBTERRANEAN STEMS OF ALOE GRAMINICOLA.

TLC analyses of successive pet. ether (60-80°C), chloroform and acetone extracts of the subterranean stems of A. graminicola showed the chloroform extract to contain at least 11 spots, while the other two extracts contained some of the spots present in the chloroform extract. The MeOH soluble fraction of the pet. ether (60-80°C) extract and the chloroform soluble fraction of the acetone extract were combined with the chloroform extract.

The combined extract was subjected to column chromatography on silica gel impregnated with oxalic acid. Through further chromatographic separations (see experimental), eight compounds were isolated and the structures determined by spectroscopic studies and in some cases by chemical transformation. They were, two pre-anthraquinones, four anthraquinones, a bianthracene derivative and a phytosterol. The colour reactions for these compounds with some spray reagents are shown in Table-6.

Table-6. Colour reactions of compounds isolated from the subterranean stems of Aloe graminicola.

Cpd	Rf(sol syst)	VIS	VIS+	KOH	F.B.S.*
1	0.73 (S-4)	yellow	brown	-	brown
2	0.52 (S-1)	yellow	yellow	red	yellow
3	0.42 (S-1)	orange	orange	red	orange
4	0.89 (S-1)	red	red	purple	red
5	0.31 (S-4)	-	brown	-	brown
6	0.82 (S-4)	orange	orange	red	brown
7	0.80 (S-3)	yellow	yellow	red	brown
8x	0.40 (S-1)	-	-	-	-

Cpd = compound

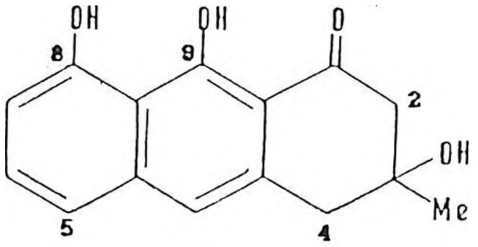
+ when seen after two weeks

* F.B.S = fast blue salt B

x detected as orange colour with H_2SO_4.

2.2.1. ALOEGRAMONOL (1).

The compound with Rf value of 0.73 (S-4) was isolated as yellow crystals, mp. 199-202°C. Yellow spot on TLC turned brown with **fast blue salt B** spray reagent, suggesting the presence of a phenolic group. Spraying with KOH did not change the yellow colour to red, ruling out a quinone skeletal structure. This compound was identified as **1-oxo-3,8,9-trihydroxy-3-methyl-1,2,3,4-tetrahydroanthracene** (1) based on the following data which are consistent with those reported in literature (Hansma et al., 1976).

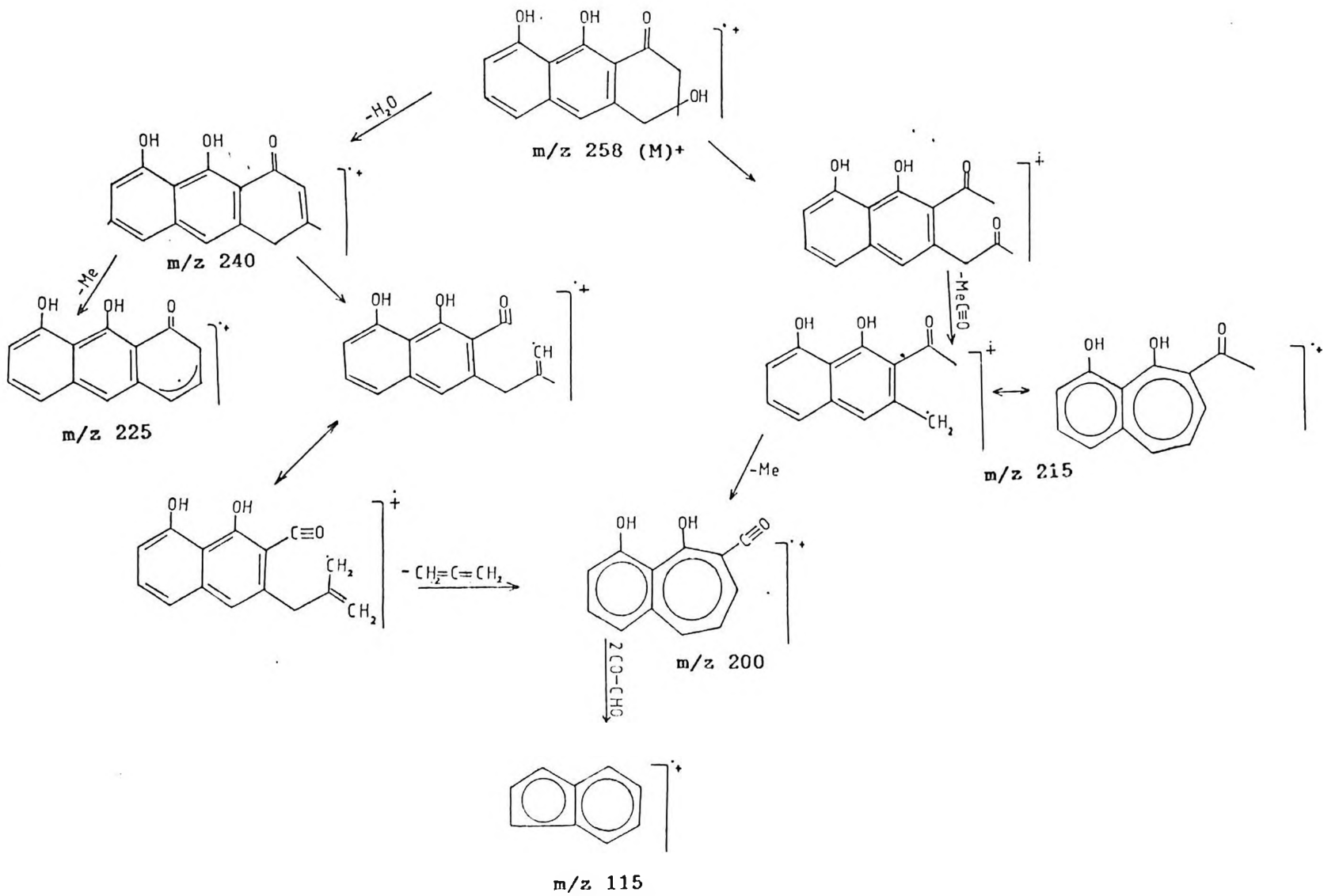


1

The **UV** spectrum in **methanol** showed absorption maxima at 268, 292, 298, 322, and 408 nm, suggesting a pre-anthraquinone skeletal structure (Yagi et al., 1977a). Addition of **AlCl3** resulted in a 38 nm bathochromic shift which was unaffected by addition of **HCl**, indicating the presence of chelation in 1. This was supported by the **IR** spectrum (KBr) which showed hydrogen bonded carbonyl absorption at 1620 cm^{-1}.

HRMS analysis showed a molecular ion peak at m/z 258.0885 corresponding to the molecular formula $C_{15}H_{14}O_4$, which requires 258.0892. EIMS analysis, showed a fragment ion peak at m/z 240 which was due to the loss of water from the molecular ion, and the peak at m/z 225 has resulted by the loss of a methyl group from the fragment ion at m/z 240. The fragment ion at m/z 200 has resulted by the loss of a C_3H_4 ($CH_2=C=CH_2$) group from the fragment ion at m/z 240. Alternatively, the fragment ion at m/z 200 could have resulted through another route which first involved the loss of $MeCO^+$ group (appeared at m/z 43) followed by the loss of a methyl group (Scheme-2).

Scheme-2, EIMS fragmentation of aloegramonol (1)



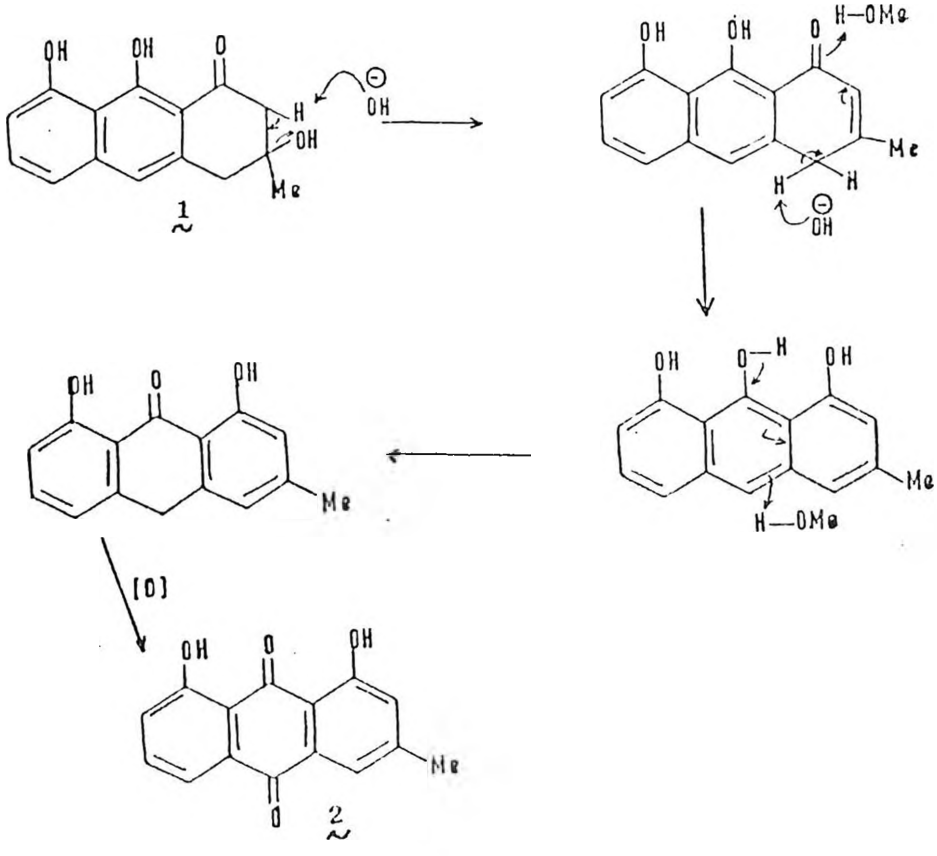
The ^{1}H NMR spectrum of compound 1 showed the presence of three aromatic protons with an ABX pattern at δ 7.39 (t, 1H, J = 8.4 Hz), 7.08 (dd, 1H, J = 8.4, 2.0 Hz), and 6.78 (dd, 1H, J = 8.4, 2.0 Hz). A singlet, at δ 6.95, indicated the presence of an isolated aromatic proton. A singlet, integrating for three protons, at δ 1.33, corresponds to the methyl group attached to an aliphatic system. Two mutually coupled doublets at δ 2.80 and 2.73 (J_{gem} = 17.5 Hz) and another two doublets at δ 3.03 and 2.97 showing similar coupling pattern (J_{gem} = 17.5 Hz) indicated the presence of two isolated methylene groups.

The ^{13}C NMR spectrum showed the presence of 15 carbon atoms which is in agreement with the MS data. A peak at δ 203.65(s) indicated the presence of a carbonyl group while, peaks at δ 157.43(s) and 164.06(s) were due to the two hydroxylated aromatic carbon atoms at C-8 and C-9. Four doublets at δ 110.64, 116.13, 118.49 and 132.39, correspond to the four methine aromatic carbon atoms. Furthermore, four singlets at δ 135.01, 139.29, 112.36 and 109.38 were due to the quaternary aromatic carbon atoms. A singlet, at δ 70.24 was due to the hydroxylated aliphatic quaternary carbon atom at C-3. Two triplets, at δ 42.99 and 51.40 correspond to the two methylene carbon atoms at C-2 and C-4, while the signal at δ 28.41(q) was due to the

aliphatic methyl carbon atom at C-3.

The final proof for this structure was obtained by dehydration and subsequent aerial oxidation of compound 1 in basic methanol, which afforded **chrysophanol** (2) (Scheme-3). This was identified by direct comparison (TLC, UV, IR and MMP) of the product with a sample of **chrysophanol** isolated from the plant.

Scheme-3. Postulated mechanism for conversion of aloegramonol (1) into chrysophanol (2).

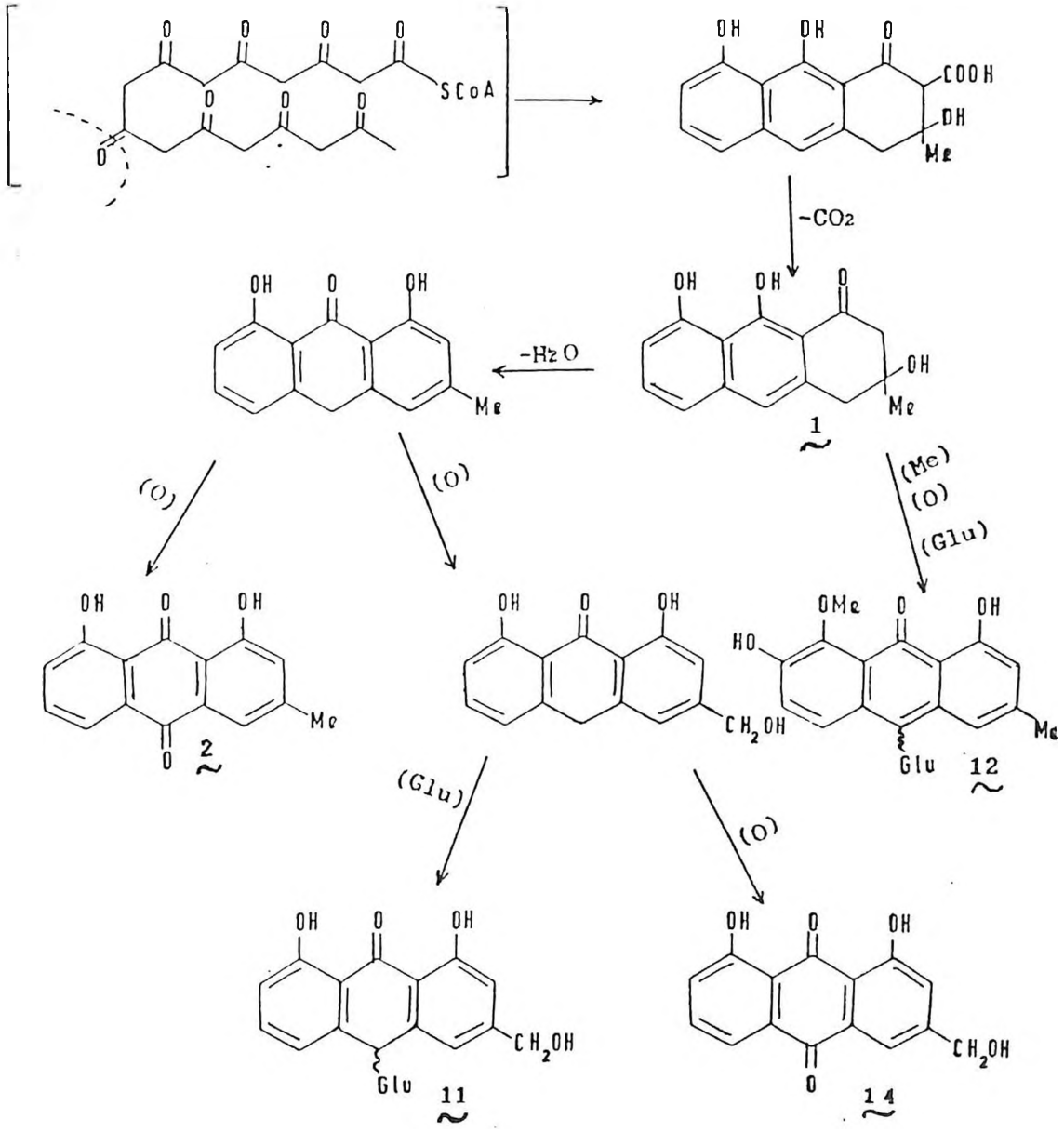


Although compound 1 has been identified as one of the intermediates during biogenetic-like synthesis of **chrysophanol** (2) (Hansma et al., 1976), as to the best of our knowledge, the compound is isolated here for the first time from nature.

The co-occurrence of **aloegramonol** (1) with **chrysophanol** (2) in the plant, might suggest that **aloegramonol** could be a precursor to **chrysophanol**, in the plant.

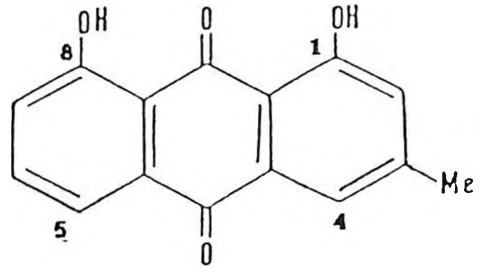
Chrysophanol (2) and related anthraquinone derivatives, such as **barbaloin** (11) and **aloe-emodin** (14), found in Aloe, lack oxygenation at C-6 position, which is predicted by the polyketide biogenetic theory (Hansma et al., 1976). The isolation of 1, which could be a precursor to **chrysophanol** (2) and also to some related anthraquinone derivatives is significant in understanding the biogenesis of such anthraqinone derivatives. Possible biogenetic relationships between **aloegramonol** (1) and some anthraquinone derivatives of Aloe are shown in Scheme-4.

Scheme-4. Postulated biogenetic relationships between aloegramonol (1) and some anthraquinone derivatives of Aloe.



2.2.2. CHRYSOPHANOL (2).

The compound with Rf value of 0.52 (S-1) was isolated as orange crystals, mp. 194-196°C (MeOH). It gave red colour with methanolic KOH spray reagent. This compound was identified as chrysophanol (2) based on spectroscopic data which are in agreement with those reported in literature (Yagi et al., 1977a).



2

The UV spectrum in methanol showed absorption bands, at 254, 276, 286, 396 428, and 444 nm. Addition of KOH shift reagent resulted a 78 nm bathochromic shift, while addition of $AlCl_3$ resulted in 86 nm bathochromic shift indicating 1,8-dihydroxy anthraquinone derivative (Thomson, 1971).

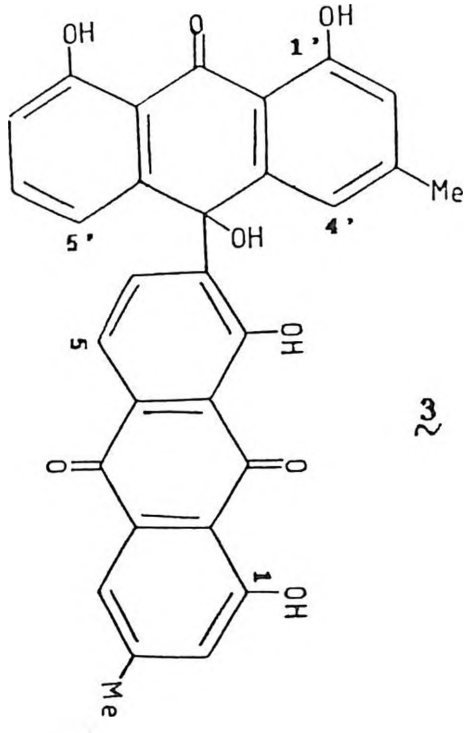
The IR spectrum showed the presence of hydroxyl (3450 cm^{-1}) and chelated carbonly group (1630 cm^{-1}). This is in support of the UV data.

EIMS analysis showed a stable molecular ion peak at m/z 254 (100%), corresponding to the molecular formula $C_{15}H_{10}O_4$. The fragment ion peaks at m/z 226 $(M-CO)^+$ 198 $(M-2CO)^+$, 197 $(M-CO-CHO)^+$ and 169 $(M-2CO-CHO)^+$, are typical of hydroxylated anthraquinones. The weak intensities of these peaks, further suggested the presence of chelation, in this compound (Thomson, 1971).

The ^1H NMR spectrum showed the presence of two chelated hydroxyl groups which showed signals at δ 12.05 and 12.15, three aromatic protons with an ABX patern at δ 7.70 (dd, J = 8.0, 2.0 Hz), 7.59 (t, J = 8.0 Hz) and 7.22 (dd, J = 8.0, 2.0 Hz), which were assigned to the aromatic protons at C-5, C-6 and C-7, respectively. Two <u>meta</u> coupled protons at δ 7.02 (1H, d, J = 2.0 Hz) and 7.56 (1H, d, J = 2.0 Hz) were due to the C-5 and C-7 protons, respectively. A singlet, at δ 2.35, integrating for three protons, corresponds to the methyl protons at C-3.

2.2.3 ASPHODELIN (3).

The compound with Rf value of 0.42 (S-1) was isolated as an orange amorphous solid, mp. 161-163°C. It turned red upon spraying with methanolic KOH spray reagent, indicating the presence of a quinoid moeity. This compound was identified as **asphodelin (3)** based on spectroscopic and chemical data which are in agreement with those reported in literature (Yagi et al., 1978a).



3

The UV spectrum in methanol showed absorption bands at 256, 286, 410, 432 and 458 nm, which is similar to that of chrysophanol (2). Addition of methanolic KOH resulted in 78 nm bathochromic shift. This further suggested the presence of a 1,8-dihydroxy anthraquinone skeletal structure.

The IR (KBr) showed the presence of hydroxyl (3500 cm^{-1}) and chelated carbonyl (1650 cm^{-1}) supporting the UV data.

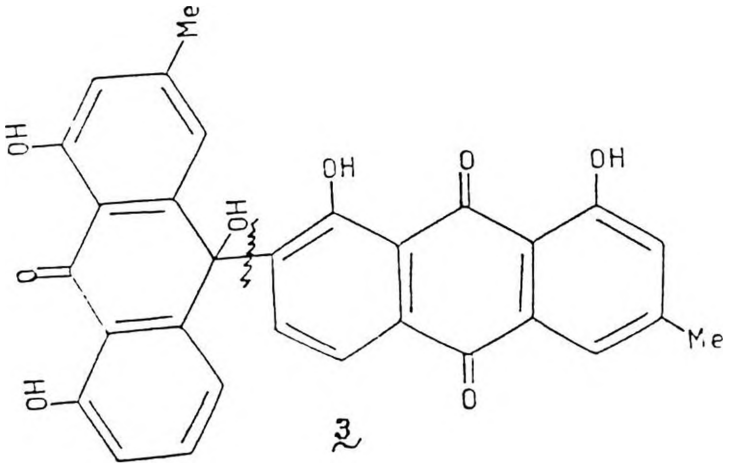
The EIMS of asphodelin (3) showed a molecular ion peak at m/z 508 corresponding to the molecular formula $C_{30}H_{20}O_8$. Compound 3 has easily lost a hydroxyl group giving rise to a stable fragment ion, at m/z 491. A fragment ion at m/z 254 corresponds to a chrysophanol molecule. This has been observed in some bi-chrysophanol derivatives (Dagne et al., 1987). This suggested, compound 3 to be a bi-chrysophanol derivative.

The ^1H NMR spectrum showed the presence of four hydrogen bonded hydroxyl groups which showed signals at δ 12.02, 12.04, 12.40 and 12.59. This further supported a bi-anthracene skeletal structure, in which each half contained a 1,8-dihydroxy substitution. Two

singlets, each integrating for three protons, at δ 2.15 and 2.50 were attributed to the two methyl groups, each belonging to one half of the molecule, i.e. at C-3' and C-3, respectively. The signal for the 3'-methyl group (i.e. δ 2.15) is upfield by .35 ppm compared with that of the other methyl group. This upfield shift appears to have been caused by the shielding effect of the **chrysophanol** half of the molecule. Two ortho coupled protons at δ 7.95 (J = 7.7 Hz) and 7.35 (J = 7.7 Hz) which were due to C-5 and C-6 protons and two meta coupled protons at δ 7.13 (J = 1.5 Hz) and 7.59 (J = 1.5 Hz) which were due to C-2 and C-4 aromatic protons, respectively, correspond to the **chrysophanol** half of the molecule. This will leave the C-7 position of the **chrysophanol** unit to be attached with the other half of the molecule. Comparison of this with the spectrum of **chrysophanol** (2) showed similar pattern except for the absence of peaks due to the C-7 protons in compound 3. The C-2' and C-4' protons of the **chrysophanol anthranol** unit appeared at δ 7.31 and 7.68, as doublets (J = 1.5 Hz), respectively. The C-5', C-6' and C-7' protons of the **chrysophanol anthranol** unit appeared, at δ 7.27 (dd, J = 7.7, 1.5 Hz), 7.59 (t, J = 7.7 Hz) and 7.25 (dd, J = 7.7, 1.5 Hz) respectively, with an **ABX** pattern. The chemical shift values for C-2' and C-4' aromatic protons were shifted down field compared to

those of C-5', C-6' and C-7' aromatic protons. This further showed the proximity of the chrysophanol half of the molecule to the right side of the chrysophanol anthranol unit.

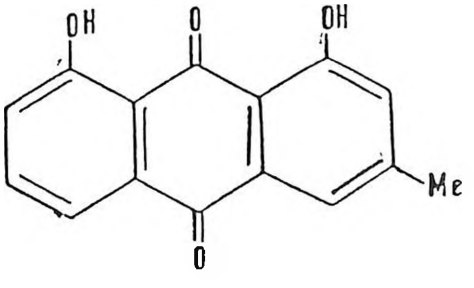
Finally, reductive cleavage of compound 3 with $Na_2S_2O_4$ gave chrysophanol (2) which was identified by comparison with authentic sample (UV, co-TLC, mmp).



3

KOH

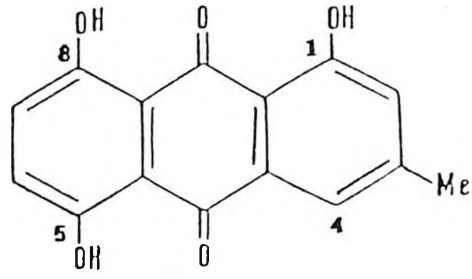
$Na_2S_2O_4$



2

2.2.4. HELMINTHOSPORIN (4).

The compound with Rf value of 0.89 (S-1) was isolated as red needles ($CHCl_3$), mp. 215-217°C. It turned purple with methanolic KOH spray reagent on TLC plate. It was identified as **helminthosporin** (4) based on spectroscopic data which are in agreement with those reported in literature (Yagi et al., 1977a).



4

The UV spectrum showed absorption bands at 252, 282, 290, 460, 474, 484, 504 and 518 nm. Addition of KOH showed a bathochromic shift of ca. 80 nm. This indicated compound 4 to be either, 1,4,8-trihydroxy or 1,5,8-trihydroxy anthraquinone derivative (Thomson, 1971).

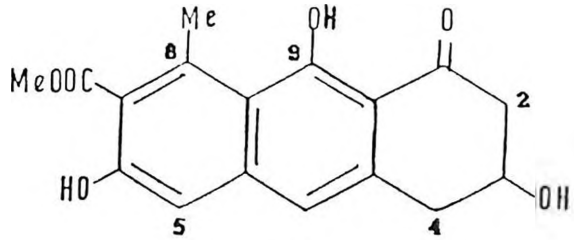
EIMS analysis of 4, showed a stable molecular ion peak at m/z 270, corresponding to the melecular formula

$C_{15}H_{10}O_5$. Weak fragment ion peaks, at m/z 253, 242, and 213 corresponed to the lose of OH, CO and CO+CHO groups from the molecular ion, respectively. These are characteristic of hydroxylated anthraquinones. The weak intensities of these fragment ions, further suggested the presence of chelation (Thomson, 1971).

The ^1H NMR spectrum showed the presence of three hydrogen bonded hydroxyl groups which appeared, at δ 12.15, 12.32 and 13.02. This is in agreement with the UV data which indicated either 1,4,8-trihydroxy or 1,5,8-trihydroxy anthraquinone derivative. The ^1H NMR spectrum also showed the presence of two ortho coupled aromatic protons, appearing at δ 7.25 (J = 8.0 Hz) and 7.28 (J = 8.0 Hz) which were due to C-6 and C-7 protons and two meta coupled aromatic protons, appearing at δ 7.11 and 7.69 which were assigned to the C-2 and C-4 protons, respectively. The methyl protons at C-3, appeared, at δ 2.45. This data corresponds to 1,5,8-trihydroxy-3-methylanthraquinone (**helmintosporin** (4)), ruling out the alternative structure which places the hydroxyl group at C-4 instead of C-5.

2.2.5. ALOESAPONOL-I (5).

The compound with Rf value of 0.31 (S-4) was isolated as light yellow needles (EtOAc), mp. > 240°C (dec.). It showed strong fluorescence under UV light at 366 nm. The fluorescence intensified when sprayed with methanolic KOH on a silica gel TLC plate. It gave brown colouration with fast blue salt B spray reagent, indicating compound 5 to be a phenolic derivative. This was identified as aloesaponol-I (5) based on spectroscopic and chemical data which are in agreement with literature data (Yagi et al., 1974).



5

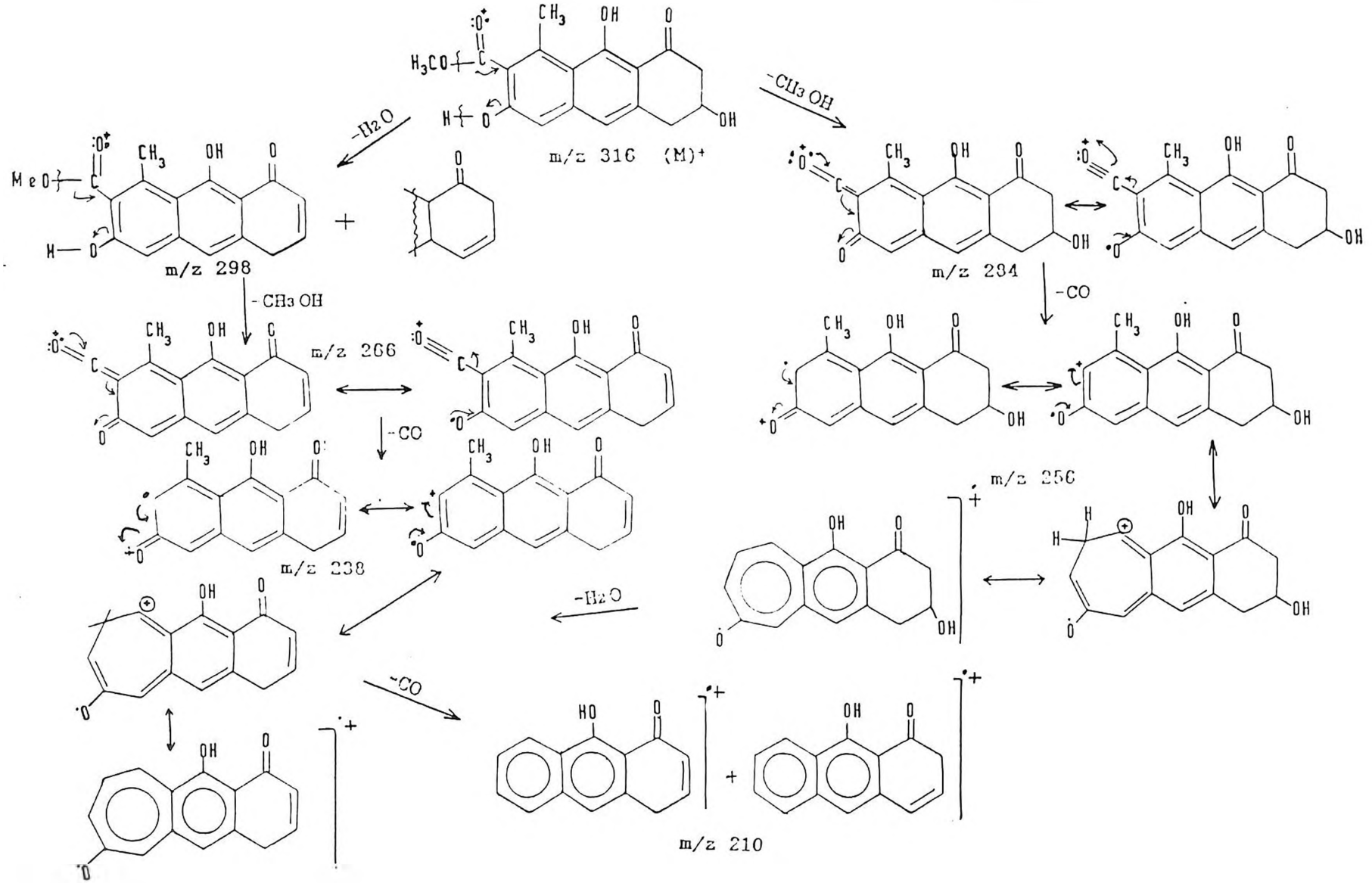
The UV spectrum in MeOH showed absorption bands at 274, 298, 304 and 390 nm. This indicated a pre-anthraquinone skeletal structure (Yagi et al., 1974). Addition of KOH resulted a 10 nm bathochromic shift. Addition of methanolic $AlCl_3$, did not show any

change when the spectrum was recorded immediately, but showed a 35 nm bathochromic shift when recorded after two days. This indicated the presence of a chelated hydroxyl group.

The IR spectrum (KBr) showed the presence of hydroxyl groups (3375 cm^{-1}), ester carbonyl (1710 cm^{-1}) and chelated carbonyl (1630 cm^{-1}).

EIMS analysis showed a stable molecular ion peak at m/z 316 corresponding to the molecular formula $C_{17}H_{16}O_6$. The fragmentation appeared to have followed two parallel routes (Scheme-5). The major fragmentation involves the loss of MeOH giving rise to the base peak at m/z 284, which is typical of ortho-hydroxy methyl benzoates (Silverstein et al., 1963). The remaining fragments on this route have resulted by repeated losses of CO from the base peak. The other fragmentation route, first involves the loss of a water molecule followed by the loss of MeOH. The remaining fragmentation in this route also involves repeated losses of CO molecule from the fragment ion at m/z 266 (Scheme-5).

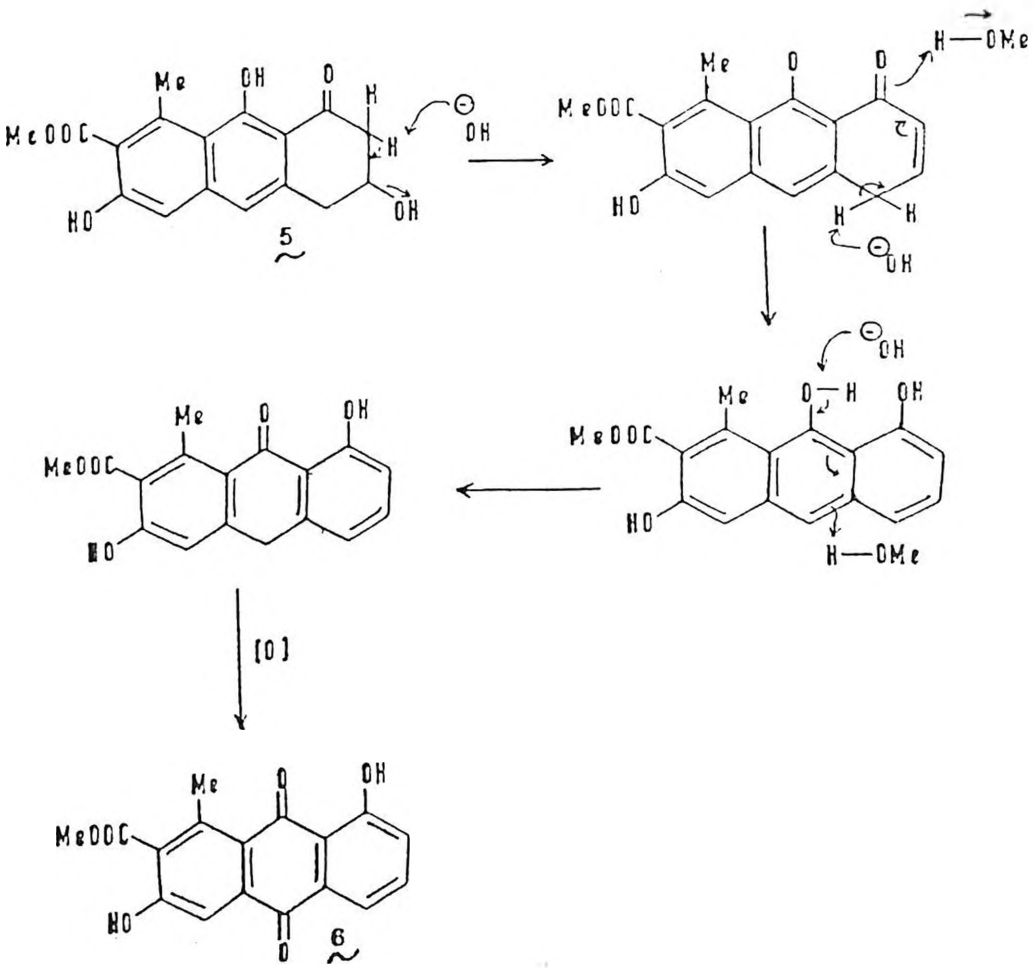
Scheme-5. EIMS fragmentation of aloesaponol-I (5)



The ^1H NMR spectrum showed two singlets each integrating for one proton, at δ 6.85, and 6.83 corresponding to C-5 and C-10 protons. The peak at δ 6.83 was broad, probably caused by a long range coupling and was assigned to the proton at C-10 which can interact through space with the C-4 methylene protons. The two methylene protons at C-4 appeared as two sets of double doublets, at δ 3.20 (dd, J=17.3, 3.7 Hz) and 3.00 (15.6, 17.3 Hz). The other two methylene protons at C-2, resonate again as two sets of double doublets at δ 3.00 (J = 3.7, 17.3 Hz) and 2.75 (J = 15.6, 17.3 Hz). The hydroxymethine proton at C-3, appeared at δ 4.35 as a multiplet. A three proton singlet, at δ 2.87 was due to the methyl protons attached to the C-8 aromatic carbon atom. The chemical shift value for the methyl group is de-shielded by about 0.3 ppm, which allows for its placement at C-8 and eliminates the alternative structure which would place the methyl group at C-6 and the hydroxyl group at C-8. The chelated phenolic proton at C-9, appeared at δ 15.20 which is characteristic for pre-anthraquinones (Takido et al., 1982). A three proton singlet at δ 3.98 was assigned to the ester methyl protons.

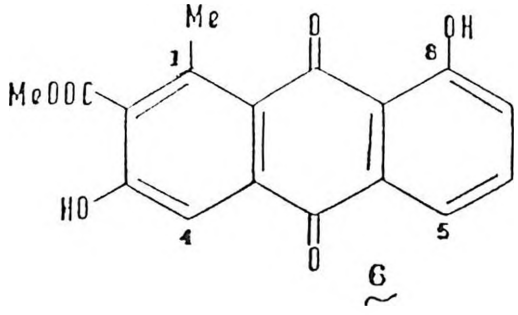
Finally, dehydration and subsequent aerial oxidation (Scheme-6) of compound 5 in basic methanol medium gave the anthraquinone, **Aloesaponarin-I** (6) which was identified by comparison (UV, co-TLC) with a sample isolated from the plant.

Scheme-6. Postulated **mechanism** for conversion of **aloesaponol-I** (5) into **aloesaponarin-I** (6).



2.2.6. ALOESAPONARIN-I (6).

The compound with Rf value of 0.82 (S-4) was isolated as orange crystals (EtOAc), mp. >197°C (dec.). It gave red colour on spraying with KOH and brown with fast blue salt B spray reagents, indicating this compound to be a hydroxylated anthraquinone derivative. This was identified as aloesaponarin-I (6) based on spectroscopic data and chemical derivatization which are consistent with those reported in literature (Yagi et al., 1974).

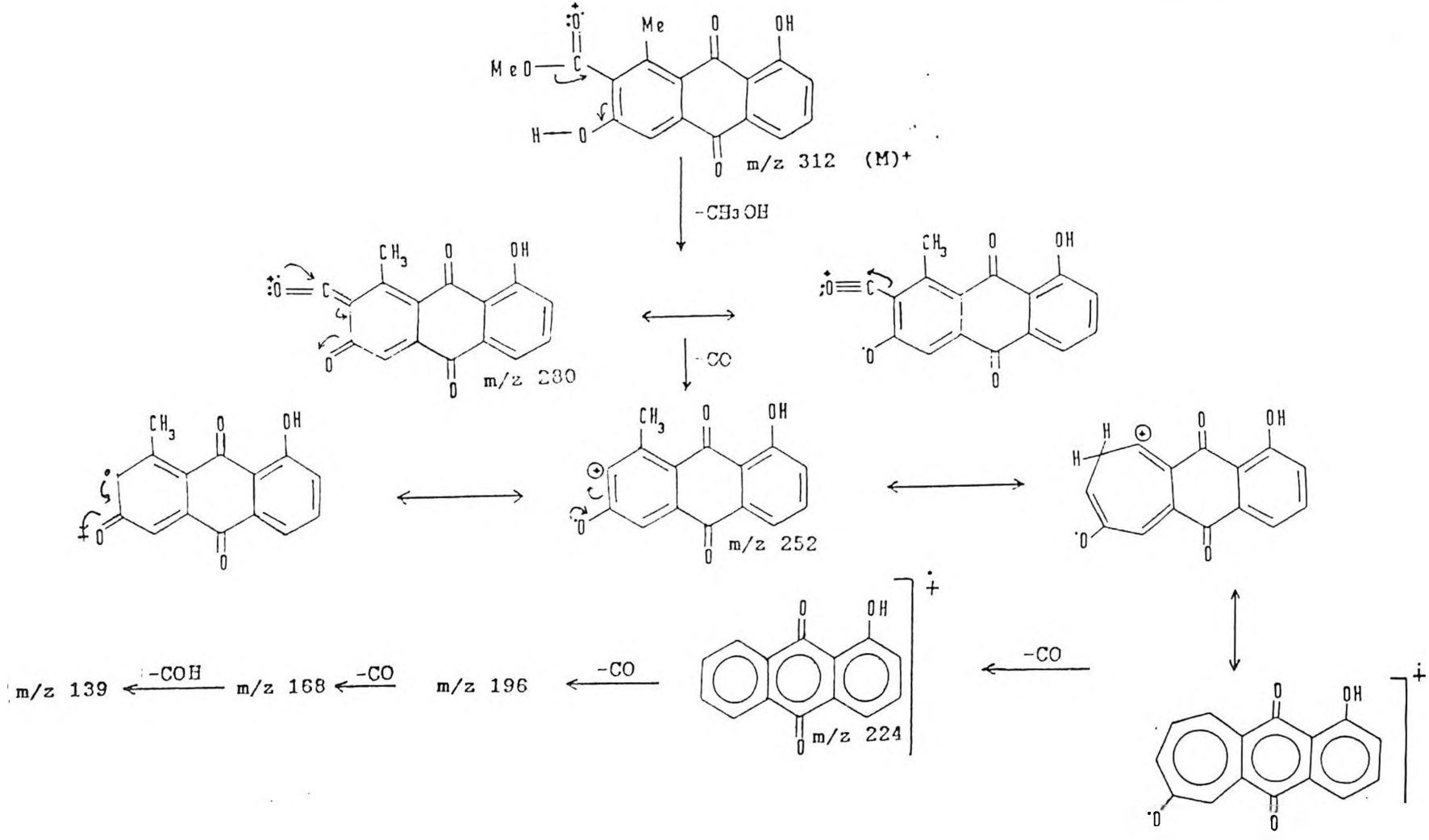


6

The UV spectrum in MeOH showed absorption maxima at 268, 274, 390, 406 and 428 nm. Addition of methanolic KOH resulted in a 74 nm bathochromic shift. This is typical of 1-hydroxy anthraquinones (Yagi et al., 1974). This was supported by the IR spectrum which showed the presence of hydroxyl (3450 cm^{-1}), free (1720 cm^{-1}) and hydrogen bonded carbonyl (1635 cm^{-1}) groups.

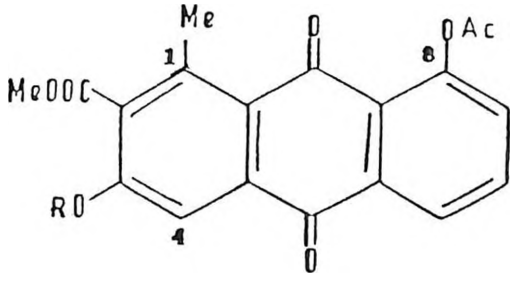
EIMS analysis showed a molecular ion peak at m/z 312, corresponding to the molecular formula $C_{17}H_{12}O_6$. In addition, the fragment ion peak, at m/z 297, was due to the loss of a CH_3 group from the molecular ion. The base peak at m/z 280 has resulted by the loss of MeOH from the molecular ion. This is characteristic of o-hydroxy methyl benzoates and is known as "ortho effect" (Silverstein, et. al., 1963). The remaining major fragment ions have resulted by a series of losses of CO molecules from the base peak. The fragmentation pattern for compound 6 is given in Scheme-7.

Scheme-7. EIMS fragmentation of alocsaponarin-I (6)



The ^1H NMR spectrum showed two singlets, each integrating for three protons, at δ 2.81 and 4.02 corresponding to the aromatic methyl protons at C-1 and the methyl ester protons, respectively. The chemical shift of the methyl protons appeared down field shifted by about 0.3 ppm. This is caused by the peri effect of the C-9 CO group. This eliminates the alternative structure that places the methyl group at C-3 and the hydroxyl group at C-1. Three sets of Peaks, with an ABX pattern, at δ 7.76 (dd, J = 7.5, 1.3 Hz), 7.62 (t, J = 7.5 Hz) and 7.25 (dd, J = 7.5,1.3 Hz) were assigned to the aromatic protons at C-5, C-6 and C-7, respectively. A singlet, at δ 7.64 corresponds to the isolated aromatic proton at C-4.

Acetylation of compound 6 with acetic anhydride and pyridine afforded two derivatives being a mono- (6a) and a di-acetate (6b) which further gave evidence for the structure.



6a R = H

6b R = Ac

2.2.6.1. 8-ACETOXYALOESAPONARIN-I (6a).

One of the derivatives (6a) was isolated as a light yellow needles (acetone) mp. 190-193°C. Spraying with KOH did not cause any colour change, while, fast blue salt B changed the yellow colour to brown. This was identified as 8-acetoxyaloesaponarin-I (6a) based on spectroscopic data.

The UV spectrum of 6a showed absorption bands at 238, 270, 322 and 364 nm. The bands due to the C-8 hydroxyl group of aloesaponarin-I (6) were absent in the acetate. Hence, addition of base did not cause any shift in the absorption bands. This indicated the absence of chelation, thus suggested, the 8-hdroxyl group in aloesaponarin-I (6) to have been acetylated.

The EIMS showed a molecular ion peak at m/z 354 which indicated a mono-acetate. Furthermore, a fragment ion at m/z 312 was due to the loss of the acetate group. The remaining fragmentation occurred from this ion and was identical to the pattern observed in aloesaponarin-I (6).

The ^{1}H NMR spectrum showed the methyl protons of the acetate group at δ 2.47. Comparison of the chemical shift values of the aromatic protons of

8-acetoxyaloesaponarin-I (6a) with those of the parent molecule, aloesaponarin-I (6) showed acetylation shifts of 0.39, 0.10 and 0.16 for the C-5, C-6 and C-7 protons, respectively (Table-7). This made it possible to place the acetate group at C-8. This derivative has not been prepared before.

Table-7. ^{1}H NMR data for compounds 6, 6a and 6b.

H	6	6a	6b
4	7.64(s)	7.70(s)	7.71(s)
5	7.76(dd)	8.15(dd)	8.15(dd)
6	7.62(t)	7.72(t)	7.73(t)
7	7.25(dd)	7.41(dd)	7.41(dd)
1-Me	2.81(s)	2.82(s)	2.82(s)
2-COOMe	4.02	4.04	4.04
8-OAc	-	2.47	2.47
3-OAc	-	-	2.18

J_{ortho} = 7.5 Hz

J_{meta} = 1.3 Hz

2.2.6.2. 3,8-DIACETOXYALOESAPONARIN-I (6b).

The other derivative (6b) was crystallized as a light yellow needles (acetone), mp 195-197°C. Spraying with KOH or fast blue salt B, did not produce colour changes. This compound was identified as 3,8-diacetoxyaloesaponarin-I (6b) based on spectroscopic data which were found to be identical with those reported by Yagi et al. (1974).

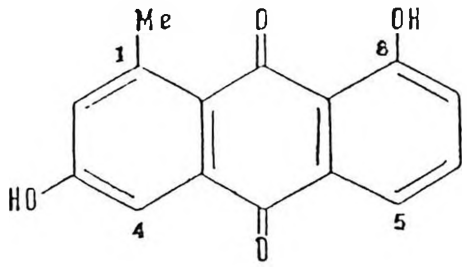
The UV spectrum showed absorption bands at 244, 274 and 337 nm. As in 6a, addition of base did not cause any shift in the absorption bands.

The EIMS analysis showed a molecular ion peak at m/z 396 indicating a di-acetate. Fragment ion peaks at m/z 354 and 312 corresponds to the loss of one and two acetate groups from the molecular ion, respectively. The remaining fragmentations were identical to those observed in aloesaponarin-I (6).

THE ^{1}H NMR spectrum, differs from 6a by the presence of additional singlet, integrating for three protons at δ 2.18 which corresponds to the methyl protons of the second acetate group at C-3 (Table-7).

2.2.7. ALOESAPONARIN-II (7).

The compound with Rf value of 0.80 (S-3) was isolated as yellow crystals (EtOAc), mp. 264-265°C. It turned red with KOH and brown with fast blue salt B spray reagents on silica gel TLC plate. This compound was identified as aloesaponarin-II (7) based on spectroscopic data which were found to be in agreement with those reported in literature (Yagi et al., 1974).



7

The UV spectra of compound 7 in MeOH and MeOH/KOH were similar to those of aloesaponarin-I (6) indicating similar skeletal structure.

EIMS analysis showed a stable molecular ion peak at m/z 254 corresponding to the molecular formula, $C_{15}H_{10}O_4$, which was also found for chrysophanol (2). The fragment ion peaks at m/z 198 $(M-2CO)^+$, 197 $(M-CO-CHO)^+$ and 169 $(M-2CO-CHO)^+$ observed in

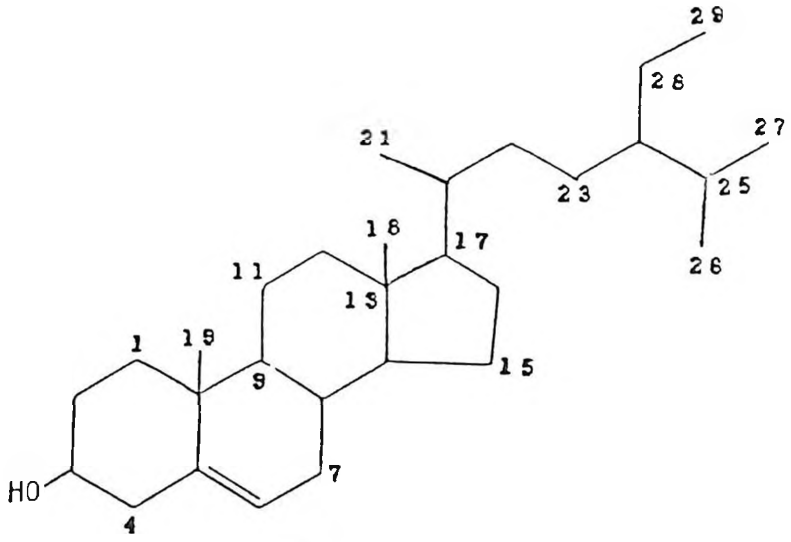
aloesaponarin-II (7) were also found in chrysophanol (2). These data suggested compound 7 could be an isomer of compound 2.

The ^1H NMR spectrum showed the presence of a deshielded methyl protons attached to an aromatic system, at δ 2.80. Three aromatic protons with an ABX pattern appeared, at δ 7. 74 (dd, J = 8.0, 2.0 Hz), 7.60 (t, J = 8.0 Hz.) and 7.28 (dd, J = 8.0, 2.0 Hz), were assigned to the C-5, C-6 and C-7 aromatic protons, respectively. Two meta coupled protons, at δ 7.00 (J = 2 Hz) and 7.56 (J = 2.0 Hz) were due to C-2 and C-4 protons, respectively. Except for the chemical shift value for the methyl protons, the ^1H NMR data of this compound were similar to those of compound 2, further indicating the two compounds to be isomers.

As in compounds 5 and 6, the position of the methyl group was fixed at C-8 based on its chemical shift, which appeared ca. 0.3 ppm deshilded as compared with the chemical shift of the methyl protons, in the isomeric structure, chrysophanol (2)). Furthermore, aloesaponarin-II (7)) was more polar than chrysophanol (2) (solubility and Rf value) suggesting one of the hydroxyl groups in aloesaponarin-II (7) to be non-chelated.

2.2.8. β-SITOSTEROL (8).

The compound with Rf value of 0.40 (S-1) was isolated as colourless needles (acetone), mp 134-136°C. This UV insensitive compound gave orange colour with conc. sulphuric acid on silica gel TLC plate, after heating at 110°C. It was identified as β-sitosterol (8) based on spectroscopic data which were in agreement with those reported in literature (Waller, 1972, Wulfaoh et al., 1964).



8

The UV spectrum did not show the presence of any conjugation, while, the IR spectrum showed the presence of hydroxyl group (3450 cm^{-1}) and an aliphatic system (2950 cm^{-1} (C-H st.), 1450 cm^{-1} and 1375 cm^{-1} (C-H bend.)).

The EIMS showed a molecular ion peak at m/z 414, corresponding to the molecular formula $C_{29}H_{50}O$. The fragment ion at m/z 396 corresponds to the loss of water from the molecular ion. Furthermore, the fragment ion at m/z 329 which could probably be due to a fragmentation between C-23 and C-24, is characteristic of phytosterols .

The ^1H NMR spectrum showed a multiplet, centered at δ 5.32 which is attributed to the vinylic proton, at C-6. Another multiplet, at δ 3.50 was assigned to the C-3 hydroxymethine proton. Two singlets, each integrating for three protons, at δ 0.68 and 0.98 were due to the methyl protons, at C-18 and C-19, respectively. A doublet, at δ 0.90 was assigned to the C-21 secondary methyl protons. Another doublet, integrating for six protons, at δ 0.82, corresponds to the two equivalent methyl protons, at C-26 and C-27. The C-29 methyl protons appeared, at δ 0.80.

2.2.9. SOLVENT EFFECTS ON 3,8-DIHYDROXY-1-METHYL ANTHRAQUINONE DERIVATIVES.

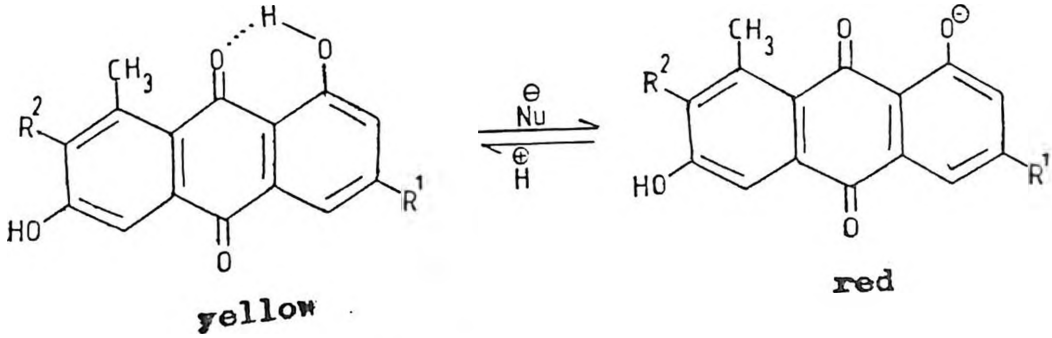
The methanolic solution of aloesaponarin-I (6) gradually changed its colour from yellow to red. The UV spectrum of the red solution (in MeOH), showed absorbtion maxima at 244, 304 and 480 nm. Addition of methanolic KOH did not show any shift in the UV spectrum of the red solution, but addition of methanolic HCl resulted in a 74 nm hypsochromic shift. The bands in MeOH/HCl were at 268, 274, 390, 406 and 428 nm, which are identical to the spectrum of 6 in MeOH, when run immediately. The 8-acetoxy derivative 6a was not affected by MeOH.

Similar effects were observed for the methanolic solution of laccaic acid-D methyl ester (10), while the yellow methanolic solution of aloesaponarin-II (7) turned orange. The orange colour remained unchanged for several days. The UV spectrum of the orange solution of aloesaponarin-II (7) showed a complex set of bands. Addition of methanolic HCl resulted in a simple spectrum which was identical with the spectrum of fresh solution of aloesaponarin-II (7) in MeOH. Addition of methanolic KOH gave a simple spectrum which was identical with MeOH/KOH spectrum of the fresh solution of aloesaponarin-I (7). This indicated

the orange solution of aloesaponarin-II (7) to be a mixture of yellow and red colours and explains the complex UV spectrum recorded for the orange solution.

Similar solvent effects were observed for these compounds when kept in MeCN, while chloroform did not show any effect.

These observations indicated that aloesaponarin-I (6), aloesaponarin-II (7) and laccaic acid-D methyl ester (10) were solvated in MeOH or MeCN. The chelated proton at C-8 position appears to have been solvated, leaving a red anionic species. The fact that 8-acetoxy-aloesaponarin-I (6a) was not solvated, indicated the involvement of the C-1 phenolic group in solvation.



yellow red

6 R^1 = H, R^2 = COOMe

7 R^1 = R^2 = H

10 R^1 = OH, R^2 = COOMe

These compounds are 3,8-dihydroxy-1-methyl anthraquinone derivatives. A parallel study on a possible solvent effect on **chrysophanol (2)** which is 1,8-dihydroxy-3-methyl anthraquinone did not show any change on the UV spectrum of this compound. This suggested the observed solvent effects on **aloesaponarin-I (6), aloesaponarin-II (7)** and **laccaic acid-D methyl ester (10)** could be related with the structures of these compounds. It appears that, through space the **methyl** electrons at C-1 push the lone pair of electrons of the C=O group at C-9 position so that they chelate more strongly with the C-8 phenolic group. This will enhance the degree of polarization of the O-H bond, hence, making the phenolic proton to be more liable to nucleophilic attack. The **methyl ester** group in **aloesaponarin-I (6)** and **laccaic acid-D methyl ester (10)** contributed to the solvation process, by stabilizing the resulting anion. This is based on the observed difference on the degree of solvation in **aloesaponarin-I (6)** and **laccaic acid-D methyl ester (10)** as compared with that of **aloesaponarin-II (7)**.

2.3. COMPARATIVE STUDY ON SOME ALOE SPECIES.

TLC comparisons of the acetone extracts of the roots of four Aloe species belonging to the Saponariae group and two more species, from other groups, namely; A. secundiflora and A. nyerensis (var. kedongensis) are shown in fig.-1a to 1d. This study also included the flowers of A. graminicola and A. nyerensis (var. kedongensis) and the subterranean stems of A. graminicola and A. secundiflora.

About 20 compounds were detected in the roots, subterranean stems and flowers of the Aloe species analysed in the present study. These compounds were labelled as Art-1 (Aloe root-1) to Art-20. Rf values in different solvent systems and colours under VIS and UV lights were used as major guidelines for identification and classification under these preliminary studies. The TLC properties of the chromatographic zones are summarized in Table-8 and the distribution of these compounds in the studied Aloe species in Table-5. Eight of these were further identified by direct comparison (co-TLC) with standard samples.

Fig-1 TLC CHROMATOGRAMS OF THE ACETONE EXTRACTS OF SOME ALOE SPECIES.*

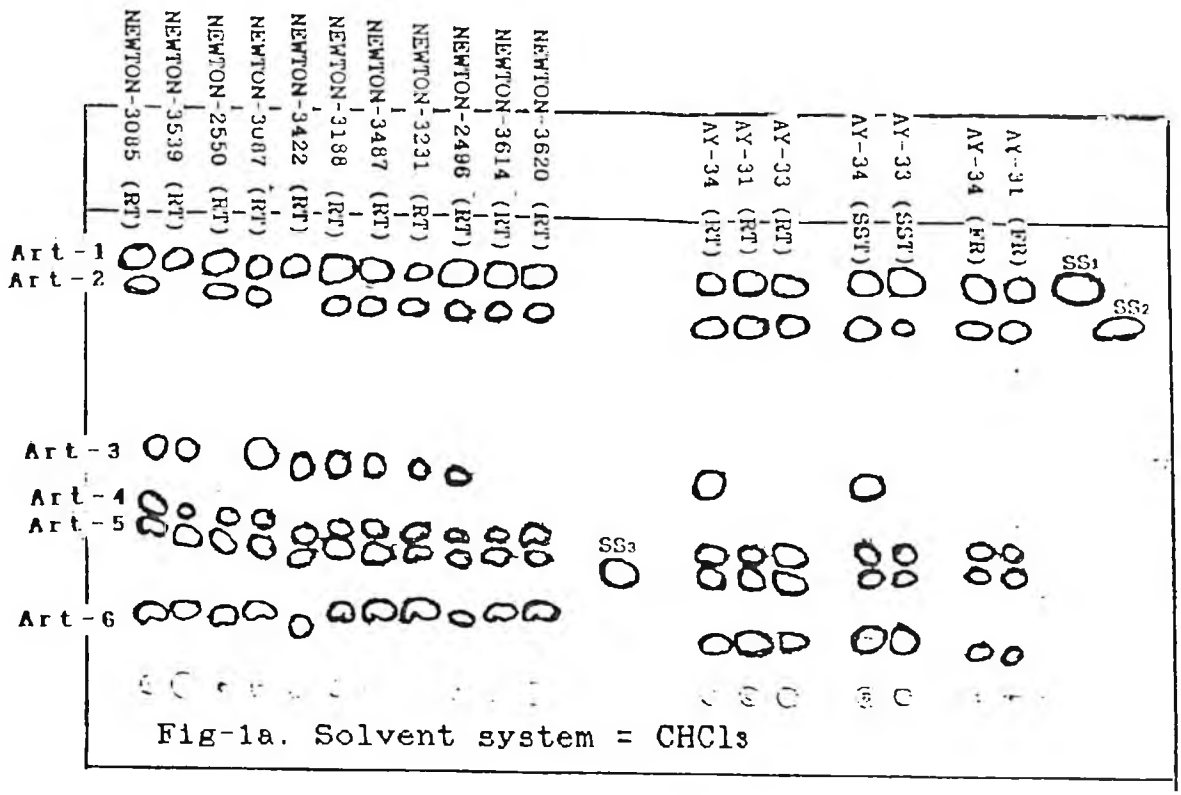


Fig-1a. Solvent system = $CHCl_3$

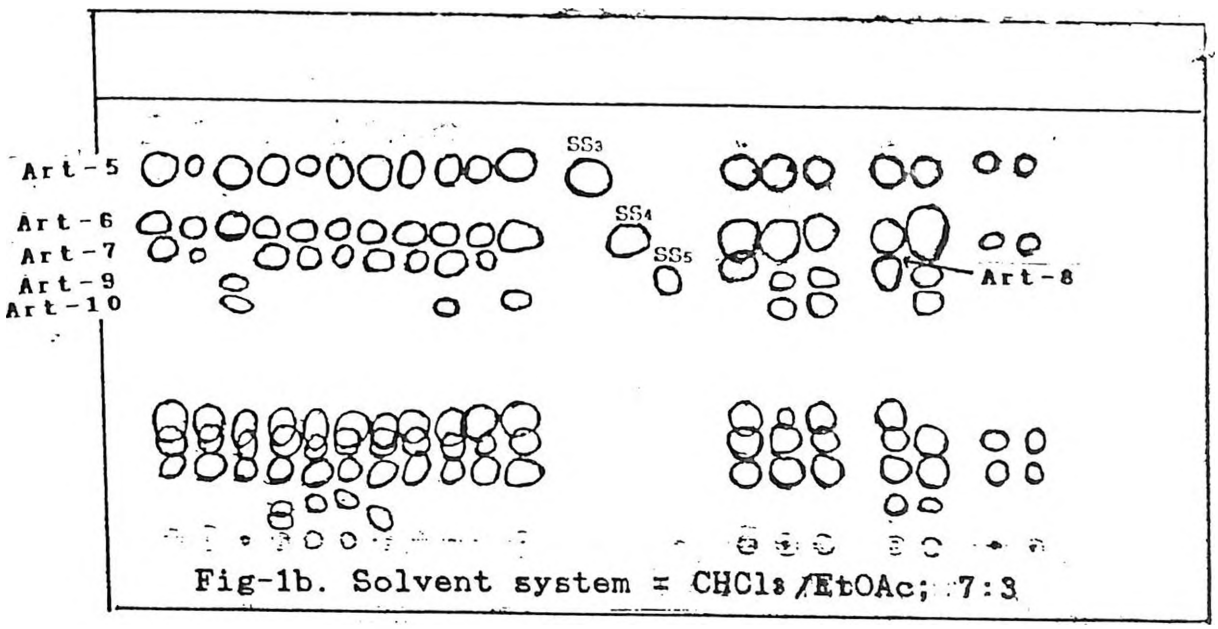


Fig-1b. Solvent system = $CHCl_3$/EtOAc; 7:3

SS_1 = Art-1 = chrysophanol (2)

SS_2 = Art-2 = asphodelin (3)

SS_3 = Art-5 = aloesaponrin-II (7)

SS_4 = Art-6 = aloesaponarin-I (6)

SS_5 = Art-8 = aloegramonol (1)

* See section 4.2. for identifications of plants.

Fig-1

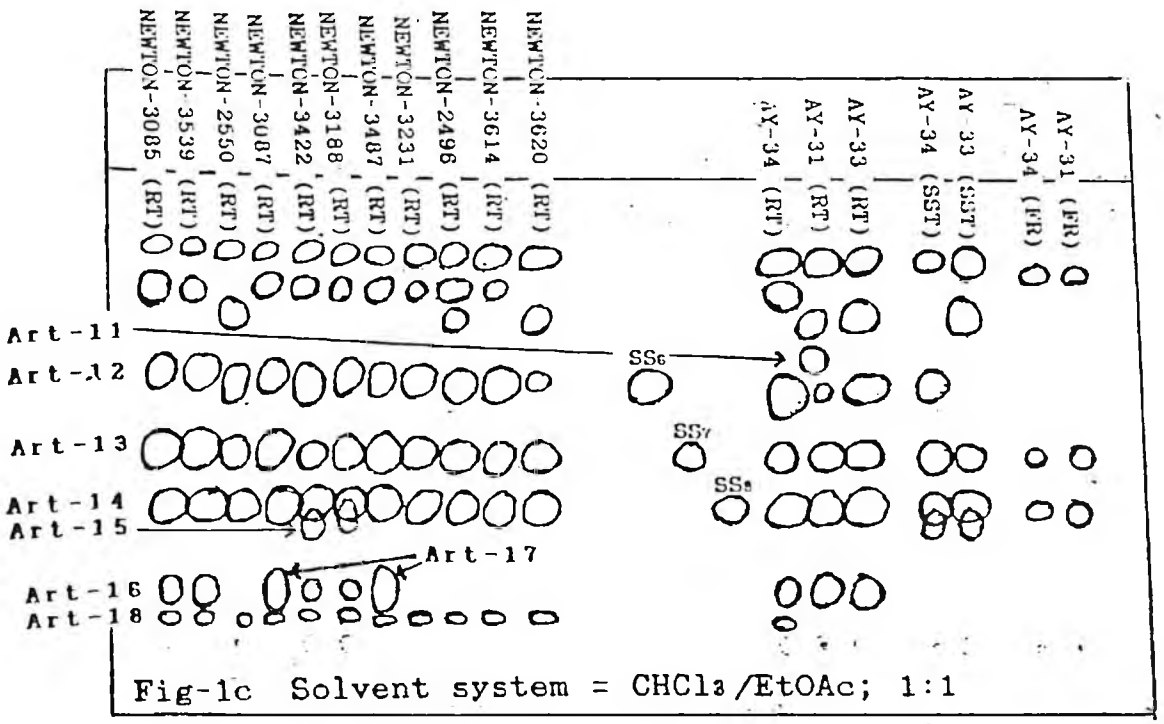


Fig-1c Solvent system = $CHCl_3$/EtOAc; 1:1

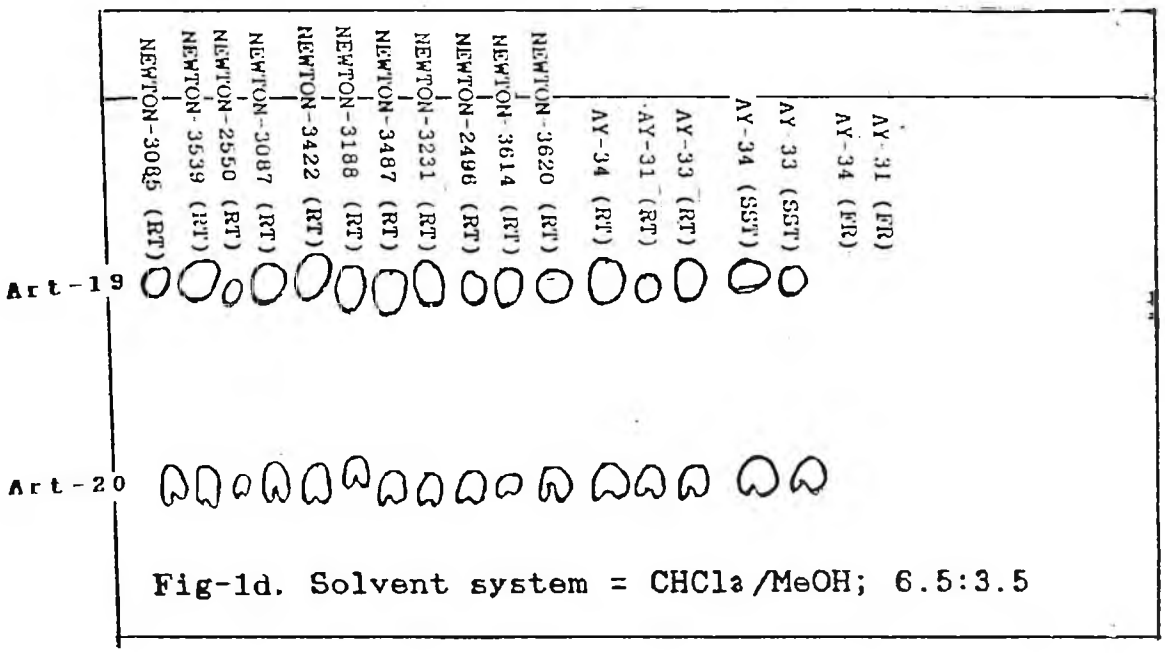


Fig-1d. Solvent system = $CHCl_3$/MeOH; 6.5:3.5

SS_6 = Art-12 = laccaic acid-D methyl ester (10)

SS_7 = Art-13 = aloesaponol-II (9)

SS_8 = Art-14 = aloesaponol-I (5)

Table- 8 TLC properties of chromatographic zones.

**	Rf value under S-2*	S-3	S-4	S-5	Colour under UV (366)	UV (254)	VIS.	Cpd
Art-1	0.85	-	-	-	yellow	brown	yellow	2
Art-2	0.20	0.79	-	-	orange	brown	orange	3
Art-3	0.42	0.88	-	-	blue	blue	-	unknown
Art-4	0.30	0.80	-	-	green	-	-	unknown
Art-5	0.25	0.80	-	-	orange	brown	yellow	7
Art-6	0.17	0.65	0.82	-	orange	brown	orange	6
Art-7	-	0.59	0.75	-	purple	-	-	unknown
Art-8	-	0.57	0.73	-	yellow	brown	yellow	1
Art-9	-	0.57	0.73	-	green	-	-	unknown
Art-10	-	0.49	0.73	-	green	-	-	unknown
Art-11	-	0.62	-	-	blue	-	-	unknown
Art-12	-	0.29	0.55	-	orange	brown	orange	10
Art-13	-	0.25	0.42	-	blue	blue	-	5
Art-14	-	0.18	0.31	-	blue	blue	-	9
Art-15	-	0.10	0.26	-	yellow	brown	yellow	unknown
Art-16	-	-	0.19	-	-	brown	-	unknown
Art-17	-	-	0.19	-	brown	brown	yellow	unknown
Art-18	-	-	0.10	-	-	brown	-	unknown
Art-19	-	-	-	0.62	blue	blue	-	unknown
Art-20	-	-	-	0.20	blue	blue	-	unknown

* Solvent system; see section 4.1.4.

** chromatographic zone

2.3.1. COMPARISON OF DIFFERENT PARTS.

2.3.1.1. TLC COMPARISON

The acetone extracts of the flowers and the subterranean stems of A. graminicola, the flowers of A. nyeriensis (var. kedongensis) and the subterranean stems of A. secundiflora were compared with the roots of these plants.

The constituents of subterranean stems of A. graminicola and A. secundiflora were similar with the roots of these plants. The subterranean stems of A. graminicola differ from the roots by the presence of **Art-8** and **Art-15** which were not detected in the roots, while **Art-7**, **Art-16** and **Art-18** found in the roots were not detected in the subterranean stems. The subterranean stems of A. secundiflora differ from the roots by the absence of **Art-12 (laccaic acid-D methyl ester (10))**, **Art-16** and **Art-18** in the former, while **Art-15** present in the subterranean stems was not detected in the roots.

The constituents of the flowers of A. graminicola and A. nyeriensis (var. kedongensis) showed some similarities with the roots and subterranean stems. Most compounds which were detected in the flowers were

found to be of low concentrations. **Chrysophanol (2)** and **asphodeline (3)** are the major compounds in the flowers of the two species analysed. The pre-anthraquinones derivatives; **Aloesaponol-I (5)** and **aloesaponol-II (9)** were detected in the flowers of A. graminicola and A. nyeriensis (var. kedongensis). These compounds were reported to be confined to the subterranean stems of A. saponaria (Yagi et al., 1983). Thus, the co-occurrence of these two pre-anthraquinones with the corresponding anthraquinones, namely; **aloesaponarin-I (6) aloesaponarin-II (7)** in the roots and subterrenean stems, and also in the flowers is biogenetically significant. To the best of our knowledge this is the first report on the chemistry of the flowers of Aloe.

2.3.1.2. HPLC COMPARISON

The constituents of the roots and subterranean stems of A. graminicola which were identified by **TLC** are of different polarities. The reversed phase **HPLC** system used could only separate the polar compounds containing free hydroxyl groups. The less polar compounds have broad peaks with high retention times.

The use of a C-18 reversed phase column with **acetonitrile/water; (1:1)** mixture for elution,

enabled us to identify aloegramonol (1), aloesaponol-I (5), aloesaponol-II (9), laccaic-D acid methyl ester (10) and asphodelin (3) in the acetone extract of the subterranean stems of A. graminicola (Fig-2a). Except aloegramonol (1) these compounds were also identified in the extract of the roots (Fig-2b). The peak with a retention time of 7.0 min found in the roots extract was not observed in the subterranean stems which instead showed a peak with a retention time of 5.0 min which was not observed in the roots.

Fig 2a HPLC CHROMATOGRAM OF THE ACETONE EXTRACT OF THE SBTERRANEAN STEMS OF A. GRAMINICOLA

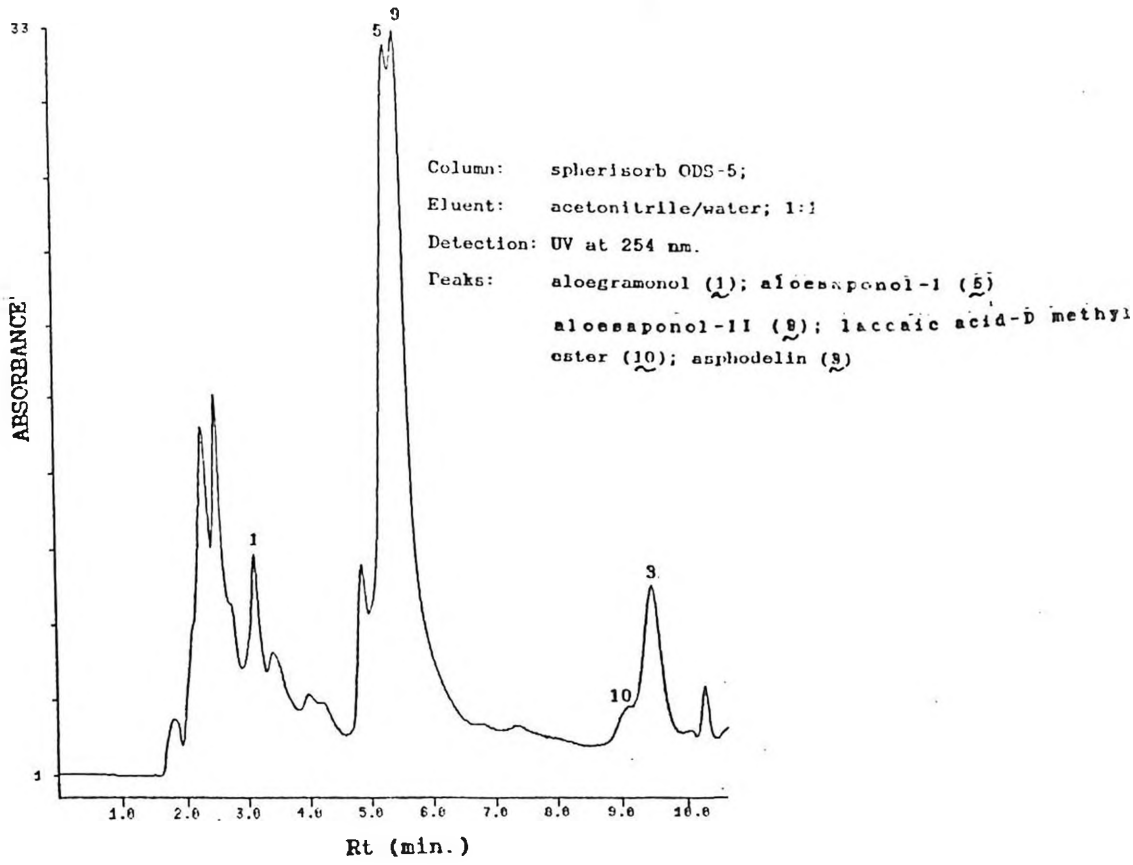
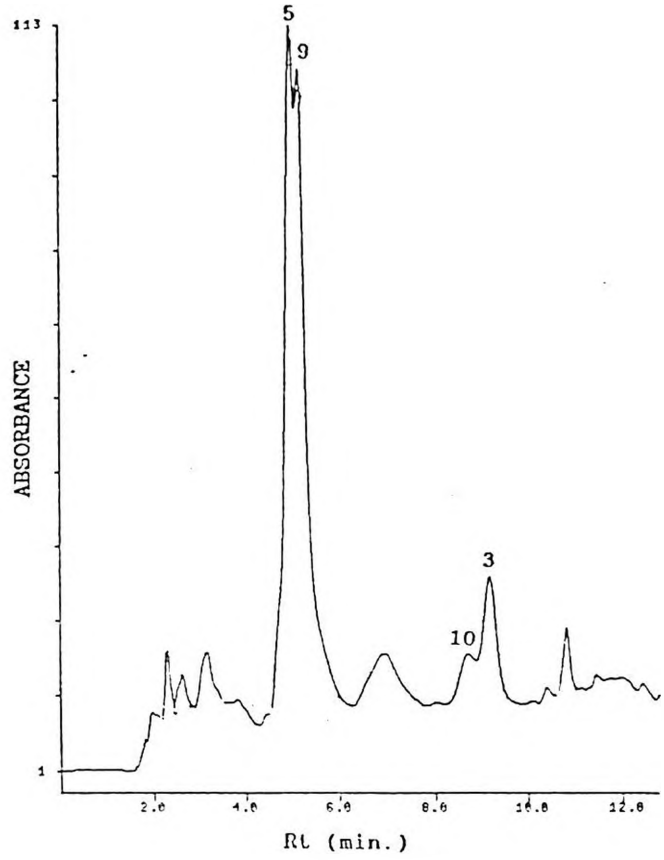


Fig-2b HPLC CHROMATOGRAM OF THE ACETONE EXTRACT OF THE ROOTS OF A. GRAMINICOLA



Condition of separation: same as given in fig. 2a

Among the compounds identified in the roots and subterranean stems of A. graminicola, aloesaponol-I (5) and aloesaponol-II (9) are the major compounds in both extracts. Based on the relative absorbance observed after injecting equal quantities of the extracts, the concentrations of the identified compounds appear to be higher in the roots than the subterranean stems.

2.3.2. COMPARISON OF SAMPLES OF A SPECIES COLLECTED FROM DIFFERENT LOCALITIES.

Of all chromatographic zones identified from the roots (Table-5), it is only **Art-2, Art-3, Art-7, Art-9, Art-10, Art-15, Art-16, Art-17** and **Ar-18** which are useful to tell the similarities and differences of the studied samples. Table-9 is extracted from Table-5 and includes only these compounds.

A. graminicola, collected along the Nairobi-Nakuru road (AY-34) was found to occur in two different forms. One has distinct spots while the other is completely devoid of any spot. Some plants which are intermediate between these two forms were also seen. All these forms were found to grow together along the Nairobi-Nakuru road. **TLC** comparison of the roots, subterranean stems and flowers of the two forms of A. graminicola did not show any difference.

The roots of unspoted leaves of Aloe (Newton-3085), which has an affinity to A. graminicola, showed identical **TLC** picture with the roots of A. graminicola (AY-34), while the roots of a related plant (Newton-3539) which also has an affinity to A. graminicola, differs from A. graminicola (AY-34) by the abscence of **asphodelin (3)** in the former.

Table-9. Compounds with taxonomic significance in the roots of few taxa.

Plant (collected from)	Compound									
	Art- 2	3	7	9	10	11	15	16	17	18
Aloe graminicola										
Newton-3085 (Burole)	+	+	+	-	-	-	-	+	-	+
Newton-3539 (Limuru)	-	+	+	-	-	-	-	+	-	+
AY-34 (Gilgil)	+	+	+	-	-	-	-	+	-	+
A. dumetorum										
Newton-3188 (Huri)	+	+	+	-	-	-	+	+	-	+
Newton-3231 (Huri)	+	+	+	-	-	-	-	-	-	+
Newton-3487 (Lolgur)	+	+	+	-	-	-	-	?	+	+
Newton-3087 (Wamba)	+	+	+	-	-	-	-	?	+	+
A. lateritia										
Newton-3422 (Hanang)	-	+	+	-	-	-	+	+	-	+
Newton-3614 (T)*	+	-	+	-	-	-	-	-	-	+
Newton-3620 (T)	+	-	-	+	-	-	-	-	-	+
A. duckeri										
Newton-2496 (T)	+	+	+	-	+	-	-	-	-	+
Newton-2550 (T)	+	-	-	+	+	-	-	-	-	+
A. secundiflora										
AY-33 (Thika)	+	-	-	+	+	-	-	-	-	-
A. nyeriensis (var. kedongensis)										
AY-31 (Gilgil)	+	-	-	+	+	+	-	-	-	-

* T = Tanzania

As the spoted and unspoted leaves of A. graminicola (AY-34) showed identical TLC pictures, and the close similarities of these two samples with A. graminicola (AY-34) could be used as additional data for identification of the plants.

Three samples of the roots of A. lateritia (which is very related and often confused with A. graminicola) were also analysed in this study. The two A. lateritia samples (Newtone-3614 and Newton-3620) showed similar TLC pattern. The only difference being, Newton-3614 contained Art-7 but not Art-9, while Newton-3620 contained Art-9 instead of Art-7. On the other hand, the third sample of A. lateritia (Newton-3422), showed a marked difference from the other two A. lateritia samples.

The root samples of A. dumetorum which were collected from different localities showed differences. The two A. duckeri samples also showed difference. These plants were reported to be variable over geographical differences (Reynolds, 1966).

Differences in chemical composition within a species collected from different localities have been reported in many species (Reynolds, 1986). This appears to be related to the previous indication by Reynolds (1966)

that states the presence of variations in physical appearance of a species due to differences in geographical locality.

2.3.3. COMPARISON OF DIFFERENT SPECIES.

The distribution of some compounds in the samples analysed here showed the presence of some pattern in the Saponariae group. **Art-18** was detected in the roots of all samples belonging to the Saponariae group but not in the two non-Saponariae species. Among the Saponariae samples, **Art-3** and **Art-7** were found in all but the two A. lateritia samples (i.e. Newton-3614 and Newton-3620) and one of the A. duckeri samples (i.e. Newton-2550). These two compounds were not detected in A. secundiflora and A. nyeriensis (var. kedongensis). Except in Newton-2550 (A. duckeri) and Newton-3620 (A. lateritia), **Art-9** and **Art-10** were not detected in the Saponariae samples. These two compounds were found in the two non-Saponariae species.

Art-11 was detected in the roots of A. nyeriensis (var. kedongensis), only, while **Art-8** was detected in the subterranean stems of A. graminicola, only. Except for these two compounds all the other compounds were detected in at least two species. In addition, the observed differences of a species due to geographical

location makes the task of correlating such preliminary chemical informations with taxonomy very difficult.

2.3.4. TAXONOMIC AND BIOGENETIC IMPLICATIONS OF COMPOUNDS FOUND IN ALL PLANTS.

The presence of some compounds, namely **Art-1**, **Art-2**, **Art-4**, **Art-5**, **Art-6**, **Art-12**, **Art-13**, **Art-14**, **Art-19** and **Art-20** in all the Saponariae plants and also in the two non-Saponariae Aloe species showed the presence of some relations between different groups.

Chrysophanol (2) was detected in all samples analysed here. This compound has been isolated from the subterranean stems of A. saponaria (Yagi et al., 1977a) and from the leaves of some Aloe species (Thomson, 1971).

Asphodelin (3) which is a **bi-chrysophanol** derivative was found in all but two samples analysed, namely; Newton-3539 (Aff. A. graminicola) and Newton-3422 (A. lateritia). This compound has been isolated from the subterranean stems of A. saponaria (Yagi et al., 1978) and earlier from Asphodelus microcarpa (Gonzalez et al., 1973). Its wide occurrence in the analysed Aloe species as well as in Asphodelus microcarpa

supports the previous suggestion which indicated taxonomical and chemical affinity between Aloinae and Asphodelinae (Rheede Van Oudtshoorn, 1963, 1964).

The co-occurrence of the two 7-methyl ester anthraquinone derivatives; **aloesaponarin-I (6)** and **laccaic acid-D methyl ester (10)** along with the pre-anthraquinone; **aloesaponol-I (5)** in all plants analysed, supports the biogenetic relationships (Scheme-1) established by Yagi et al., (1978b), for these compounds.

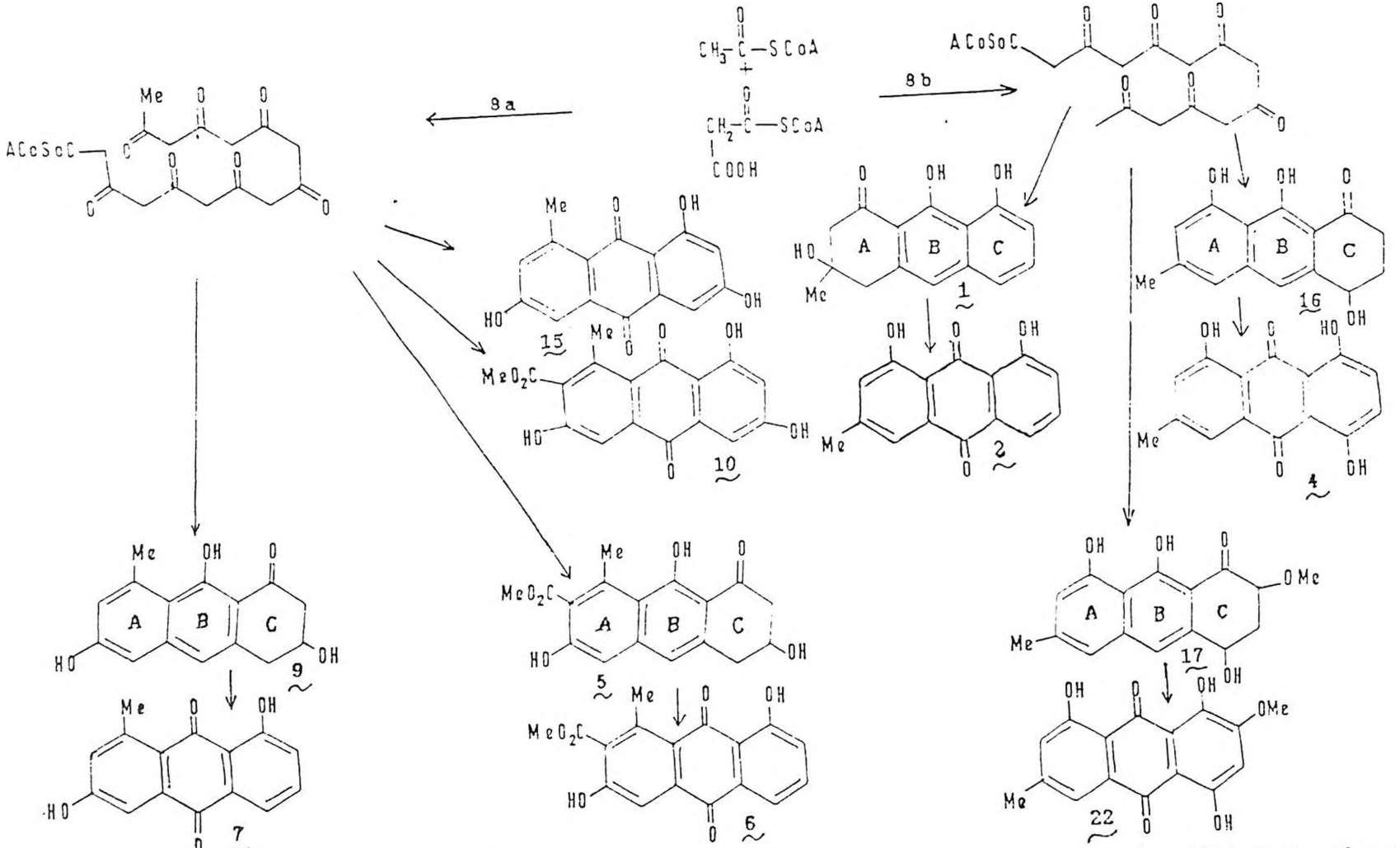
Similarly, the co-occurrence of **aloesaponol-II (9)** and **aloesaponarin-II (7)**, in all samples analysed here as well as in A. saponaria (Yagi et al., 1974), might suggest similar biogenetic relationship as the one shown in Scheme-1.

It is worth noting that, apart from Aloe, the pre-anthraquinone derivatives; **aloesaponol-I (5)** and **aloesaponol-II (9)**, and the anthraquinone derivatives; **aloesaponarin-I (6)**, **aloesaponarin-II (7)** and **laccaic acid-D methyl ester (10)** were not reported from any other source. In particular, 7-methyl ester anthraquinone derivatives appear to be restricted to the genus. As there is no compound so far reported from Aloe which is a marker of the genus, some of

these compounds could very well be markers of the taxa, provided that the distribution of these compounds in Aloe species could be investigated further.

The anthraquinone derivatives detected in the present study and also from A. saponaria (Yagi et al., 1974, 1977a, 1977b, 1978a), are of two kinds; 3,8-dihydroxy-1-methyl and 1,8-dihydroxy-3-methyl derivatives. These two appear to have been formed by two parallel routes differing by the way the octaketide chain was folded (Scheme-8) (Leistner 1973).

Scheme-8. The two different folding of octaketide chain in Aloe and possible biogenetic relationships.



Aloesaponol-I (5), **aloesaponarin-I** (6) and **laccaic acid-D methyl ester** (10) having a methyl ester substitution at C-2 are the major compounds among the 3,8-dihydroxy-1-methyl derivatives. It appears that ring-A (scheme-8a) has been aromatized directly from the octaketide chain with esterification. Hence decarboxylation is not favoured. This probably accounts for their presence in high concentrations.

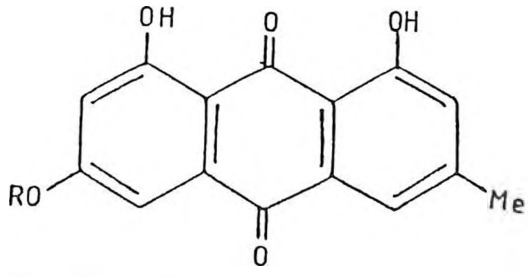
Chrysophanol (2) and **asphodelin** (3) (which is a **bi-chrysophanol** derivative) are the major compounds among the 1,8-dihydroxy-3-methyl derivatives. It is worth noting that, among the 1,8-dihydroxy-3-methyl derivatives so far reported from Aloe or any other source, to the best of our knowledge, no compound with a methyl ester derivative had been reported. Even the carboxylic acid derivatives are rare. Unlike the 3,8-dihydroxy-1-methyl derivatives during the formation of **chrysophanol**-like anthraquinones, aromatization on the ring containing carboxylic acid (ring-A) has not occurred directly from the polyketide chain. Instead 1-keto acid was first formed which undergoes decarboxylation to give pre-anthraquinones (Scheme-4).

Helminthosporin (4) isolated from the subterranean stems of A. graminicola was not detected in any of the other samples analysed. Even in A. graminicola, it was detected after partial purification and isolated in a small quantity. This compound was also isolated from the subterranean stems of A saponaria (Yagi et. al., 1977b). **Helminthosporin (4)** and **isoxanthorin (22)** (which was isolated from the subterranean stems of A. saponaria (Yagi et al., 1977b), appear to have been formed according to scheme-8b. Unlike **chrysophanol (2)**, during the formation of these compounds aromatization of ring-A has first occurred directly from the octaketide chain, giving rise to the formation of pre-anthraquinone derivatives which were then converted to the corresponding anthraquinones.

The anthraquinones derived from both routes were found in different parts of Aloe. Those derived according to Scheme-8b are most concentrated in the flowers. **Barbaloin (11)** and **homonataloin (12)** which are the major anthraquinone derivatives in the leaves of Aloe (Reynolds, 1985a) were formed according to Scheme-8b. While, those derived according to Scheme-8a are most concentrated in the roots.

Barbaloin (11) and **homonataloin** (12) could not be detected in any of the samples analysed, in this study. It is worth noting that these compounds were not reported from the subterranean stems of A. saponaria. This probably indicates that glycoxylation at C-10 which is dominant in the leaves is not favoured in other parts of the plants. In addition, the absence of these compounds in the flowers, roots and subterranean stems appear to explain the absence of any report on purgative uses on these parts, unlike the extensive uses of the leaves.

Physion (28) and **emodin** (29) which are widely distributed in higher plants and fungi, often with **chrysophanol** (2), were not detected in all samples analysed. These two compounds are oxygenated at C-6 position as required biogenetically. On the other hand all the anthraquinones so far reported from Aloe, which are biogenetically related to **chrysophanol** (2), lack oxygenation at this position.



28 R = Me

29 R = H

2.3. ANTIBACTERIAL ACTIVITIES OF THE ROOTS OF ALOE GRAMINICOLA

The acetone extract of the roots of A. graminicola showed significant activities against E. coli and B. subtilis at concentrations of 500 and 250 µg/ml and without activities at 125 µg/ml. The corresponding extracts of the flowers and subterranean stems did not show any activity against the same bacteria at these concentrations. All extracts were in-active against S. albus and S. cerevicie.

Aloesaponarin-I (6) and laccaic acid-D methyl ester (10) which were reported to have antimicrobial activities (Yagi et al., 1983) were identified from the roots of A. graminicola. These compounds could be responsible for the observed activities. It is worth noting that these compounds were detected in the roots of all samples analysed and their presence could be responsible for some of the traditional uses of these plants.

CHAPTER 3.

CONCLUSION AND RECOMMENDATION.

3.1. CONCLUSION.

Anthraquinone derivatives are the major constituents of the flowers, roots and the subterrenean stems of the Aloe species analysed. These appear to have been derivatived through the polyketide path way and they were formed by two routes differing by the way the octaketide chain was folded.

The isolation of the new natural product, namely; **aloegramonol (1)** along with **chrysophanol (2)** from the subterranean stems of A. graminicola is biogenetically significant. It could reasonably be suggested that **chrysophanol**-like anthraqunone derivatives, which are commonly found in Aloe, could have been biosynthesised through the polyketide pathway involving **aloegramonol** as one of the precursors.

The presence of **aloesaponol-I (5)**, **aloesaponol-II (9)**, **aloesaponarin-I (6)**, **aloesaponarin-II (7)** and **laccaic acid-D methyl ester (10)** in all plants and different parts of the analysed plants as well as in A. saponaria (Yagi et al., 1974) is of taxonomic

values for the taxa Aloe, as these compounds have never been reported from any other source. In addition, the co-occurrence of these compounds in all plants analysed suggested the presence of biogenetic relationships, some of which have already been established by Yagi et al. (1983).

The presence of some compounds in the analysed Aloe species belonging to the Saponariae group as well as in the two non-Saponariae, Aloe species, suggested the presence of inter-group relationship in Aloe. The distribution of some compounds has also showed that the chemical information obtained here could be used in taxonomic studies.

The observed antibacterial activity in the roots of A. graminicola, might partly explain, the traditional uses of the plant. In addition the presence of the two antimicrobial compounds, namely; **aloesaponarin-I (6) and laccaic acid-D methyl ester (10)** in the Aloe species analysed could be responsible for some of the traditional uses.

The absence of **barbaloin (11)** and **homonataloin (12)** in the flowers, roots and the subterranean stems of the analysed samples, appear to explain the absence of any report on the purgative uses of these parts of Aloe.

3.2. RECOMMENDATION

The 8-methyl anthraquinone derivatives could be markers of the genus. Further studies on the presence or absence of these compounds in other Aloe species and some related plants could give useful taxonomical information.

Most of the chromatographic zones detected in the Aloe species analysed in the present study were not chemically identified. Further phytochemical studies could lead to the identification of more compounds which could be used as markers for taxonomic classifications.

CHAPTER 4

EXPERIMENTAL

4.1. GENERAL

MPs were determined on a GALLENKAMP melting point apparatus and are uncorrected. Distilled solvents were used for extractions and chromatographic separations.

4.1.1. SPECTROSCOPY

EIMS were recorded at 70 ev, on a VARIAN 311 machine. ^{1}H NMR were recorded at 250 MHz or 400 MHz on a BRUKER WM and BRUKER AM spectrometers. ^{13}C NMR was recorded at 90.56 MHz on BRUKER AM spectrometer. TMS was used as internal standard in all NMR analysis. UV were recorded on a PYE UNICAM SP8-150 UV/VIS Spectrophotometer using UV grade solvents. IR were recorded on a PERKIN-ELIMER Spectrophotometer.

4.1.2. HPLC

LC SYSTEM: a BRUKER (LC-21) liquid chromatographic system with three solvent delivery units and an automatic mixer was used.

INJECTION: a RHEDYNE injection valve was used.
DETECTION: BRUKER LC-313I UV/VIS variable wave length monitor set at 254 nm was used.

SOLVENT PROGRAMMING AND DATA HANDLING: a microcomputer (EPSON QX10) with BRUKER LC 41 software package was interphased with the detecter and the LC system.
SOLVENT SYSTEM: Elutions were carried out with acetonitrile/water (1:1) mixture. HPLC grade acetonitrile and double distilled water were used for elution. The solvents were degassed by passing them through a helium degasser, before reaching the pumps.

4.1.3. CHROMATOGRAPHIC MATERIALS: The silica gel for column chromatography (MERCK, 60 G, 70-230 mesh) was impregnated with 3% oxalic acid. Analytical TLC were performed on pre-coated silica gel (MERCK).

4.1.4. TLC SOLVENT SYSTEMS:

S-1	Pet. ether/$CHCl_3$; 1:1
S-2	$CHCl_3$
S-3	$CHCl_3$/EtOAc; 7:3
S-4	$CHCl_3$/EtOAc; 1:1
S-5	$CHCl_3$/MeOH; 6.5:3.5

4.1.5. DETECTION OF CHROMATOGRAPHIC ZONES: chromatographic zones were detected under UV (254, 366 nm) and VIS lights. A 5% methanolic KOH and 10% Fast Blue Salt B spray reagents were also used.

4.2. PLANT MATERIALS.

Aloe graminicola Reynolds was collected at 140 km on the road from Nairobi to Nakuru,Kenya (AY-34).

Two Aloe plants with unspoted leaves (Aff. A. graminicola) were collected from Burole Mt, West of Moyale, Kenya (Newton-3085) and from escarpment road below Limuru, Kenya (Newton-3539).

A. dumetorum Mathew & Branham was collected from Wamba pass, Kenya (Newton-3087), Huri Hills, Kenya (Newton-3188, Newton-3231) and Ol Doinyo Lolgurgu, Kenya (Newton-3487).

A. lateritia Engler was collected from Hanang Mountain, Tanzania (Newton-3422), 12 km NE of Mafinga-Tanzania (Newton-3614) and Mbaya Mountain range on the road to Chunyo, ca. 30 km from Mbeya, Tanzania (Newton-3620).

A. duckeri **Christian** was collected south of Laela, towards Sumbawanga, Tanzania (Newton-2496, Newton-2550)

A. nyeriensis (var. kedongensis) **(Reynolds) S. Carter** was collected at 120 km from Nairobi to Nakuru, Kenya (AY-31).

A. secundiflora **Engler** was collected from Mevaloni hills, around the town of Thika, Kenya (AY-33).

All samples for analyses were collected in June 1990 (AY-34 was also collected in June 1989). Herbarium specimen are deposited at the Herbarium, Botany Departement, University of Nairobi and the National Museums of Kenya. Some are also planted at the Garden, Botany Department, Kenyatta University.

4.3. COMPARATIVE TLC ANALYSES OF SOME ALOE SPECIES.

Air dried and grounded roots, subterranean stems and flowers (1 gm in each case) of the Aloe species indicated in Section 4.2. were extracted with acetone (2X30 ml), by percolation at room temp., for 48 hrs. The extracts were concentrated under reduced pressure.

25 µg of each of the extracts were applied on pre-coated silica gel plates (10X20 cm) and developed using solvent systems (S-1 to S-5). The chromatographic zones were identified by their Rf values and colours under VIS. and UV lights. Some of the chromatographic zones were further identified by direct comparison with authentic samples. The results are summarized in Table-5.

4.4. HPLC ANALYSES.

Air dried and finely grounded roots and subterranean stems of A. graminicola (5 gm in each case) were extracted by cold percolation in acetone for 48 hrs. The extracts were concentrated under reduced pressure and were purified by passing aliquot amounts through silica gel (5 gm) eluting with MeCN, each time.

Samples for analyses were prepared by dissolving 1 mg of the extracts in 1 ml of MeCN and 20 µl of these were injected into the liquid chromatographic system. Peaks were identified by spicking with authentic samples, which resulted in enhencment of peaks (Table-10).

Table-10. HPLC Identification of compouds from *A. graminicola*.

Compound	Rt	Plant Part
Aloegramonol (1)	3.1	SST
Aloesaponol-I (5)	5.3	SST, RT
Aloesaponol-II (9)	5.5	SST, RT
Laccaic acid-D methyl ester (10)	9.1	SST, RT
Asphodelin (3)	9.6	SST, RT

SST = subterranean stems;

RT = roots;

Rt = retention time.

4.5. EXTRACTION OF THE SUBTERRANEAN STEMS OF A. GRAMINICOLA.

Air dried and powdered subterranean stems (700 gm) of A. graminicola were extracted successively, with pet. ether (60-80°C) (3X500 ml), chloroform (3X500 ml) and acetone, (3X500 ml) by percolation at room temprature for 48 hrs, in each case. The extracts were filtered and concentrated under reduced pressure to give 4.5, 18 and 7 gm, respectively.

The methanol soluble portion of the pet. ether (60-80°C) extract and the chloroform soluble portion of the acetone extracts were combined with the chloroform extract and subjected to chromatographic separation. The insoluble portions of the extracts were not followed further.

4.5.1. ISOLATION OF COMPOUNDS.

The combined extract (20 gm) was dissolved in chloroform/methanol (1:1) mixture and the mixture was transfered to a round bottomed flask containing 20 gm of silica gel impregnated with oxalic acid. The mixture was homogenized and the solvent evaporated off under reduced pressure to give dry and uniformly

adsorbed extract. The pre-adsorbed material was carefully transfered to a column packed with silica gel impregnated with 3% oxalic acid (400 gm). Elutions were carried out with mixtures of pet. ether (60-80°C)/dichloromethane then with dichloromethane/ ethyl acetate.

A total of 16 fractions (labeled A-P) each containing about 250 ml were collected, by monitoring with TLC (silica gel), developing with solvent systems S-1 to S-5.

4.5.1.1. PURIFICATIONS OF FRACTIONS AND STRUCTURAL DATA OF COMPOUNDS.

The fractions obtained from the first column chromatography contained more than one compound. The fractions containing major compounds were further purified.

Fraction A which was eluted with pet. ether (60-80°C) was discarded as no noticeable amoun of compounds were detected.

Fraction B which was eluted with pet. ether (60-80°C)/CH_2Cl_2 (19:1) showed one spot on TLC (S-1).

The solvent free fraction was washed with pet. ether (60-80°C) and chromatographed over a small column (15 gm of silica gel impregenated with oxalic acid), eluting with pet. ether (60-80°C/dichloromethane, 7:3 mixture and recrystallization from $CHCl_3$ afforded red crystals of 4 (3 mg), $C_{15}H_{10}O_5$, mp. 215-217°C, lit. mp. 210-212°C (Yagi et al., 1977a), Rf 0.89 (S-1), turned purple with KOH spray reagent;

UV λ_{max} (MeOH) (log ε) nm: 230(5.1), 252(4.6), 282(4.3), 290(4.2), 460(4.3), 474(4.4), 484(4.4), 504(4.3), 518(4.3);

UV λ_{max} (MeOH/KOH) nm: 230, 268, 282, 290, 510, 515, 550, 600;

EIMS m/z (rel. int.): 270 (M^+, 100), 253 ($(M-OH)^+$, 4), 242 ($(M-CO)^+$, 3), 213 ($(M-CO-CHO)^+$, 4), 135 (8), 128 (39);

^1H NMR ($CDCl_3$, 400 MHz): δ 13.02, 12.32, 12.15 (3H, 3s, OH-1, OH-5, OH-8), 7.69 (1H, d, J = 2.0 Hz, H-4), 7.28 (1H, d, J = 8.0 Hz, H-7(or H-6), 7.25 (1H, d, J = 8.0 Hz, H-6(or H-7), 7.11 (1H, d, J = 2.0 Hz, H-2), 2.45 (3H, s, Me-3).

Fraction C, which was eluted with pet. ether (60-80°C)/dichloromethane (17:1) showed one spot on TLC (S-1). Crystallaization of this fraction (MeOH) afforded orange crystals of chrysophanol (2) (15 mg), $C_{15}H_{10}O_4$, mp. 194-196°C, lit. mp. 197°C, (Yagi et al.,

1977a), Rf 0.52 (S-1), yellow spot turned red on spraying with KOH;

UV λ_{max}(MeOH) (log ε) nm: 226(5.0), 254(4.7), 276(4.4), 286(4.4), 396(4.3), 428(4.4), 444(4.3);

UV λ_{max}(MeOH/KOH) nm: 226, 248, 276, 286, 506;

UV λ_{max}(MeOH/$AlCl_3$) nm: 230, 252, 266, 274, 460, 488,514;

IR $\nu_{max(KBr)}$ cm^{-1}: 3500 (OH), 1630 (chelated CO);

EIMS m/z (rel. int.): 254 (M^+, 100), 226 (16), 198 ($(M-2CO)^+$, 10), 197 ($(M-CO-CHO)^+$, 10), 169 ($(M-2CO-CHO)^+$, 6), 149 (23);

^{1}H NMR ($CDCl_3$/MeOH; 1:1, 400 MHz): δ 7.70 (1H, dd, J = 8.0, 2.0 Hz, H-5), 7.69 (1H, t, J = 8.0 Hz, H-6), 7.56 (1H, d, J = 2.0 Hz, H-4), 7.22 (1H, dd, J = 8.0, 2.0 Hz, H-7), 7.02 (1H, d, J = 2.0 Hz, H-2), 2.35 (3H, s, Me-3). (12.05, 12.15 (2H, 2s, OH-1, OH-8), observed when run in $CDCl_3$)).

Further elution of the column with pet. ether (60-80°C)/dichloromethane (17:2, 17:3, 17:4, 17:5) gave four fractions (D, E, F, and G, respectively), which contained minor compounds and were not followed further.

Fraction H which was eluted with pet. ether (60-80°C)/CH_2Cl_2 (13:7), showed a major spot orange in colour and two minor, colourless spots on TLC (S-2). On addition of methanol to this fraction, a fourth colourless and UV inert compound precipitated. The precipitate was recrystallized from acetone to give colourless crystals of β-sitosterol (8) (50 mg), $C_{29}H_{50}O$, mp. 134-136°C, Rf 0.40 (S-1), gave orange colour with conc. H_2SO_4 spray reagent after heating at 110°C;

IR ν_{max} (KBr) cm^{-1}: 3450 (OH), 2950 (C-H str), 1450 (C-H bend.), 1375 (C-H bend.), 1060;

EIMS m/z (rel int): 414 (M^+, 100), 396 ($(M-H_2O)^+$, 32), 381 (17), 329 ($(M-C_6H_{13})^+$, 21), 303 (16), 255 (12), 231 (7), 213 (12) 43 ($(MeCO)^+$, 25);

^1H NMR ($CDCl_3$, 400 MHz): δ 5.32 (1H, m, H-6), 3.50 (1H, m, H-3), 0.98 (3H, s, CH_3-19), 0.90 (3H, d, J = 6.4 Hz, CH_3-21), 0.82 (6H, d, J = 6.4 Hz, CH_3-26,27), 0.80 (3H, t, J = 6.4 Hz, CH_3-29), 0.68 (3H, s, CH_3-18).

The methanol soluble part of fraction H was applied on silica gel PTLC (S-2) to give orange amorphous solid of asphodelin (3) (27 mg), $C_{30}H_{20}O_8$, mp. 161-163°C, Rf 0.42 (S-1), turned red on spraying with KOH;

UV λ_{max} (MeOH) (log ε) nm: 228(5.3), 256(5.2), 286(4.8), 410(4.8), 432(4.9), 458(4.8);

UV λ_{max} (MeOH/KOH) nm: 220, 234, 250, 286, 510 nm;

IR ν_{max} (KBr) cm^{-1}: 3500(OH), 2950, 1650 (chelated CO), 1640, 1300;

EIMS m/z (rel int): 508 ($(M)^+$, 23), 491 ($(M-OH)^+$, 100), 477 (16), 461 (29), 254 ($(C_{15}H_{10}O_4)^+$, 47);

^1H NMR ($CDCl_3$, 250 MHz): δ 12.59, 12.40, 12.04, 12.02 (4H, 4s, OH-1, OH-1', OH-8, OH-8'), 7.13 (1H, d, J = 1.5 Hz, H-2), 7.59 (1H, d, J = 1.5 Hz, H-4), 7.95 (1H, d, J = 7.7 Hz, H-5), 7.35 (1H, d, J = 7.7 Hz, H-6), 7.31 (1H, d, J = 1.5 Hz, H-2'), 7.68 (1H, d, J = 1.5 Hz, H-4'), 7.27 (1H, dd, J = 1.5, 7.7 Hz, H-5') 7.59 (1H, t, J = 7.7 Hz, H-6'), 7.25 (1H, dd, J = 1.5, 7.7 Hz), 2.50, 2.15 (6H, 2s, Me-3, Me-3').

Fraction I which was eluted with pet. ether 60-80°C)/dichloromethane (1:1) contained trace amounts of compounds and was not followed further.

Fraction J which was eluted with pet. ether 60-80°C)/dichloromethane (2:3) showed two major spots on TLC (S-3). Rechromatography on silica gel impregnated with oxalic acid (50 gm), eluting with CH_2Cl_2 gave yellow crystals of aloegramonol (1) (42 mg), $C_{15}H_{14}O_4$, mp. 199-202°C (MeOH), lit. mp. 203-206°C (Hansma et al., 1976), Rf 0.73 (S-4), the

yellow colour changed to brown on spraying with fast blue salt B;

UV λ_{max} (MeOH) (log ε) nm: 218(4.4), 268(4.7), 292(3.6), 298(3.6), 322(3.6), 408 (3.9);

UV λ_{max} (MeOH/$AlCl_3$) nm: 222, 274, 288, 446;

UV λ_{max} (MeOH/$AlCl_3$/HCl) nm: 222, 274, 288, 446;

IR ν_{max} (KBr) cm^{-1}: 3400br (OH), 2950, 1620 (chelated CO), 1590, 1445, 1410;

HRMS: M^+ found 258.0885 corresponding to $C_{15}H_{14}O_4$, requires 258.0892;

EIMS m/z (rel. int.): 258 (100, M^+), 240 ($(M-H_2O)^+$, 82), 225 ($(M-H_2O-CH_3)^+$, 8), 215 ($(M-MeCO)^+$, 18), 200 ($(M-C_3H_6O)^+$, 41), 187 (5), 173 (10), 155 (7), 144 (15), 127 (11), 115 (47), 43 ($(MeCO)^+$, 27);

^1H NMR ($CDCl_3$/MeOH-d_4; 1:1, 400 MHz): δ 7.39 (1H, t, J = 8.4 Hz, H-6), 7.08 (1H, dd, J = 8.4, 2.0 Hz, H-5), 6.95 (1H, s, H-10), 6.78 (1H, dd, J = 8.4, 2.0 Hz, H-7), 3.03 (1H, d, J_{gem} = 17.5 Hz, CH_2-4), 2.97 (1H, d, J_{gem} = 17.5 Hz, CH_2-4), 2.80 (1H, d, J_{gem} = 17.5 Hz, CH_2-2), 2.73 (1H, d, J_{gem} = 17.5 Hz, CH_2-2), 1.33 (3H, S, Me-3);

^{13}C NMR ($CDCl_3$/MeOH-d_4; 1:1, 90.56 MHz): δ 203.65 (C-1), 164.06, 157.43 (C-9, C-8), 139.29, 135.01 (C-4a, C-10a), 132.39 (C-6), 118.49, 116.13 (C-5, C-10), 112.36, 109.38 (C-8a, C-9a), 110.64 (C-7), 70.24 (C-3), 51.40, 42.99 (C-2, C-4), 28.41 (Me-3).

Rechromatography of fraction J on silica gel impregnated with oxalic acid and eluting with dichloromethane also gave orange crystals of aloesaponarin-I (6) (60 mg), $C_{17}H_{12}O_6$, mp. >197°C (dec.) (EtOAc), lit. mp. 199-203°C (Yagi et. al., 1974), Rf 0.82 (S-4), turned red on spraying with KOH and brown with fast blue salt B. Yellow solution in methanol turns red after six hrs;

UV λ_{max} (MeOH run immediately) (log ε) nm: 220(5.2), 268(5.3), 274(5.3), 390(4.5), 406(4.6), 428(4.5);

UV λ_{max} (MeOH, run after six hrs) nm: 244, 304, 480;

UV λ_{max} (MeOH/HCl, run by adding HCl after keeping the sample in MeOH for six hrs) nm: 220, 268, 274, 390, 406, 428;

UV λ_{max} (MeOH/KOH) nm: 244, 304, 480;

UV λ_{max} ($CHCl_3$, run after six hrs) nm: 238, 276, 282, 390, 410, 428;

UV λ_{max} (MeCN, run after six hrs) nm: 226, 270, 282(sh), 354, 322, 524;

IR ν_{max} (KBr) cm^{-1}: 3450 (OH), 1720 (CO), 1635 (chelated CO), 1590.

EIMS m/z (rel int.): 312 (M^+, 60), 297 ($(M-CH_3)^+$, 13), 280 ($(M-MeOH)^+$, 100), 252 ($(M-MeOH-CO)^+$, 23), 224 ($(M-MeOH-2CO)^+$, 30), 196 ($(M-MeOH-3CO)^+$, 13), 168 ($(M-MeOH-4CO)^+$, 24), 139 ($(M-MeOH-4CO-COH)^+$, 33);

1H NMR ($CDCl_3$/MeOH-d_4; 1:1, 250 Mz): δ 7.76 (1H, dd, J = 7.5, 1.3 Hz, H-5), 7.64 (1H, s, H-4), 7.62 (1H, t, J = 7.5 Hz, H-6), 7.25 (1H, dd, J = 7.5, 1.3 Hz, H-7), 4.02 (3H, s, COOMe-2), 2.81 (3H, s, Me-1).

Fraction K which was eluted with pet. ether (60-80°C)/dichloromethane (3:7), showed one major spot on TLC (S-3). Rechromatography on silica gel impregnated with oxalic acid and eluting with CH_2Cl_2 gave yellow crystals of alosaponarin-II (7) (8 mg), $C_{15}H_{10}O_4$, mp. 264-265°C, (EtOAc), lit. mp. 250-254°C (Yagi et al., 1974), Rf 0.80 (S-3), turned red on spraying with KOH and brown with fast blue salt B spray reagents. Yellow methanolic solution gradually changed to orange;

UV λ_{max} (MeOH, run immediately) (log ε) nm: 220(5.2), 244(4.8) 268(4.9), 280(5.0), 410(4.4), 428(4.5), 462(4.4);

UV λ_{max} (MeOH, run after seven days) nm: 220, 254, 258, 280, 294, 410, 428, 462, 480;

UV λ_{max} (MeOH/KOH) nm: 270, 294, 480;

UV λ_{max} (MeOH/HCl, run by adding HCl after keeping sample in MeOH for seven days) nm: 220,244, 268, 280, 294, 410, 428, 462;

UV λ_{max} ($CHCl_3$, run after seven days) nm: 268, 278, 294, 410, 428, 462;

UV λ_{max} (MeCN run after seven days) nm: 208, 232, 258, 276, 330, 390, 406, 426, 530;

EIMS m/z (rel int.): 254 (M^+, 100), 198 ($(M-2CO)^+$, 30), 197 ($(M-CO-CHO)^+$, 45), 169 ($(M-2CO-CHO)^+$, 26), 152 (29), 115 (39);

^1H NMR ($CDCl_3$/MeOH-d_4; 1:1, 250 MHz): δ 7.74 (1H, dd, J = 8.0, 2.0 Hzs, H-5), 7.55 (1H, d, J = 2.0 Hz, H-4), 7.60 (1H, t, J = 8.0 Hz, H-6), 7.28 (1H, dd, J = 8.0, 2.0 Hz, H-7), 7.00 (1H, d, J = 2.0 Hz, H-2), 2.80 (3H, s, Me-1).

Fractions L and M, which were eluted with pet. ether 60-80°C)/dichloromethane (1:4, 1:9, respectively), fraction N eluted with dichloromethane and fraction O eluted with CH_2Cl_2/EtOAc (19:1) contained trace amounts of compounds and were not followed further.

Fraction P which was eluted with dichloromethane/EtOAc (17:3), showed three spots on TLC (S-4). Fractional crystallization from EtOAc afforded light yellow crystals of aloesaponol-I (5) (60 mg). $C_{17}H_{16}O_6$, mp. >240°C (dec.) (EtOAc), lit. mp. 248-250°C (Yagi et al., 1974), Rf 0.31 (S-4), it showed blue fluorescence under UV light (366 nm), which intensified with KOH spray reagent, also gave brown colour with fast blue salt B spray reagent;

UV λ_{max}(MeOH) (log ε) nm: 220(4.7), 274(5.0), 298(3.8), 304(3.6), 390(4.6);

UV λ_{max}(MeOH/KOH) nm: 226, 274, 298, 400;

UV λ_{max}(MeOH/$AlCl_3$, run immediately) nm: 220, 274, 298, 304, 390;

UV λ_{max}(MeOH/$AlCl_3$, after three days) nm: 220, 278, 284, 402, 425;

IR ν_{max}(KBr) cm^{-1}: 3375(OH), 2950, 2600, 1710 (ester CO), 1630 (chelated CO), 1610, 1580;

EIMS m/z (rel int.): 316 (M^+, 31), 298 ($(M-H_2O)^+$, 6), 284 $(M-MeOH)^+$, 100), 266 ($(M-H_2O-MeOH)^+$, 9), 256 ($(M-MeOH-CO)^+$, 28), 228 $(M-MeOH-2CO)^+$, 22), 210 ($(M-MeOH-2CO-H_2O)^+$, 20), 128 (30), 43 ($(MeCO)^+$, 20);

^1H NMR ($CDCl_3$/MeOH-d_4; 1:1, 250 MHz): δ 6.85 (1H, s, H-5), 6.83 (1H, s, H-10),4.35 (1H, m, H-3), 3.20 (1H, dd, J = 17.3, 3.7 Hz, CH_2-4), 3.00 (1H, dd, J = 17.3, 15.6 Hz, CH_2-4), 3.00 (1H, dd, J = 17.3, 3.7 Hz, CH_2-2), 2.75 (1H, dd, J = 17.3, 15.6 Hz, CH_2-2), 3.98 (3H, S, COOMe-7), 2.87 (3H, s, Me-8), (15.20 (1H, s, OH-9) observed when run in DMSO-d_6).

Fractional crystallization of fraction P from EtOAc also gave orange crystals of laccaic acid-D methyl ester (10) (1.5 mg), $C_{17}H_{12}O_6$, mp. >258°C (dec.) (EtOAc), lit. mp. 270-275°C (Yagi et al., 1974), Rf 0.55 (S-4), turned red on spraying with KOH and brown with fast blue salt B. Yellow solution in methanol

turned red after six hrs;

UV λ_{max} (MeOH, run immediately) (log ε) nm: 220(5.2), 268(5.3), 282(5.2), 336(3.5) 396(4.5), 420(4.6), 435(4.5);

UV λ_{max} (MeOH run after six hrs) nm: 228, 306, 380, 508;

UV λ_{max} (MeOH/HCl, run by adding HCl after keeping the sample in MeOH for six hrs) nm: 220, 268, 282, 336,396, 420, 435;

UV λ_{max} (MeOH/KOH) nm: 228, 306, 380, 508;

UV λ_{max} ($CHCl_3$, run after six hrs) nm: 238, 278, 282, 336, 390, 410, 435.

UV λ_{max} (MeCN, run after six hrs) nm: 224, 252, 274, 282, 322, 360, 524. This compound was further identified by direct comparison with authentic sample (UV, co-TLC, HPLC).

4.6. CONVERSION OF ALOEGRAMONOL (1) INTO CHRYSOPHANOL (2).

Aloegramonol (1) (10 mg) was dissolved in 5% methanolic KOH and the solution was allowed to stand at room temp., for three days. The solvent was removed under reduced pressure and the resulting residue was diluted with 10 ml water. The solution was then neutralized with dil. HCl and extracted with CH_2Cl_2

(3X15 ml). The extract was dried (anhydrous Na_2SO_4) and the solvent evaporated under reduced pressure. The product was finally purified on silica gel PTLC (S-1) and identified as **chrysophanol** (2) (6 mg), by direct comparison (UV, IR, mmp co-TLC) with a sample isolated from the plant.

4.7. REDUCTIVE CLEAVAGE OF ASPHODELIN (3).

In a round bottom flask containing **asphodelin** (3) (15 mg), 5 ml of 5% KOH and $Na_2S_2O_4$ (20 mg) were added and the mixture refluxed at 80°C for 1 hr. The product was then allowed to cool to room temp., acidified and extracted into **dichloromethane**. The extract was then dried (anhydrous Na_2SO_4) and the solvent removed under reduced pressure. The product was purified by PTLC and recrystallized from MeOH to give **chrysophanol** (2) (9 mg) and identified by direct comparison (co-TLC, UV, mmp) with a sample isolated from the plant.

4.8. CONVERSION OF ALOESAPONOL-I (5) INTO ALOESAPONARIN-I (6).

Aloesaponol-I (2) (10 mg) was dissolved in 5% **methanolic** KOH and the solution was allowed to stand for three days at room temp. The colourless solution gradually turned red. After the **methanol** was removed,

turned red after six hrs;

UV λ_{max} (MeOH, run immediately) (log ε) nm: 220(5.2), 268(5.3), 282(5.2), 336(3.5) 396(4.5), 420(4.6), 435(4.5);

UV λ_{max} (MeOH run after six hrs) nm: 228, 306, 380, 508;

UV λ_{max} (MeOH/HCl, run by adding HCl after keeping the sample in MeOH for six hrs) nm: 220, 268, 282, 336,396, 420, 435;

UV λ_{max} (MeOH/KOH) nm: 228, 306, 380, 508;

UV λ_{max} ($CHCl_3$, run after six hrs) nm: 238, 278, 282, 336, 390, 410, 435.

UV λ_{max} (MeCN, run after six hrs) nm: 224, 252, 274, 282, 322, 360, 524. This compound was further identified by direct comparison with authentic sample (UV, co-TLC, HPLC).

4.6. CONVERSION OF ALOEGRAMONOL (1) INTO CHRYSOPHANOL (2).

Aloegramonol (1) (10 mg) was dissolved in 5% methanolic KOH and the solution was allowed to stand at room temp., for three days. The solvent was removed under reduced pressure and the resulting residue was diluted with 10 ml water. The solution was then neutralized with dil. HCl and extracted with CH_2Cl_2

(3X15 ml). The extract was dried (anhydrous Na_2SO_4) and the solvent evaporated under reduced pressure. The product was finally purified on silica gel PTLC (S-1) and identified as **chrysophanol** (2) (6 mg), by direct comparison (UV, IR, mmp co-TLC) with a sample isolated from the plant.

4.7. REDUCTIVE CLEAVAGE OF ASPHODELIN (3).

In a round bottom flask containing **asphodelin** (3) (15 mg), 5 ml of 5% KOH and $Na_2S_2O_4$ (20 mg) were added and the mixture refluxed at 80°C for 1 hr. The product was then allowed to cool to room temp., acidified and extracted into **dichloromethane**. The extract was then dried (anhydrous Na_2SO_4) and the solvent removed under reduced pressure. The product was purified by PTLC and recrystallized from MeOH to give **chrysophanol** (2) (9 mg) and identified by direct comparison (co-TLC, UV, mmp) with a sample isolated from the plant.

4.8. CONVERSION OF ALOESAPONOL-I (5) INTO ALOESAPONARIN-I (6).

Aloesaponol-I (2) (10 mg) was dissolved in 5% **methanolic** KOH and the solution was allowed to stand for three days at room temp. The colourless solution gradually turned red. After the **methanol** was removed,

the residue was diluted with water and neutralized with dil. HCl. The product was then extracted into dichloromethane (3x15 ml). The residue obtained, after removing the solvent was crystallized from EtOAc. It was identified as aloesaponarin-I (6) (7 mg) by direct comparison (UV, co-TLC) with a sample isolated from the plant.

4.9. ACETYLATION OF ALOESAPONARIN-I (6).

In a 25 ml round bottom flask containing compound 6 (20 mg), acetic anhydride (4 ml) and pyridine (three drops) were added. The mixture was refluxed on water bath at 80°C for two hours, which was then allowed to cool to room temperature and poured over ice cold water. The resulting precipitate was filtered, dissolved in chloroform and dried (anhydrous Na_2SO_4). Fractional crystallization of the product from acetone gave light yellow needles of 8-acetoxyaloesaponarin-I (6a) (8mg), $C_{19}H_{14}O_7$, mp. 190-193°C;

UV λ_{max}(MeOH) (log ε) nm: 238(5.2), 270(5.4), 322(4.6), 364(4.4);

EIMS m/z (rel. int.): 354 (M^+, 45), 336 ($(M-H_2O)^+$, 7), 312 ($(M-Ac)^+$, 76), 297 ($(M-Ac-CH_3)^+$, 25), 280 ($(M-Ac-MeOH)^+$, 100), 252 ($(M-Ac-MeOH-CO)^+$, 32), 224 ($(M-Ac-MeOH-2CO)^+$, 29), 196 ($(M-Ac-MeOH-3CO)^+$, 11), 168 ($(M-Ac-MeOH-4CO)^+$, 19), 139 ($(M-Ac-MeOH-5CO)^+$, 45);

^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.15 (1H, dd, J = 7.5, 1.3 Hz, H-5), 7.70 (1H, s, H-4), 7.72 (1H, t, J = 7.76 Hz, H-6), 7.41 (1H, dd, J = 7.5, 1.3 Hz, H-7), 4.04 (3H, s, COO<u>Me</u>-2), 2.82 (3H, s, Me-1), 2.47 (3H, s, OAc-8).

Fractional crystallization of the acetylation product from acetone also gave light yellow crystals of 3,8-diacetoxyaloesaponarin-I (<u>6b</u>)(9 mg) $C_{21}H_{16}O_8$, mp. 195-197 ^{0}C , lit. mp. 203-206^{0}C);

UV λ_{max}(MeOH) (log ε) nm: 244(5.3), 274(5.5), 337(4.2);

EIMS m/z (rel int.): 396 (M^+, 18), 354 ($(M-Ac)^+$, 27), 312 ($(M-2Ac)^+$, 52), 297 ($(M-2Ac-Me)^+$, 14), 280 ($(M-2Ac-MeOH)^+$, 100), 252 ($(M-2Ac-MeOH-CO)^+$, 224 ($(M-2Ac-MeOH-2CO)^+$, 13), 196 ($(M-2Ac-MeOH-3CO)^+$, 5), 168 ($(M-2Ac-MeOH-4CO)^+$, 7), 139 ($(M-2Ac-MeOH-5CO)^+$, 15);

^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.15 (1H, dd, J = 7.5, 1.3 Hz, H-5), 7.71 (1H, s, H-4), 7.73 (1H, t, J = 7.7 Hz, H-6), 7.41 (1H, dd, J = 7.5, 1.3 Hz, H-7), 4.04 (3H, s, COO<u>Me</u>-2), 2.82 (3H, s, Me-1), 2.47 (3H, s, OAc-8), 2.18 (3H, s, OAc-3).

4.10. ANTIBACTERIAL TESTS.

4.10.1. SAMPLES PREPARATION: 500 µg of the acetone extracts of the roots, flowers and subterranean stems of <u>A. graminicola</u> were dissolved in 1 ml of acetone

and serially diluted to give 500, 250, and 125 µg/ml solutions. Blank disks (diameter 0.5 cm) were soaked in these solutions until saturation and allowed to dry in open air. Control disks were prepared by applying 5 µl, from a 500 µg/ml solution of streptomycin. The extracts were tested against four species of bacteria, namely, E. coli, S. albus, S. cereviciae and B. subtilis.

4.10.2. MEDIA PREPARATION: 28 gm of nutrient agar was mixed with 1000 ml of double distilled water and heated to boiling. The hot mixture was then sterilized in an autoclave. The sterilized agar was transfered into 115 petridish each containing 10 ml of the agar. The dishes were left to cool and settle in a laminar flow cabinet before they were stored at 4°C.

The nutrient agar was then streaked with the bacteria and the disks containing the extracts and the control were placed on the surface. It was then incubated at 30°C and antibacterial activities were checked after 24 hrs. The tests were done in four replicates. The inhibition of growth of bacteria around the disk containing the extract was taken as a positive antibacterial activity. The results are summarized in Table-11.

Table-11. Results of antibacterial tests on *A. graminicola*.

Sample	Conc.	1	2	3	4
RT	500 µg/ml	+	+	-	-
"	250 µg/ml	+	+	-	-
"	125 µg/ml	-	-	-	-
SST	500 µg/ml	-	-	-	-
"	250 µg/ml	-	-	-	-
"	125 µg/ml	-	-	-	-
FR	500 µg/ml	-	-	-	-
"	250 µg/ml	-	-	-	-
"	125 µg/ml	-	-	-	-

1 = *E. coli*, 2 = *B. subtilis*,

3 = *S. albus*, 4 = *S. cerevicie*

RT = roots, SST = subterranean stems,

FR = flowers.

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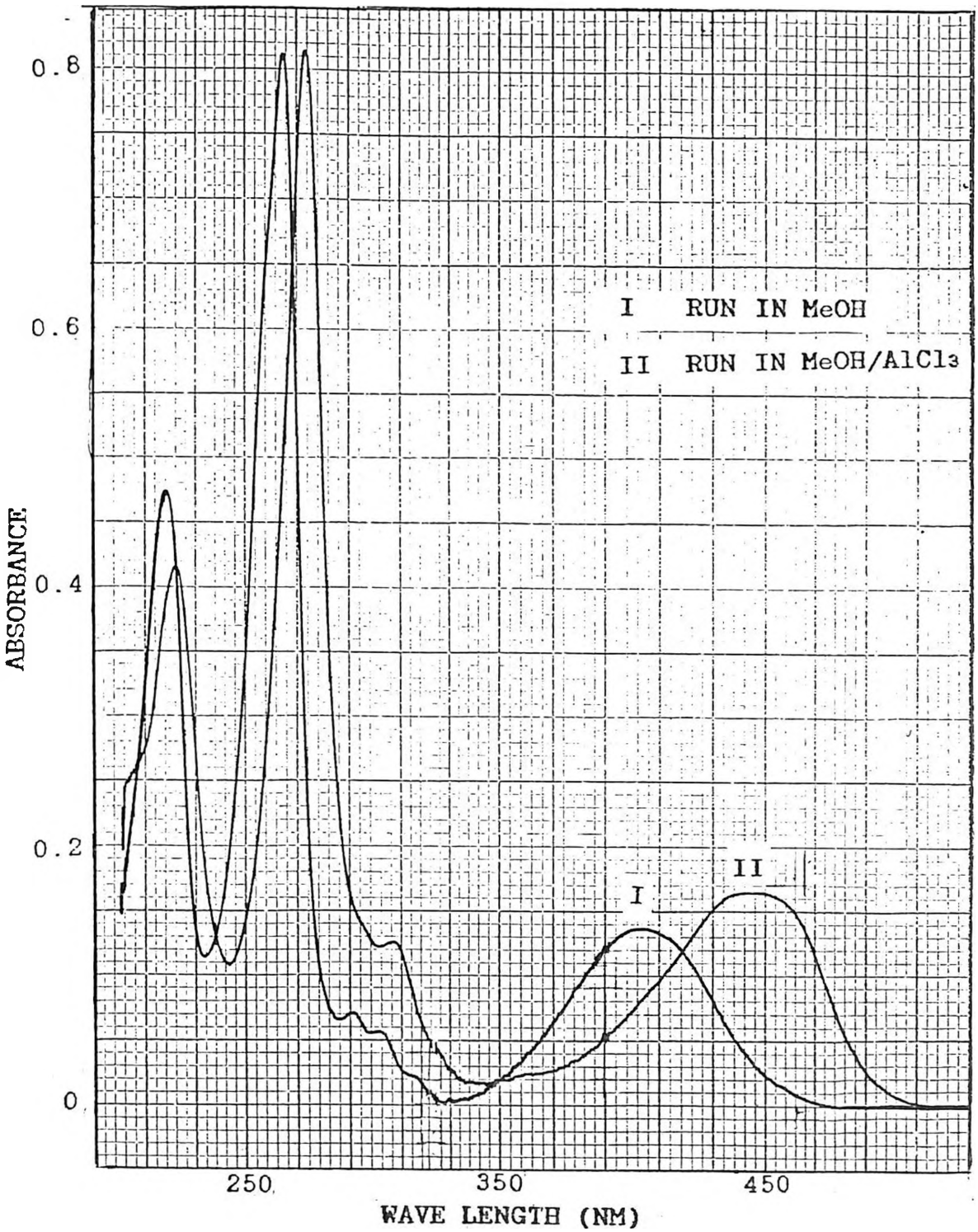
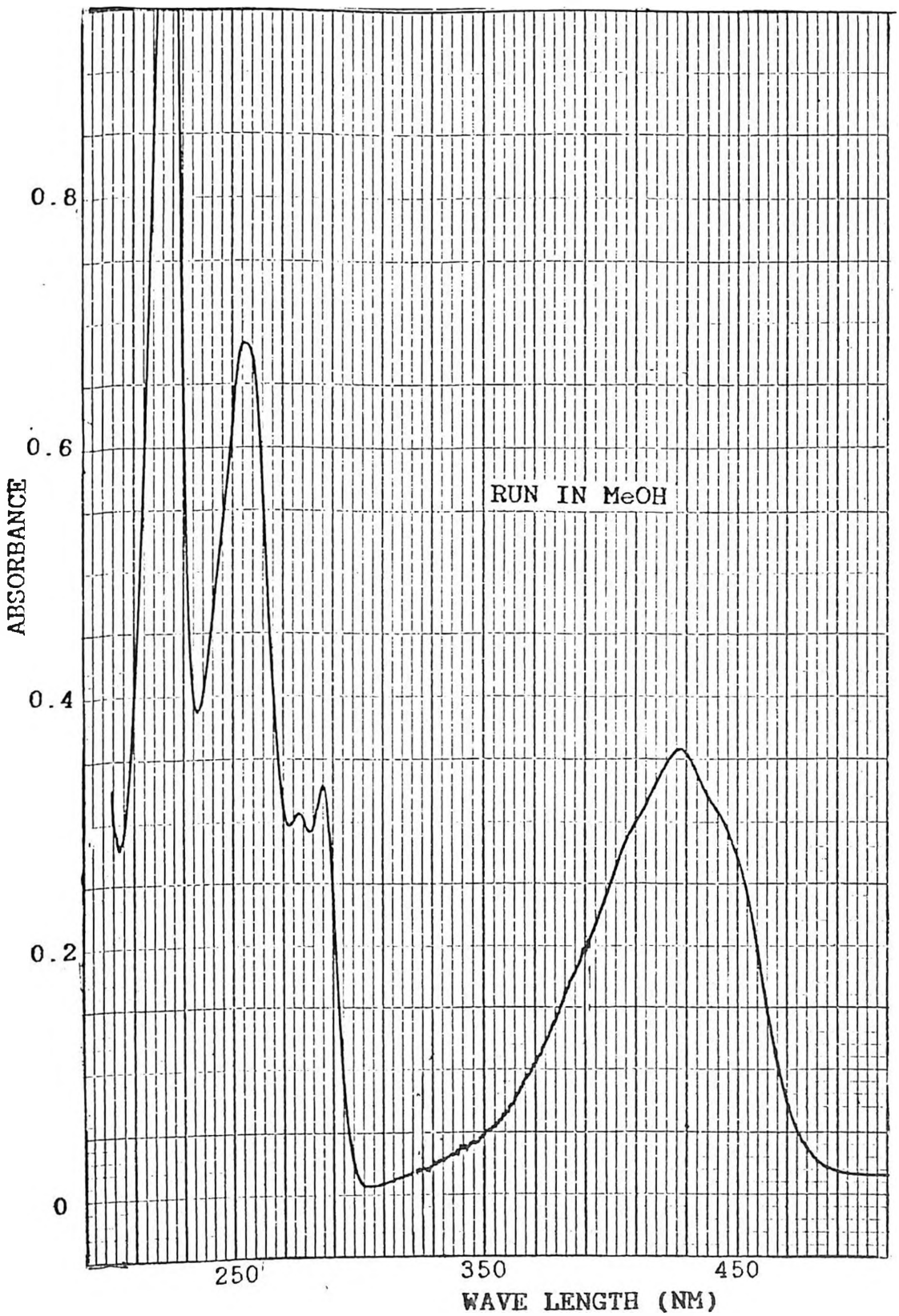
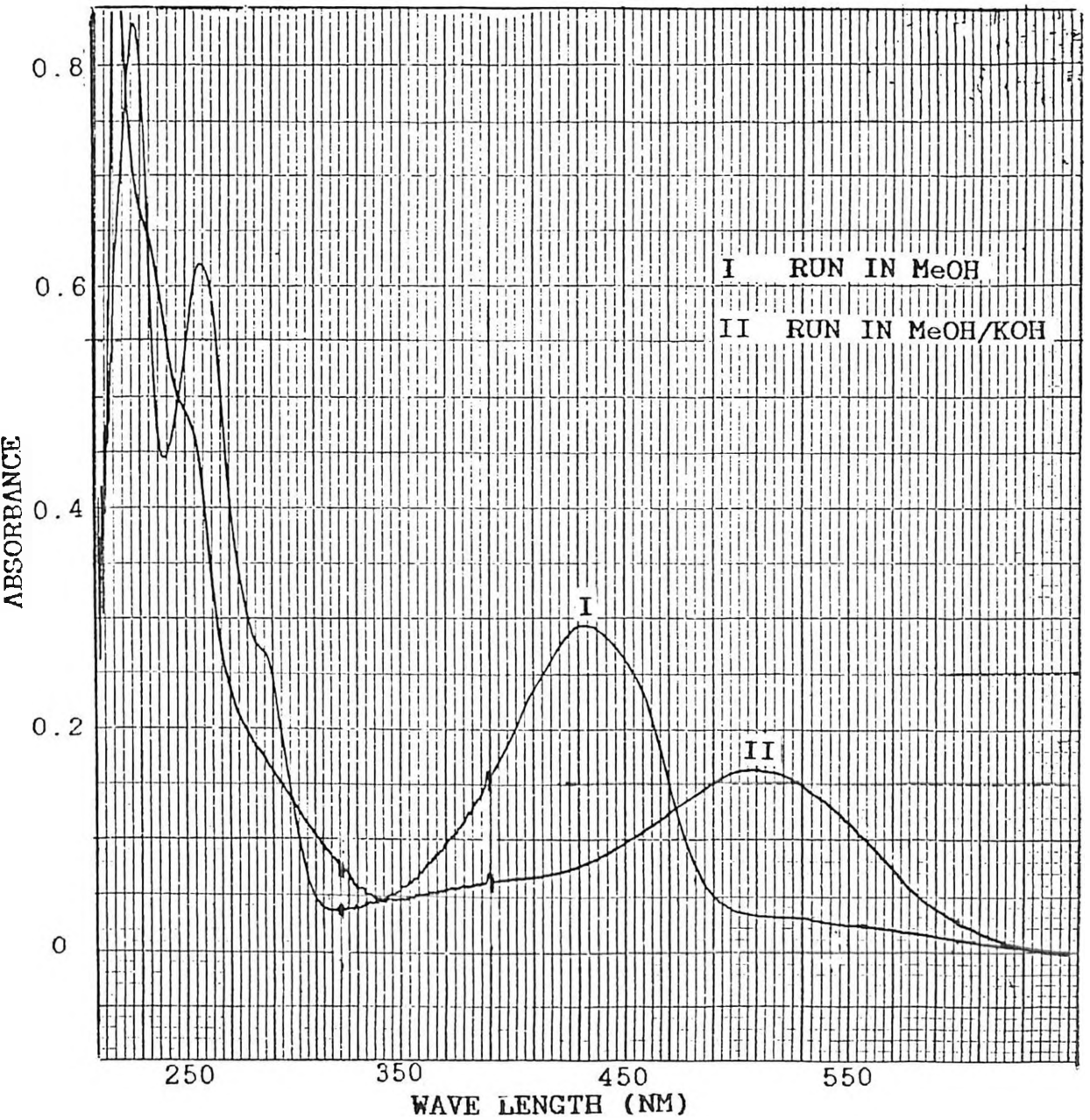
UV SPECTRUM OF ALOEGRAMONOL (1)



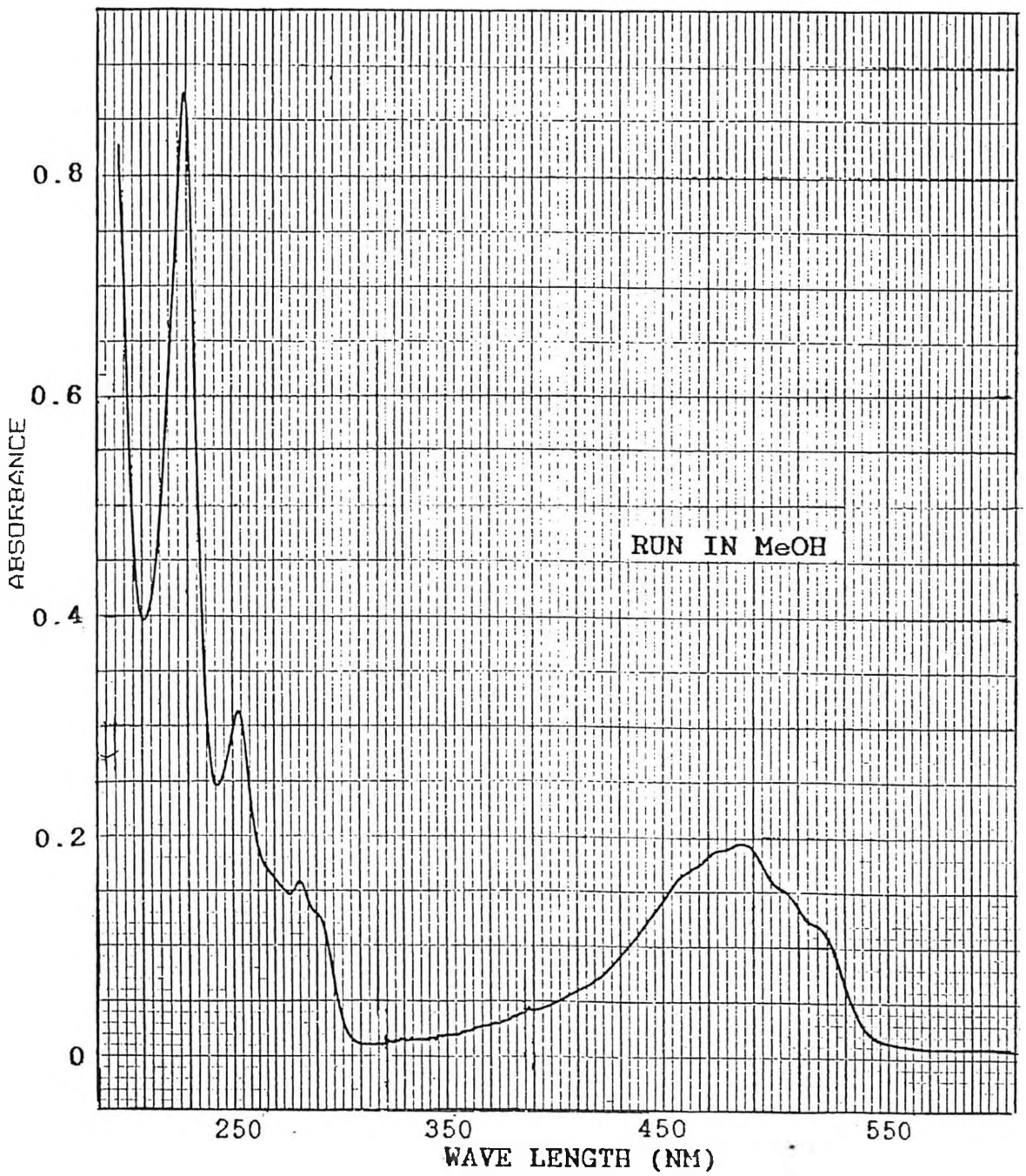
UV SPECTRUM OF CHRYSOPHANOL (2)



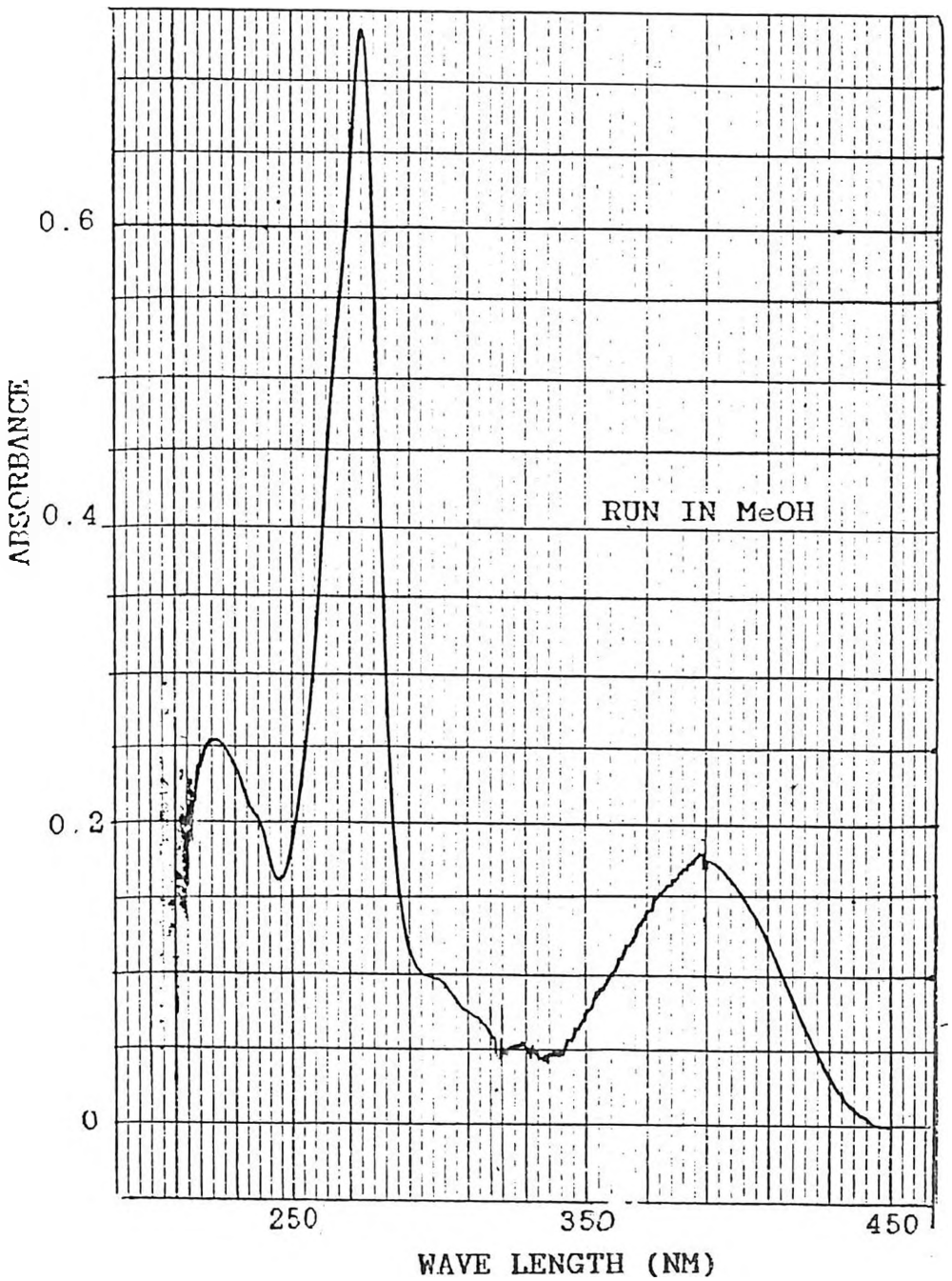
UV SPECTRUM OF ASPHODELIN (3)



UV SPECTRUM OF HELMINTHOSPORIN (4)



UV SPECTRUM OF ALOESAPONOL-I (5)



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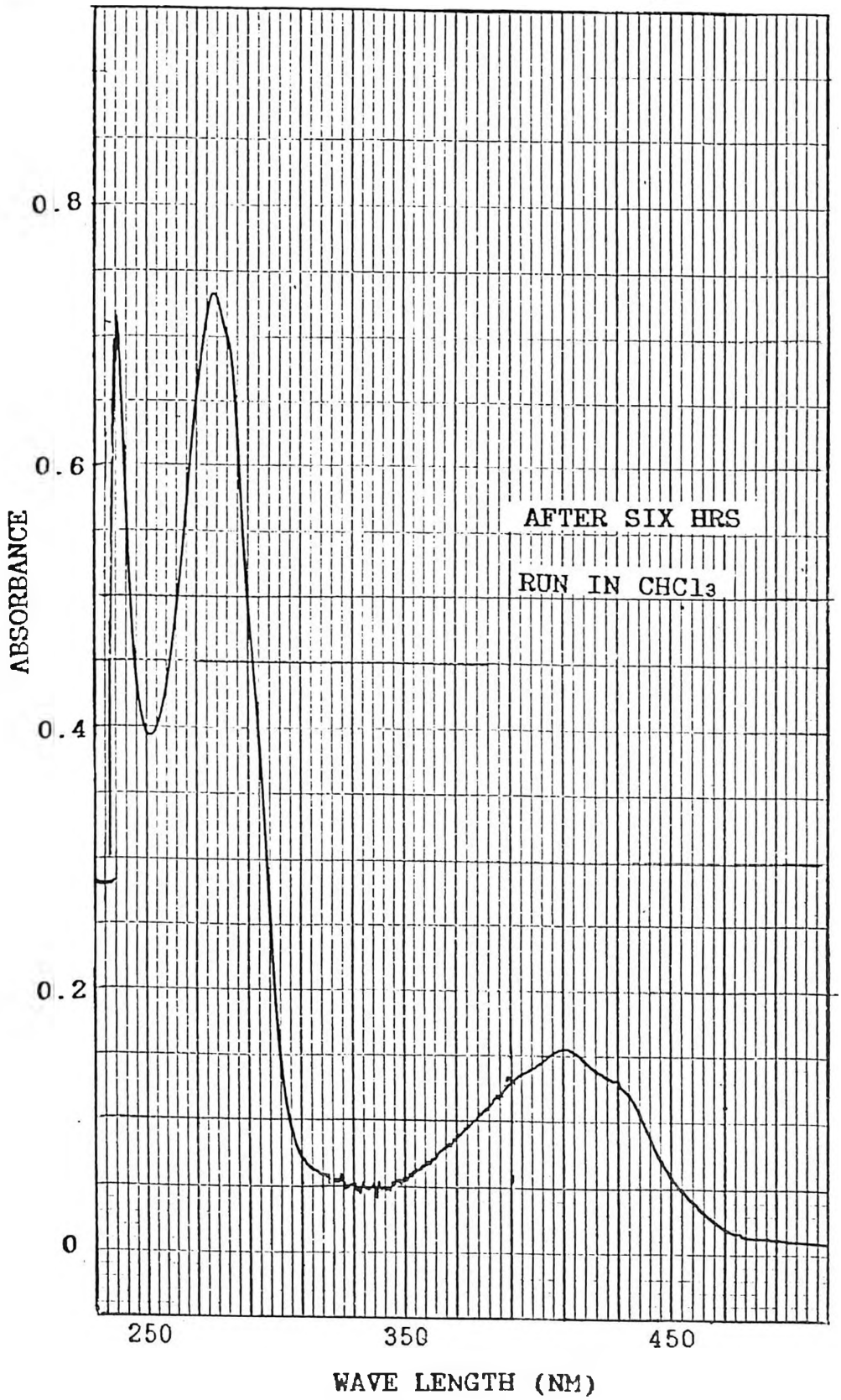

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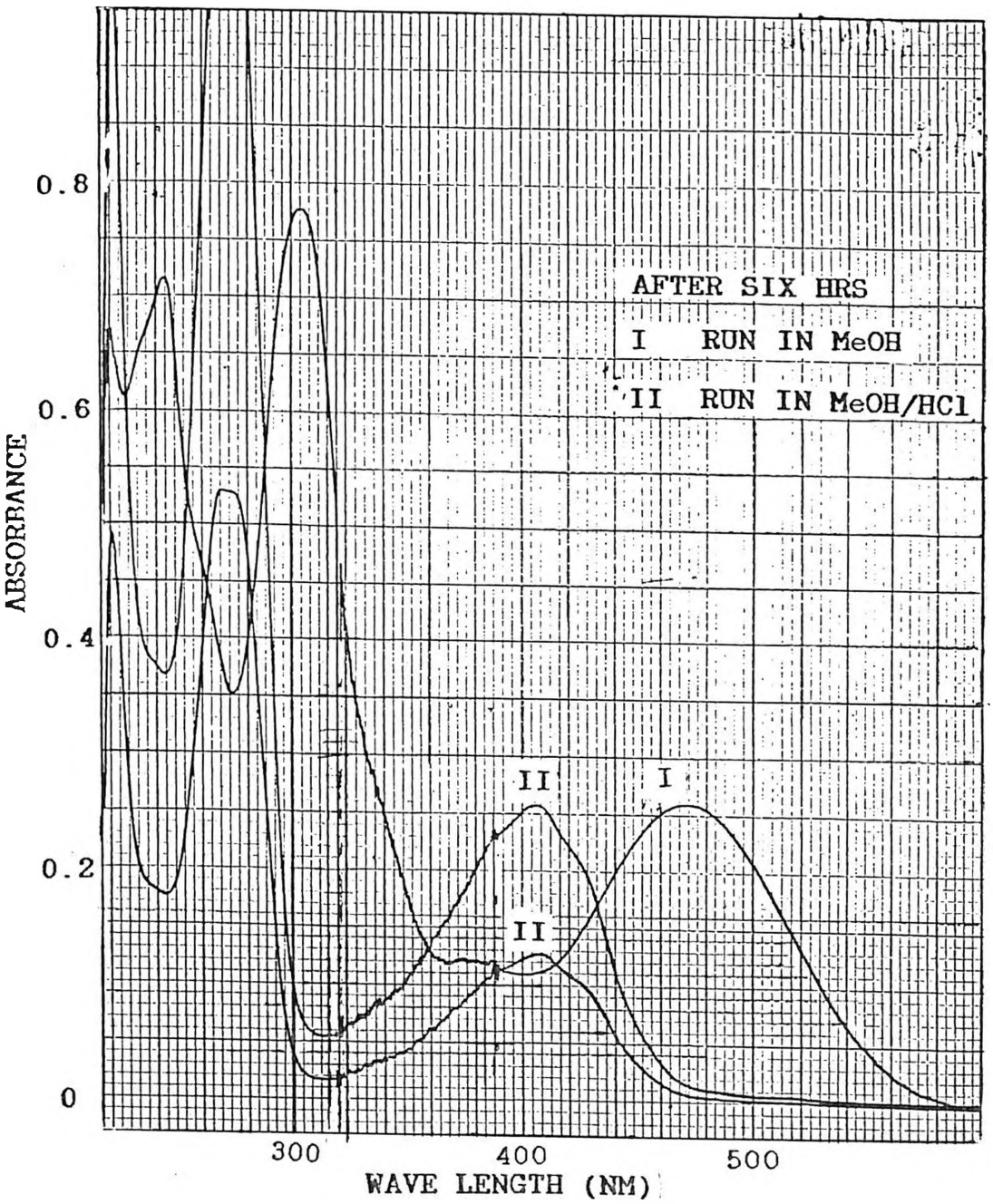
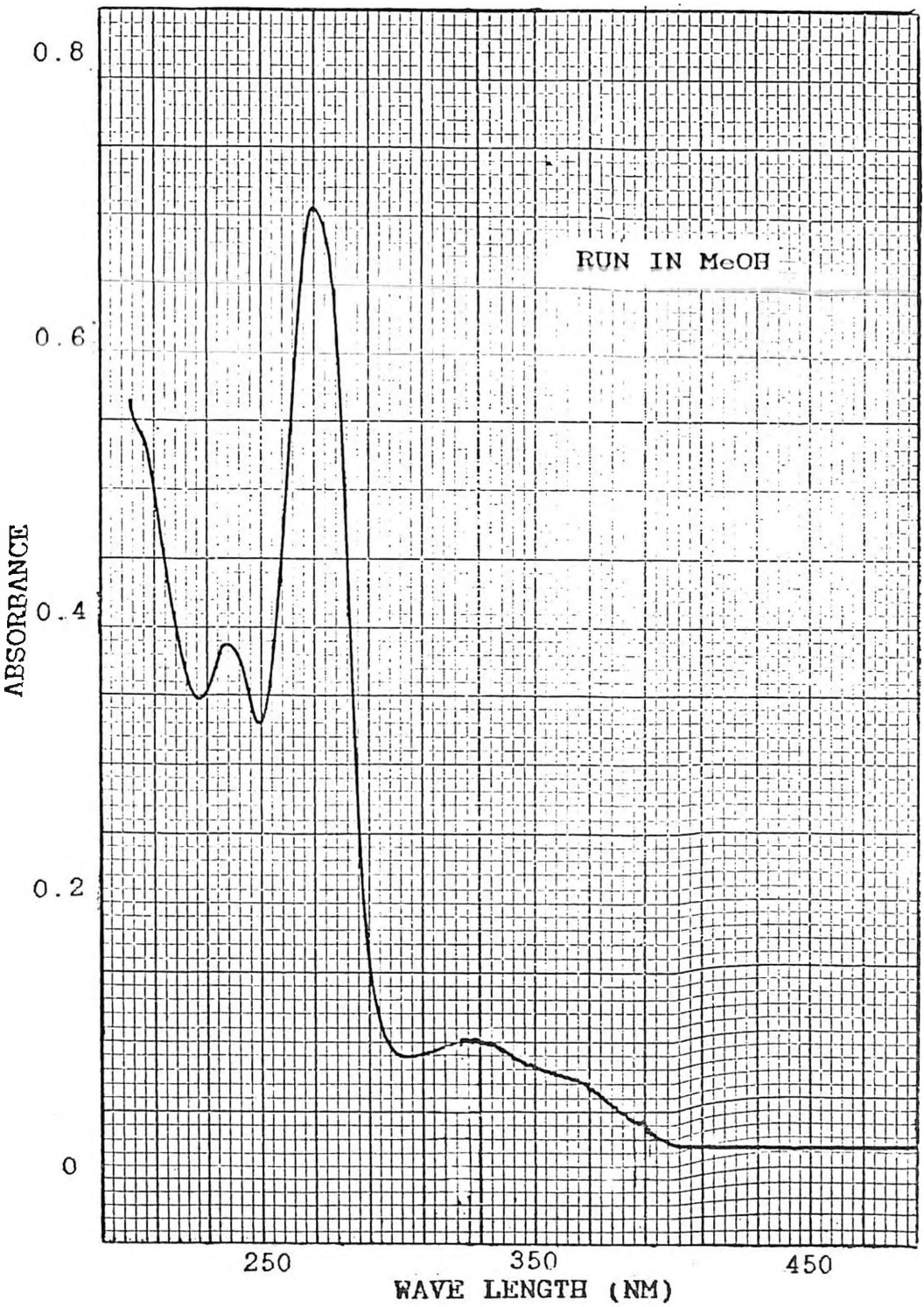


UV SPECTRUM OF 6-ACETOXYALOESAPONARIN-I (6a)



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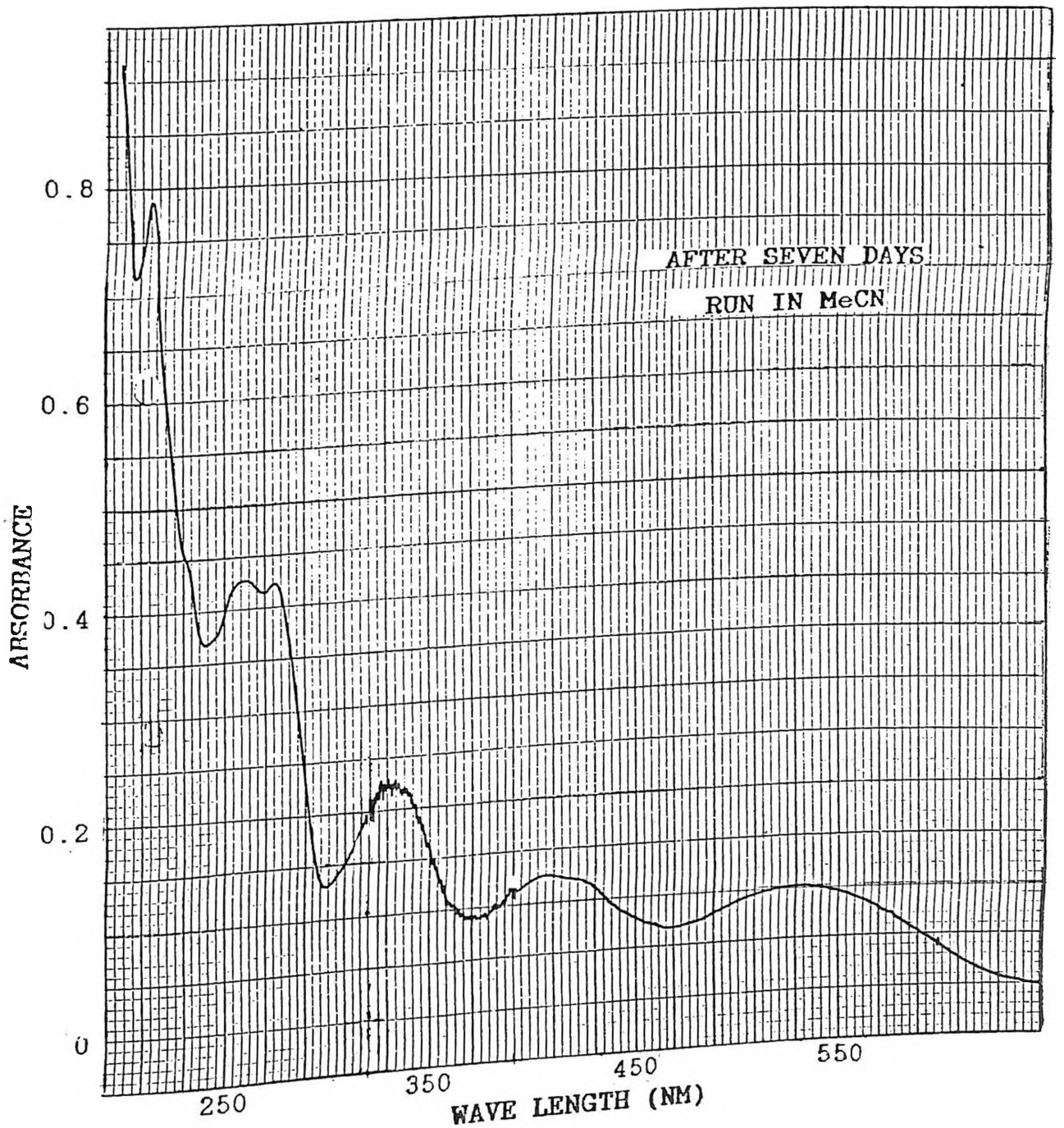

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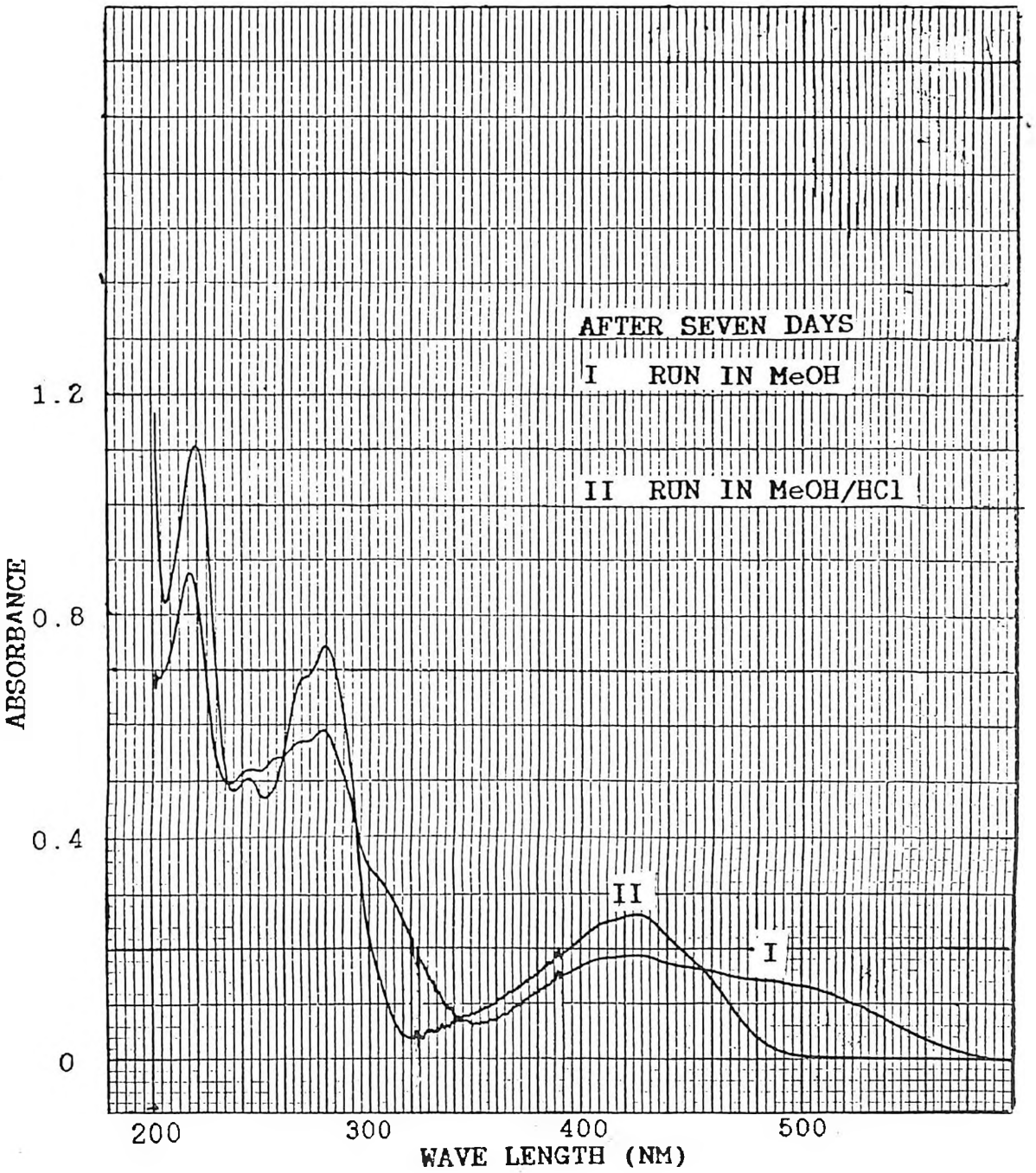
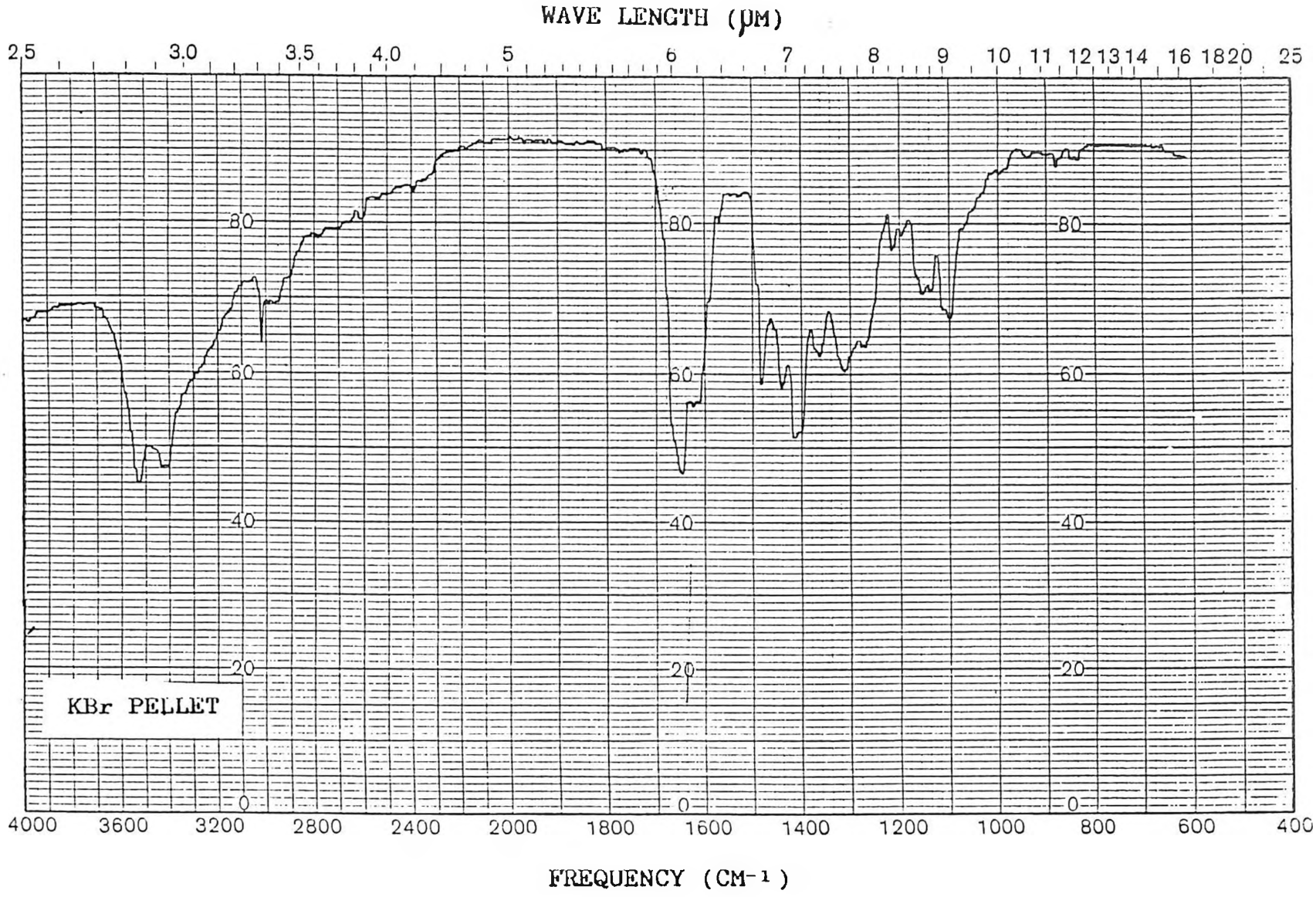
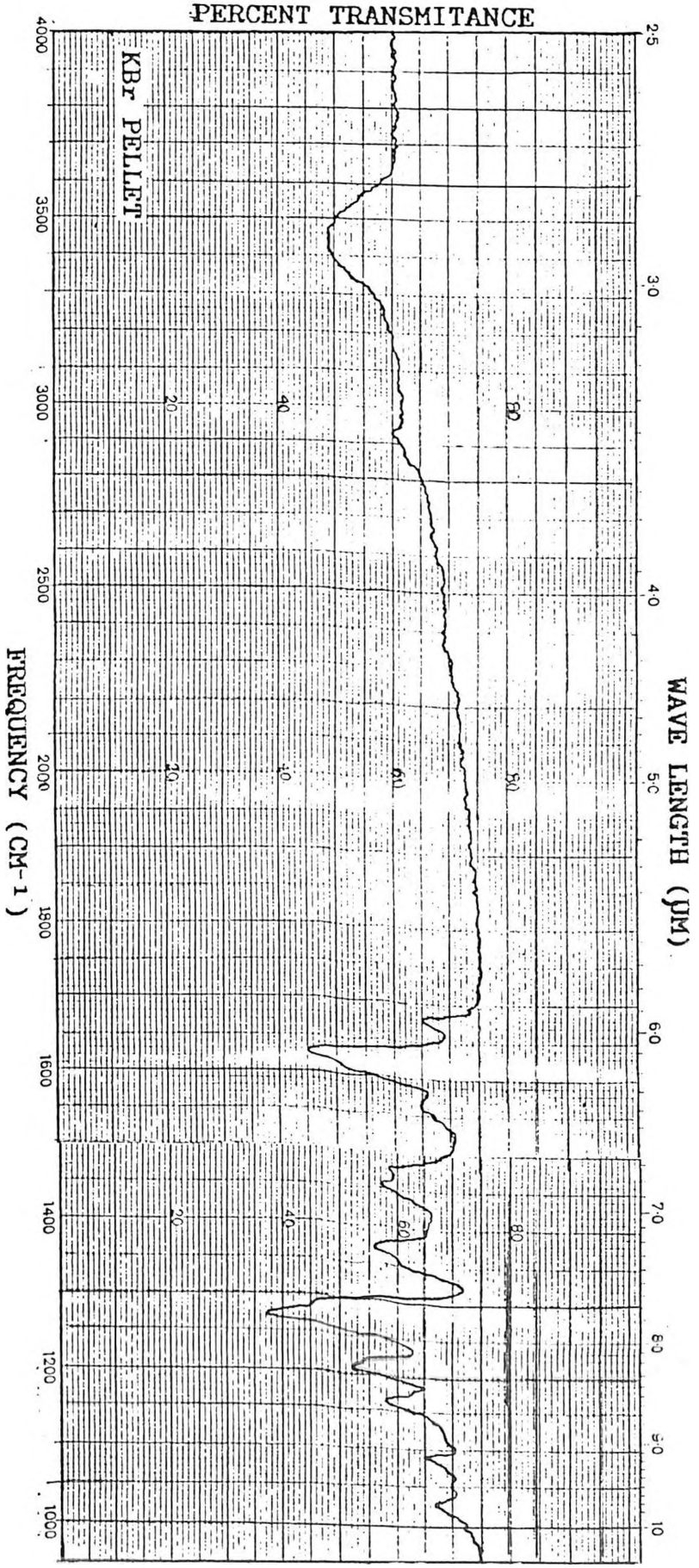


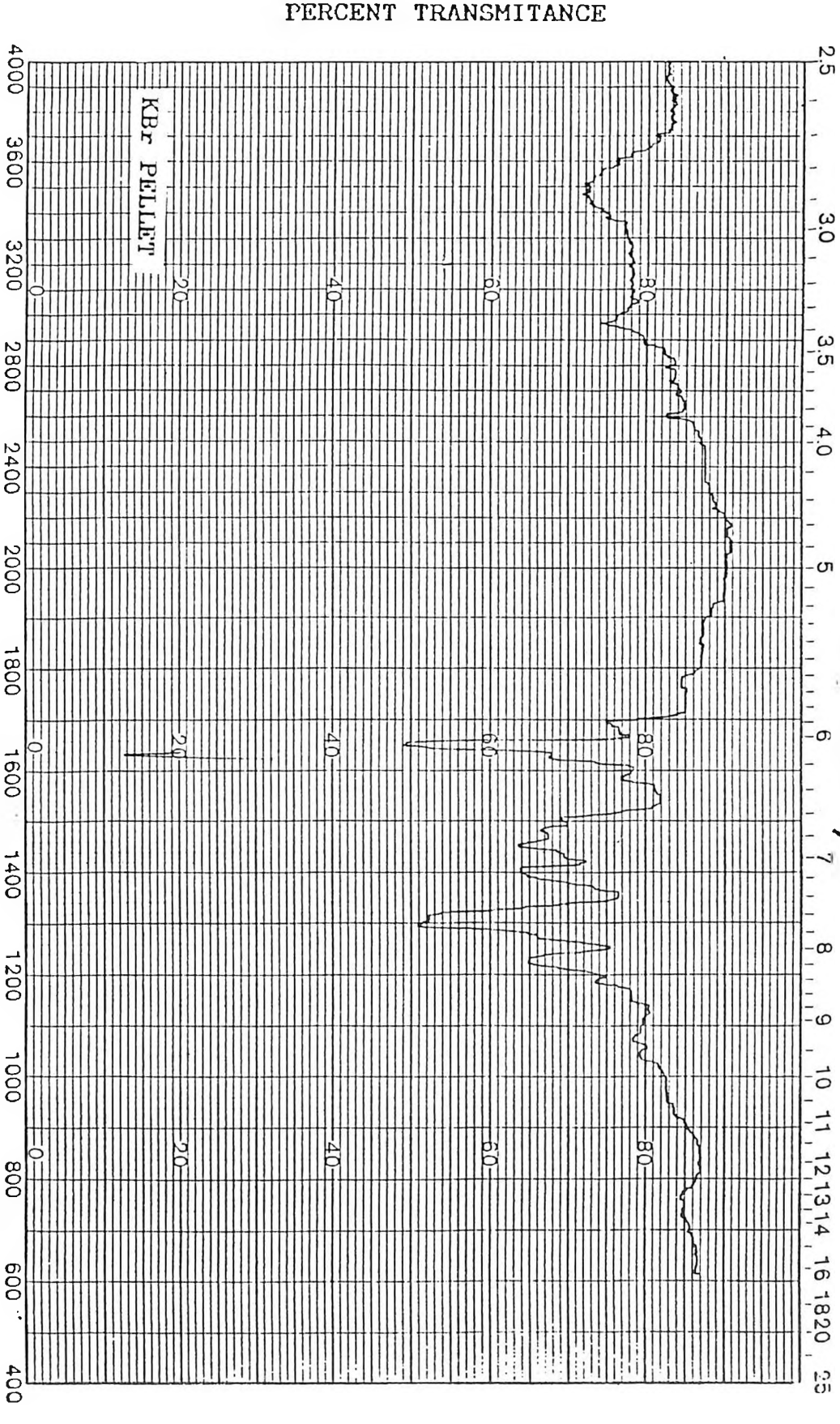
IR SPECTRUM OF ALOEGRAMONOL (1)



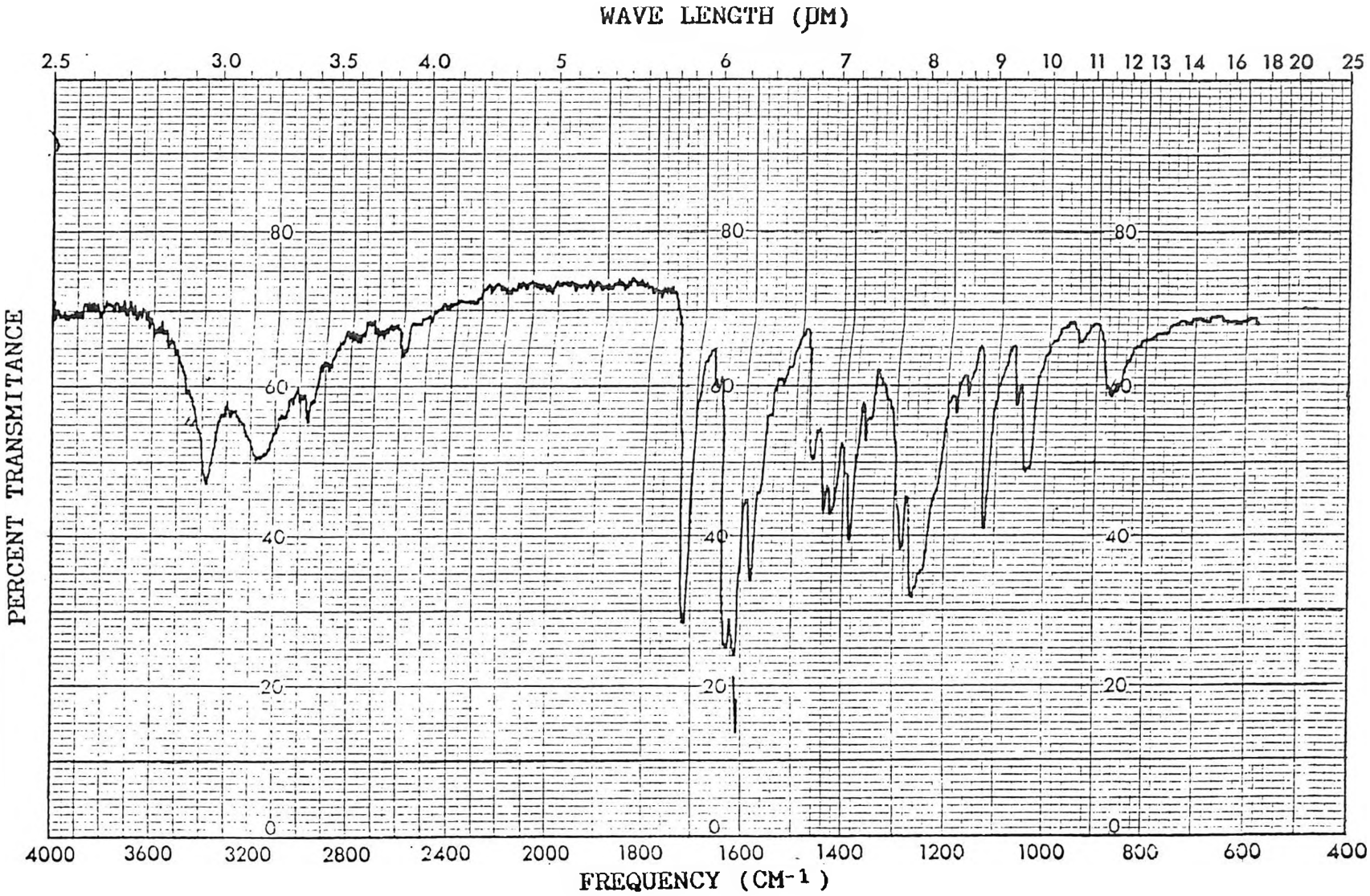
IR SPECTRUM OF CHRYSOPHANOL (2)



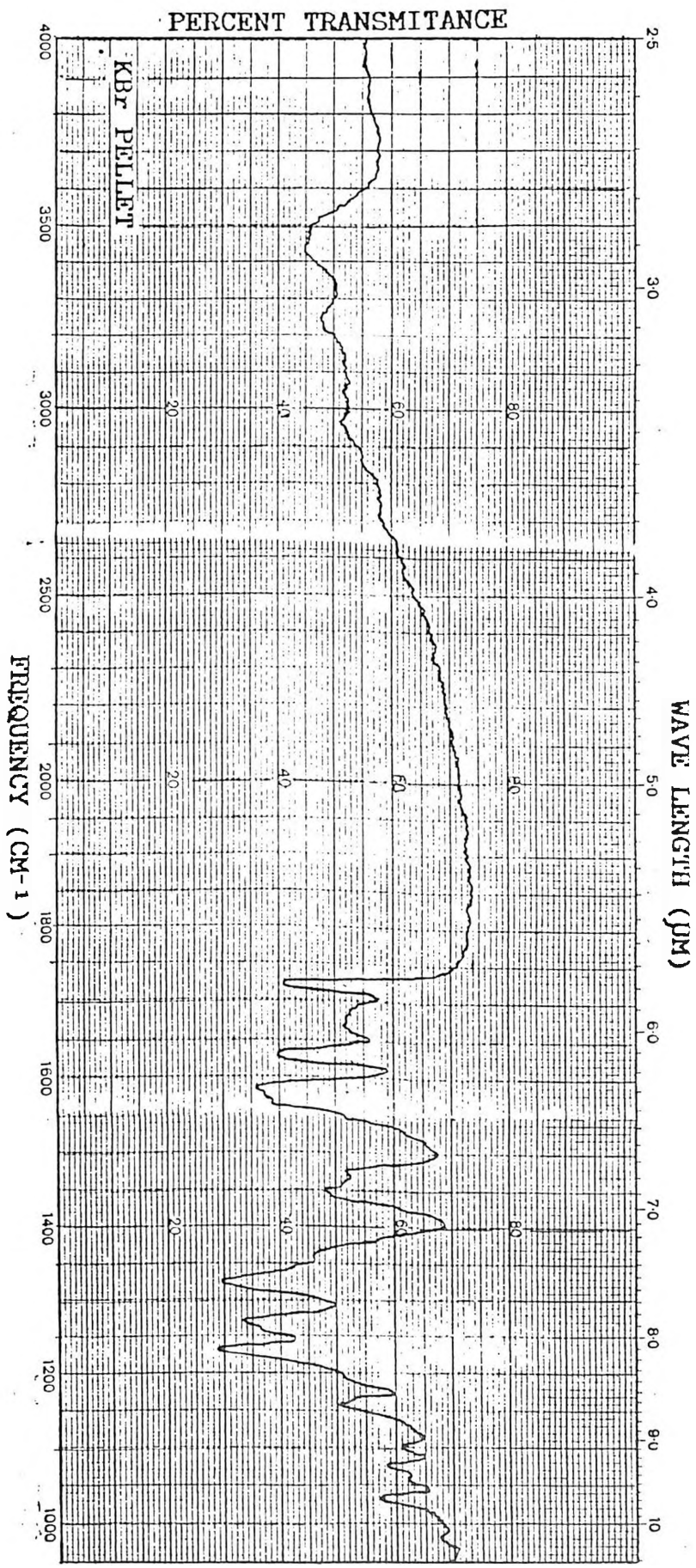
IR SPECTRUM OF ASPHODELIN (3)



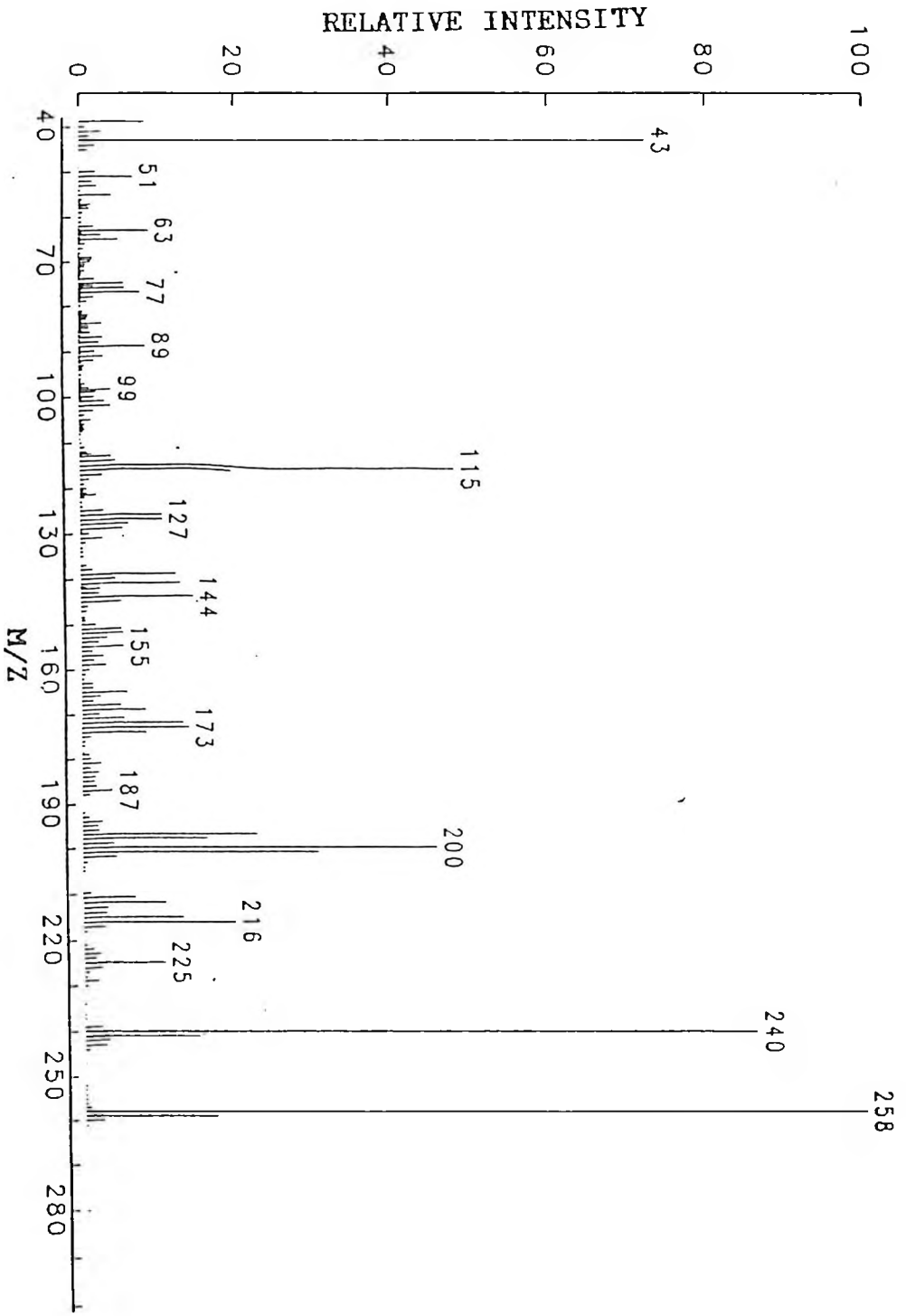
IR SPECTRUM OF ALOESAPONOL-I (5)



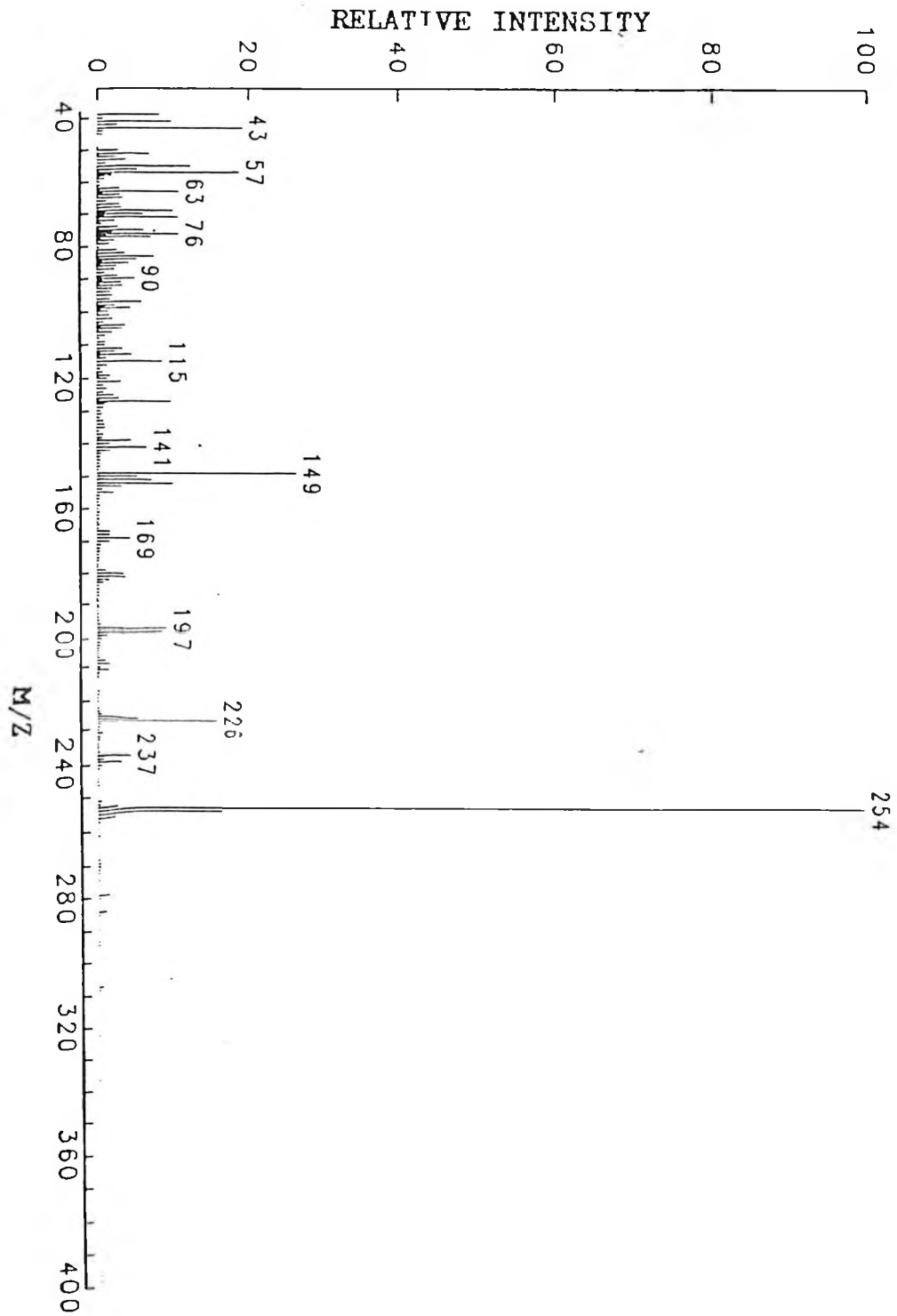
IR SPECTRUM OF ALOESAPONARIN-I (6)



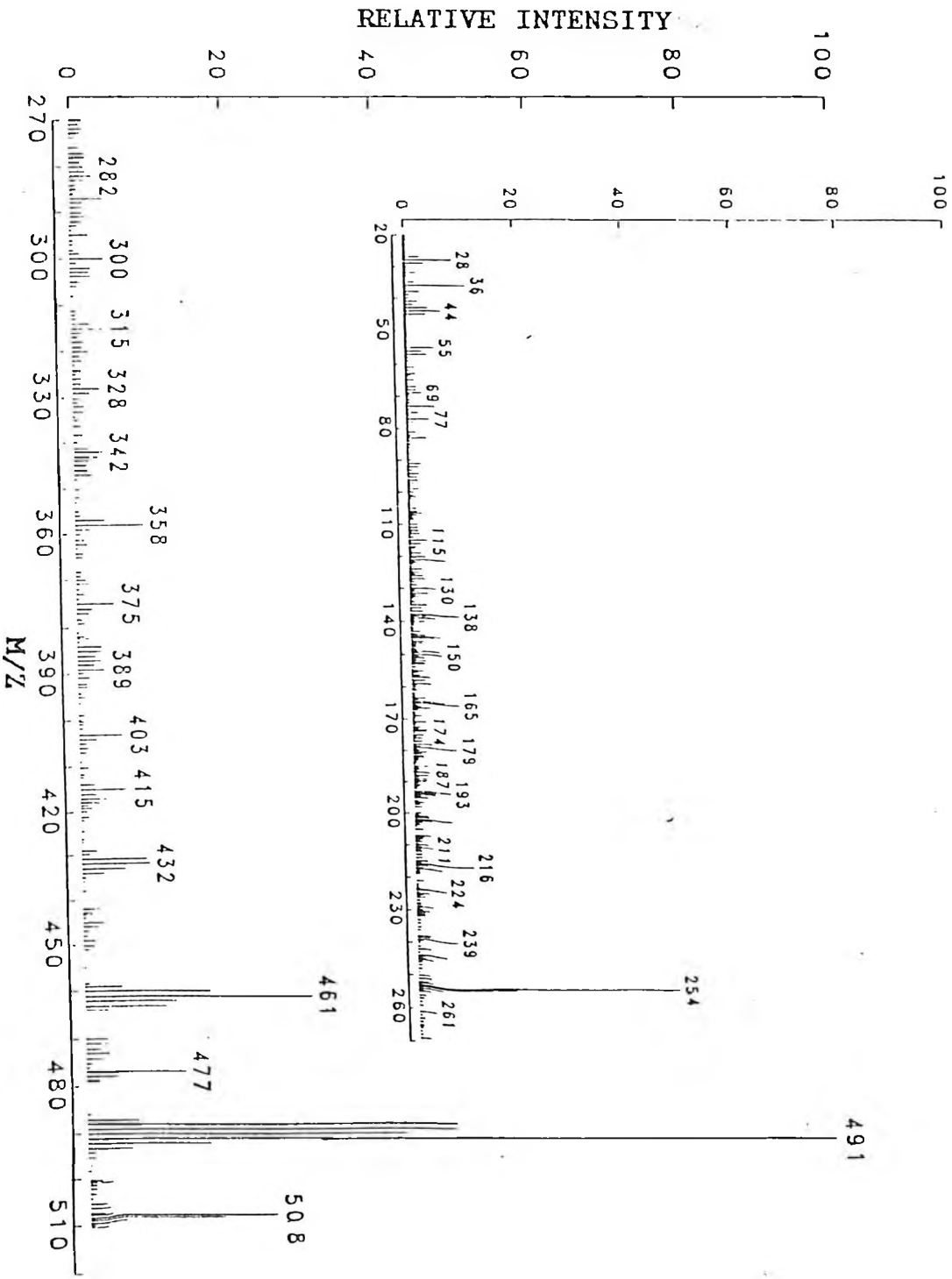
EIMS OF ALOEGRAMONOL (1)



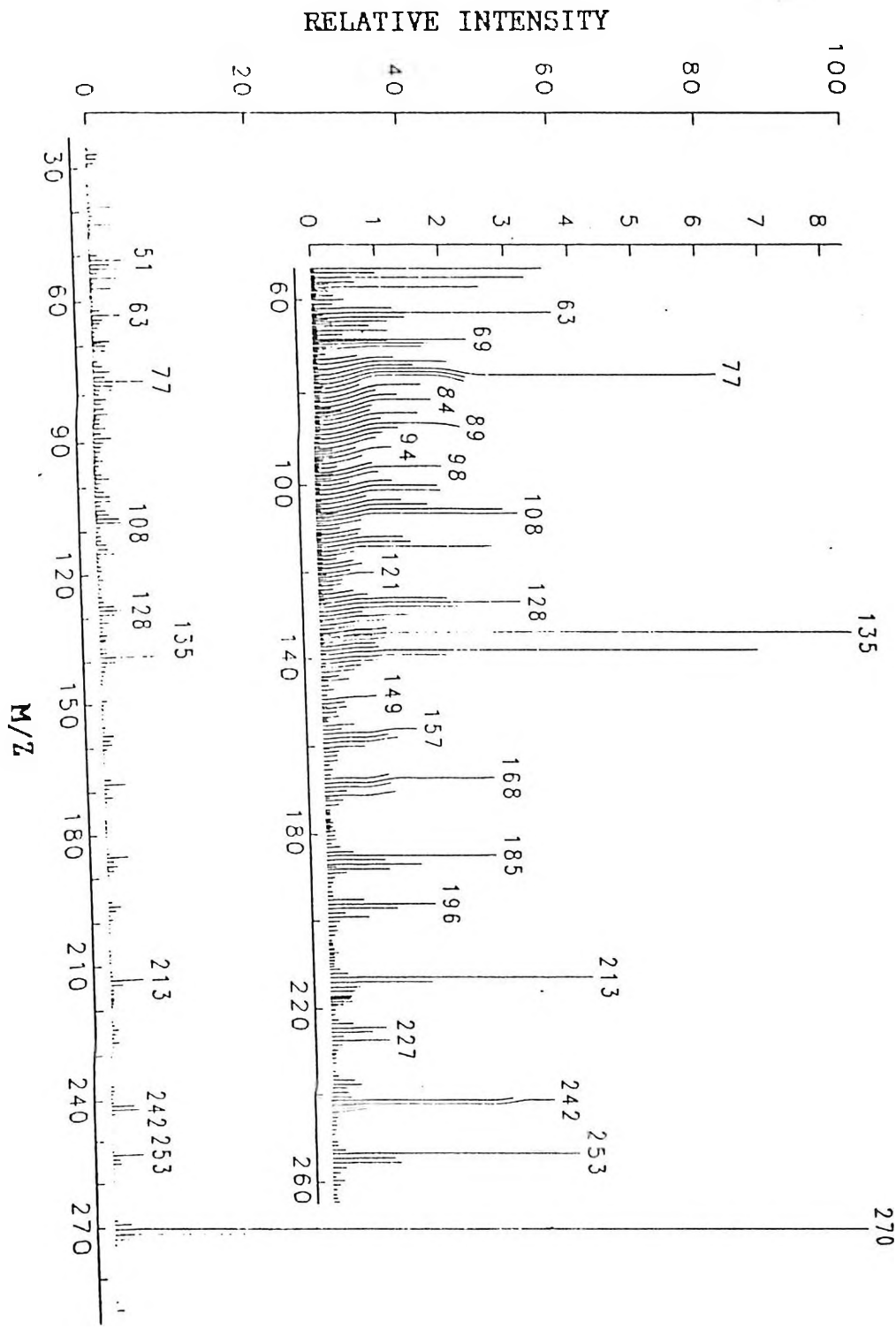
EIMS OF CHRYSOPHANOL (2)



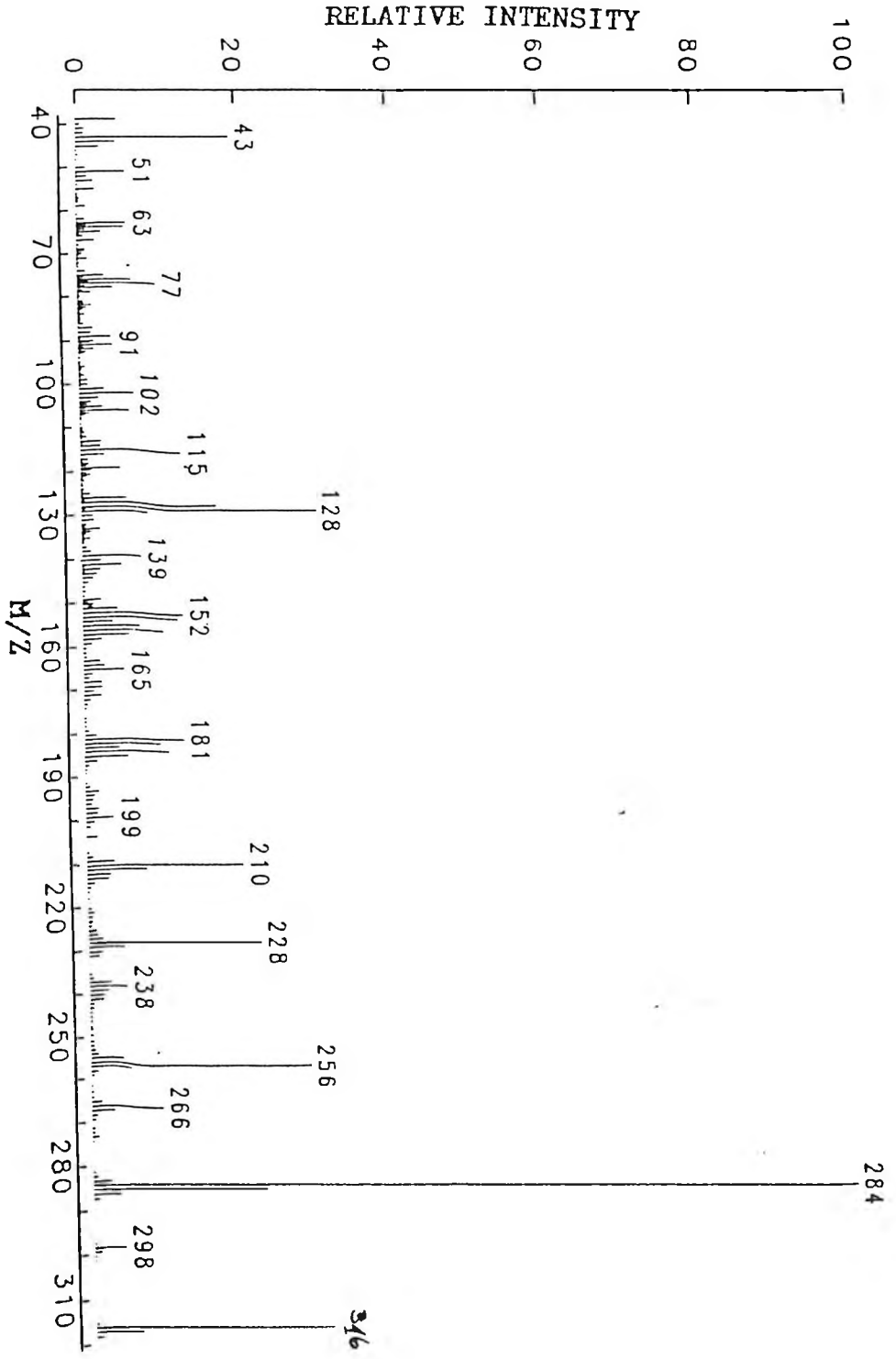
EIMS OF ASPHODELIN (3)



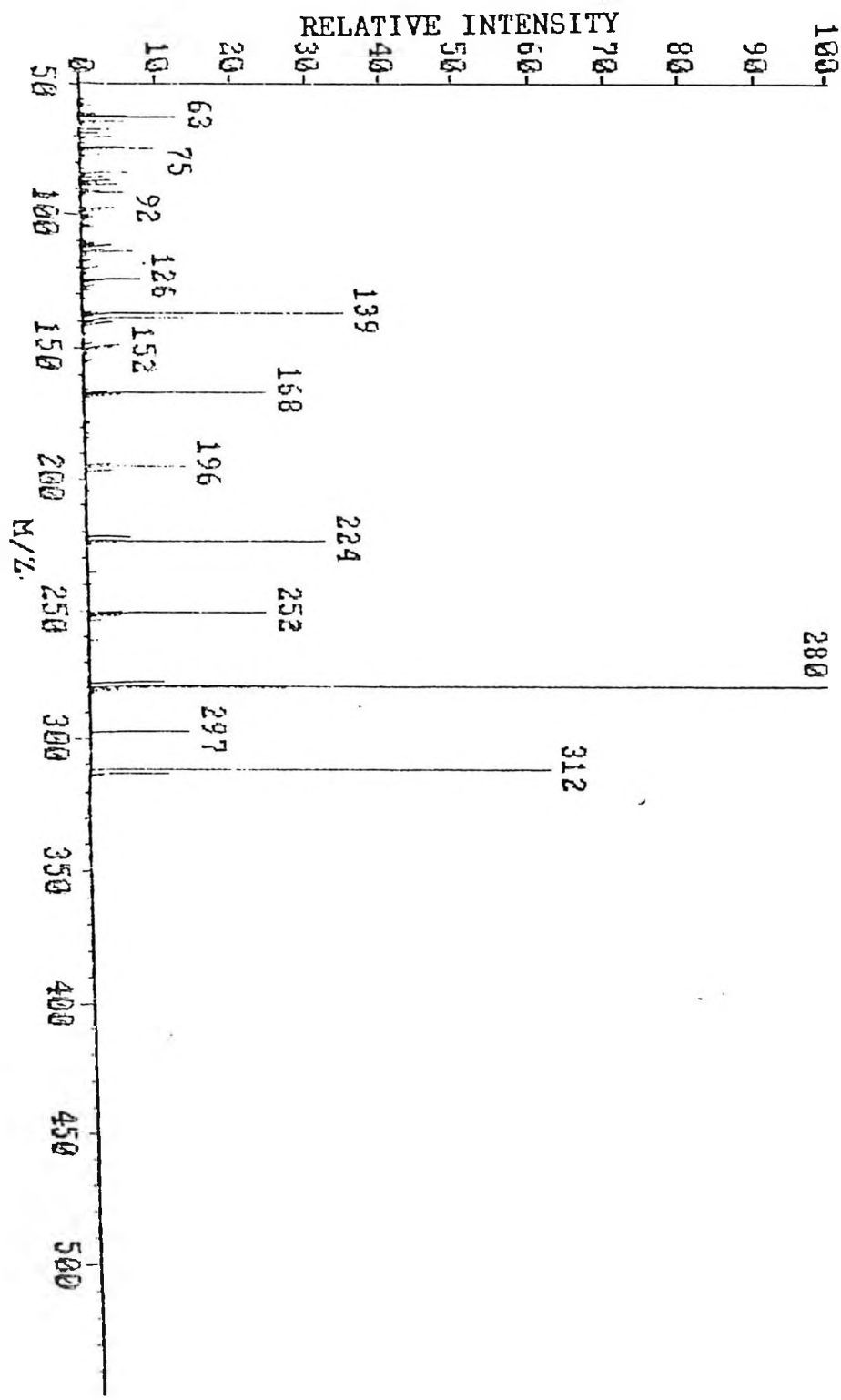
EIMS OF HELMINTHOSPORIN (4)



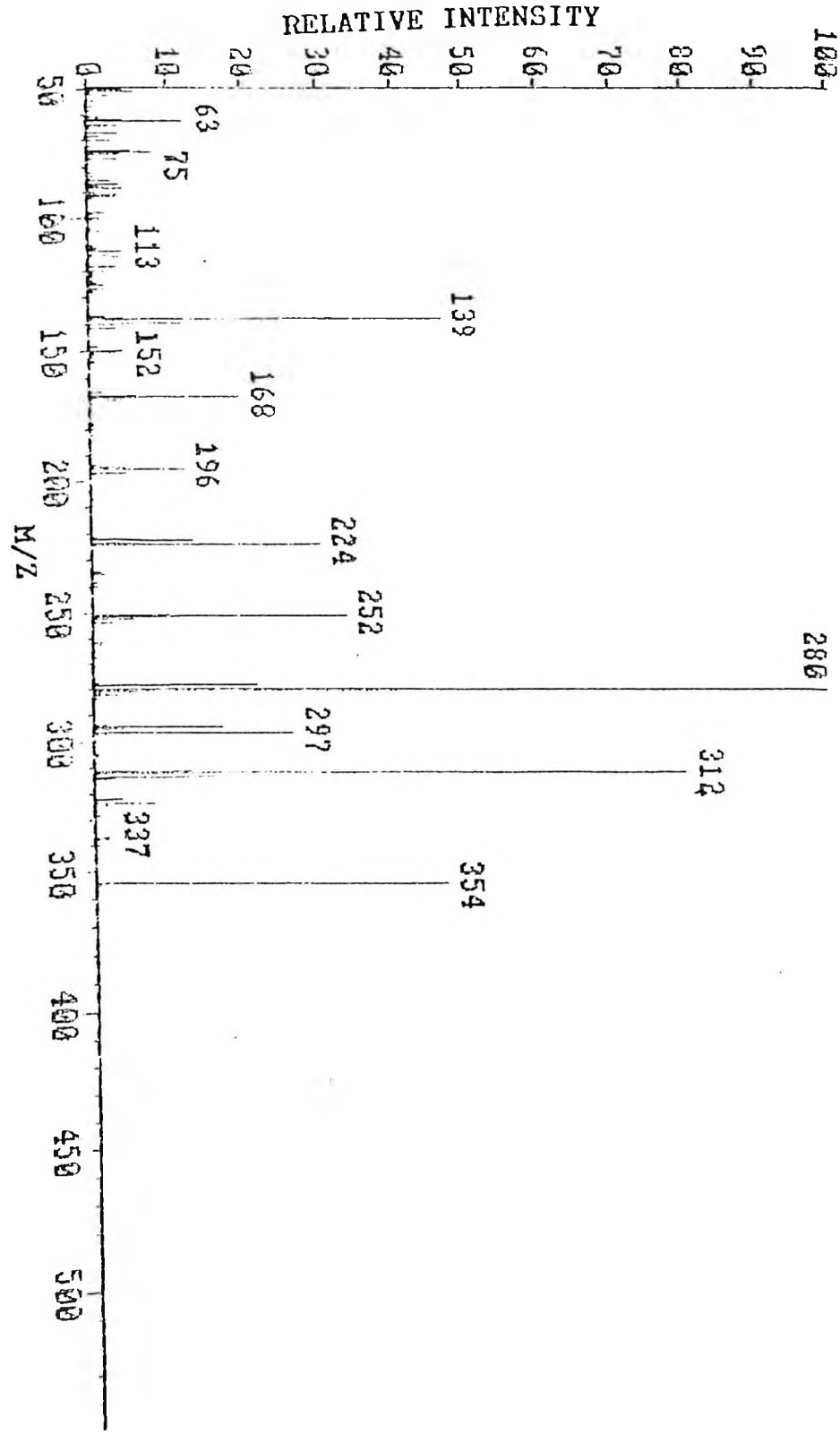
EIMS OF ALOESAPONOL-I (5)



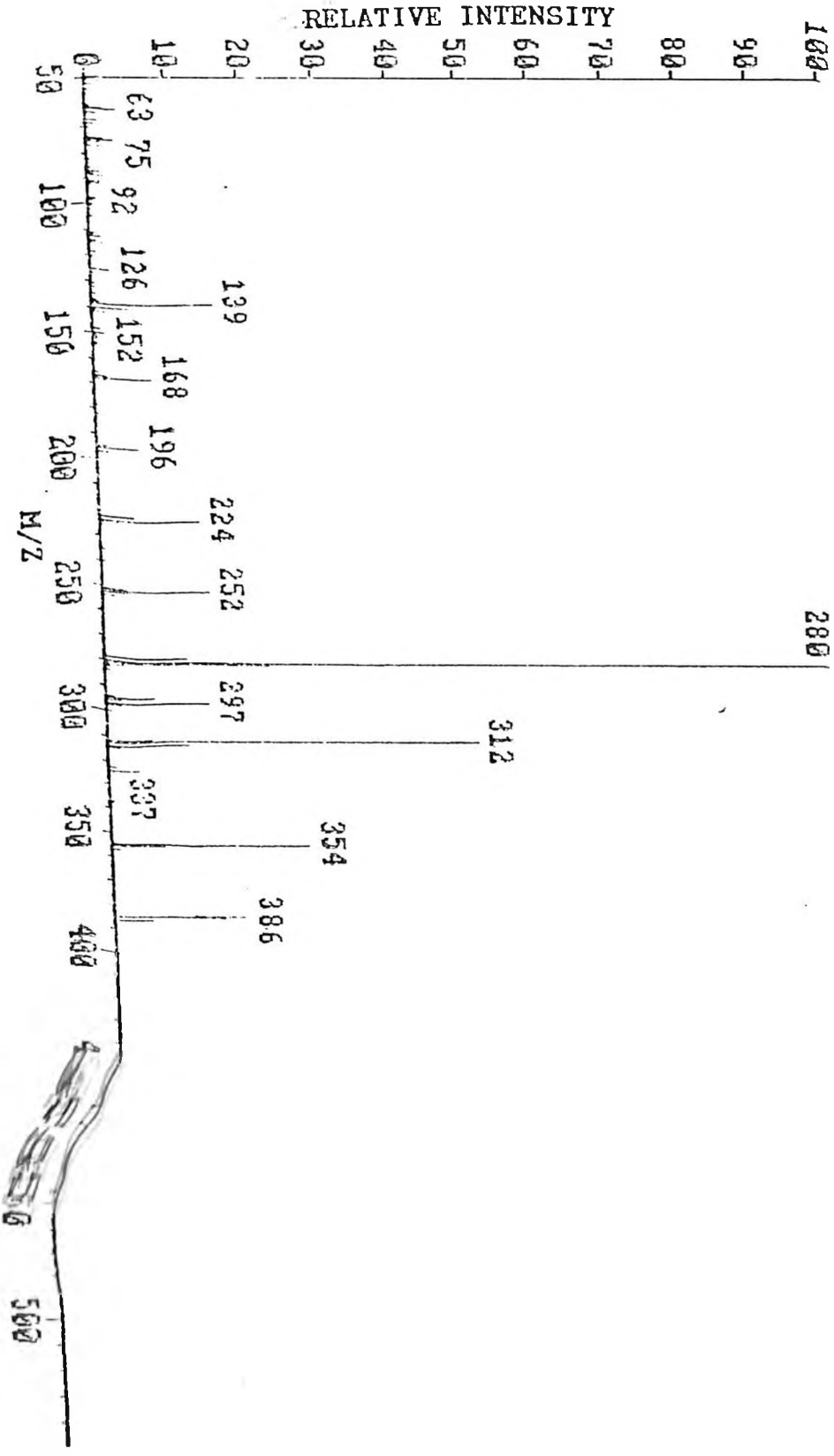
EIMS OF ALOESAPONARIN-I (6)



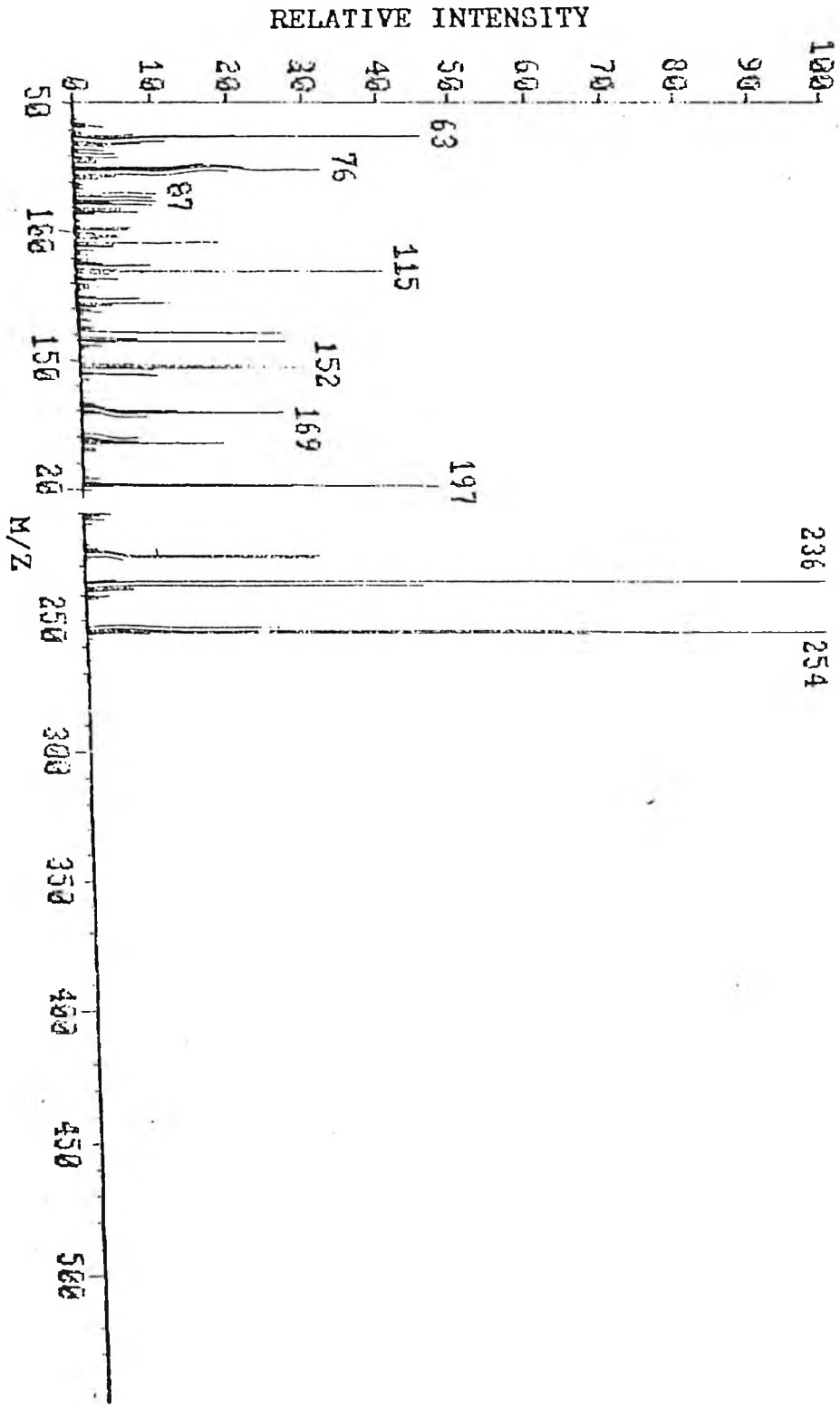
EIMS OF 8-ACETOXYALOESAPONARIN-I (6a)



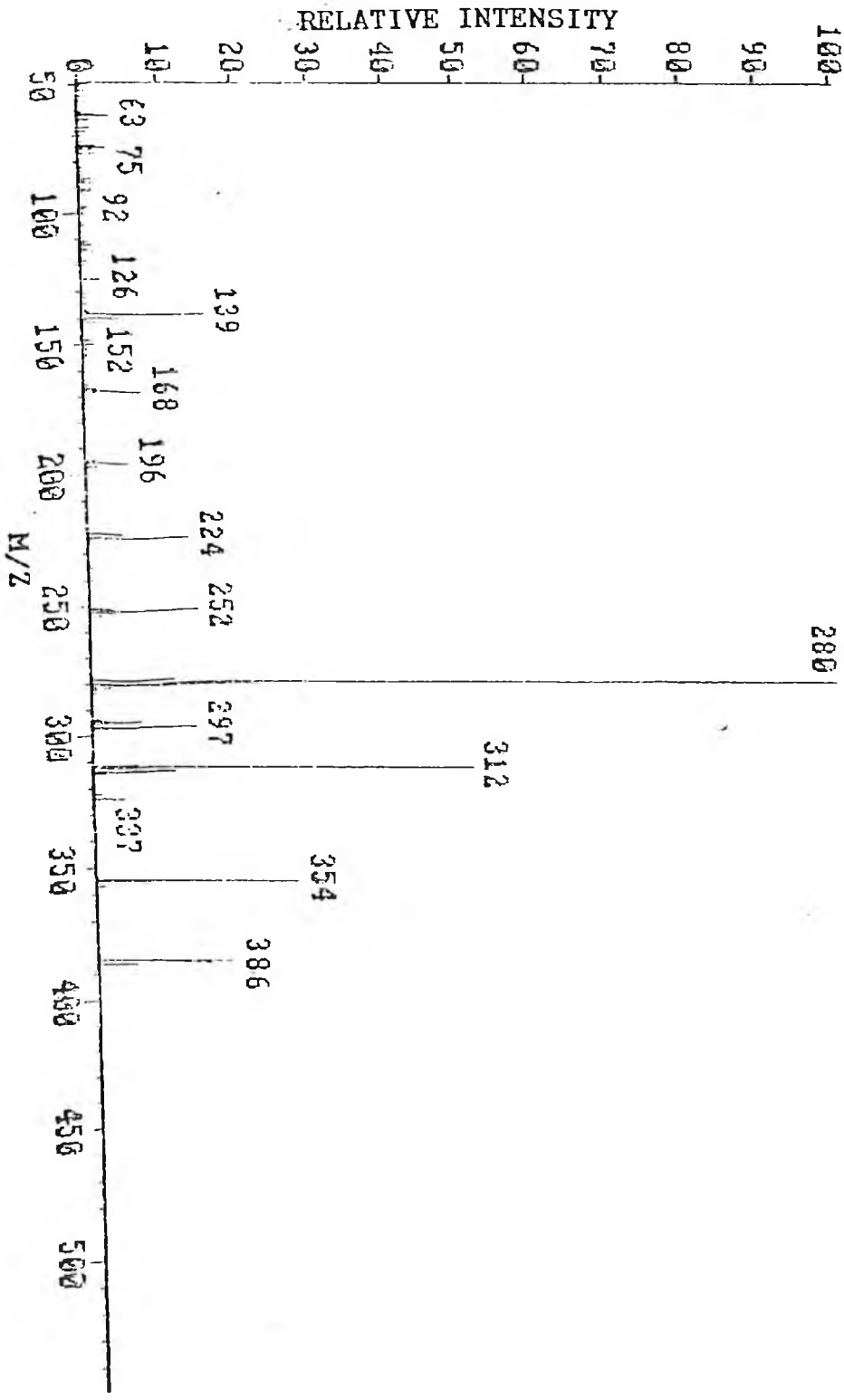
EIMS OF 3,8-DIACETOXYALOESAPONARIN-I (6b)



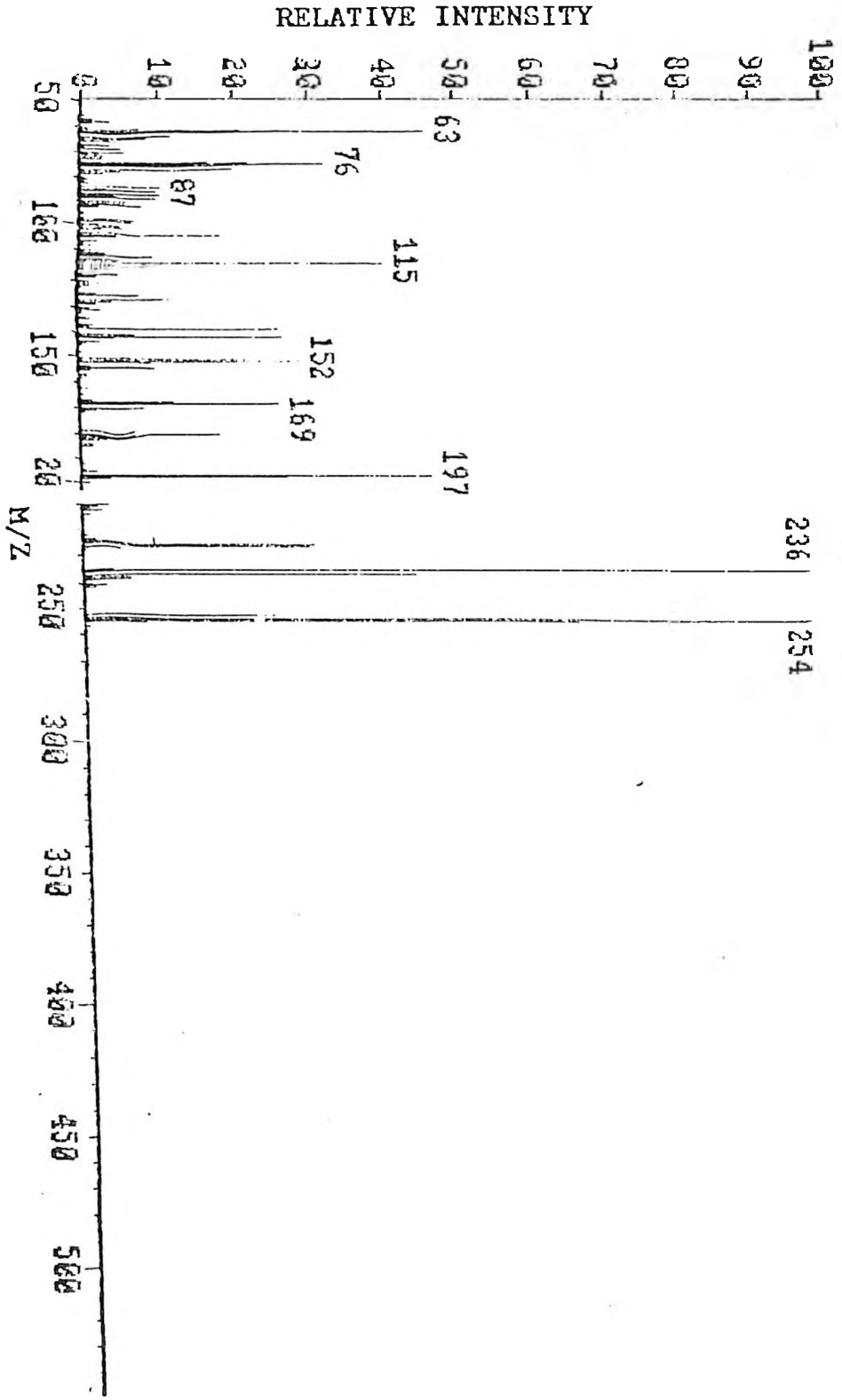
EIMS OF ALOESAPONARIN-II (7)



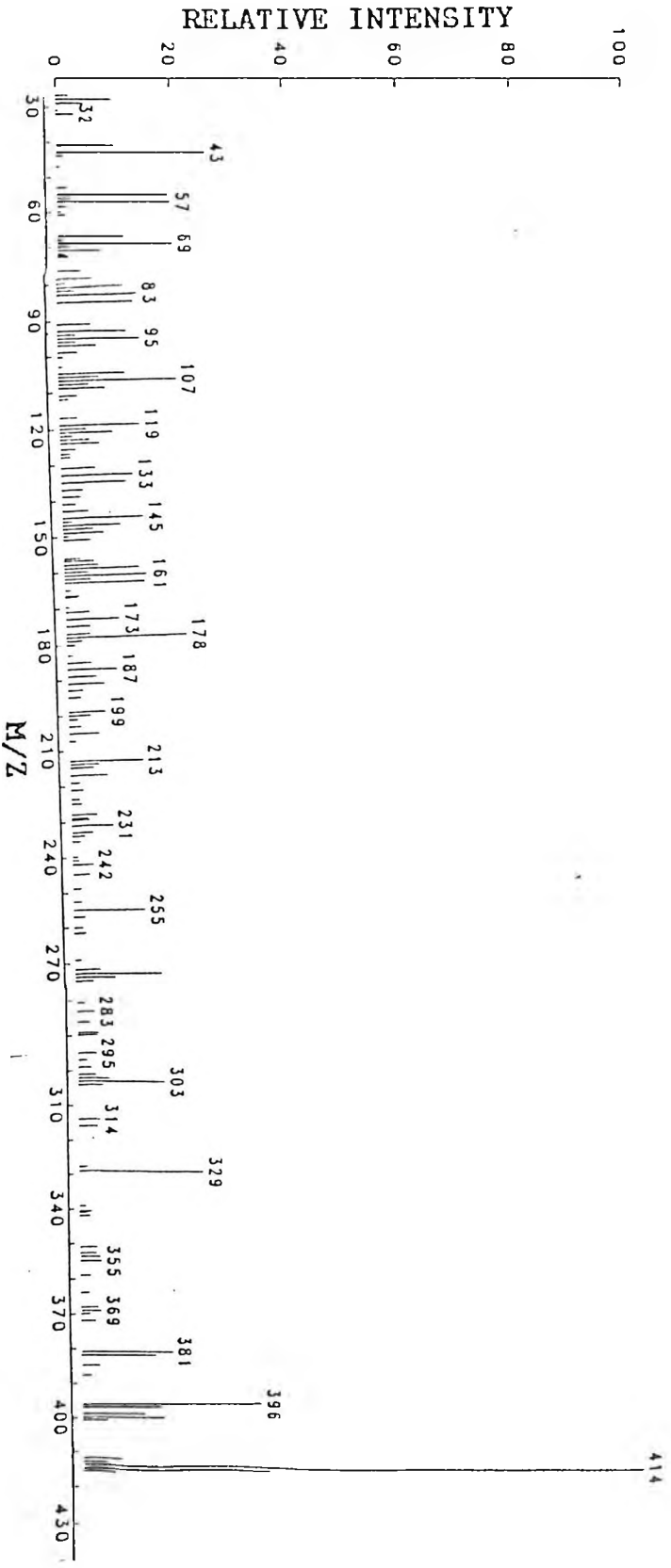
EIMS OF 3,8-DIACETOXYALOESAPONARIN-I (6b)



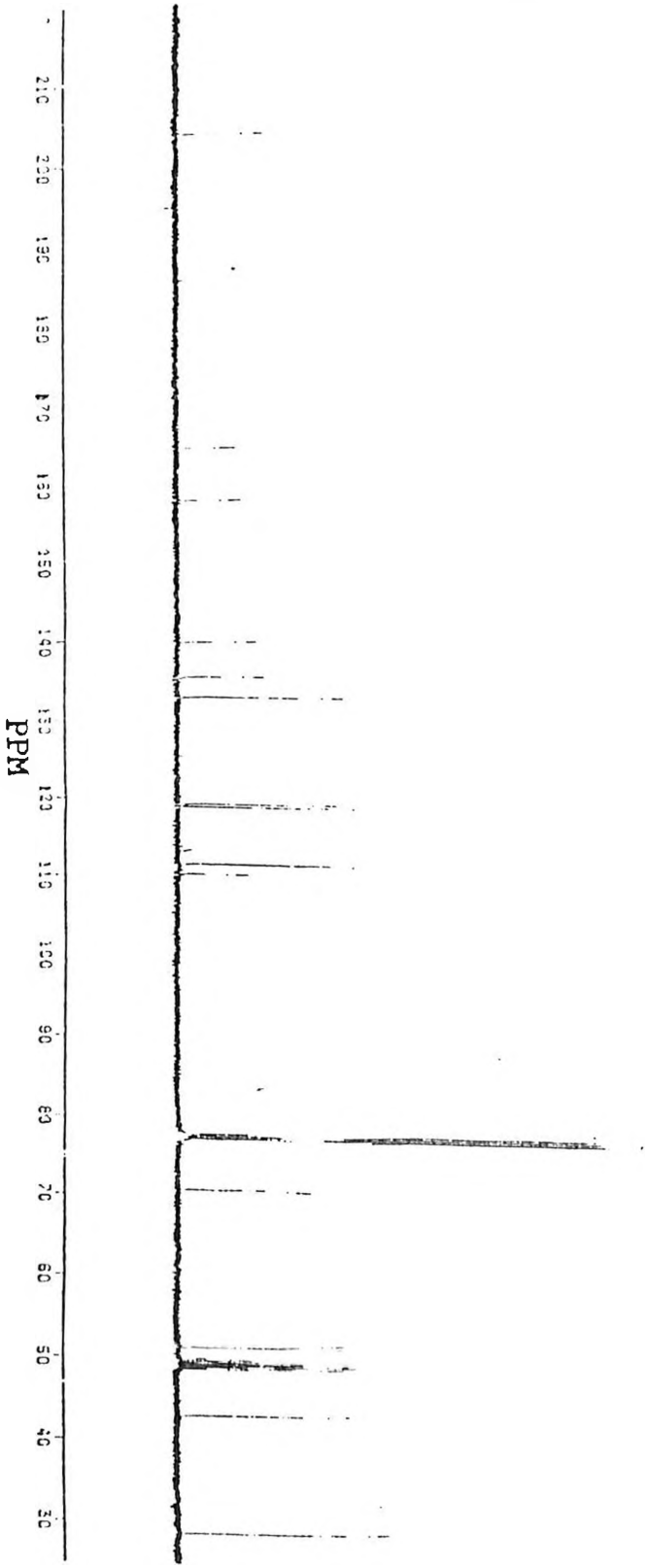
EIMS OF ALOESAPONARIN-II (7)



EIMS OF β-SITOSTEROL (8)

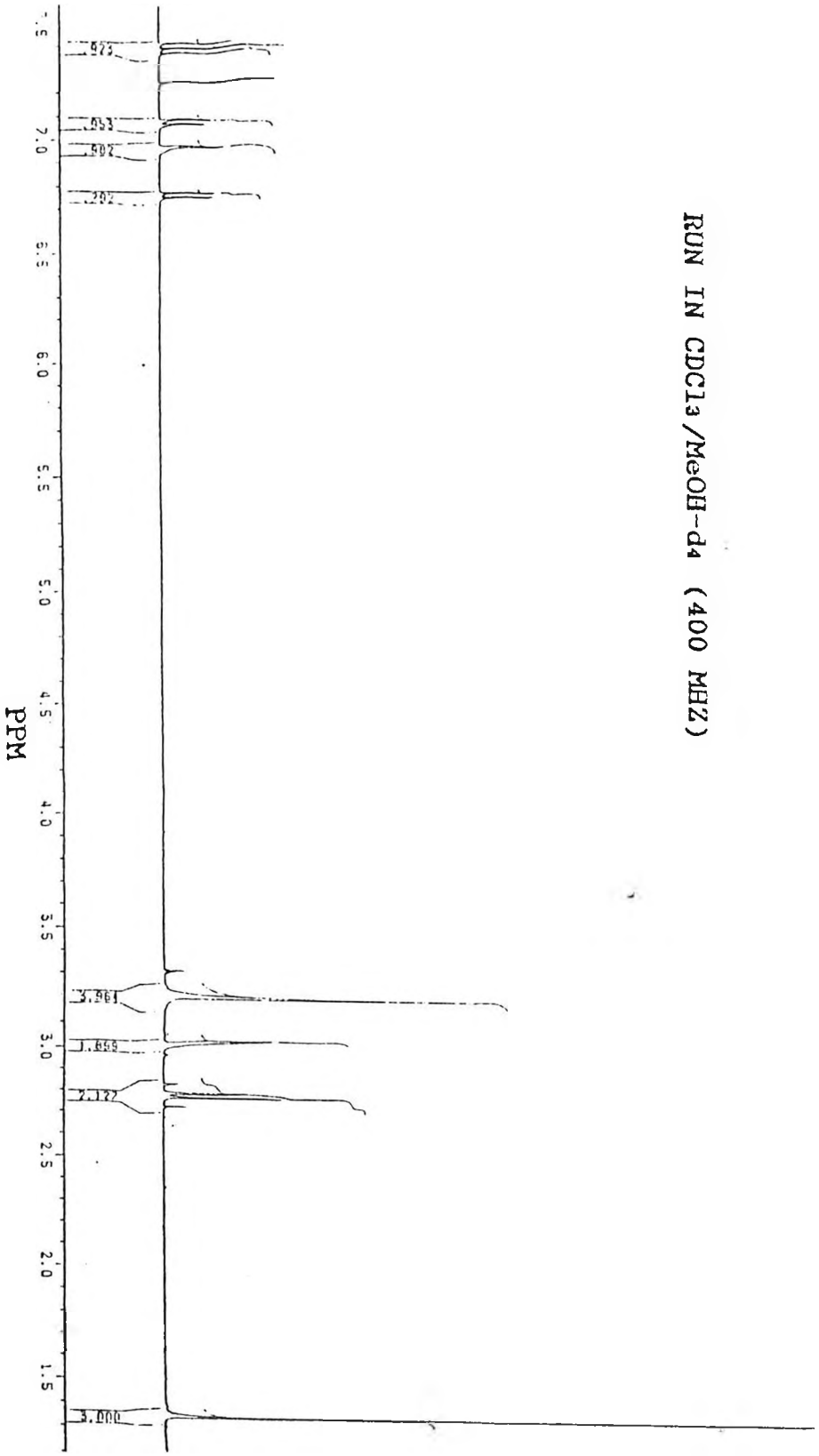


^{13}C NMR SPECTRUM OF ALOEGRAMONOL (1)

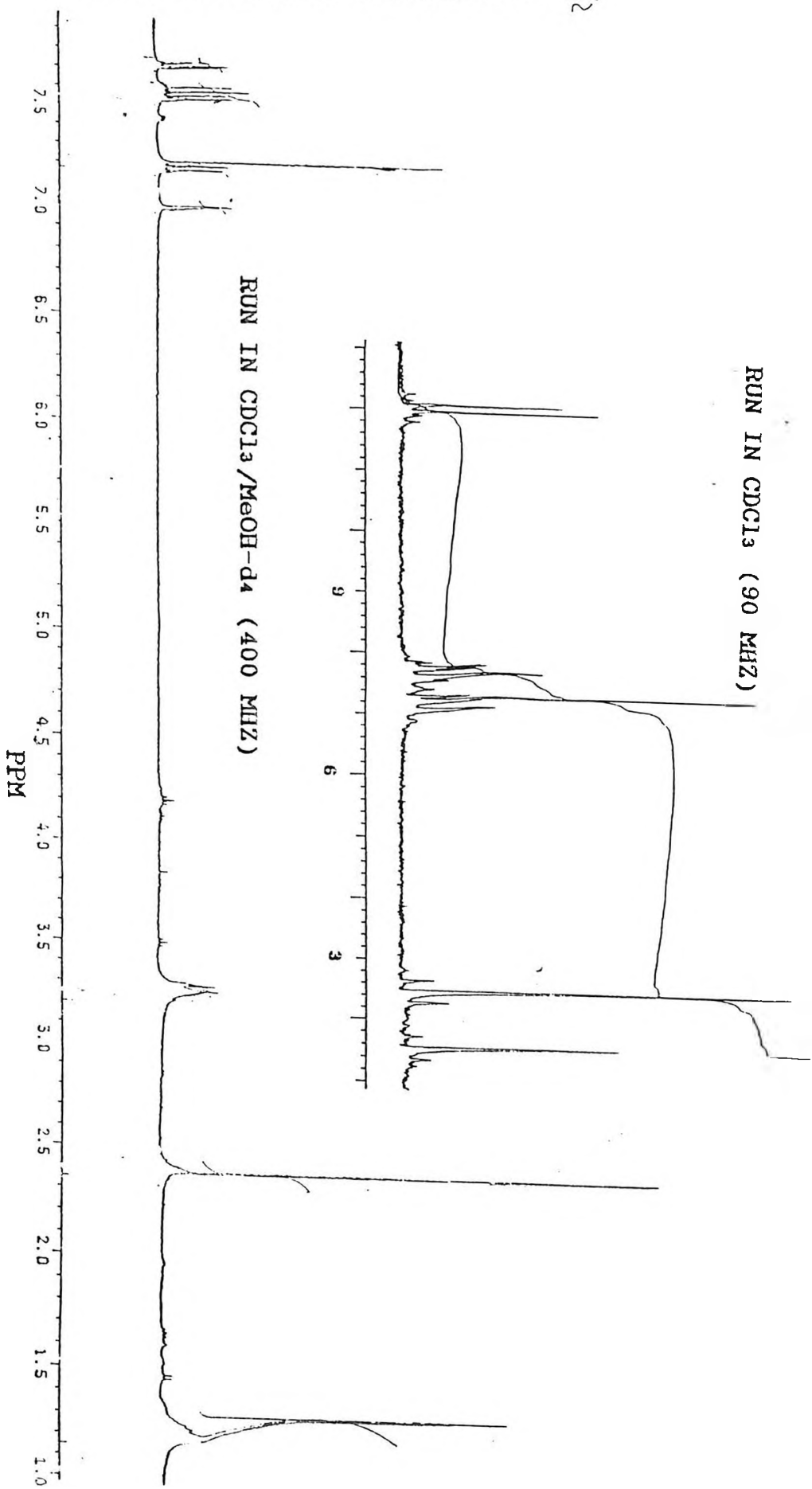


RUN IN $CDCl_3$/MeOH-d_4 (90 MHZ)

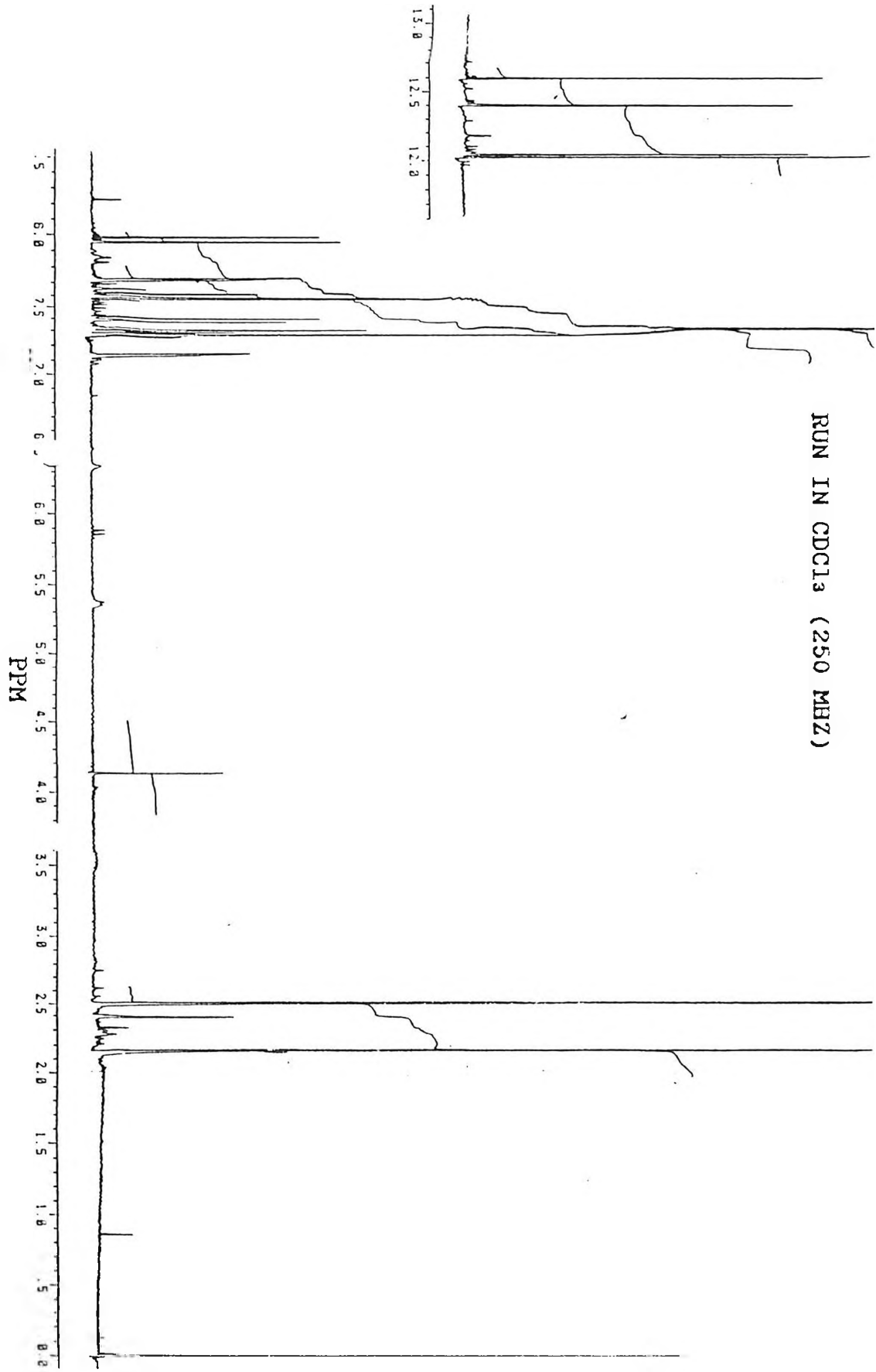
^{1}H NMR SPECTRUM OF ALOEGRAMONOL (1)



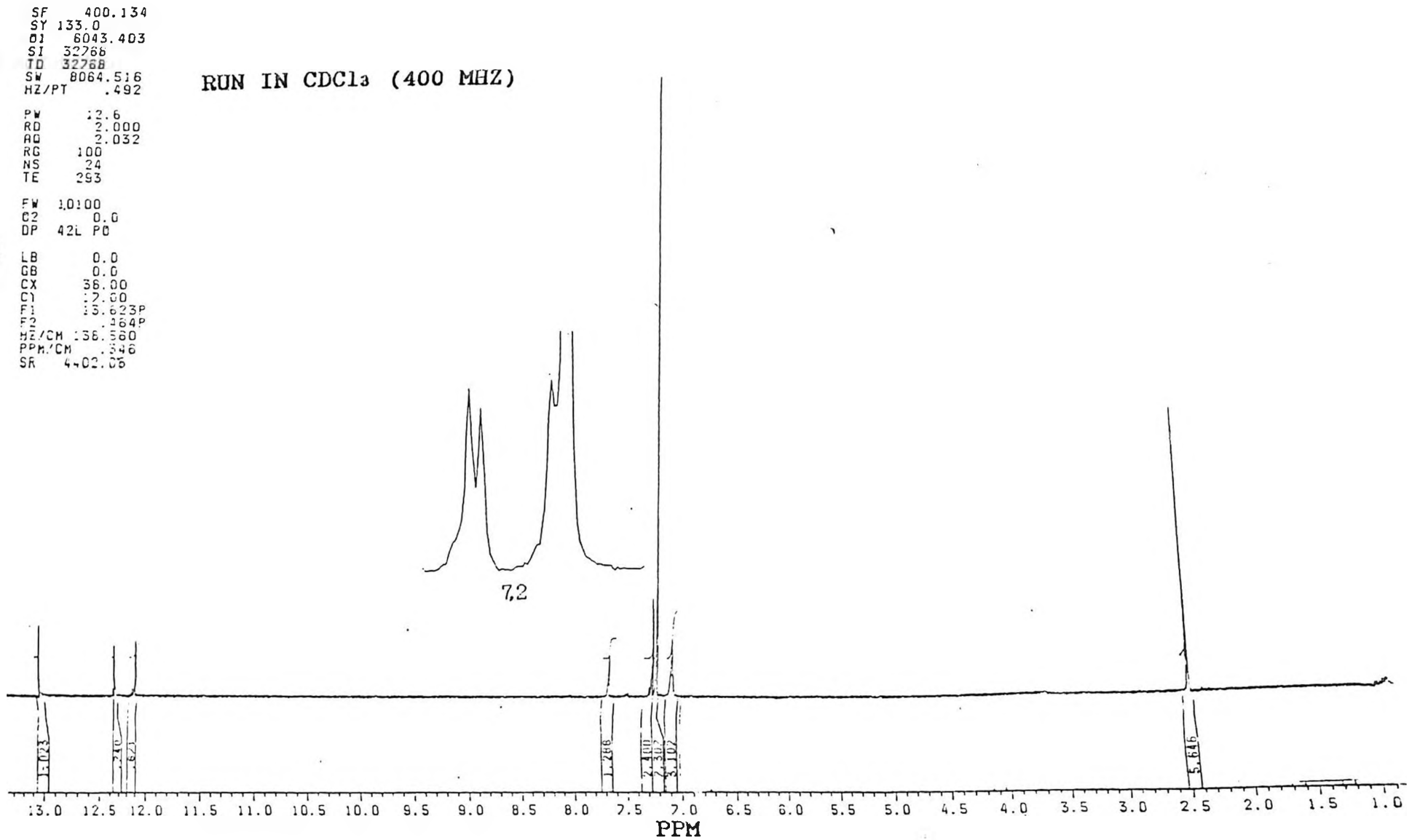
^1H NMR SPECTRUM OF CHRYSOPHANOL (2)



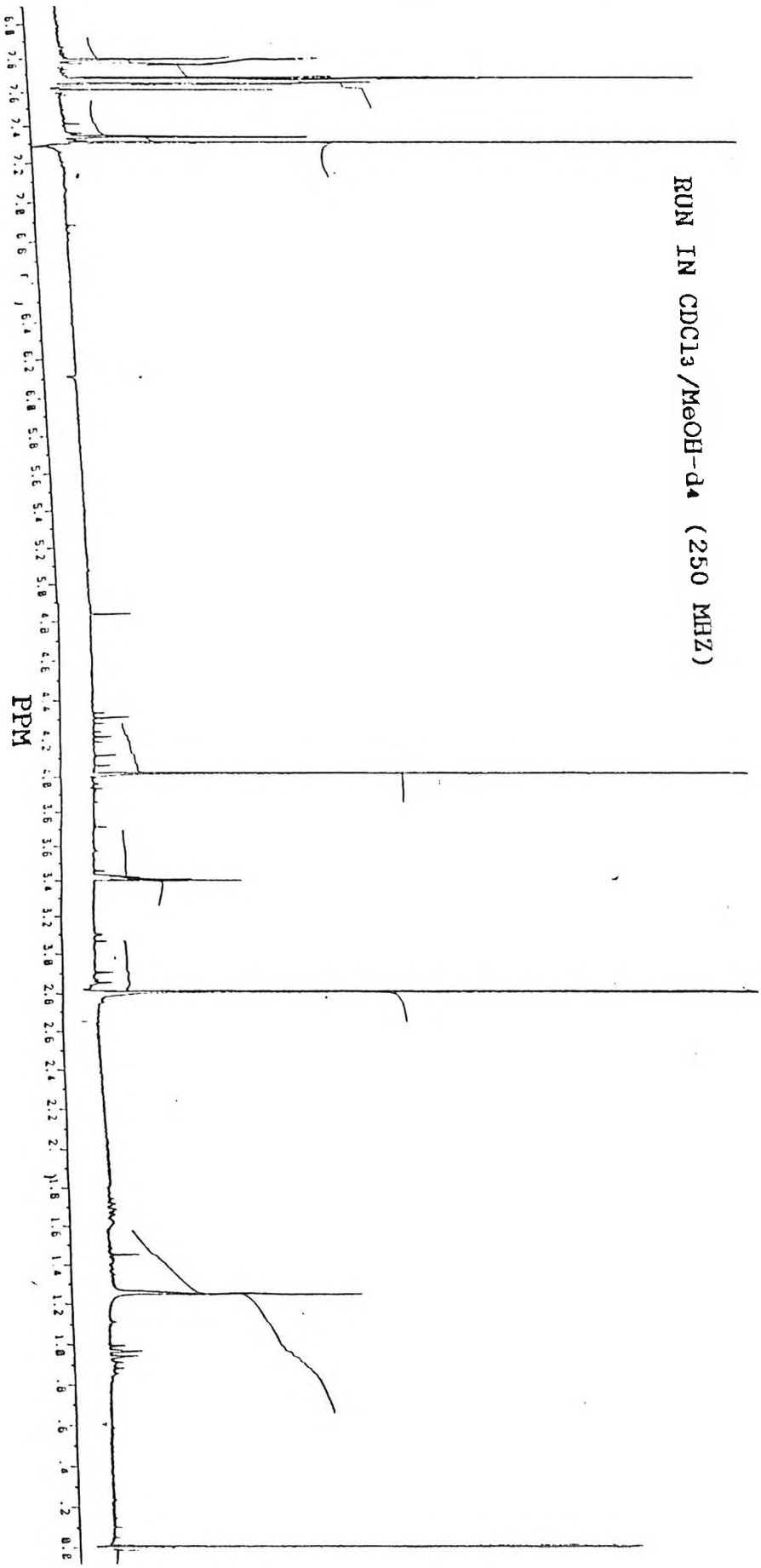
^{1}H NMR SPECTRUM OF ASPHODELIN (3)



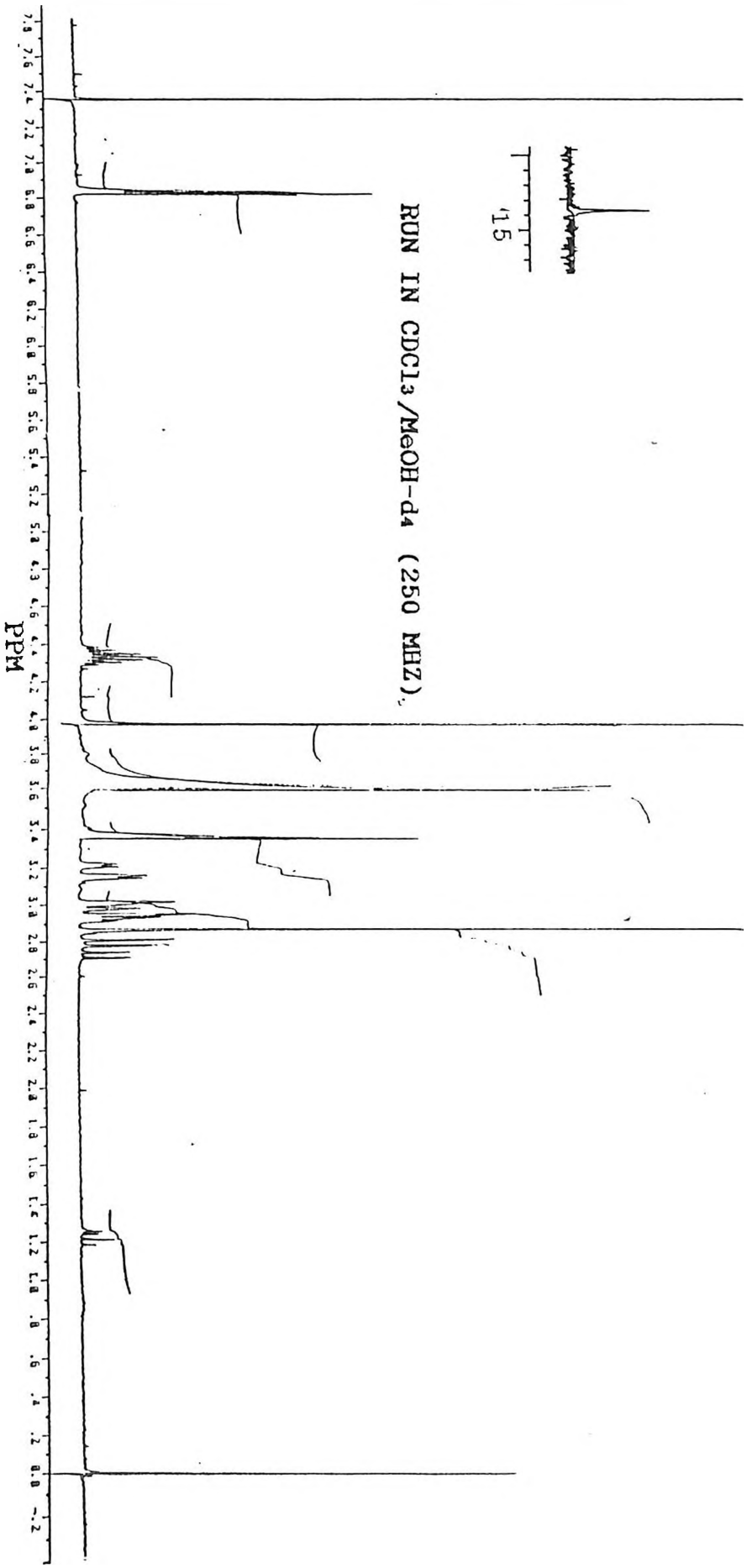
^{1}H NMR SPECTRUM OF HELMINTHOSPORIN (4)



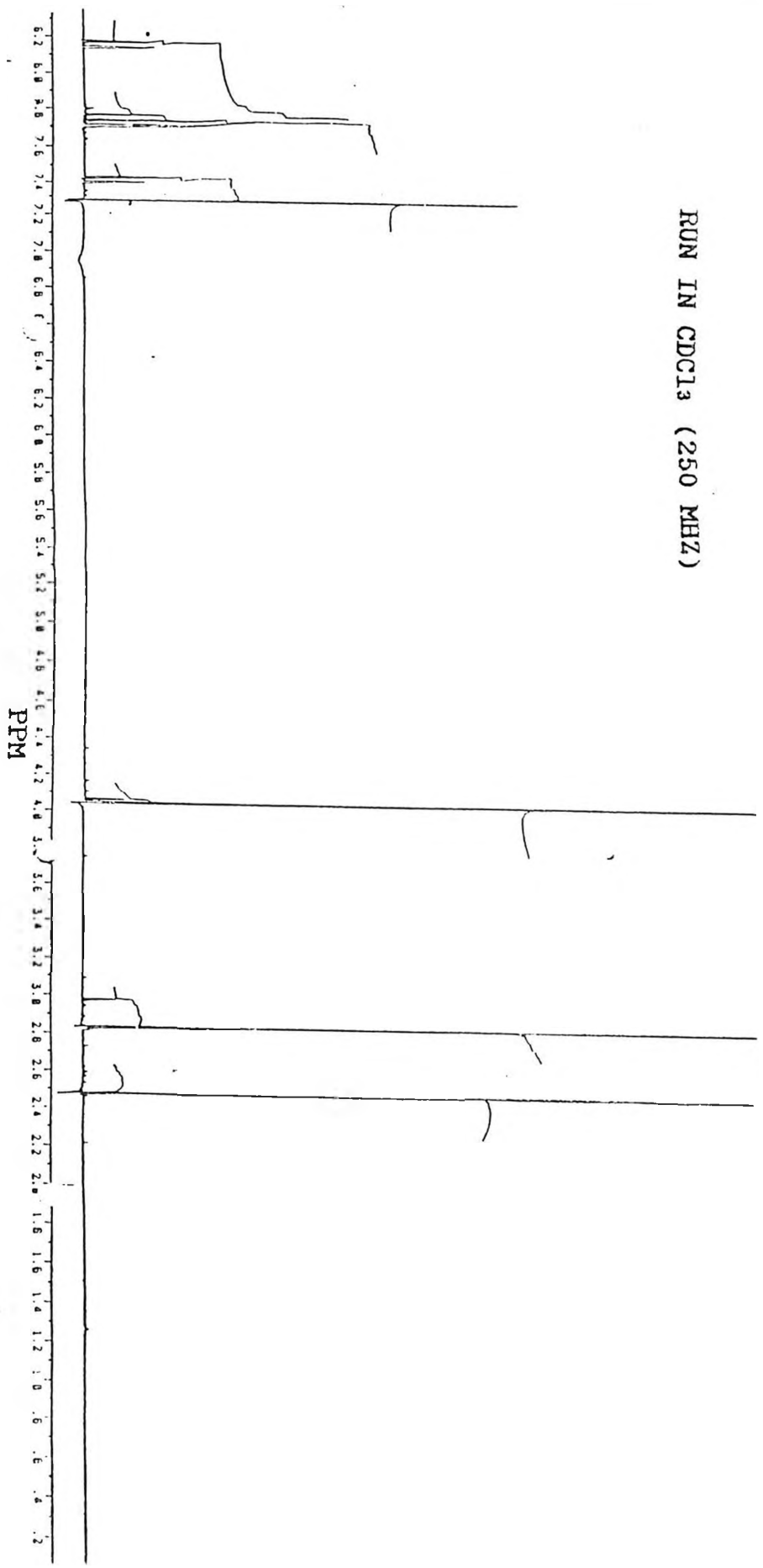
^{1}H NMR SPECTRUM OF ALOESAPONARIN-I (6)



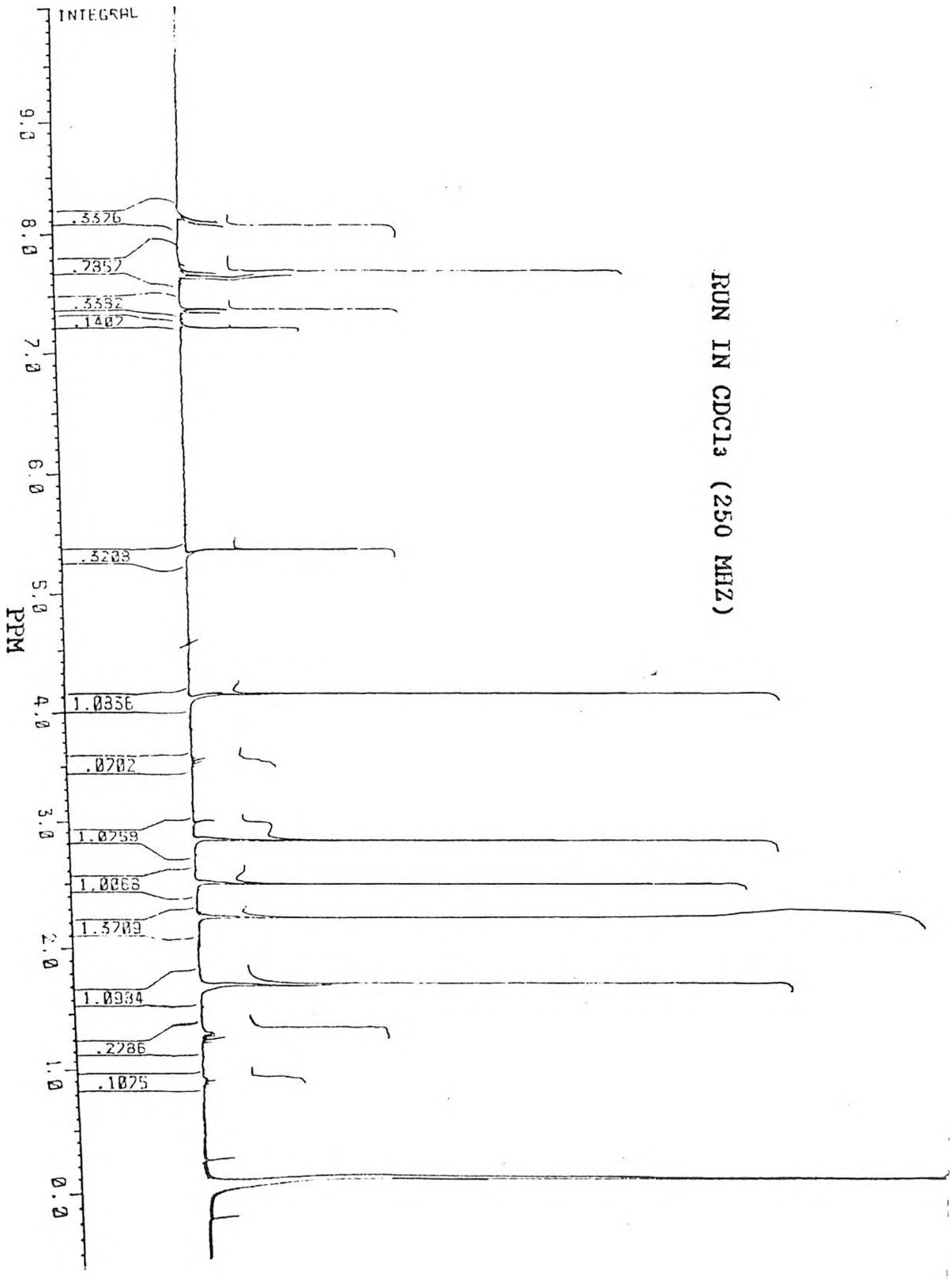
^{1}H NMR SPECTRUM OF ALOESAPONOL-I (5)



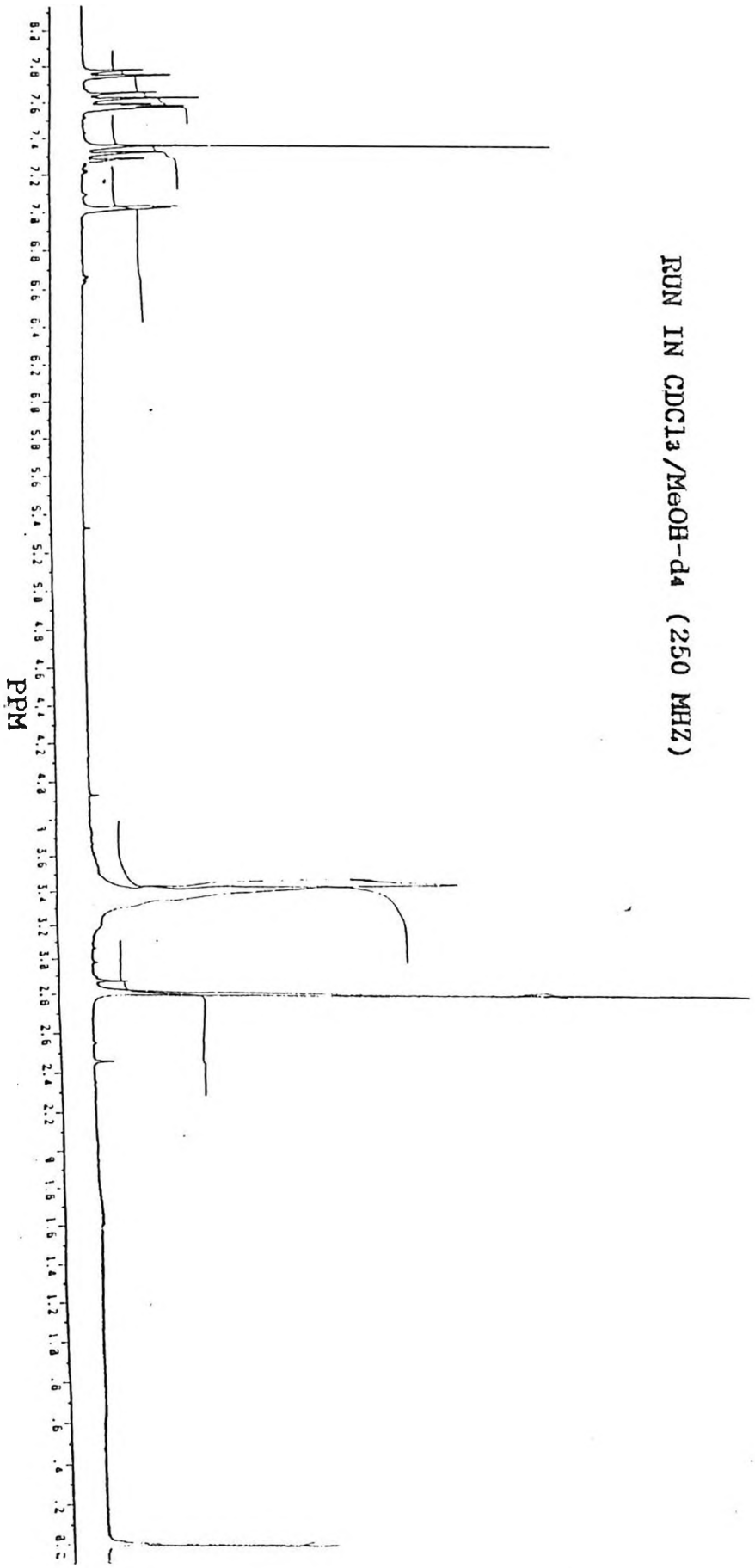
^{1}H NMR SPECTRUM OF 8-ACETOXYALOESAPONARIN-I (6a)



^{1}H NMR SPECTRUM OF 3,8-DIACETOXYALOESAPONARIN-I (6b)



^{1}H NMR SPECTRUM ALOESAPONARIN-II (7)



^{1}H NMR SPECTRUM OF B-SITOSTEROL (8)