PHYTOCHEMICAL ANALYSIS OF KENYAN MEDICINAL HYPERICACEAE PLANTS

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A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN THE UNIVERSITY OF NAIROBI

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

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This thesis is dedicated to all the members of my family.

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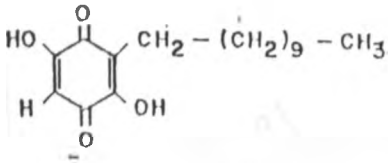
SUMMARY

There are five members of the Myrsinaceae in Kenya, four of which, Embelia schimperi, Myrsine africana, Rapanea melanphloes and Maesa lanceolata are applied broadly in our traditional medicine and at least one part of each plant is invariably used for anthelminitic purposes.

Chemical screening of these plants was performed on their stem and root barks, fruits and leaves by a process of soxhlet extractive fractionation with non-polar solvents (petroleum ether and chloroform) and a polar solvent (methanol) followed by silica gel chromatographic separation and spectroscopic analysis using UV, IR, ^{1}H NMR and mass spectrometers. Benzoquinone pigments were found in all the portions of the plants analysed and in all extract fractions. The methanol fraction usually contained benzoquinone glycosides but these were transformed to the aglycones by acid hydrolysis. The concentration of total aglycones varied over a wide range depending on the species and part studied or more than but with as much as 12.1% in the fruits of M.lanceolata and R. melanphloes. Leaves had the least amount of the compounds while the stem and root barks had intermediate but relatively high concentrations reaching upto 9.3% in the root bark of R. melanphloes . Qualitatively, E. schimperi, R. melanphloes and M. africana contain in most parts studied, embelin (5), rapanone (6), macrophyllin (12) and a compound of yet indeterminate structure (35). M. lanceolata on the other hand, was found to contain maesaquinone (7), acetylmaesaquinone (14), maesanin (15), 2, 5-dihydroxy-3-pentadecenylbenzoquinone (39) and 2, 5-dihydroxy-3-pentadecyl-

benzoquinone (40), the latter two representing novel natural products.

Pharmacological testing on embelin and rapanone indicate that they have larvicidal (from tests using mosquito larvae) and antimicrobial (against gram-positive streptococci) activities. Tests with embelin, maesaquinone and rapanone also show that embelin may exert antifertility action in male rabbits with the varying testosterone levels as the criterion of fertility.



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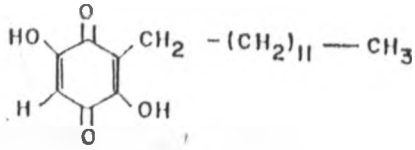

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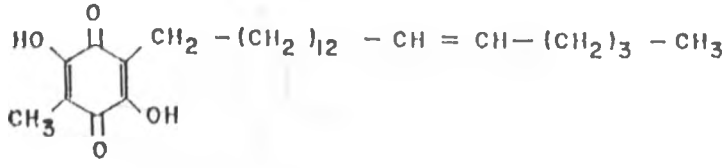

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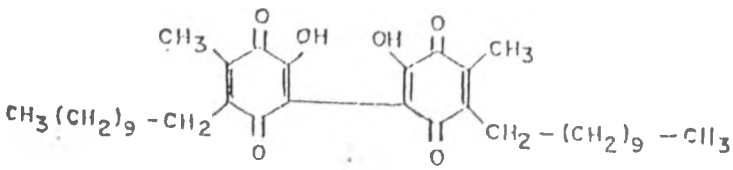

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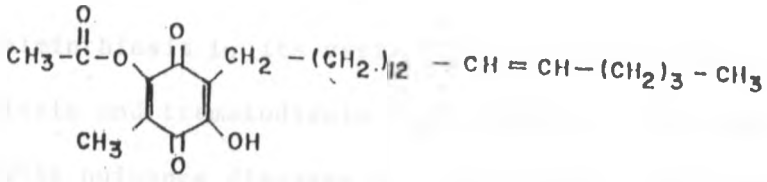

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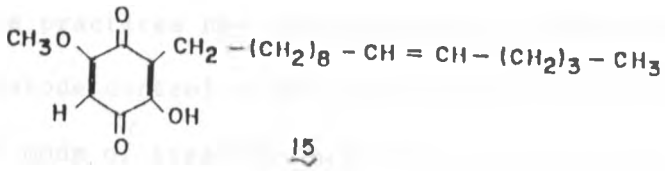

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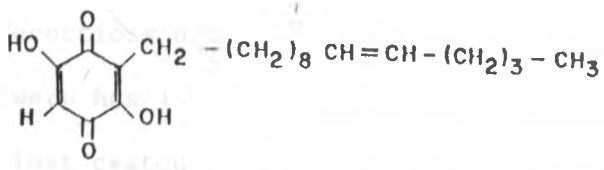

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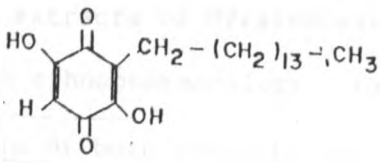

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CHAPTER 1

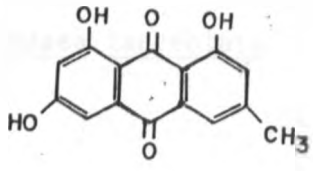
1.0 INTRODUCTION

Helminthiasis in its various forms: cestodiasis, nematodiasis and trematodiasis ranks as some of the most intractable nuisance diseases of the tropical third world. They are hygiene problems which are still endemic in some parts of Africa. Control of cestodes and nematodes along with good hygiene practices has been achieved in the developed world; trematode control however has proved more intractable; the present mode of treatment entailing use of rather toxic drugs (Islip, 1979).

Traditional control of cestodes and nematodes in this country uses decoctions of plants from several indigenous families. Kokwaro has listed 120 plants as having anthelmintic properties against cestodes, nematodes and trematodes. In the highland regions, encompassing the environs of the capital, the roots and seeds of some Polygonaceae and the fruits, root and stem bark extracts of Myrsinaceae have been used extensively in ethnopharmacology for cestodal and nematodal control. It is of both economic and chemical interest to delineate the active principles in these plants. Such knowledge may serve to lay ground for drug development in form of galenicals or tablets from these plants, or the structures elucidated thereof used as templates in structure activity relationship studies.

Some Polygonaceae species of Kenya have been studied rather extensively .

Members of this family, Rumex spp contain high concentrations of 1,8 - dihydroxyanthraquinones, emodin (1), physcion (2) and chrysophanol (3) and some of them contain along with these, high concentrations of nepodin (4). All these compounds have tested anthelmintic efficacy and infact comprise formulations of laxative or purgative drugs in most modern world Pharmacopeae. In other words there is good reason for their use in traditional medicine.



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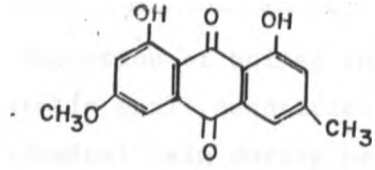

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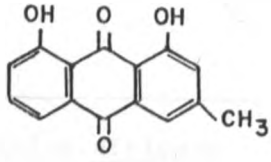

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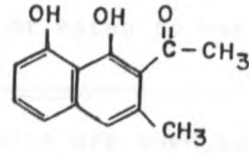

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This type of correspondence between traditional use and proven efficacy is recorded for species in many other families against other diseases (Sofowora, 1982). Therefore investigation of traditional anthelmintic plants is a useful endeavour indeed, especially if the efforts are concentrated on widely used varieties. Myrsinaceae are an example of plants which have large ethno-pharmacological oral literature and reputation in this country. It was therefore deemed possible that correspondence mentioned above would be encountered and thereby open-up interesting possibilities chemically and pharmacologically.

TABLE 1. MEDICINAL APPLICATIONS OF THE MYRSINACEAE PLANTS

Plant	Medicinal use
Embelia schimperi	The root is boiled or soaked in water and the infusion drunk for intestinal worms. The leaves are pounded and used as linament for swollen breasts. The fruits are used both as purgative and vermifuge
Maesa lanceolata	A decoction of boiled roots is taken warm in small quantities for lower abdominal pain during pregnancy, for stomach problems in children and for infantile convulsions. The fruits are used as purgative, as a remedy for sore throat or eaten to cure tapeworm.
Myrsine africana	The fruits are used as purgative and for roundworm, tapeworm treatment and for other stomach ailments. They are also used as a remedy for chest pains.
Rapanea melanphloes	The fruits are used as anthelmintics especially for tapeworms.

There are five Myrsinaceae species in Kenya, four of which find application in traditional anthelmintic treatment. These plants are Embelia schimperi, Maesa lanceolata, Myrsine africana and Rapanea melanphloes. The traditional mode of usage have been recorded in Kokwaro's book and are collected in table 1. The other plant which is not noted as anthelmintic but is in this country is Embelia keniensis found around Mt. Kenya (Dale and Greenway, 1961). This plant was not studied in this work.

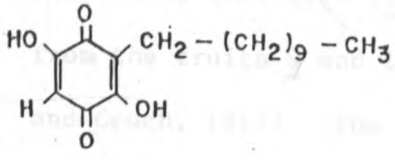
1.1 Distribution and Description

Myrsinaceae belongs to the Primulales and the plant species in this family have been classified into 33 genera (Ogawa and Natori, 1968). These are either large trees, shrubs or scramblers. Rapanea melanphloes is a large tree generally 15 metres in height and commonly found in highland areas as in Mts. Elgon, Aberdares and Kenya, Kithembe hills in Machakos District, Kikuyu escarpment, Naivasha and Kapsabet. Maesa lanceolata is a tree, usually 9 metres tall, also found in highland areas as in Mts. Aberdares, Kenya, at the foot of Mt. Elgon and around Kitale. Myrsine africana is a small evergreen shrub found in the same areas with Rapanea melanphloes. Lastly, Embelia schimperi is a scrumbler generally over 4 metres. It is commonly found in the same habitat as the species above (Dale and Greenway, 1961).

1.2 LITERATURE SURVEY

The chemical analysis and economic development of the Myrsinaceae plants elsewhere started rather early this century.

Gokhale and Paranjpe in 1933 isolated the benzoquinone, embelin (5) from the dried fruits of both Embelia ribes (Burm) and Embelia robusta (Roxb), the compound was determined to be effective against tapeworm. Three years later, the berries of Myrsine africana were used as anthelmintic and in preparation of ointments for ringworm and skin diseases (Krishna and Varma, 1936). Further evidence about the pharmacological activity of Myrsinaceae plants was observed when embelin isolated from the berries of Embelia robusta, Myrsine semiserrata and Myrsine capitellata was found to be anthelmintic (Krishna and Varma, 1937). Later Rapanea maximowiczi (Koidz) was studied and observed to contain rapanone (6) which was a more potent anthelmintic (Kawamura and Hokoku, 1937).



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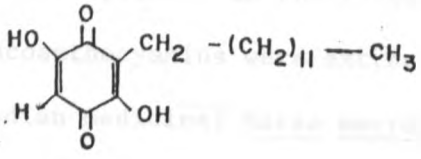

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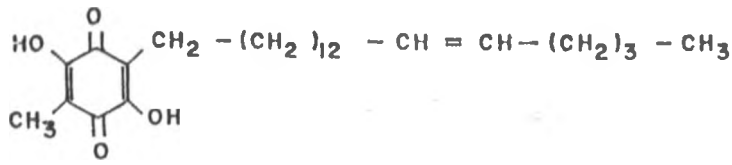
Harimoto (1939) tested the fruits of Maesa japonica (Moritz) against pathogenic Gram-positive and Gram-negative bacteria and found it effective to both. The activity was attributed to maesaquinone (7). In another study, Merrian and Schlitter (1948) screened the dried fruits of E. kilimandscharica, M. africana and R. neurophylla and found them to contain embelin in 7.55%, 2.47% and 3.96% respectively. Two Madagascan Myrsinaceae plants, were studied; Maesa emirnesis contained 7 (2.1%), sterols, tannins and saponins, while from the roots of E. berbeyana, 5 (4.1%) and catechol tannins were extracted (Paris and Rabenoro, 1950).



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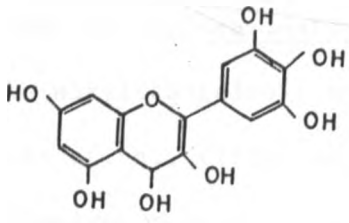
In 1961, Wilkinson in his biological and phytochemical evaluation of Rapanea pulchra extracted 0.39% of 5 from the berries while from both the root and stem barks, 6 was isolated in 2.8% and 1.2% respectively. Ardisia macrocarpa (Wall Roxb) which is indigenous to India has been used in traditional medicine as febrifuge, in diarrhoea treatment and also applied externally on ulcers. A phytochemical screening of the stem bark and hardwood of the plant gave rapanone and 3,4,5,7, 3', 4', 5' - heptahydroflavan (8) (Murthy et. al., 1965). Myrsine australis when examined showed that vilangin (9) and the flavonoid, quercitin (10) were present in the flowers while from the fruits 9 and leucoanthocyanins were extracted (Cambie and Couch, 1967). The Indian medicinal Maesa macrophylla contains the benzoquinone, bhogatin (11) in the leaves (Chandrasekhar et. al., 1970). In the course of continuation with the work on M. macrophylla, Prabhu and Venkateswarlu (1971) extracted another new benzoquinone, macrophyllin (12) together with quercitin (10) and kaempherol (13) from the leaves of the same plant.



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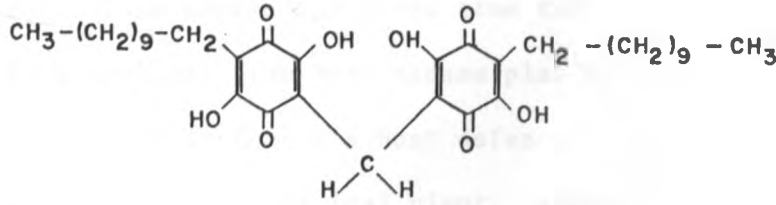

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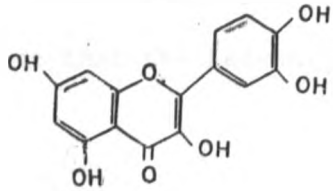

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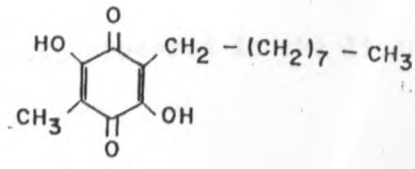

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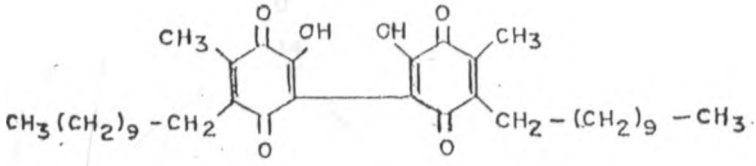

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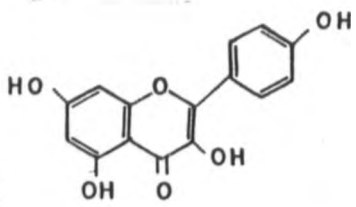

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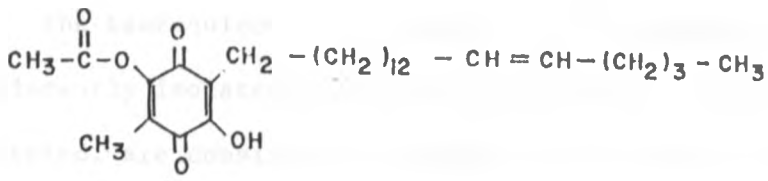
Ogawa and Natori (1968) cited several hydroxybenzoquinones of the Japanese Myrsinaceae species and asserted the chemotaxonomical association of these compounds with the family. The compounds reported from three genera in this family, Ardisia, Maesa and Myrsine were acetylmaesaquinone (14), 2-hydroxy-5-methoxy-3-pentadecylbenzoquinone or maesanin (15), bisbenzoquinones, ardisiaquinones, A(16), B(17), C(18), 2-hydroxy-5-methoxy-3-tridecylbenzoquinone (19), 2-hydroxy-5-methoxy-3-tridecenyl benzoquinone (20) together (5), (6), (7), (9), (11) and (12). Taiwanese Maesa formosana used as an antibacterial in traditional medicine was found to contain (7) and (14) in the unripe fruits while α-spinasterol and catechol tannins were isolated from the twig (Russel et. al., 1976). In 1977 Madrigal et. al., reported the isolation of 5-alkylresorcinols together with embelin and rapanones from the seeds of Rapanea laetevirens. This marked the first time that the alkylresorcinols had been isolated from Myrsinaceae plants. Kubo et. al. (1983) reported the isolation of a host defence stimulant maesanin (15) from an African medicinal plant, Maesa lanceolata. Its structural elucidation agreed with what was proposed by Ogawa and Natori in 1968. This marked the first time that the Kenyan M. lanceolata appeared in the literature.



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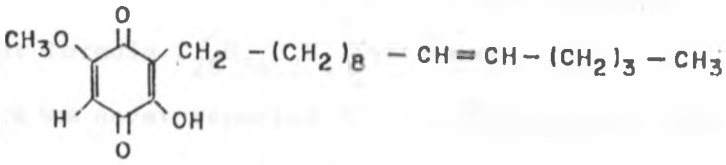

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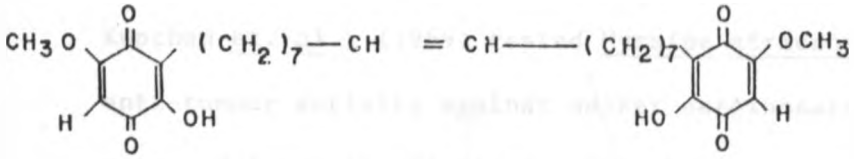

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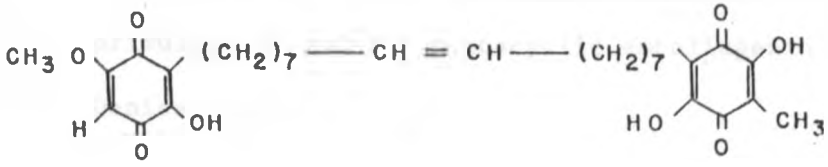

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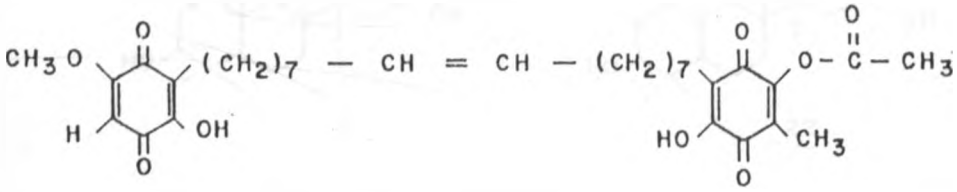

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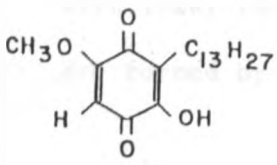

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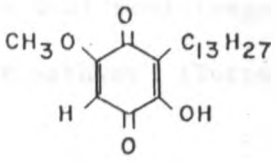

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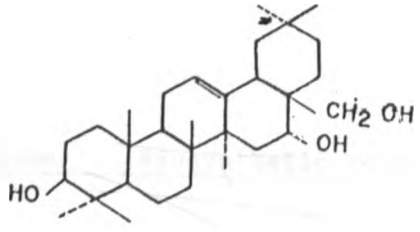
The benzoquinones are therefore the secondary metabolites consistently isolated from Myrsinaceae plants, and have been indicated, are considered as biogenetic markers of the family (Yoshihira and Natori, 1966). The more divergent secondary metabolite from this family is however an alkaloid reported in the leaves of Hongkong Maesa perlarius with a molecular formula $C_{26}H_{54}O_2N_4$ (Arthur et. al., 1966); its structure was never reported in the literature. With the search for anti-cancer agents accelerating over the past three decades some Myrsinaceae plants have been screened for this activity. Kupchan et. al., (1969) tested Myrsine africana leaves for anti-tumour activity against Walker carcinosarcoma-256 in rats and found it effective. The chief component of the active leaf saponin hydrolysate was the triterpene primulagenin (21). The leaves and shoots of Embelia concinna have also yielded primulagenin and the pentacyclic embeliagenin (22) (Heltz and Denise, 1973).



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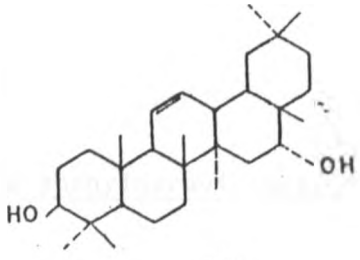

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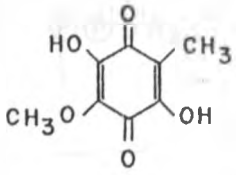
1.3 BIOGENESIS OF BENZOQUINONES

Studies on benzoquinones biogenesis have been carried out with lower fungi . on evidence that most fungal benzoquinones are formed by the acetate-malonate pathway (Turner 1971).

For instance, spinulosin (23) arises from a tetraketide by way of orsellinic acid and orcinol which then undergoes further hydroxylation. Packter (1969) found that tyrosine was effectively incorporated into coprinin (24), the benzene ring providing the quinone ring and the c-methyl group arising from β -carbon atom of tyrosine. An earlier study of volucrisporin (25) (Chandra et. al., 1966) showed that shikimic acid, phenylalanine, phenyllactic acid and m-hydroxytyrosine were incorporated, the labelling pattern suggesting the biosynthetic route via phenylpyruvic acid shown in scheme 1.



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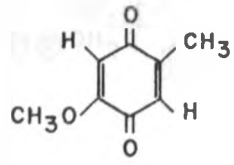

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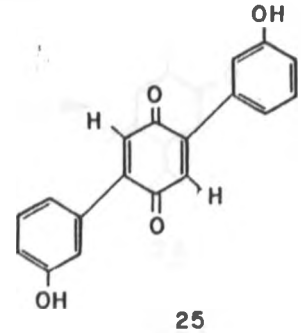

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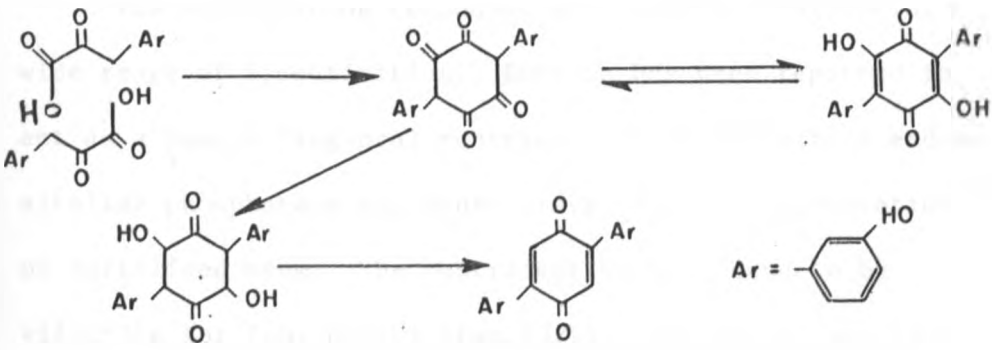
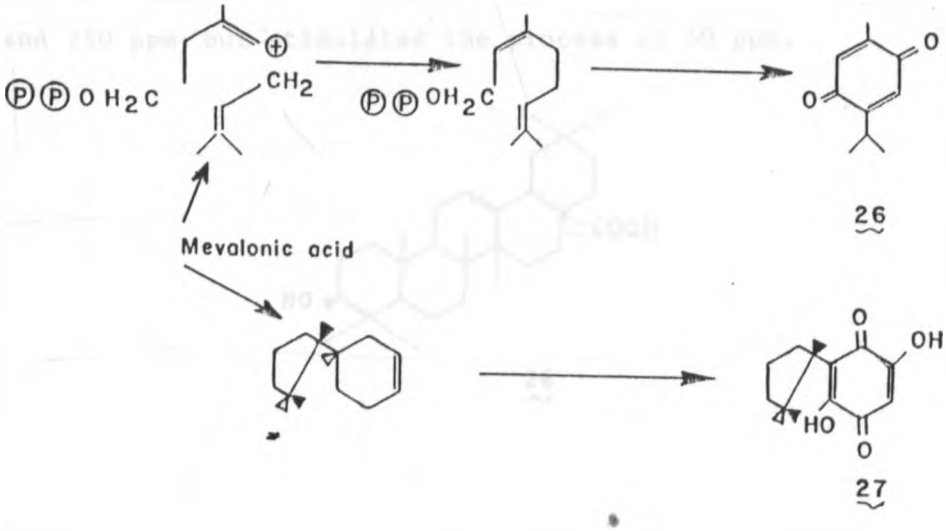
Scheme 1. Biosynthetic route via phenylpyruvic acid



25

Benzoquinones can also be derived from mevalonic acid (scheme 2). Dimethylallylpyrophosphate and isopentenylpyrophosphate are believed to be the precursors of thymoquinone (26) whereas helicobasidin (27), the first fungal benzoquinone which has been demonstrated not to be acetate-derived, is likely to be formed from farnesylpyrophosphate (Fischer, 1980).

SCHEME 2. BIOSYNTHETIC ROUTE VIA MEVALONIC ACID

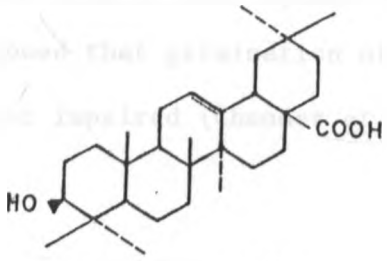


1.4 BIOLOGICAL ACTIVITY

The benzoquinone compounds are capable of eliciting a wide range of bioactivities. Embelin has been reported to act as a long acting oral contraceptive by inhibiting endometrial alkaline phosphatase and hence preventing the implantation of fertilised ovum. The contraceptive was found to be effective for four months (Das, 1976). Gurjar et. al. (1979) reported that embelin administered at a dose of 100 mg per

day for 3 months to male monkeys adversely affected the quantity and quality of semen. They also observed that circulating testosterone levels were reduced. The reduction in testosterone levels in the circulation was responsible for the reduced secretory activity of the accessory glands which in turn results in a decrease in the volume of semen.

In 1981 Gupta et. al. studied the effects of oleanolic acid (28) isolated from Parthenium hypsterophorus and that of embelin on maize and cowpea germination at 22°C. It was found that at 50-520 ppm the acid markedly reduces the germination while embelin also reduced germination at 100 and 250 ppm but stimulated the process at 50 ppm.



28

In another study embelin was tested on rats and mice for its analgesic activity. The drug was effective orally and by central routes and the results compared well with that of morphine. Although the drug acts centrally to produce analgesia, its effect was not antagonised by naloxone indicating that the central site of action was different from that of opiates. It was observed that there is no precipitation of abstrience syndrome as observed with morphine.

A peripheral site of action of the drug was ruled out as it lacks any demonstrable anti-inflamatory action. The high efficacy and non-narcotic properties make embelin more acceptable than morphine (Atal et. al., 1984).

The efficacy of embelin against red flour beettle Tribolium castaneum, Herbst has been widely studied. The drug mixed with wheat samples at 0.19% concentration was found to exhibit high efficacy by bringing about adult mortality even after 8 months of storage. Productivity of the insect was reduced significantly in treatment at different intervals as compared to control. It was also observed that significant reduction in progecy after 8 months of storage was due to larvicidal action of embelin. Further research showed that germination of wheat treated with the drug was not impaired (Chander et. al., 1985).

1.5 OBJECTIVES

From the foregoing, it is clear that Myrsinaceae plants contain compounds which have economic and scientific interest. Chemical analysis of our indigenous species with a view to establishing levels of their benzoquinones, along with bioactivity tests was therefore deemed appropriate and is the objective of this thesis.

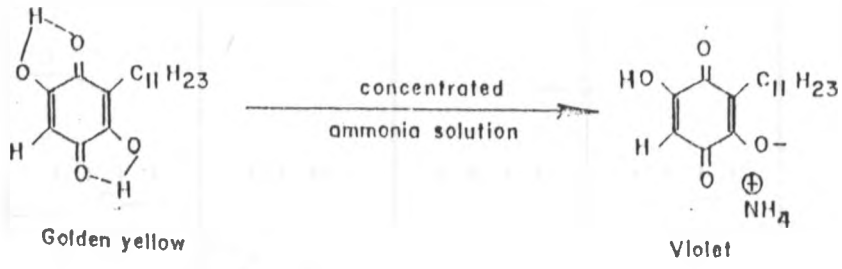
CHAPTER 2

2.0 RESULTS AND DISCUSSION

2.1 Preliminary Study of Myrsinaceae Plants

Micro-chemical tests as detailed in the experimental section revealed that benzoquinones are the most common secondary metabolites in the Myrsinaceae in line with earlier assertions (Ogawa and Natori, 1968). Extracts of all the parts of the plants gave violet colour with concentrated ammonia solution due to ionisation of the hydroxyl groups. Such deprotonation of the acidic enolic group leads to absorption at a longer wavelength than the usual absorption which gives rise to orange coloration (Scheme 3).

SCHEME 3: Reaction of ammonia and 2, 5-dihydroxy-1, 4-benzoquinone



The tests for alkaloids, flavonoids, saponins, sterols and terpenes showed positive results in at least one part of each plant examined except for E. schimperi which was devoid of alkaloids. These tests were performed according to the procedures of Ayoub and Kingston (1982). Table 2 shows the results of phytochemical screening of the Kenyan Myrsinaceae species studied.

TABLE 2

RESULTS OF PRELIMINARY STUDY OF MYRSINACEAE PLANTS

Botanical names	Plant parts	alkaloids	flavonoids	quinones	saponins	sterols	terpenes
Embelia schimperi	root bark	-	-	+ +	+	-	-
	stem bark	-	+	+ +	+	-	-
	leaves	-	+	+ +	+	+	-
	fruits	-	+	+ +	+	-	+
Maesa lanceolata	root bark	-	+	+ +	+	-	+
	stem bark	-	+	+ +	+	+	-
	leaves	+	+	+ +	+	-	-
	fruits	-	+	+ +	+	-	+
Myrsine africana	root bark	-	-	+ +	+	+	-
	stem bark	+	+	+ +	-	-	+
	leaves	-	+	+ +	+	-	-
	fruits	+	+	+ +	-	+	-
Rapanea melanphloes	root bark	+	-	+ +	-	-	+
	stem bark	-	+	+ +	+	-	-
	leaves	-	+	+ +	+	-	+
	fruits	-	+	+ +	+	+	-

KEY: (-) = negative, (+) = positive and (++) very positive

From the table it can be seen that alkaloids are the least common compounds detected followed by sterols and terpenes. Flavonoids and saponins were second to benzoquinones in frequency but they are of lower concentrations in the respective parts of the plants studied. Considering the various benzoquinones that have been previously isolated from the Myrsinaceae plants together with the overwhelming positive ammonia test, it was decided to isolate and characterise them and if possible perform the bioactivities of some constituents.

For separation of the compounds, sequential extraction was carried out using petroleum ether (40-60), chloroform and methanol. The methanol extracts were suspected to be glycosidated and were usually subjected to acid hydrolysis before being chromatographed. The various extracts were examined by silica gel thin layer chromatography plates impregnated with 3% oxalic acid solution and the solvent system n-hexane-ethylacetate-acetic acid (85:10:5) to establish their constitution.

2.2. E. shimperi, M. africana and R. melanphloes

In all parts of three plants above were isolated three orange bands which tested positive for benzoquinones. Using the t.l.c system described above, one band had an R_f 0.80 (35) and found to consist of one compound by mass spectrometry (vide infra).

The second band had an R_f of 0.40 and was found in most cases to be a mixture of benzoquinones, embelin (5) and rapanone (6) but in the case of root bark of Myrsine africana it was consisting of only 6, while in Rapanea melanphloes root bark there was only 5. The last band had an R_f of 0.33 and consisted of one substance most likely with the structure 12 . The gross concentration of the benzoquinones were relatively high (Tables 14 - 17). The highest concentrations were in the fruits: those of Rapanea melanphloes were more than 12% benzoquinones. These were followed by root and stem barks in that order, with the least concentration being found in the leaves. Clearly, benzoquinones are products of secondary metabolism in the leaves and transported for storage in the fruits and the bark. The concentrations in the fruits are higher than here-to-fore reported values for any Myrsinaceae plants.

The band corresponding to R_f 0.40 was retrieved in highest amounts and therefore was studied first. The melting point of the powder 140-142°c corresponding to embelin (5) and or raponone (6) (Lit. 141-142°c, Ogawa and Natori, 1968). The presence of the molecular ions m/e 294 and 322 in the mass spectrum confirmed the presence of two compounds. These were separated into embelin and rapanone with preparative h.p.l.c. using a silica gel packed column eluted with methanol. Compound 5 was received first from this procedure. This is a golden yellow compound with mp 140 - 142°c (Lit. 143°c, Rao and Venkateswarlu, 1962). The spectra for the compounds are at

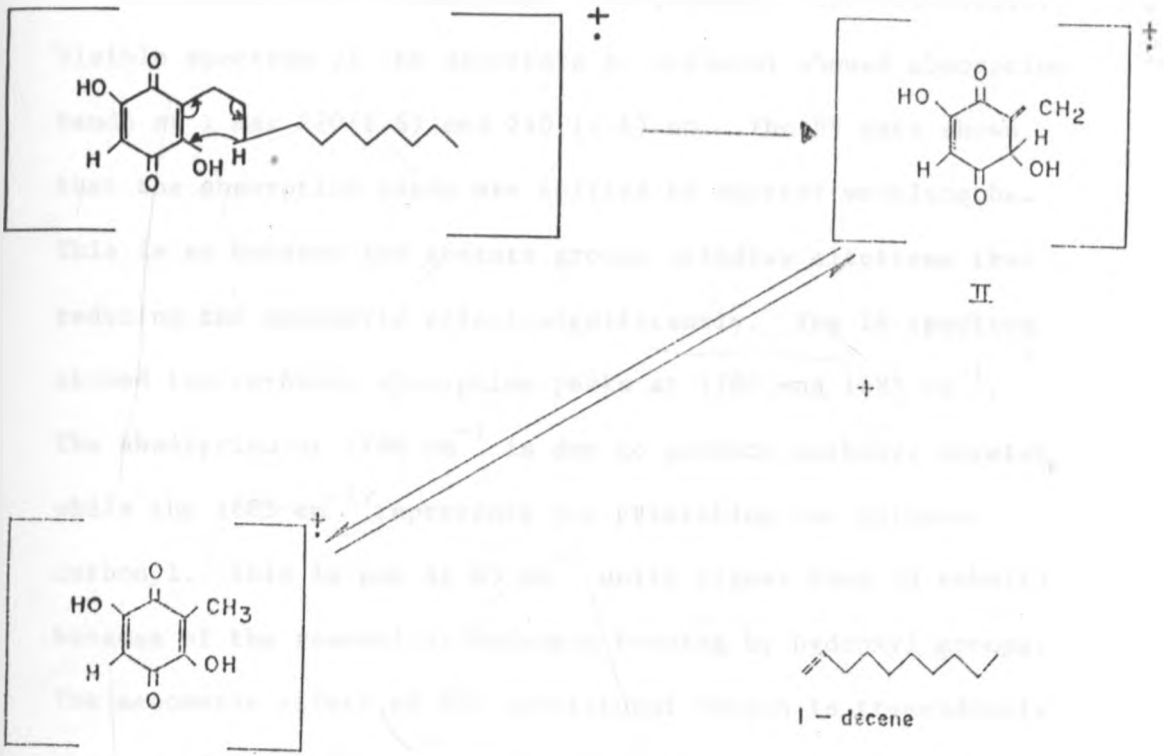
the end of the thesis and marked accordingly.

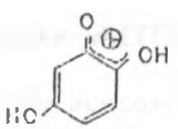
The UV/Visible spectrum of the compound in methanol gave absorption bands at λ max 425 (ε , 2.53) and 292(ε , 4.24) nm. indicating that the chromophore is a dihydroxy-p-benzoquinone. This agrees with literature data (Nakata et. al., 1964). The infrared spectrum run as potassium bromide pellet showed an absorption frequency at 3300 cm^{-1} representing intramolecularly hydrogen bonded hydroxyl groups. The IR spectrum has a strong carbonyl absorption frequency at 1620 cm^{-1} which is attributed to strongly hydrogen bonded carbonyl group stretch. The band showing at 710 cm^{-1} is due to methylene rocking vibrations in which all of them rock in one phase. This is characteristic of straight chain alkanes with seven or more carbon atoms (Silverstein et. al., 1981). The NMR ($CDCl_3$) spectrum of the compound showed a singlet at δ 7.66. This is due to protons of the hydroxyl groups on the ring. Integration of the peak indicates the presence of two such groups. A sharp singlet centred at δ 6.00 (H) is due to vinyl proton on the ring while the protons giving triplet appearing at δ 2.45 (2H) are the methylene protons on the first carbon substituting the ring. A multiplet observed at δ 1.45(2H) is attributed to methylene protons on the second carbon from the ring. A peak at δ 1.25(16H) represents methylenes of the alkyl side chain and lastly the triplet peak at δ 0.88(3H) is due to terminal methyl group of alkyl side chain. The mass spectrum shows significant peaks at m/e 294, 154 and 153.

The peak at m/e 294 is due to the molecular ion. Simple fission of the α,β -bond of the side chain gives a low abundance peak at m/e 153 due to even electron I. Apparently, the more favoured event is the ene reaction of the molecular ion leading to elimination of a neutral olefinic side chain and leaving a base peak at m/e 154 due to the even electron species II, according to scheme 4. The eliminated 1-decene fragments in the ionisation chamber in an expected fashion for straight chain hydrocarbon providing a homologous series of peaks at m/e 97, 83, 69, 55 and 41 due to simple fission processes. Scission of benzoquinone ring α -carbon bond together with oxygen loss leads to a low abundance but diagnostic peak at m/e 125 is due to the ion III.

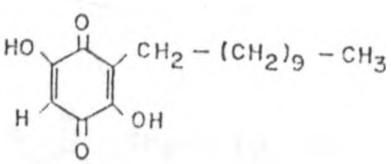
SCHEME 4: Fragmentation processes of embelin

I



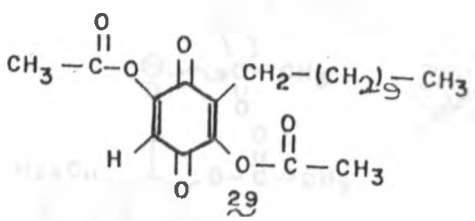


III



5

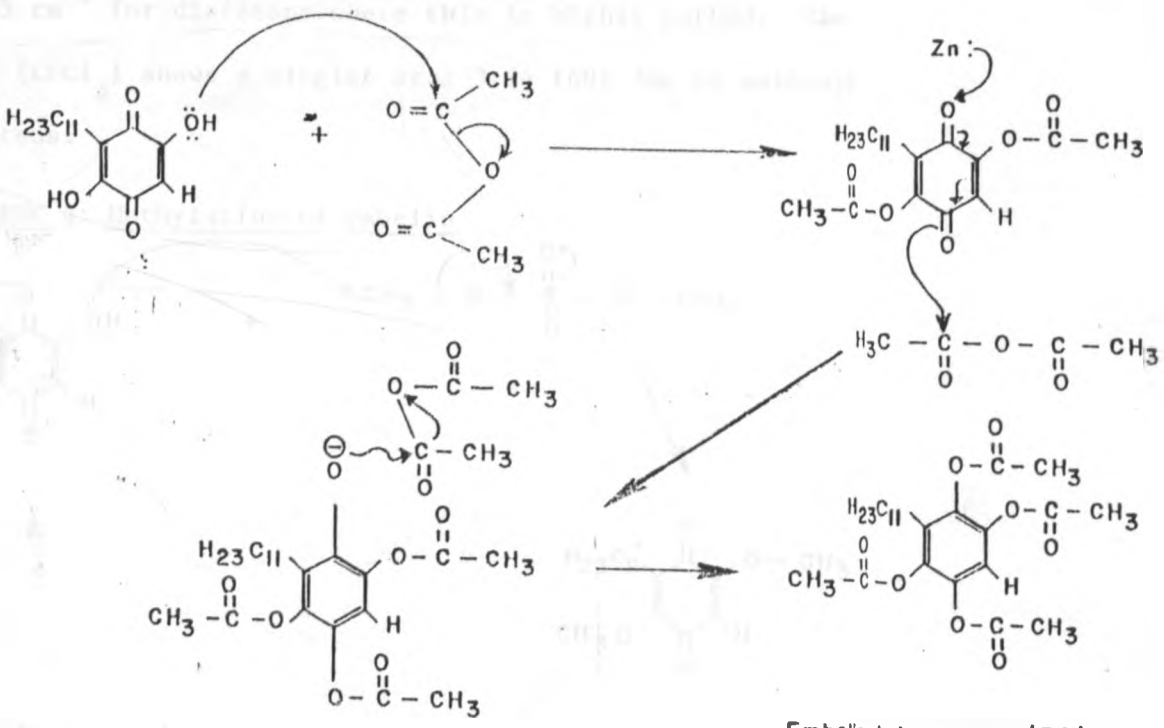
Diacetylation of the compound using acetic anhydride and pyridine at 25°c for 48 hrs gave embelin diacetate (29) with mp 52-54°c (Lit. 54°c, Krishna and Varma, 1936). The Ultraviolet/ Visible spectrum of the diacetate in methanol showed absorption bands at λ max 270(1.6) and 210 (2.6) nm. The UV data shows that the absorption bands are shifted to shorter wavelengths. This is so because the acetate groups withdraw electrons thus reducing the mesomeric effect significantly. The IR spectrum showed two carbonyl absorption peaks at 1780 and 1685 cm^{-1}. The absorption at 1780 cm^{-1} is due to acetate carbonyl stretch while the 1685 cm^{-1} represents the stretching for quinone carbonyl. This is now at 65 cm^{-1} units higher than in embelin because of the removal of hydrogen bonding by hydroxyl groups. The mesomeric effect of the substituent oxygen is tremendously reduced due to electronegativity of the acetate group. The NMR ($CDCl_3$) spectrum showed a singlet at δ 7.05(H) which on integration gave one proton. This is vinyl proton on the ring which is shifted downfield due to electron depletion by the two acetoxy groups. The singlet at δ 2.25 (6H) is attributed to the diacetate methyl groups.



29

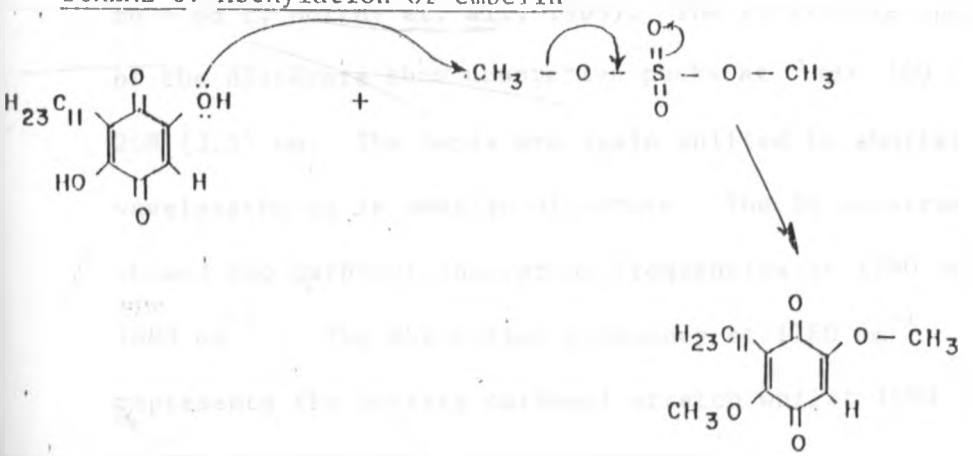
Tetraacetylation (according to scheme 5) of the compound carried out with boiling acetic anhydride and zinc dust gave colourless embelin tetraacetate (30) which melted at 121-123°c (Lit., 123 -124°c, Merrian and Schlittler, 1948). Tetraacetate formation ascertained the existence of two carbonyl and two hydroxyl groups. The UV/Visible spectrum in methanol showed absorption bands at λ max 265 (1.2) and 208 (2.2) nm. The hypsochromic effect is now exacarbated by four acetoxy groups to the extent that the compound becomes colourless. In the IR (KBr) a carbonyl absorption frequency at 1780 cm^{-1} which is due to acetate carbonyl stretch appeared while the peak for quinonoid carbonyl was altogether absent. The NMR ($CDCl_3$) spectrum showed a singlet at δ 7.09 (1H) which on integration gave one proton. This is due to the vinyl proton on the ring. The singlet centred at δ 2.25 (12H) originated from the four acetate methyl groups.

SCHEME 5: Tetraacetylation of embelin



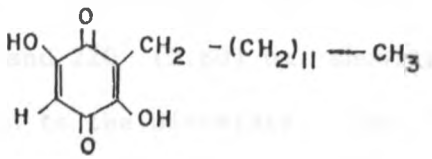
Methylation of the compound using dimethylsulphate in anhydrous acetone gave the expected dimethylether (31) according to scheme 6 with mp 55 - 56°c (Lit. 55 - 59°c, Krishna and Varma, 1936). The UV/Visible spectrum in methanol showed absorption peaks at λ max 280 (1.6) and 220 (2.6) nm. The absorption peaks are shifted to shorter wavelengths due to reduction in mesomeric effect of the alkoxy oxygen. However, the shifts are less pronounced compared to the diacetate compound. The infrared spectrum showed a carbonyl absorption frequency at 1680 cm^{-1} which is due to quinone carbonyl stretch. This shows that the occurrence of the carbonyl peak at the low value of 1620 cm^{-1} for the parent compound is mostly due to bond strength reduction by hydrogen bonding rather than by mesomerism. With dimethyl ether mesomerism is still allowed because of positive rather than negative inductive effect of the methyl group but the frequency of absorption compares well with 1685 cm^{-1} for diacetate where this is highly curbed. The NMR ($CDCl_3$) shows a singlet at δ 3.84 (6H) due to methoxyl protons.

SCHEME 6: Methylation of embelin



Embelin dimethylether (31)

The other golden yellow compound R_f 0.4, (6) from these plants had a melting point of 140-142°C. The compound is intimately associated with embelin and was separated from it by preparative silica gel h.p.l.c. as already described. It was also isolated uncontaminated with embelin from the root bark of Myrsine africana. The spectroscopic characteristics, like the mp were identical to those of embelin to a large extent. However, the ^1H NMR spectrum methylene absorption at δ 1.26 integrated to 20H rather than 16H as for embelin. Furthermore, the molecular ion was observed at m/e 322 rather than 294. These differences suggest that the compound is a homologue of embelin in which the alkyl side chain is increased by two methylene groups and is therefore rapanone (6) (Murthy et. al., 1965).

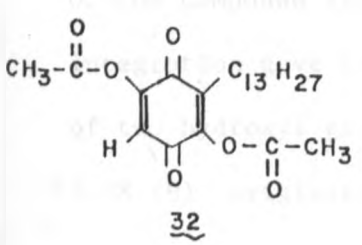


6

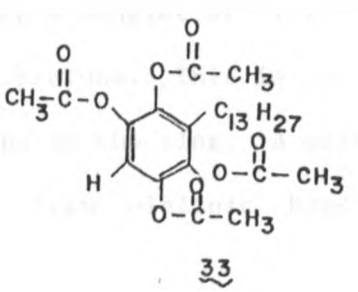
With acetic anhydride and pyridine at room temperature the compound gives a diacetate (32) with mp 63 -64°c (Lit. 64 - 66°c, Murthy et. al., 1965). The UV/Visible spectrum of the diacetate show absorption peaks at λmax 280 (1.4) 208 (3.5) nm. The bands are again shifted to shorter wavelengths as in embelin diacetate. The IR spectrum showed two carbonyl absorption frequencies at 1780 and 1680 cm^{-1}. The absorption frequency at 1780 cm^{-1} represents the acetate carbonyl stretch whilst 1680 cm^{-1} is due to quinonoid carbonyl stretch.

The NMR ($CDCl_3$) peaks for diacetate correspond to those of 5 diacetate. Reductive acetylation of the compound with boiling acetic anhydride and zinc dust yielded a colourless tetraacetate (33) which melted at 114°c - 116°c (Lit. 117°c, Murthy et. al., 1965). The UV/Visible spectrum of the tetraacetate in methanol showed absorption bands at λ max 260 (1.2) and 208 (3.2) nm. exhibiting an hypsochromic shift of bands as expected. The peak at 1780.cm^{-1} in the IR is for acetate carbonyls. The NRM ($CDCl_3$) showed a singlet at δ 2.25 (12H) attributed to four acetate methyl groups. The rest of the peaks are as expected.

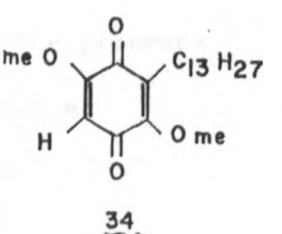
Methylation of the compound done as for embelin gave a dimethyl ether (34) with mp 63-65°c (Lit. 64-65°c, Aiyr et. al., 1964). The UV/Visible spectrum showed absorption bands at λ max 290 (1.4) and 220 (2.80) nm. showing reduced hypsochromic shift compared to the diacetate. The IR (ν_{max} KBr) spectrum exhibited olefinic carbon-hydrogen bond absorption frequency at 3050 cm^{-1} and the quinone carbonyl stretch at 1680 cm^{-1}. The NMR Spectrum recorded using $CDCl_3$ as a solvent confirmed dimethylation with a peak at δ 3.86 (3H).



32



33



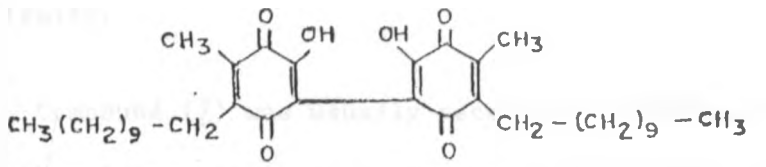
34

In all parts of the three plants studied the compound (35) with mp 119 - 121°c was isolated. Its elution from the column was effected with n-hexane-benzene (4:1) solvent mixture. The UV/Visible spectrum of the compound in methanol gave absorption bands at λ max 420 and 280 with log ε values 2.45 and 4.20 respectively. The absorption band at 420 nm. indicates the presence of a dihydroxy-p-benzoquinone chromophore. (Beynon and Williams, 1968, Nakata et. al., 1964). The IR spectrum of the compound run as potassium bromide pellet has a characteristic absorption frequency for intramolecularly hydrogen bonded hydroxyl stretch at 3325 cm^{-1}. The bands exhibited at 2940 and 2860 cm^{-1} represent doubly split absorption frequencies characterising alkane carbon - hydrogen bond stretches. A strong absorption for the carbonyl bond is observed at 1650 cm^{-1}. Since the carbonyl has increased intensity like in 5 and 6, the two hydroxyl groups probably are at the 2,5 - positions. The band at 720 cm^{-1} for long chain alkyl substituent is also present. The NMR ($CDCl_3$) spectrum of the compound showed a singlet at δ7.72 (2H) which on integration gave two protons. This is due to the presence of two hydroxyl groups on the ring. A multiplet at δ5.38 (H) originated from olefinic bond in

the side chain and the triplet displayed at δ 4.28 (H) is assigned to a proton on the olefinic carbon substituting the ring. Another triplet at δ 2.40 (2H) represents methylene on the ring. The multiplet centred at δ 2.0 (2H) is attributed to methylene substituting the olefinic bond in the side chain while the broad peak at δ 1.25 (10H) represents methylenes of the side chain. Lastly, a triplet observed at δ0.88 with intergration intensity six is for terminal methyls of the side chain. The mass spectrum exhibited the molecular ion peak at m/e 294. The ions at m/e 153, 154 and 125 are due to the ions I, II and III as for embelin. This confirms the dihydroxy substitution pattern for the benzoquinone ring of the compound. The compound formed a tetraacetate confirming tetra-oxygenation in the benzoquinone ring. However, the structure of the compound still remained unresolved. Its spectroscopic properties resemble those of embelin to a large extent. The one glaring difference is the carbonyl peak in the infrared spectrum which for embelin shows at 1620 cm^{-1} while in this compound, it is at 1650 cm^{-1}. Furthermore, despite the fact that mass spectometry data suggests tetra-substitution of the ring, the NMR does not indicate an absorption for the supposed lone vinyl proton on the ring. This is probably fortuitously coincident with chloroform peak but it is not revealed in the NMR of the tetraacetate. It is essential to retrieve better spectroscopic data along with chemical degradative information to allow a suggestion of the structure of this compound. Lack of material prevented these events.

The other compound to come out of the columns for the three plants is an orange pigment with mp 66 - 68°c and R_f 0.8. Its elution from column was effected with benzene. The UV/Visible spectrum determined in methanol showed absorption bands at λ max 420 (2.80) and 280 (4.50) nm. The UV bands are characteristic of p-benzoquinone with one or more hydroxyl group substituents. The IR spectrum showed an absorption frequency at 3275 cm^{-1} indicating intramolecularly hydrogen bonded hydroxyl group. The absorption centred at 1620 cm^{-1} is for chelated carbonyl stretch. The low field NMR region of the compound did not have any major peaks except for a small broad bump around the chloroform peak attributable to hydroxy protons. The high field region on the other hand had peaks at δ 2.45 (4H, triplet), a sharp singlet at δ 1.83 (6H, $2CH_3$) due to a methyl group on the quinonoid ring, δ 1.5 (multiplet), δ 1.25 (multiplet of long chain) and a triplet at δ 0.88(6H) due to end methyl groups of the alkyl side chains. The peak at δ2.45 collapsed to a singlet on irradiation at δ1.5 implying coupling between these sets of signals. This experiment confirmed that the methylene group absorbing at δ 2.45 is attached to the quinone ring. The NMR is devoid of any quinonoid proton signal, thus it was fully substituted. The mass spectrum had peaks for the expected alkyl side chain and other significant peaks at m/e 582, 178 and 154. The first peak probably represents the molecular ion. This leads to the tentative proposition that the compound is the symmetrical dimer macrophyllin (12) (Prabhu and Venkateswarlu, 1971).

The possible structure is shown below:



12

Lack of sufficient material prevented confirmation of this structure by further spectroscopic and synthetic studies. This compound is found in highest concentration in R. melanphloes fruit. A further collection of larger amounts of material and extraction should lead to the confirmation of the structure.

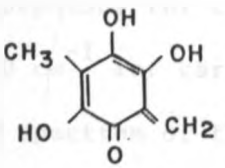
2.3 Maesa lanceolata

Maesa lanceolata, as opposed to the other three Myrsinaceae plants, gave a different set of benzoquinones. With silica gel plates impregnated with 3% oxalic acid solution and developed with the solvent system, n-hexane-ethylacetate-acetic acid (85:10:5) as before, led to realisation of components with the following R_f values; in the leaves, 0.63 (7), 0.50 (14) and 0.20 (15); in all the other parts, these compounds and others appearing on the chromatogram at R_f 0.48 (39) and 0.35 (40). Again the concentration of some of the benzoquinones was quite impressive in this plant. The concentration was highest in the fruit (11.3% for 7) but lowest in the leaves with intermediate quantities in the stem and root barks.

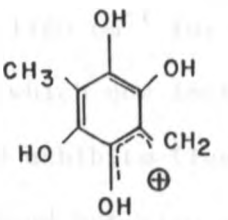
Again this probably reflects the procession of secondary metabolism in the leaves with subsequent storage in the bark and fruits.

Compound (7) was usually received in highest amount compared to the other four. It is an orange compound with mp 122 - 124°c. The UV/Visible spectrum of the compound in methanol gave absorption bands at λ max 440 and 294 nm with log ε values 2.40 and 4.40 respectively indicating the existence of a dihydroxy-p-benzoquinone chromophore (Chandrasekhar et. al., 1970). The IR spectrum determined as potassium bromide pellet showed absorption frequency at 3300 cm^{-1} which is attributed to intramolecularly hydrogen bonded hydroxyl group stretch. The other significant peaks included a broadened strong peak at 1620 cm^{-1} representing the hydrogen bonded α , β -unsaturated hydroxybenzoquinone carbonyl as has been noted for cases above. The base of this peak is rather broad probably encompassing a weak olefinic bond otherwise another weak peak is observable at 1470 cm^{-1} attributable to the benzoquinone olefinic stretch. The 710 cm^{-1} band diagnostic of a long side chain is also observed. The NMR spectrum of the compound determined in $CDCl_3$ showed a singlet at δ 7.59 (2H) which is due to hydroxyl protons on the quinonoid ring. A multiplet at δ 5.38 (2H) is attributed to vinyl protons of an olefin possibly in the alkyl side chain and the triplet at δ 2.40 (2H) represents the methylene group attached to the quinone ring. The multiplet at δ 2.0 (4H) represents the methylene groups substituting the olefinic bond in the alkyl side chain while the singlet centred at δ 1.94 (3H) shows the presence of

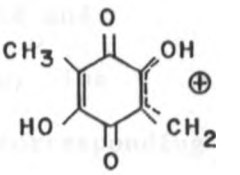
a methyl substituent on the quinonoid ring. The huge peak at δ1.26 (26H) is due to side chain methylenes. Lastly, a triplet at δ 0.90 (3H) is attributed to side chain end methyl group. The mass spectrum shows a molecular ion peak at m/e 418. The base peak results from an ene reaction of the molecular ion resulting in the ion IV while peaks at m/e 169, 167 and 139 are due to the daughter ions V, VI and VII.



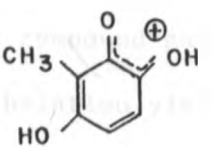
IV



V

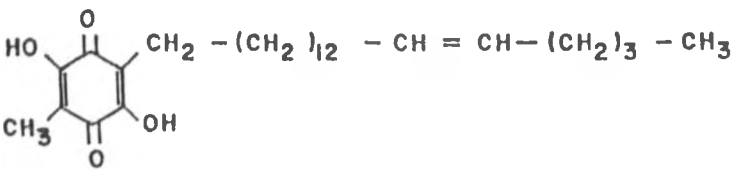


VI



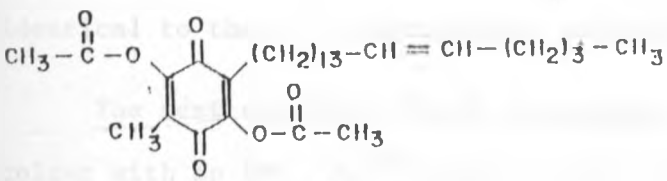
VII

These MS data and those of other spectra correspond to those reported for maesaquinone (7) in the literature (Ogawa and Natori, 1968). Infact the mp in the literature is also quite close (123°c, Ogawa and Natori, 1968) to that obtained for compound (7). It was therefore established as maesaquinone shown below.

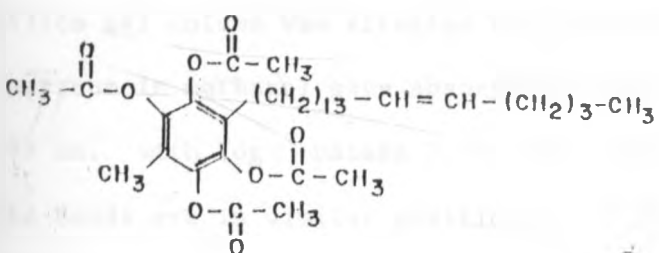


7

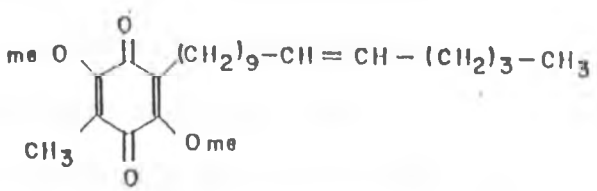
Diacetylation of the compound by the usual method gave a diacetate (36) which melted at 72 - 74°c (Lit. 74 - 76°c, Harimoto, 1939). The UV spectrum in methanol showed absorption peaks at λ max 260 and 220 with log ε values 2.45 and 1.45 respectively. The bands are shifted to shorter wavelength compared to maesaquihone due to withdrawal of electrons by the acetate groups thereby reducing the mesomeric effect of the oxo-substituent. The IR spectrum showed a characteristic absorptions for carbonyl at 1780 cm^{-1} for the acetate and 1670 cm^{-1} for carbonyl peak which now lack chelation. The NMR spectrum of the compound exhibits five signals corresponding to peaks in the parent compound but also notebly the singlet at δ 2.28 (6H) due to acetate methyl groups. Reductive acetylation of the compound gave a tetraacetate (37) as expected while methylation yielded dimethyl ether (38).



36

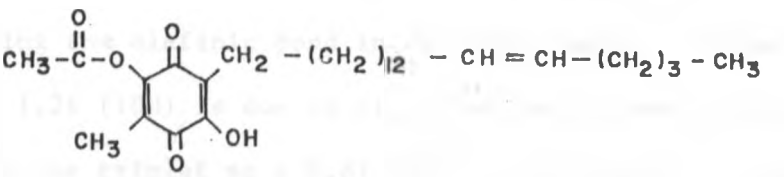


37



38

The second compound to elute from the column after 7, from all parts of Maesa lanceolata, was an orange compound with mp 25 - 38°c and Rf 0.50 (14) on silica gel analytical plate impregnated with 3% oxalic acid solution and developed with n-hexane-ethylacetate-acetic acid (85:10:5). Its spectroscopic properties were similar to those of maesaquinone except for peaks in various spectra signifying the presence of an acetate.



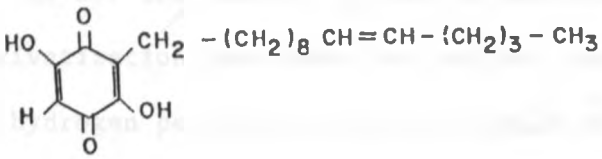
14

Tetraacetylation of the compound using similar procedures as for maesaquinone (7) gave its tetraacetate as crystals with mp 80 - 82°c. Its spectroscopic data and mp were identical to those of maesaquinone tetraacetate (37).

The next compound from M. lanceolata was orange in colour with mp 104 - 106°c and R_f 0.48 (39) on T.L.C. with the usual solvent system. Its elution from the treated silica gel column was effected with benzene. The UV/Visible spectrum in methanol gave absorption bands at λ max 440 and 285 nm. with log ϵ values 2.70 and 4.20 respectively. The bands are in similar positions to those of maesaquinone (7) indicating the existence of a dihydroxy-p-benzoquinone. The IR spectrum showed a characteristic absorption for intramolecularly hydrogen bonded hydroxyl group at 3350 cm^{-1} in a KBr pellet. A weak absorption frequency at 3000 cm^{-1} is assigned to olefinic carbon-hydrogen bond stretch.

The spectrum exhibits a carbonyl absorption frequency at 1625 cm^{-1} which is attributed to strongly hydrogen bonded carbonyl group. The NMR ($CDCl_3$) spectrum showed the presence of two hydroxyl groups at δ 7.65 (2H) and the vinyl proton singlet at δ 6.0 (1H) all are on the ring. A multiplet at δ 5.35 (2H) represents protons on an olefinic bond in the alkyl side chain and the triplet at δ 2.44 (2H) is due to protons on methylene substituting the ring. A multiplet at δ 2.0 (4H) represents two methylenes substituting the olefinic bond in the side chain. A broad peak at δ 1.26 (18H) is due to side chain methylene protons and lastly the triplet at δ 0.87 (3H) is attributed to terminal methyl protons. The mass spectrum showed a molecular ion peak at m/e 348. The peak at m/e 153, 154 and 125 are due to ions I, II and III which have the same origins as for embelin. All these data suggest a tentative structure as shown below. Although the benzoquinone ring substitution pattern may be considered confirmed because of mass spectrometry fragmentation peaks together with UV and IR data correlation, the position of substitution of the double bond in the side chain is not confirmed. This can be reached by chemical degradation using sodium periodate/potassium permanganate and subsequent analysis of the resultant carboxylic acids. This was not possible at this stage because of scarcity of material. The double bond positioning in this proposed structure is based on comparison with maesaquinone and maesanin, the other compounds with unsaturated alkyl side chains from this plant, which invariably have the double bonds at carbon-5 from the end of the chain.

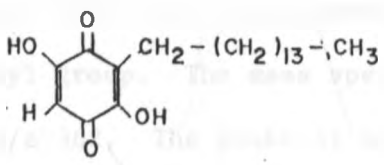
Lack of material also prevented derivative formation which would have further confirmed the structure of this otherwise novel benzoquinone structure.



39

From Maesa lanceolata extracts of root bark, stem bark and fruits another orange compound with R_f 0.35 (40) and melting point 130 - 132°c was isolated. It was eluted from column with chloroform. The UV/Visible spectrum in methanol exhibited absorption bands at λ max 420 and 290 nm. with log ε values 2.40 and 4.20 respectively typical of 2, 5-dihydroxybenzoquinone. The IR spectrum run as potassium bromide pellet showed an absorption frequency at 3300 cm^{-1} which is characteristic of hydrogen bonded hydroxyl stretch. An absorption frequency at 1620 cm^{-1} is due to strongly hydrogen bonded carbonyl stretch and an alkyl side chain is indicated by a peak at 720 cm^{-1}. The NMR ($CDCl_3$) spectrum showed a singlet at δ 7.66 representing the two hydroxyl protons on the quinonoid ring and the triplet at δ 2.45 (2H) represents the methylene substituting the ring. The broad peak displayed at δ1.25 (26H) is attributed to side chain methylene protons and lastly the triplet at δ0.88 (3H) is attributed to the side chain end methyl group.

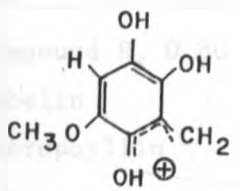
The mass spectrum exhibited a molecular ion peak at m/e 350. Other peaks appeared at m/e 155, 154, 142 and 125 with good abundances corresponding to those already discussed for all the 2,5-dihydroxy-benzoquinones and thus confirming this moeity. The tentative structure proposed which will most likely to be correct is 2, 5-dihydroxy-3-pentadecylbenzo-quinone (40). As for the case of 39 lack of material barred any derivatization reactions for further characterisation. Mild alkaline hydrogen peroxide oxidation should lead to easily identifiable pentadecanoic acid (Rao and Venkateswarlu, 1962).



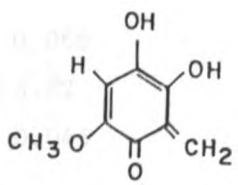
40

The last compound to elute from the column was a yellow pigment with mp 67 - 69°c. Its UV/Visible spectrum in methanol gave absorption bands at λ max 420 and 280 nm with log ε values 2.70 and 4.20 respectively. The IR spectrum showed an absorption frequency at 3325 cm^{-1} which is attributed to an intramolecularly hydrogen bonded hydroxyl group. The absorption frequency displayed at 3000 cm^{-1} represents olefinic carbon-hydrogen bond stretch. The IR spectrum exhibits bsorption peaks at 1650 and 1610 cm^{-1} indicating two carbonyl moeities with different bond strengths. The peak at 1650 cm^{-1} is attributed to unchelated quinonoid carbonyl stretch while the 1610 cm^{-1} is due to chelated carbonyl stretch.

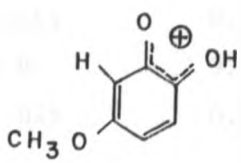
The NMR ($CDCl_3$) spectrum showed a hydroxyl group at δ 7.2 (1H) and a singlet at δ 5.94 (1H) is due to vinyl proton on the quinone ring. A multiplet at δ 5.35 which on integration gave two protons is due to vinyl protons on the olefinic bond in the side chain. The singlet displayed at δ 3.85 (3H) represents the methoxyl group substituting the quinonoid ring and the triplet signal at δ 2.40 (2H) is attributed to protons of methylene substituting the ring. The multiplet at δ 2.0 (4H) represents two methylenes substituting the olefinic bond in the alkyl side chain and the broad peak at 1.26 (18H) is for side chain methylene protons. Lastly, the triplet at δ 0.87 (3H) is assigned to the side chain terminal methyl group. The mass spectrum showed a molecular ion peak at m/e 362. The peaks at m/e 169, 168 and 139 are are due to ions XI, XII and XIII typical of benzoquinones as already discussed.



XI

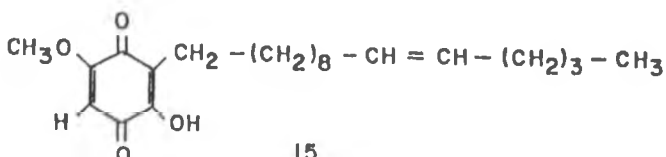


XII



XIII

The data generated here correspond to those of maesanin (Lit. mp 69-73 °c, Ogawa and Natori, 1968).



15

Table 3. Level of benzoquinones in the root bark of Myrsinaceae plants

	A Free aglycones (in grams)	B O-glycosides (in grams)	A+B	Aglycones as % root bark weight
Embelia schimperi				
Compound R_f 0.80	0.022	-	0.022	0.14
embelin (rapanone)	3.36	0.019	3.38	2.25
macrophyllin	0.013	-	0.013	0.008
Maesa lanceolata				
maesaquinone	3.79	0.25	4.04	2.69
acetylmaesaquinone	0.763	0.015	0.778	0.519
2,5-dihydroxy-3-pentadecenylbenzoquinone	trace	-	-	-
2,5-dihydroxy-3-pentadecylbenzoquinone	trace	-	-	-
maesanin	0.082	0.015	0.097	0.065
Myrisine africana				
Compound R_f 0.80	0.023	-	0.023	0.015
rapanone	3.43	0.014	3.44	2.29
macrophyllin	0.014	-	0.014	0.001
Rapanea melanphloes				
Compound R_f 0.80	0.066	-	0.066	0.044
embelin	13.82	0.08	13.9	9.27
macrophyllin	0.045	-	0.045	0.003

Table 4. Level of benzoquinones in stem bark of Myrsinaceae plants

	A Free aglycones (in grams)	B O-glycosides (in grams)	A+B (in grams)	Aglycones as % stem weight
Embelia schimperi				
Compound R_f 0.80	0.009	0.003	0.012	0.009
embelin (rapanone)	2.15	0.026	2.18	1.45
macrophyllin	0.010	-	0.010	0.007
Maesa lanceolata				
maesaquinone	3.51	0.014	3.52	2.34
acetylmaesaquinone	0.073	trace	0.073	0.41
2,5-dihydroxy-3-pentadecenylbenzoquinone	trace	-	-	-
2,5-dihydroxy-3-pentadecylbenzoquinone	trace	-	-	-
maesanin	0.039	-	0.039	0.026
Myrsine africana				
Compound R_f 0.80	0.011	-	0.011	0.007
embelin (rapanone)	2.23	0.02	2.25	-
macrophyllin	0.014	-	0.014	0.009
Rapanea melanphloes				
Compound R_f 0.80	0.05	-	0.05	0.03
embelin (rapanone)	3.95	0.003	3.95	2.63
macrophyllin	0.027	-	0.027	0.047

Table 5. Level of benzoquinones in the leaves of Myrsinaceae plants

	A Free aglycones (in grams)	B 0-glycosides (in grams)	A+B	Aglycones as % root bark weight
Embelia schimperi				
Compound R_f 0.80	0.002	-	0.002	0.001
embelin (rapanone)	1.88	0.008	1.89	1.26
macrophyllin	0.001	-	0.001	0.001
Maesa lanceolata				
maesaquinone	2.02	0.002	2.02	1.35
acetylmaesaquinone	0.065	-	0.065	0.04
2,5-dihydroxy-3-pentadecenylbenzoquinone	-	-	-	-
2,5-dihydroxy-3-pentadecylbenzoquinone	-	-	-	-
maesanin	0.032	-	0.032	0.02
Myrsine africana				
Compound R_f 0.80	0.01	-	0.01	0.007
embelin (rapanone)	2.69	0.034	2.72	1.81
macrophyllin	0.002	-	0.002	0.001
Rapanea melanphloes				
Compound R_f 0.80	0.04	-	0.04	0.02
embelin (rapanone)	3.75	0.005	3.75	2.50
macrophyllin	0.014	-	0.014	0.009

Table 6. Level of benzoquinones in the fruits of Myrsinaceae plants

	A	B	A+B	
	Free aglycones (in gram)	O-glycosides (in gram)	(in gram)	Aglycones as % fruits weight
Embelia schimperi				
Compound R_f 0.80	0.025	-	0.025	0.017
embelin (rapanone)	6.47	0.004	6.47	4.32
macrophyllin	0.015	-	0.015	0.01
Maesa lanceolata				
maesaquinone	16.68	0.31	16.99	11.33
acetylmaesaquinone	0.95	-	0.95	0.63
2,5-dihydroxy-3-pentadecenylbenzoquinone	0.005	-	0.002	0.002
2,5-dihydroxy-3-pentadecylbenzoquinone	0.029	-	0.029	0.020
maesanin	0.163	0.003	0.166	0.11
Myrsine africana				
Compound R_f 0.80	0.026	-	0.026	0.017
embelin (rapanone)	6.09	0.003	6.093	4.06
macrophyllin	0.03	-	0.003	0.02
Rapanea melanphloes				
Compound R_f 0.80	0.34	-	0.34	0.23
embelin (rapanone)	18.18	0.057	0.057	0.038

2.4 CONCLUSIONS AND COMMENTS

It has been shown in this thesis that Myrsinaceae of this country, *Embelia schimperi*, *Myrsine africana*, *Rapanea melanphloes* and *Maesa lanceolata* contain long side chain benzoquinone derivatives. The concentration of these materials in *R. melanphloes* root (9.3%) and stem bark (2.7%) and particularly fruits (12.4%) suggest that they can be harvested from this plant. The concentration in the fruits is higher than any here-to-fore reported levels of these compounds from any Myrsinaceae. The chief constituents of the extract mixture from *Rapanea melanphloes* are embelin and rapanone. The former shows anti-fertility effects while both indicate promising bacteriostatic and mosquito larvicidal effects. The concentration of the compounds have been observed which do not correspond to those known to date in *E. schimperi*, *R. melanphloes* and *M. africana*. Another two which were not known before are also observed in *M. lanceolata* along with its maesaquinones. The maesaquinones found in this species in high yield are interesting compounds in themselves since their 2, 3-oxiranyl derivative have anti-cancer activity (Otsuka, 1982) amongst other effects already noted. Clearly further chemical work on these plants, especially leading to their economic exploitation is warranted.

CHAPTER 3

EXPERIMENTAL

3.1.0 GENERAL

Melting points were determined using Gallenkamp melting point apparatus and are uncorrected. The Ultraviolet/Visible spectra were obtained using Beckman Du-50 spectrophotometer. Infrared spectra were run as potassium bromide pellets using a Perkin Elmer infrared 720 spectrometer. NMR spectra were determined at the University of Calgary, Canada on a Varian 200 MHz spectrometer. Mass spectra were run on a Masslab VG 12-250. H.P.L.C. preparative separation was performed on a Varian 5000 liquid chromatograph .

All the reagents and solvents used were of analytical grade. Merck silica gel 60G (0.063 - 0.2 mm/70 - 230 mesh A.S.T.M.) was used for column chromatography and silica gel 60 G was used for both t.l.c. and preparative t.l.c.

Plants were collected from different parts of the country, dried in shade and ground to powder using a Willey mill. Embelia schimperi was collected from Ngong hills at 2400 m above sea level. Maesa lanceolata and Rapanea melanphloes were collected from the top of Mt. Aberdares (2400 - 3000 m above sea level). Myrsine africana was collected from Kithembe hills in Machakos District at 2000 m above sea level. All the plants used were identified at the Botany Department, University of Nairobi and the National Museum herbaria. The spectra are at the end of the thesis. ^{1}H NMR spectral assignment tables along with tables for other physical

characteristics of compounds are also provided for quick reference.

3.2.0 PRELIMINARY STUDIES

Preliminary studies were carried out using conventional methods (Ayoub and Kingston, 1982) to determine the presence of alkaloids, flavonoids, quinones, saponins, sterols and terpenes.

3.2.0.1 Embelia shimperi root bark

Dry powder (50 g) was exhaustively extracted with 300 ml of 95% ethanol in a soxhlet extractor for 24 hrs until the solvent in the siphon arm was colourless. The extract was filtered while hot under vacuum and concentrated using a rotary evaporator leaving a dark red syrup (12.0g).

3.2.0.2 Test for alkaloids in the root bark of E. schimperi

(i) In this experiment 1.0g of the above extract was mixed with 10 ml of 2m hydrochloric acid and the mixture heated in a boiling water bath for 15 minutes. The mixture was filtered while hot and to the filtrate a few drops of Mayer's reagent were added. Turbidity or white precipitates expected of alkaloids were not observed.

(ii) Approximately 1.0g of the extract was heated with 10ml of methanol-chloroform (1:1) mixture. The solution was spotted on three silca gel t.l.c. plates and developed separately using chloroform-methanol (9:1), chloroform-ethylacetate (4:1) and methanol-ammonia (10:3). Dragendorff's reagent was sprayed onto the plates. Yellow spots expected of alkaloids were not observed.

3.2.0.3 Test for flavonoids in the root bark of E. schimperi

The extract (2.0 g) was defatted with petroleum ether (40-60) and the solid residue dissolved in 30 ml of 80% methanol. The solution was filtered and the filtrate used for the following tests:

(i) To 3 ml of the filtrate in a test-tube 4.0 ml of 1% aluminium chloride in methanol was added. A yellow colour expected of flavonoid compounds was not observed.

(ii) To 3 ml of the filtrate in a test-tube 4 ml of 1% potassium hydroxide solution was added. A dark yellow colour expected of flavonoids was not observed.

3.2.0.4 Test for hydroxybenzoquinones in the root bark of E. schimperi

A sample of the extract (2.0 g) was boiled with 20 ml of 1% hydrochloric acid for 5 minutes and then filtered while hot. The filtrate was extracted with 5 ml of benzene. The organic layer was separated and 5 ml of 10% aqueous ammonium hydroxide solution were added to it. A violet colour was observed in the alkaline medium indicating the presence of hydroxy-benzoquinones.

3.2.0.5 Test for saponins in the root bark of E. schimperi

Approximately 1.0 g of the extract was vigorously shaken with water in a test-tube. Froth occured and persisted for over 24 hrs. This was taken as a positive test for saponins.

3.2.0.6 Test for sterols and terpenes in the root bark of E. schimperi

Some 2.0 g sample of the extract was defatted with petroleum ether (40-60). The defatted solid residue was then extracted with 20 ml of chloroform and the solution dried over anhydrous magnesium sulphate. A 5 ml portion of the chloroform solution was mixed with 0.5 ml of acetic anhydride followed by two drops of concentrated sulphuric acid. A gradual appearance of green to blue colours taken as an indication for the presence of sterols was not observed. Also a change of colour from pink to purple which is an indication for the presence of terpenes was not observed.

Other parts of E. schimperi and those of M. lanceolata, M. africana and R. melanphloes were screened in a similar manner. The results are shown in table II.

3.3.0 EXTRACTIONS AND ISOLATIONS OF BENZOQUINONES

3.3.1 Embelia schimperi root bark

3.3.1.1. Petroleum ether (40-60) extraction

Dry powdered root bark (150 g) was exhaustively extracted with 1000 ml of petroleum ether (40-60) in a soxhlet extractor for 24 hours. The extract was filtered while hot and the filtrate concentrated using a rotary evaporator leaving a dark brown solid weighing 2.76 g. Analysis on silica gel t.l.c. plate impregnated with 3% oxalic acid solution and eluted with n-hexane-ethylacetate-acetic acid (85:10:5) gave three yellow spots of R_f values 0.80 (35), 0.40 (5 and 6) and 0.33 (12).

All turned violet on exposure of the plate to concentrated ammonia solution. UV light and iodine vapour development did not reveal any more spots. The extract weighing 2.5 g was pre-adsorbed on dry acid washed silica gel and then introduced into a glass column 3.5 cm diameter packed with 100 g of the acid washed silica gel. The column was packed under n-hexane and successively eluted with n-hexane-benzene (4:1), n-hexane-chloroform (4:1) and benzene. The first orange band was eluted out by passing n-hexane-benzene (4:1) through the column and fractions of 100 ml each were collected. This n-hexane-benzene (4:1) elution gave a single orange compound and the fractions were combined and evaporated to dryness. Recrystallisation from methanol-chloroform (4:1) gave orange crystals (35) (21.7 mg) with mp 119-121°c. UV/ Visible λ max (meOH) 420 (2.45) and 280 (4.20) nm. IR υ max (KBr) 3325 (O-H stretch), 2940, 2860 (alkane C-H stretch), 1650 (C=O stretch), 1480, 1150, 800, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.72 s (2H, OH), δ 5.38 m (H, CH=C), δ4.28 t (H, CH=C), δ 2.40 t (2H, CH_2), δ 2.0 m (2H, CH_2), δ1.25 m (1OH, $(CH_2)_5$) and δ 0.88 t (6H, $2CH_3$). MS m/e M+ 294(7.9), 169(1.0), 168(2.7), 167(1.0), 155(19.5), 154(61.6), 153(12.4), 142(12.1), 125(7.2), 11(5.4), 97(12.4), 85(17.4), 69(35.8), 57(66.3), 43(100) and 41(64.2).

Subsequent elution of the column with n-hexane-chloroform (4:1) washed out the second orange band containing compounds 5 and 6 as shown by h.p.l.c. (vide infra). Elution with benzene gave further a compound of R_f 0.33 (12, trace).

3.3.1.1.1. H.P.L.C. Separation of the second component from E. schimperi root bark

The mixture with R_f 0.40 (5 and 6) was recrystallised from benzene giving golden yellow plates (1.49 g) with mp 140-142°c. When 50 mg of the sample dissolved in methanol was

injected into analytical h.p.l.c., two peaks were observed indicating the presence of two compounds. These were separated by silica gel normal phase preparative h.p.l.c. eluting with methanol into two compounds, 5, 10 mg and 6, 23 mg.

Compound 6 melted at 140 - 142°c and had the following spectrophotometric figures: UV λ max (meOH) 425 (2.53) and 292 (4.24) nm. IR ν max (KBr) 3310(O-H stretch), 2900, 2850 (alkane C-H stretch), 1623 (chelated C=O stretch), 1480, 1380, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.66 s (2H, 2OH), δ 6.00 s (H, vinyl proton), δ 2.45t (2H, CH_2), δ 1.45 m (2H, CH_2), δ 1.26 m (20H, $(CH_2)_{10}$) and δ0.88t (3H, terminal CH_3). Mass spectrum m/e values M^+ 322 (19.4), 155(32.0), 154(100), 153(19.8), 142(20.5), 125(9.1).

Compound 5 had mp 140 - 142°c; its spectral figures were as follows: UV λ max (meOH) 425(2.53) and 292(4.24) nm. IR ν max (KBr) 3300 (O-H stretch), 2900, 2850 (alkane C-H stretch), 1620 (chelated C=O stretch), 710 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.66s (2H, 2OH), δ6.00s (H, vinyl proton), δ 2.45t (2H, CH_2), δ 1.45 m (2H, CH_2), δ1.25 (16H, $(CH_2)_8$) and δ 0.88t (3H, terminal CH_3). MS m/e values M^+ 294(6.8), 155(31.5), 154(100), 153(19.5), 142(20.1), 125(9.1).

3.3.1.2 Chloroform extract of E. schimperi root bark

The root bark residue from petroleum ether extraction was further extracted with 1000 ml of chloroform for 24 hrs giving a brown solid (2.50 g) on evaporation of the solvent.

T.l.c. analysis showed the presence of two yellow spots with R_f values 0.40 (5 and 6) and 0.33(12). Both spots turned violet on exposure to concentrated ammonia solution. A sample of the extract weighing 2.45 g was pre-adsorbed on dry acid washed silica gel and eluted through 60 g of the acid washed silica gel. The column was successively washed with n-hexane, n-hexane-chloroform (4:1) and benzene. An orange band (t.l.c., R_f 0.40) was eluted out by passing n-hexane-chloroform (4:1) through the column and 26 fractions were collected. These were combined and evaporated to dryness. After crystallisation in benzene, the component yielded 1.90 g (5 and 6). Subsequent elution of the column with benzene gave eight fractions of 100 ml each, containing two components of R_f values 0.40 and 0.33. These were combined, evaporated and then charged on dry preparative t.l.c. plates impregnated with 3% oxalic acid solution. Development of the plates performed using n-hexane-ethylacetate-acetic acid (85:10:5) gave two yellow bands which were scrapped off carefully and dissolved in chloroform. The silica gel was filtered off and evaporative removal of chloroform afforded the individual components of R_f values 0.4 (5 and 6, 5 mg) and 0.33 (12, trace).

3.3.1.3 Methanol extract of E. schimperi root bark

The root bark residue from chloroform extraction was extracted with 1000 ml of methanol for 36 hrs until the solvent in the extractor was colourless. The extract was concentrated in vacuo giving a dark red gummy material (4.49 g). The whole crude produce was gently hydrolysed with 300 ml of 3m hydrochloric acid for 3 hrs.

The dark red hydrolysate was poured into 100 ml of cold water, filtered and the solid recovered dried in open air to give 2.80 g. T.l.c. analysis of the dry solid showed a yellow spot of R_f value 0.40 which turned pink on exposure to concentrated ammonia solution. The dry solid sample weighing 2.75 g was chromatographed on a 3.5 cm diameter glass column packed with 80 g of dry acid washed silica gel. The column was successively eluted with n-hexane and n-hexane-chloroform (4:1). An orange band was eluted out by passing n-hexane-chloroform (4:1) through the column and collecting two fractions of 100 ml each. The eluants were monitored by t.l.c. plate developed with n-hexane-ethylacetate-acetic acid (85: 10:5) giving a single spot, R_f 0.40. These were combined and the solvent evaporated to dryness yielding 19 mg of the component (5 and 6).

3.3.2.0 Petroleum ether (40-60) extract of E. schimperi stem bark

A 150 g portion of the stem bark was extracted with 1000 ml of petroleum ether (40-60) and the resultant extract (2.91 g) obtained was handled as in 4.3.1.1 above to yield three components: R_f 0.80 (yellow, 3, 9.5 mg), R_f 0.40 (yellow, 5 and 6, by mass spectrometer analysis, 180 mg) and R_f 0.33 (yellow, 12, trace).

3.3.2.1 Chloroform extract of E. schimperi stem bark

The stem bark residue from petroleum ether extraction was further extracted with 1000 ml of chloroform for 48 hrs until the solvent in the extractor was colourless. The

extract obtained was concentrated *in vacuo* leaving a dark brown solid of mass 1.80 g. The extract was handled as in 4.3.1.2 above to yield two components: R_f 0.40 (yellow, 5 and 6, 1.5 g) and R_f 0.33 (yellow, 12, 10 mg, mp 66-68°c).

3.3.2.2 Methanol extract of E. schimperi stem bark

The stem bark residue in the soxhlet extractor was finally extracted with 1000 ml of methanol for 48 hrs. The extract was concentrated using a rotary evaporator leaving a dark red gummy material weighing 2.40 g. The resultant extract obtained was similarly handled as in 4.3.1.3 above to give two components: R_f 0.8 (35, 3 mg) and R_f 0.40 (5 and 6, 26 mg).

3.3.3.0 Petroleum ether (40-60) extract of E. schimperi leaves

The ground air dried leaves weighing 150 g were exhaustively extracted with 1000 ml of petroleum ether (40-60) in a soxhlet extractor for 24 hrs. The resultant extract (5.0 g) was worked on as in 4.3.1.1 above giving three compounds: R_f 0.80 (35, 1.3 mg), R_f 0.40 (5 and 6, by mass spectrometer analysis, 1.40 g), and R_f 0.33 (12, trace).

3.3.3.1 Chloroform extract of E. schimperi leaves

The leaf residue from petroleum ether extraction was further extracted with 1000 ml of chloroform for 48 hrs until the solvent in the extractor was colourless. The extract obtained was evaporated *in vacuo* to give a dark green syrup of mass 2.76 g. The whole extract was treated as in 4.3.1.2 above to yield two components: R_f 0.40 (5 and 6, 200 mg) and R_f 0.33 (12, 2 mg).

3.3.3.2 Methanol extract of E. schimperi leaves

After extraction with chloroform, the leaf residue was finally exhaustively extracted with 1000 ml of methanol for 48 hrs. The extract was concentrated in vacuo giving a dark green gummy material (4.40 g). The resultant extract when handled as in 4.3.1.3 above yielded a component with R_f 0.40 (5 and 6) in 8 mg.

3.3.4.0 Petroleum ether (40-60) extract of E. schimperi fruits

The powdered material weighing 150 g was exhaustively extracted with 1000 ml of petroleum ether (40-60) for 24 hrs. The extract was filtered while hot under vacuum and the filtrate evaporated in vacuo leaving a brown solid of mass 6.14 g which was handled as in 4.3.1.1 above to give three compounds: R_f 0.80(35, 25 mg), R_f 0.40 (5 and 6, 4.97 g) and R_f 0.33 (12, trace).

3.3.4.1 Chloroform extract of E. schimperi fruits

Further extraction of the fruit residue with 1000 ml of chloroform for 48 hrs yielded a brown solid (2.46 g) after evaporation of the solvent using a rotary evaporator. The extract obtained was similarly treated as in 4.3.1.2 above resulting into two components: R_f 0.40 (5 and 6, 1.50 g) and R_f 0.33 (12, 15 mg). The latter was recrystallised in methanol to give orange crystals with mp 66-68°c. Its spectral figures are as follows: UV/Visible λmax (meOH) 420 (2.80) and 289 (4.50) nm. IR ν max (KBr) 3275 (O-H stretch), 2900, 2850 (alkane C-H stretch), 1620 (chelated

C=O stretch), 1470, 1400, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.22 s (2H, 2OH), δ 2.45t (4H, $(CH_2)_2$), δ 1.83s (6H, $2CH_3$), δ 1.5 m (4H, $2CH_2$), δ 1.25 m (32 H, 16 CH_2) and δ0.88t (6H, $2CH_3$). Mass spectrum m/e values M^+ 582 (3.2), 294(3.3), 179(16.0), 178(20.6), 177(4.7), 155(12.0), 154(35.6), 153(8.0), 142(7.9), 125(4.5), 69(49.1), 57(52.4) and 43(100).

3.3.4.2 Methanol extract of E. schimperi fruits

After successive extraction with petroleum ether (40-60) and chloroform, the fruit residue in the soxhlet was finally extracted with 1000 ml of methanol for 36 hrs. The extract gave a red gummy material (4.0g) after evaporation of the solvent using a rotary evaporator. This extract was hydrolysed as in 4.3.1.3 above yielding a solid residue weighing 2.56 g when dried in open air. T.l.c. analysis of the solid showed a yellow spot of R_f 0.40 (5 and 6) which turned violet on exposure to concentrated ammonia solution. The whole extract was subjected to column chromatography on a 3.5 cm diameter glass column packed with 80 g of dry acid washed silica gel. The column was eluted like in 4.3.1.3 above giving 3 mg of the mixture (5) and (6).

3.4.0 Petroleum ether (40-60) extract of Myrsine africana root bark

A 150g sample of the dry powdered root bark was exhaustively extracted in a soxhlet extractor with 1000 ml of the above solvent for 24 hrs. The extract obtained was filtered while hot and the filtrate concentrated in vacuo to give a dark brown solid (2.08g). This extract was subjected to a similar treatment as in 4.3.1.1. above yielding 13 mg of the compound corresponding to R_f 0.80 (35). The other component with R_f 0.40 was isolated

in 1.50 g. This compound was found to be composed of only 6, as determined by mass spectrometer. Component with R_f 0.33 (12) was in trace amount.

3.4.1 Chloroform extract of M. africana root bark

Further extraction of the root bark residue with 1000 ml of chloroform was exhaustively carried out for 24 hrs. A brown solid weighing 1.10 g was obtained after evaporation of the solvent using a rotary evaporator. The extract was similarly handled as in 4.3.1.2 above to yield two components: R_f 0.40 (6, by mass spectrometer analysis, 0.51 g) and R_f 0.33 (12, 15 mg).

3.4.2 Methanol extract of M. africana root bark

After chloroform extraction, the root bark residue was further exhaustively extracted with 1000 ml of the above solvent for 48 hrs. The extract obtained was concentrated in vacuo leaving a dark red gummy material (4.40 g) as crude product. The whole extract was hydrolysed as in 4.3.1.3 above to give a resultant extract of mass 1.75g. A 1.50 g portion of the solid was subjected to column chromatography on a 3.5 cm diameter glass column packed with 80 g of dry acid washed silica gel. Elution of the column with n-hexane and n-hexane-chloroform (4:1) successively eluted out a component with R_f 0.40 (6) in 4 mg.

3.4.3 Petroleum ether (40-60) extract of M. africana stem bark

Some powdered material (150 g) was extracted with 1000 ml of petroleum ether (40-60) in a soxhlet extractor for 24 hrs until the solvent in the extractor was colourless. The extract obtained was filtered while hot and the filtrate concentrated in vacuo to yield 2.31g. T.l.c. analysis of the extract showed

three spots of R_f values 0.80 (yellow), 0.40 (yellow), and 0.33 (yellow) which turned violet on exposure to concentrated ammonia solution. The whole extract was chromatographed as in 4.3.1.1 above giving three components: R_f 0.80 (35, 11 mg), R_f 0.40 (5 and 6, by mass spectrometer analysis, 1.44 g) and R_f 0.33 (12, 1.2 mg).

3.4.4 Chloroform extract of M. africana stem bark

Subsequent extraction of the stem bark with 1000 ml of chloroform was exhaustively carried out for 12 hrs and the resultant extract (1.08g) was subjected to column chromatography on a 3.5 cm diameter glass column packed with 60 g of dry acid washed silica gel. Elution of the column was performed as in 4.3.1.2 above to give two components: R_f 0.40 (5 and 6, (0.69g) and R_f 0.33 (12, 16 mg).

3.4.5 Methanol extract of M. africana stem bark

The stem bark residue after chloroform extraction was finally extracted with 1000 ml of methanol until the solvent in the extractor was colourless. The extract obtained was concentrated under reduced pressure leaving a dark red gummy material of mass 5.84 g. The material was hydrolysed like in 4.3.1.3 above and the resultant extract was chromatographed on a glass column of diameter 3.5 cm packed with 100 g of dry acid washed silica gel. Elution of the column with n-hexane and h-hexane-chloroform (4:1) successively washed out the compound with R_f value 0.4 (5 and 6) in 20 mg.

3.4.6 Petroleum ether (40-60) extract of M. africana leaves

Powdered ground air dried leaves weighing 150 g were exhaustively extracted in a soxhlet extractor with 1000 ml of the above solvent for 24 hrs. The obtained extract was filtered while hot and the filtrate evaporated in vacuo to give a dark green syrup (7.68 g). The whole extract was handled as in 4.3.1.1 above yielding two components: R_f 0.80 (35, 10mg) and R_f 0.40 (5 and 6, by mass spectrometer, analysis, 1.20g).

3.4.7 Chloroform extract of M. africana leaves

After petroleum ether (40-60) extraction, the leaf residue was further extracted with 1000 ml of chloroform for 48 hrs. Evaporation of the solvent using a rotary evaporator yielded a dark green syrup of mass 2.56 g. T.l.c. analysis of the extract showed two violet spots of R_f values 0.40 and 0.33 on exposure to concentrated ammonia solution. The extract was subjected to column chromatography on 3.5 cm diameter column packed with 80 g of dry acid washed silica gel. Elution of the column carried out as in 4.3.1.2 above gave the component, R_f value 0.4(5 and 6) 1.49 g and the component with R_f 0.33 (12) 2 mg.

3.4.8 Methanol extract of M. africana leaves

Lastly the leaf residue was exhaustively extracted with 1000 ml of methanol for 48 hrs and the resultant extract weighing 4.5 g was treated like in 4.3.1.3 above to give a component with R_f 0.4 (5 and 6) 34 mg.

3.4.9 Petroleum ether (40-60) extract of M. africana fruits

Dry powdered material (150 g) was extracted in a soxhlet extractor with 1000 ml of the above solvent for 24 hrs. The extract was filtered while hot and then the filtrate concentrated in vacuo to give a brown solid weighing 5.40 g. Analysis of the extract by t.l.c. gave three yellow spots of R_f values 0.80, 0.40 and 0.33 which all turned violet on exposure to concentrated ammonia solution. The extract was handled as in 4.3.1.1 above to yield three compounds: R_f 0.80(35, 26 mg), R_f 0.40 (5 and 6, by mass spectrometer analysis, 4.59g) and R_f 0.33 (12, 26mg).

3.4.10 Chloroform extract of M. africana fruits

The fruit residue was further extracted with 1000 ml of chloroform for 24 hrs. The extract after concentration under reduced pressure gave a brown solid (1.98g) which was later treated like in 4.3.1.2 above to yield two compounds R_f 0.40 (5 and 6, 1.50g) and R_f 0.33 (12, 6mg).

3.4.11 Methanol extract of M. africana fruits

Lastly, the fruit residue was exhaustively extracted with 1000 ml of methanol in a soxhlet extractor for 48 hrs. Concentration of the extract using a rotary evaporator left a dark red gummy material weighing 4.13 g. The extract was hydrolysed as in 4.3.1.3 above and the resultant extract was chromatographed on a glass column of diameter 3.5 cm packed with 80 g of dry acid washed silica gel. The chromatogram was eluted out with 350 ml of n-hexane-chloroform (4:1) to give 6 mg (5 and 6).

3.5.0 Petroleum ether (40-60) extract of R. melanphloes root bark

In this experiment dry powder material weighing 150 g was exhaustively extracted in a soxhlet extractor with 1000 ml of petroleum ether (40-60) for 24 hrs. The extract obtained was concentrated in vacuo to give a brown solid (4.75 g) which was then handled as in 4.3.1.1 above to yield three components: R_f 0.88 (35, 25 mg) R_f 0.4 (5, by mass spectrometer analysis, 4.40 g) and R_f 0.33(12, 5 mg).

3.5.1 Chloroform extract of R. melanphloes root bark

Further extraction of the root bark residue with 1000 ml of chloroform was carried out in a soxhlet till exhaustion. A brown solid (10.14 g) was obtained after evaporation of the solvent using a rotary evaporator. A 7.0 g portion of the extract was subjected to column chromatography on a 3.5 cm diameter column packed with 200 g of dry acid washed silica gel. Elution of the column performed as in 4.3.1.2 above yielded two components: R_f 0.40 (5, 6.50 g) and R_f 0.33 (12, 20mg).

3.5.2 Methanol extract of R. melanphloes root bark

The root bark residue was finally extracted with 1000 ml of methanol for 36 hrs. Concentration of the extract under reduced pressure gave a dark red gummy material of mass 4.15 g which was treated like in 4.3.1.3 above to give a component with R_f 0.4(5) in 80 mg.

3.5.3 Petroleum ether (40-60) extract of R. melanphloes stem bark

Some 150 g of powdered stem bark was extracted with 1000 ml of the above solvent in a soxhlet extractor till exhaustion. The extract was filtered while hot using suction filtration and the filtrate concentrated in vacuo leaving a brown solid (4.12 g). The extract obtained was subjected to a similar treatment as in 4.3.1.1 above to yield three components: R_f 0.80 (35, 5 mg), R_f 0.40 (5 and 6, by mass spectrometer analysis, 3.45 g) and R_f 0.33 (12, 4 mg).

3.5.4 Chloroform extract of R. melanphloes stem bark

The stem bark residue in the soxhlet was further extracted with 1000 ml of chloroform for 24 hrs. The extract obtained was concentrated in vacuo to give 1.69 g. This was chromatographed on a glass column diameter 3.5 cm packed with 80 g of dry acid washed silica gel. The column was run like in 4.3.1.2 above yielding two components: R_f 0.40 (5 and 6, 0.5 g) and R_f 0.33 (12, 23 mg).

3.5.5 Methanol extract of R. melanphloes stem bark

The stem bark residue was finally extracted with 1000 ml of methanol for 48 hrs until the solvent in the extractor was colourless. The extract gave 5.45 g after concentration of the solvent using a rotary evaporator. The extract was hydrolysed and later chromatographed as in 4.3.1.3 above to yield a component with R_f 0.40 (5 and 6) in 3 mg.

3.5.6 Petroleum ether (40-60) extract of R. melanphloes leaves

A 150 g portion of ground air dried leaves was extracted with 1000 ml of the above solvent for 24 hrs and the resultant extract of mass 5.1 g was subjected to column chromatography on a 3.5 cm diameter column packed with 150 g of dry acid washed silica gel. The column was eluted like in 4.3.1.1. above to give three compounds: R_f 0.8(35, 3mg), R_f 0.40 (5 and 6, by mass spectrometer analysis, 3.35 g) and R_f 0.33 (12, trace).

3.5.7 Chloroform extract of R. melanphloes leaves

Subsequent extraction of the leaf residue with 1000 ml of chloroform was carried out for 24 hrs. The extract obtained was concentrated in vacuo leaving a dark green syrup (2.29 g) which was chromatographed on a glass column diameter 3.5 cm packed with 80 g of dry acid washed silica gel. Elution of the column was performed as in 4.3.1.2 above to yield two compounds: R_f 0.40 (5 and 6, 0.40 g) and R_f 0.33 (12, 14 mg).

3.5.8 Methanol extract of R. melanphloes leaves

The leaf residue was further extracted with 1000 ml of methanol in a soxhlet extractor till exhaustion. The extract obtained was evaporated under reduced pressure to give a dark red syrup (5.20 g) which was later handled as in 4.3.1.3 above to yield a component with R_f 0.40 (5 and 6, 5 mg).

3.5.9 Petroleum ether (40-60) extract of R. melanphloes fruits

Powdered material of mass 150 g was exhaustively extracted with 1000 ml of petroleum ether (40-60) in a soxhlet extractor for 24 hrs. The extract was filtered while hot and the filtrate concentrated in vacuo to give 12.3 g. A 8.50 g portion of the extract was treated like in 4.3.1.1 above and three compounds were afforded: R_f 0.80(35, 220mg), R_f 0.40 (5 and 6, by mass spectrometer analysis, 7.40 g).and R_f 0.33 (12, 4.8 mg).

3.5.10 Chloroform extract of R. melanphloes fruits

The fruit residue in the extractor was further exhaustively extracted with 1000 ml of chloroform for 24 hrs. The resultant dark brown solid (8.5 g) obtained was chromatographed on a 3.5 cm diameter glass column packed with dry acid washed silica gel (200 g). The column was run like 4.3.1.2 above to yield two components: R_f 0.40 (5 and 6, 6.80 g) and R_f 0.33 (12, 17mg).

3.5.11 Methanol extract of R. melanphloes fruits

Lastly, the fruit extract was extracted with 1000 ml of the above solvent for 48 hrs. The extract obtained was evaporated under reduced pressure leaving a dark red gummy material weighing 9.50 g which was subsequently handled as in 4.3.1.3 above. A compound with R_f 0.40 (5 and 6) 25mg.

3.5.12 Tetraacetylation of 35

The compound 35 (100 mg) was suspended in a mixture of 6 ml acetic anhydride and 6 ml of pyridine. To the mixture, 500 mg of zinc dust was added and the mixture gently refluxed for 3 hrs. It was then cooled and poured into ice-water giving a white precipitate which was filtered and recrystallised from methanol to give 80 mg of colourless plates with mp 74-76°c. UV λ max (meOH) 280 (2.80) and 208 (3.0) nm. IR λ max (KBr) 2900, 2825 (alkane C-H stretch), 1470, 1380, 1330, 1050, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ5.38 m (H, C=C-H), δ 4.16 t (H, C=C-H), δ2.22 s (12H, 4 ester methyls), δ 2.0 m (2H, CH_2), δ 1.25 m (1OH, $(CH_2)_5$) and 0.88t (6H, $2CH_3$).

3.5.13 Diacetylation of 6

A 0.5 g of 6 was dissolved into a mixture of 6 ml acetic anhydride and 6 ml of pyridine. The mixture was stirred for 48 hrs at room temperature after which poured into ice-water giving pale yellow solids. The solids were filtered by suction filtration and recrystallised in benzene to give pale-yellow plates (0.38 g) with mp 63-64°c. UV λ max (meOH) 280(1.4) and 208 (3.5) nm. IR ν max (KBr) 2900, 2825 (alkane C-H stretch), 1780 (ester C=O stretch), 1680 (quinone C=O stretch), 1475, 1380, 1120, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.06s (H, vinyl proton), δ 2.42t (2H, CH_2), δ 2.25 s (6H, $(COOCH_3)_2$), δ 1.26 m (22H, $(CH_2)_{11}$) and δ 0.89 t (3H, terminal CH_3).

3.5.14 Tetraacetylation of 6

A mixture of 0.5g of 6 and 0.5g of zinc dust in 15 ml of acetic anhydride was handled as in 4.5.12 above to give colourless plates (0.35g) with mp 114-116°c after crystallisation in n-hexane. UV λ max (meOH) 260 (1.2) and 208 (3.2) nm. IR ν max (KBr) 2900, 2850 (alkane, C-H stretch), 1780 (ester C=O stretch), 1480, 1380, 1120, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.09 s (H, vinyl proton), δ 2.40 t (2H, CH_2), δ2.25 s (12H, 4 ester methyls), δ 1.26 m (22H, $(CH_2)_{11}$) and δ 0.88t (3H, terminal CH_3).

3.5.15 Methylation of 6

A 0.25 g of the compound was gently refluxed with 2.0 ml of dimethylsulphate and 5g of anhydrous potassium carbonate in dry acetone (150 ml) for 30 hrs. The solvent was evaporated in vacuo and the residue poured into ice-water giving a yellow solid.

The solid was filtered, washed several times with cold water and then dried in open air. The dried yellow compound was recrystallised in petroleum ether (40-60) to give pale yellow plates (0.12g) with mp 63-65°c. UV λ max (meOH) 290 (1.40) and 220 (2.80) nm. IR ν max (KBr) 3050 (olefinic (C-H stretch), 2900, 2850 (alkanes C-H stretch), 1680 (C=0 stretch), 1480, 1370, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.06s (H, vinyl H), δ 3.86s (6H, $(COCH_3)_2$), δ 2.40t (2H, CH_2), δ 1.25 m (22H, $(CH_2)_{11}$) and δ 0.88t (3H, terminal CH_3).

3.5.16 Diacetylation of 5

The compound (0.5 g) was diacetylated as in 4.5.13 above and the resultant product recrystallised in benzene to give pale yellow crystals (0.33 g) with mp 52 - 54°c. UV λ max (meOH) 270 (1.6) and 210 (2.6) nm. The IR ν max (KBr) 2900, 2825 (alkane C-H stretch) 1780 (ester carbonyl stretch), 1685 (C=0 stretch). 1480, 1380, 1120, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ7.05 s (H, vinyl proton), δ 2.44t (2H, CH_2), δ 2.25s (6H, $(CH_3)_2$), δ 1.25 m (18H, $(CH_2)_9$) and δ 0.88t (3H, terminal CH_3).

3.5.17 Tetraacetylation of 5

A portion of 1.0 g of the sample was suspended in a mixture of 15 ml acetic anhydride and 5 ml of pyridine. To the mixture, 0.5 g of zinc dust was added and the whole content gently refluxed for 3 hrs. The product obtained was handled as in 4.5.12 above to give colourless crystals (0.80g) with mp 121 - 123°c. UV λ max (meOH) 265 (1.2) and 208 (2.2) nm.

IR ν max (KBr) 2900, 2850 (alkane C-H stretch), 1780 (ester carbonyl stretch), 1480, 1370, 1200, 1100, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.09 s (H, vinyl proton), δ 2.40 t (2H, CH_2), δ 2.25 s (12H, 4 ester methyls), δ 1.26 m (18H, $(CH_2)_9$), and δ 0.88 t (3H, terminal CH_3).

3.5.18 Methylation of 5

A 2.0 of the compound was methylated as in 4.5.16 above and the resultant product recrystallised in benzene to give pale yellow crystals (0.33 g) with mp 55-56°C. UV λ max (meOH) 280 (1.6) and 220 (2.6) nm. The IR ν max (KBr) 2900, 2825 (alkane C-H stretch), 1680 (C=O stretch), 1480, 1380, 1120, 720 cm^{-1}. ^1H ($CDCl_3$) δ 7.05 s (H, vinyl proton), δ 2.44 t (2H, CH_2), δ 3.84 s (6H, $(OCH_3)_2$), δ 1.25 m (18H, $(CH_2)_9$) and 0.88t (3H, terminal CH_3).

3.6.0 Petroleum ether (40-60) extract of Maesa lanceolata root bark

Dry powdered root bark weighing 150 g was exhaustively extracted with 1000 ml of petroleum ether (40-60) in a soxhlet extractor for 24 hrs. The extract obtained was filtered while hot under vacuum and the filtrate concentrated in vacuo leaving a dark brown syrup of mass 3.12 g. T.l.c. analysis of the extract showed five yellow spots of R_f 0.63 (7), 0.50 (14), 0.48 (39), 0.35 (40) and 0.20 (15). All turned violet on exposure to concentrated ammonia solution. The whole extract was pre-absorbed on dry acid washed silica gel and then chromatographed on a glass column 3.5 cm diameter packed with 100 g of the silica gel. Elution of the column was in the

usual manner with n-hexane, n-hexane-benzene (4:1), benzene, chloroform, ethylacetate and methanol. n-hexane was run through the column until the first orange band started coming out. At this juncture two orange bands were observed in the column. The first was eluted out with n-hexane-benzene (4:1) giving 19 fractions of 100 ml each. The eluants collected were analysed by t.l.c. and all gave a single spot of R_f value 0.63. These were combined and the solvent evaporated. Subsequent elution of the column with benzene washed out the second orange band giving eight fractions of 100 ml each. Analysis of the fractions with t.l.c. showed three compounds of R_f values 0.63, 0.50 and 0.48. Further elution with chloroform gave five fractions (100 ml each) containing two compounds of R_f values 0.48 and 0.35 as a mixture. Introduction of ethylacetate as the next eluting solvent eluted out a yellow band giving three fractions of 100 ml each, which were detected by t.l.c. to be another single component of R_f 0.20. Fractions containing mixture of compounds were combined, evaporated and then spotted on silica gel preparative t.l.c. plates impregnated with 3% oxalic acid solution. Development of the plates was performed using n-hexane-ethylacetate-acetic acid (85:10:5) yielding three yellow bands which were scrapped off carefully and then dissolved in chloroform. The silica gel was filtered off and evaporative removal of chloroform afforded the individual components with R_f 0.63, 0.5, 0.48 and 0.35 respectively.

The compound corresponding to R_f 0.63 (7) was crystallised from methanol to give orange crystals (2.56 g) with mp 122-124°c. Other data showed its structure to be 7. The UV λ max (meOH) 440 (2.40) and 294 (4.40) nm. IR ν max (KBr) 3300 (O-H stretch), 2900, 2850 (alkane C-H stretch), 1620 (chelated C=O stretch), 1470, 1370, 1120, 710 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.59 s (2H, 2OH), δ5.38 m (2H, CH=CH), δ 2.40t (2H, CH_2), δ 2.0 m (4H, $(CH_2)_2$), δ1.94 s (3H, CH_3), δ1.26 m (26H, $(CH_2)_{13}$), and δ0.90 t (3H, terminal CH_3). MS m/e values M+ 418 (34.9), 390 (17.4), 364 (16.8), 169 (65), 168 (100), 167 (32.7), 156 (13.0), 153 (3.7), 139 (23).

The component with R_f 0.50 (14) was isolated in 0.63 g with mp 25-38°c. Its spectral figures are as follow: UV λ max (meOH) 410 (2.85) and 275 (4.20) nm. IR ν max (KBr) 3300 (olefinic O-H stretch), 2900, 2850 (alkane C-H stretch), 1780 (ester C=O stretch), 1680 (C=O stretch), 1620 (chelated C=O stretch), 1480, 1380, 1320, 1200, 1140, 1020, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.05 s (H, OH), δ 5.38 m (2H, CH=CH), δ2.40 t (2H, CH_2) δ 2.25 s (3H, ester CH_3), δ2.0 m (4H, $(CH_2)_2$), δ 1.9t (3H, CH_3), δ 1.26 m (26H, $(CH_2)_{13}$) and δ 0.89 t (3H, terminal CH_3). MS m/e values M+ 460 (11.5), 418(13), 390(4.0), 363(100), 362(26.9), 169(38.4), 168(83.8), 167(11.7), 156(17.5), 139(8.7), 95(5.8), 83(15.3), 69(13.5), 55(47.1), 43(88).

The components with R_f values 0.48 (39) and 0.35 (40) were in trace amounts. However, the component with R_f value 0.20 (15) was recrystallised in methanol to give yellow crystals (72 mg) with mp 67-68°c. UV λmax (meOH) 420(2.70) and 280(4.20) nm.

IP ν max (KBr) 3325 (O-H stretch), 3000 (olefinic C-H stretch), 2910, 2840 (alkane C-H stretch), 1650 (C=O stretch), 1610 (chelated C=O stretch), 1480, 1400, 1200, 850, 710 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.21 s (H, OH), δ 5.94 s (H, vinyl proton), δ5.35 m (2H, CH=CH), δ 3.85 s (3H, OCH_3), δ2.40 t (2H, CH_2), δ2.0 m (4H, $(CH_2)_2$), δ 1.26 m (18H, $(CH_2)_9$ and δ0.87 t (3H, terminal CH_3). MS m/e values M^+ 362 (60.2), 169(11.5), 168(100), 167(30), 156(14), 153(11.5), 125(8.9), 109(7.5), 95(14.1), 81(18.9), 69(25.8), 55(76.8), 43(52.9).

3.6.1 Chloroform extract of M. lanceolata root bark

The root bark residue in the soxhlet was further extracted with 1000 ml of chloroform till exhaustion and the resultant extract (2.4 g) obtained was handled as in 4.6.0 above to yield the following components: R_f 0.63 (7, 1.23g), R_f 0.5 (14, 0.13g), R_f values 0.48 (39) and 0.35 (40) were in trace amounts and R_f value 0.20 (15) was received in 10 mg.

3.6.2 Methanol extract of M. lanceolata root bark

Lastly root bark residue was extracted with 1000 ml of methanol for 36 hrs. Concentration of the the extract under reduced pressure gave a dark gummy material of mass 2.1 g. Hydrolysis of the extract in the usual way produced a red solid (1.30 g) which was later chromatographed on a 3.5 cm diameter glass column packed with 80 g of dry acid washed silica gel. Elution of the column with n-hexane, n-hexane-benzene (4:1), n-hexane-chloroform (4:1) and ethylacetate afforded two compounds: R_f 0.63 (7, 250 mg) and R_f 0.20 (15, 15 mg).

3.6.3 Petroleum ether (40-60) extract of M. lanceolata stem bark

A 150 g portion of dried ground stem bark was exhaustively extracted with 1000 ml of the above solvent for 24 hrs and the resultant extract (3.96 g) obtained was treated like in 4.6.0 above resulting into five components: R_f 0.63 (7, 2.50 g), R_f 0.5 (14, 50 mg), while R_f values 0.48 (39), 0.35 (40) and R_f 0.20 (15) were in trace quantities.

3.6.4 Chloroform extract of M. lanceolata stem bark

Subsequent extraction of the stem bark residue was further carried out with 1000 ml of chloroform until the solvent in the extractor was colourless. The extract obtained was as usual concentrated under reduced pressure giving a dark brown solid (1.38 g) which was then handled as in 4.6.0 above to give the components as follows: R_f 0.63 (7, 0.71 g), R_f 0.50 (14, 13 mg), R_f values 0.48 (39), R_f 0.35 (40) and R_f 0.20 (15) were in trace amounts.

3.6.5 Methanol extract of M. lanceolata stem bark

The stem bark residue was finally extracted with 1000 ml of methanol until exhaustion. The resulting extract was concentrated in vacuo giving a red syrup weighing 3.13 g. This was hydrolysed as in 4.3.1.3 above yielding a dark red hydrolysate which was filtered and the recovered extract dried in open air gave 1.50 g. The whole extract was introduced into a column of diameter 3.5 cm packed with 70 g of dry acid washed silica gel. Elution of the column with n-hexane-benzene (4:1) eluted out the component with R_f 0.63 (7) in 14 mg.

3.6.6 Petroleum ether (40-60) extract of M. lanceolata leaves

Ground air dried leaves (150 g) were exhaustively extracted in a soxhlet with 1000 ml of petroleum ether (40-60) for 24 hrs. The extract obtained was filtered while hot and then concentrated in vacuo leaving a dark green syrup (3.82 g) which was similarly handled as in 4.6.0 above resulting into threee components: R_f 0.63 (7, 1.40 g), R_f 0.50 (14, 15 mg) and R_f 0.2 (15, 20 mg).

3.6.7 Chloroform extract of M. Lanceolata leaves

After extraction with petroleum ether (40-60), the leaf residue in the soxhlet was subsequently extracted with 1000 ml of the above solvent for 24 hrs and the resultant extract (2.0g) was treated like in 4.6.0 above to yield three compounds: The compound with R_f 0.63 (7) 0.62 g, the other one with R_f 0.50 (14) 50 mg and the component corresponding to R_f 0.20 (15) 12 mg.

3.6.8 Methanol extract of M. Lanceolata leaves

Further extraction of the leaf residue with 1000 ml of methanol for 48 hrs gave a resultant extract of 6.0 g. This was treated like in 4.3.1.3 above and a component of R_f value 0.63 (7) 20 mg. was obtained.

3.6.9 Petrolem ether (40-60) extract of M. lanceolata fruits

Dry powdered material weighing 150 g was extracted with 1000 ml of petroleum ether (40-60) in a soxhlet extractor for 24 hrs. The extract obtained was filtered while hot and the filtrate concentrated in vacuo leaving a dark yellow-green syrup (13.97 g). This was handled as in 4.6.0 above to yield

five components: R_f 0.63 (7, 11.88 g) , R_f 0.50 (14, 0.71 g), R_f 0.2 (15, 27 mg).

The component with R_f 0.48 (39) was recrystallised from methanol to give crystals (30 mg) with mp 104-106°c. Its spectral figures are as follows: UV λ max (meOH) 440 (2.70) and 285 (4.20) nm. IR υ max (KBr) 3350 (O-H stretch), 3000 (olefinic C-H stretch), 2950, 2850 (alkane C-H stretch), 1625 (chelated C=O stretch), 1480, 1380, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.65 s (2H, 2OH), δ 6.00 s (H, vinyl proton), δ5.35 m (2H, CH=CH), δ 2.44 t (2H, CH_2), δ2.0 (4H), δ 1.26 m (18H, $(CH_2)_9$ and δ 0.87 t (3H, terminal CH_3). Mass spectrum m/e M^+ 348(5.5), 155(53.5), 154(100), 153(30.0), 142(15), 125(18.0).

The compound with R_f 0.35 (40) gave orange crystals weighing 50 mg with mp 130-132°c after crystallisation from methanol. UV λ max (meOH) 420 (2.40) and 290(4.20) nm. IR υ max (KBr) 3300 (O-H stretch), 2950, 2850 (alkane C-H) stretch), 1620 (chelated C=O stretch), 1480, 1370, 1120, 720 cm^{-1}. ^{1}HNMR (CDCl) δ7.66 s (2H, 2OH), δ 6.00 s (H, vinyl proton), δ 2.45 t (2H, CH_2), δ1.25 m (26H, $(CH_2)_{13}$) and 0.88 t (3H, terminal methyl). Mass spectrum m/e values 350 (10.9), 155(36.2), 154(100), 153(21.1), 142(22.5). 125(7.8), 83(6.6), 69(23.9), 55(33.3), 43(67.4).

3.6.10 Chloroform extract of M. lanceolata fruits

The fruit residue was subsequently extracted with 1000ml of chloroform till exhausion. Evaporation of the extract using a rotary evaporator yielded a brown solid (5.60 g) which was later subjected to a column chromatography on a 3.5 cm

diameter column packed with 150 g of dry acid washed silica gel. Elution of the column was performed as in 4.6.0 above to give the components of the extract as follows: R_f 0.63 (7, 4.32 g), R_f 0.50 (14, 40 mg), R_f 0.48 (39, 20 mg), R_f 0.35 (40, 100 mg) and R_f 0.2 (15, 32 mg).

3.6.11 Methanol extract of M. lanceolata fruits

After extraction with chloroform, the fruit residue in the soxhlet was further extracted with 1000 ml of methanol for 48 hrs and the extract obtained was evaporated under reduced pressure to give a red gummy material (4.96 g). The extract was then hydrolysed as in 4.3.1.3 above yielding a resultant extract of 2.0 g. This when introduced into a 3.5 cm diameter column packed with 80 g of dry acid washed silica gel and the column run like in 4.3.1.1 above gave two components: R_f 0.63 (7, 0.31 g) and R_f 0.20 (15, 3mg).

3.6.12 Diacetylation of 7

A 0.5 g of the compound was diacetylated as in 4.5.13 above to yield pale yellow crystals (0.35 g) with mp 72 -74°c after crystallisation in methanol. Its spectral figures were as follows: UV λ max (meOH) 260 (2.45) and 220 (1.45) nm. IR ν max (KBr) 2900, 2850 (alkane C-H stretch), 1670 (free quinone C=O stretch), 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.35 m (2H, CH=CH), δ 2.40 t (2H, CH_2), δ 2.28 s (6H, 2 ester methyls), δ 2.0 m (4H, $(CH_2)_2$), δ 1.94 s (3H, CH_3), δ1.26 m (26H, $(CH_2)_{13}$) and δ 0.89 t (3H, terminal CH_3).

3.6.13 Tetraacetylation of 7

Tetraacetylation of the compound (0.5g) was carried out as in 4.5.12 above to yield colourless crystals weighing 0.40 g with mp 80 - 82°c on crystallisation in methanol. Its spectral data are as follows: UV λmax (meOH) 290 (3.20) and 220 (2.40) nm. IR λ max (KBr) 2900, 2850 (alkane C-H stretch), 1780 (ester carbonyl stretch), 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.38 m (2H, CH=CH), δ2.38 t (2H, CH_2), δ2.26 s (12H, 4 ester methyls), δ2.0 m (4H, $(CH_2)_2$), δ1.94 s (3H, CH_3), δ 1.26 m (26H, $(CH_2)_{13}$) and δ0.89 t (3H, terminal CH_3).

3.6.14 Methylation of 7

Methylation of 7 performed on 1.0 g was handled as in 4.5.15 above to give a yellow precipitate which recrystallised in methanol to yield yellow crystals (0.75 g) with mp 48-50°c. UV λ max (meOH) 280 (1.2) and 210 (1.0) nm. IR υ max (KBr) 2915, 2850 (alkane C-H stretch), 1680 (C=O stretch), 1480, 1380, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.35 m (2H, CH=CH), δ 3.86 s (6H, $(COCH_3)_2$, δ1.94 s (3H, CH_3), δ 1.26 m (26 H, $(CH_2)_{13}$), δ0.90 t (3H, terminal CH_3).

3.6.15 Tetraacetylation of 14

This experiment was carried out with 0.2 g of the compound as in 4.5.12 above to give colourless crystals of mass 24 mg with mp 80-82°c after crystallisation in methanol. UV λ max (meOH) 290 (3.20) and 220 (2.40) nm. IR υ max (KBr) 2900 2850 (alkane C-H stretch), 1780 (ester C=O stretch), 1480, 1370, 1220, 1190, 1060, 1020, 950, 720 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.38 m (2H, CH=CH), 2.38 t (2H, CH_2), δ 2.26 s (12H, 4 ester methyls), δ 2.0 m (4H $(CH_2)_2$), δ1.96 s (3H, CH_3), 1.26 m (26 H, $(CH_2)_{13}$) and δ0.88 t (3H, terminal CH_3).

A P P E N D I X

PRELIMINARY BIOASSAYS

Larvicidal tests

The tests for activities of crude methanol extracts of *E. schimperi*, *M. lanceolata*, *M. africana* and *R. melanphloes* together with pure isolates embelin (5), rapanone (6), maesaquinone (7) and acetylmaesaquinone (14) against *Aedes aegepti* mosquito larvae were performed according to method outlined in Wood and Silverstein (1974) and summarised here. The test samples were at concentrations 2 mg cm^{-3}, 1.0 mg cm^{-3} and 0.5 mg cm^{-3} respectively. Twenty larvae were used in each experiment and the mortality rate observed after 8, 16 and 24 hrs as indicated on tables 3-8. It was observed that embelin (5) and rapanone (6) as well as crude methanol extracts of *E. schimperi*, *M. africana* and *R. melanphloes* inhibited a larvicidal activity against the insects. The test solutions at concentrations 1 mg cm^{-3} and 2 mg cm^{-3} were repeated for embelin and rapanone with 20 larvae and the mortality rate observed after 4,8,10,12,16,20 and 24 hrs. For the results see Table 9-10.

Bacteriostatic tests

The experiment was performed to determine the bacteriostatic activities of methanol extracts of Myrsinaceae plants and of pure compounds, embelin, rapanone, maesaquinone and acetyl maesaquinone against Gram-positive and Gram-negative bacteria,

Bacillus subtilis, Escherichia coli, Streptococci and Staphyloccus aureus. The tests were carried out according to the method in Bailey and Scott (1970). The methanol extracts (2 mg cm^{-3}) and the pure compounds (1 mg cm^{-3}) were applied to the bacteria cultured in discs under controlled conditions. The methanol extracts of E. schimperi, M. africana and R. melanphloes together with embelin and rapanone exhibited antibacterial activities on Gram-positive Streptococci. The results are displayed on tables 11 and 12.

Antifertility tests

Six male rabbits were injected intramuscularly with 125 mg cm^{-3} of the pure compounds embelin, rapanone and maesaquinone. Thereafter 5 cm^3 of blood samples were withdrawn from the test samples and the control rabbits every two weeks for six weeks to determine the testosterone levels. The control rabbits were not injected with anything. The experimental procedure followed was according to Sufi et. al. (1986). The results of the experiment are in table 13.

BIOASSAY RESULTS

Larvicidal Activity

The results of larvicidal tests shown in tables 3-10 indicated that the compounds embelin and rapanone as well as the crude methanol extracts of E. schimperi, M. africana and R. melanphloes are larvicidal agents against the mosquito Aedes aegepti larvae. A comparative study of the samples at concentrations 2 mg cm^{-3}, 1 mg cm^{-3} and 0.5 mg cm^{-3} performed for 8, 16 and 24 hrs indicated that in 24 hrs all the larvae in each solution were dead except those for methanol crude extract of Maesa lanceolata and its pure isolates, maesaquinone and acetylamaesaquinone. Comparing embelin and rapanone, it is observed that the former is more active at all concentrations. It is interesting that the Maesa lanceolata extracts and maesaquinone and its acetate are not active in this test. It is most unlikely that the double bond in the long alkyl chain is the reason for this disparity although this cannot be ruled out. The more likely reason could be the alkyl side chain length which is shorter in the cases of embelin and rapanone but longer in the chief constituents of Maesa lanceolata.

Table 7. Activity of methanol extracts (0.5 mg cm^{-3}) on mortality rate of mosquito larvae

Extracts	No tested	Dead 8 hrs	Dead 16 hrs	Dead 24 hrs
E. Schimperi	20	2	11	all
M. africana	20	1	10	all
M. lanceolata	20	0	0	0
R. melanphloes	20	3	12	all

Table 8. Activity of methanol extracts (1.0 mg cm^{-3}) on mortality of mosquito larvae

Extracts	No tested	Dead 2 hrs	Dead 16 hrs	Dead 24 hrs
E. schimperi	20	4	15	all
M. lanceolata	20	0	0	0
M. africana	20	3	13	all
R. melanphloes	20	4	16	all

Table 9. Activity of methanol extracts (2 mg cm^{-3}) on mortality of mosquito larvae

Extracts	No tested	Dead 8 hrs	Dead 16 hrs	Dead 24 hrs
E. schimperi	20	6	18	all
M. lanceolata	20	0	0	0
M. africana	20	4	16	all
R. melanphloes	20	5	19	all

Table 10. Activity of pure compounds (2 mg cm^{-3}) on mortality rate of mosquito larvae

Compounds	No tested	Dead 8hrs	Dead 16hrs	Dead 24hrs
Embelin	20	10	18	all
Rapanone	20	8	16	all
Maesa-quinone	20	0	0	0
Acetyl-maesa quinone	20	0	0	0

Table 11. Activity of pure compounds (1 mg cm^{-3}) on mortality rate of mosquito larvae

Compounds	No tested	Dead 8hrs	Dead 16hrs	Dead 24 hrs
Embelin	20	6	14	all
Rapanone	20	2	10	all
Maesa-quinone	20	0	0	0
Acetyl-maesa quinone	20	0	0	0

Table 12. Activity of pure compounds (0.5 mg cm^{-3}) on mortality rate of mosquito larvae

Compounds	No tested	Dead 8hrs	Dead 16hrs	Dead 24hrs
Embelin	20	3	7	all
Rapanone	20	0	4	all
Maesa-quinone	20	0	0	0
Acetyl-maesa quinone	20	0	0	0

Table 13. Activity of embelin (2 mg cm^{-3} and 1.0 mg cm^{-3}) on mortality rate of mosquito larvae

Conc.	No tested	4hrs	8hrs	12hrs	16hrs	20hrs	24hrs
2 $mgcm^{-3}$	20	5	9	15	18	19	all
1 $mgcm^{-3}$	20	3	6	13	14	17	all

Table 14. Activity of rapanone (2 mg cm^{-3} and 1.0 mg cm^{-3}) on mortality rate of mosquito larvae

Conc.	No tested	4hrs	8hrs	12hrs	16hrs	20hrs	24hrs
2 $mgcm^{-3}$	20	4	8	14	16	18	all
1 $mgcm^{-3}$	20	0	2	8	10	15	all

Bacteriostatic results

The organisms tested were not selected at random but chosen as bacteria known to show high resistance to most antibiotics in common use. From the bacteriocidal results shown in tables 11 and 12 below, it is observed that the Gram-positive, streptococci showed sensitivity to methanol extracts of E. schimperi, M. africana and R. melanphloes. The bacterium was also found to be sensitive to pure compounds, embelin and rapanone as compared to other Gram-positive species. Bacillus subtilis , staphylococcus aureus and the Gram-negative Escherichia coli. The anti-bacterial activities was shown by inhibition of growth of bacteria around the antimicrobial zones. The susceptibility tests showed that the crude methanol extracts of the three plants are anti-microbial agents. Embelin and rapanone were also found to be active anti-microbial agents therefore it is probable that these are the active ingredients in the crude extracts. However, methanol extract of M. lanceolata fruit and pure compounds, maesaquinone and acetylmaesaquinone were unable to inhibit microbial activities. This further proves that the active ingradients could be embelin and rapanone. In this case the two compounds were not extracted from this plant. From these observations we drew the conclusion that embelin and rapanone are the anti-bacterial agents.

Table 15. Bacteriostatic activities of methanol extracts of Myrsinaceae plants

Extracts	TYPE OF BACTERIA			
	B. subtilis	E. coli	strepto.	S. aureus
E. schimperi	(-)	(-)	(+)	(-)
M. lanceolata	(-)	(-)	(-)	(-)
M. africana	(-)	(-)	(+)	(-)
R. melanphloes	(-)	(-)	(+)	(-)

Table 16. Bacteriostatic activities of pure compounds

Compounds	B. subtilis	E. coli	strepto.	S. aureus
Embelin	(-)	(-)	(+)	(-)
Rapanone	(-)	(-)	(+)	(-)
Maesaquinone	(-)	(-)	(-)	(-)
acetyl-maesaquinone	(-)	(-)	(-)	(-)

KEY: B. subtilis = Bacillus subtilis , E. coli = Escherichia coli, strepto. = streptococci, S. aureus = Staphylococcus aureus, (+) = active and (-) = inactive.

Antifertility Results

The results of antifertility effects of embelin, rapanone and maesaquinone shown in table 13 indicate that embelin has some activity. The experiments were carried out with male rabbits to determine the testosterone levels after separate

administration of 125 $mgcm^{-3}$ of the compound intramuscularly into the test animals. The controls represent 5 cm^3 of blood samples from each animals not injected with anything and the tests are also 5 cm^3 of blood samples from each animal treated with the compounds. The blood samples were each centrifuged separately to isolated the plasma which were stored in separate containers at $-20^{o}c$ for future use. The control and the test blood samples were withdrawn by syringe from the respective animals every two weeks for a period of six weeks. The respective samples were similarly centrifuged for their plasma. The levels of testosterone in each blood sample collected were determined using a radio-immunoassay technique

TABLE 17. Anti-fertility levels of some benzoquinones

Pure compounds	Testosterone level in $mgcm^{-3}$			
	0 week	2nd week	4th week	6th week
Embelin	14.11	9.13	10.81	11.40
control	14.65	14.66	14.65	14.64
rapanone	14.96	16.82	16.80	18.16
control	14.80	14.81	14.82	14.78
maesaquinone	15.41	15.83	17.01	17.87
control	15.60	15.63	15.64	15.65

From the results it can be inferred that embelin is an antifertility agent since adverse reduction in testosterone level was observed in the second week after injection. After the second week, a gradual increase in testosterone level was

noted for the fourth and the sixth weeks respectively. A reduction in testosterone level obviously affects the fertility since it is a factor determining normal re-production activities. Embelin could be a targeted contraceptive. The compounds rapanone and maesaquinone did not show any reduction testosterone level. Instead there were slight increments in levels and hence could perhaps be used in fertility regulatory exercise especially where aggrevation is required. The control solutions were pure blood samples thereby giving testosterone levels which were fairly constant.

Table 18. ^{1}H NMR peak positions for benzoquinone compounds

Position of carbon	5	6	7	12	14	15	35	39	40
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-
6	6.00	6.00	-	-	-	5.94	-	6.00	6.00
1^{1}	2.45	2.45	2.40	2.45	2.40	2.45	-	-	-
O-H	7.66	7.66	7.59	7.22	7.05	7.21	7.72	7.65	7.66
5-OCH_3	-	-	-	-	-	-	-	-	-
Methylene long chain	1.25	1.26	1.26	1.25	1.26	1.26	1.25	1.25	1.25
5-OAC	-	-	-	-	2.25	-	-	-	-
-CH=CH-	-	-	5.38	-	5.38	5.35	-	5.35	-
6-CH_3	-	-	1.94	-	1.96	-	-	-	-
Vinyl methylene	-	-	2.0	-	2.0	2.0	2.0	2.0	-
terminal CH_3	0.88	0.88	0.90	0.88	0.89	0.87	0.88	0.87	0.88

Table 19: ^{1}H NMR peak positions for benzoquinone derivatives

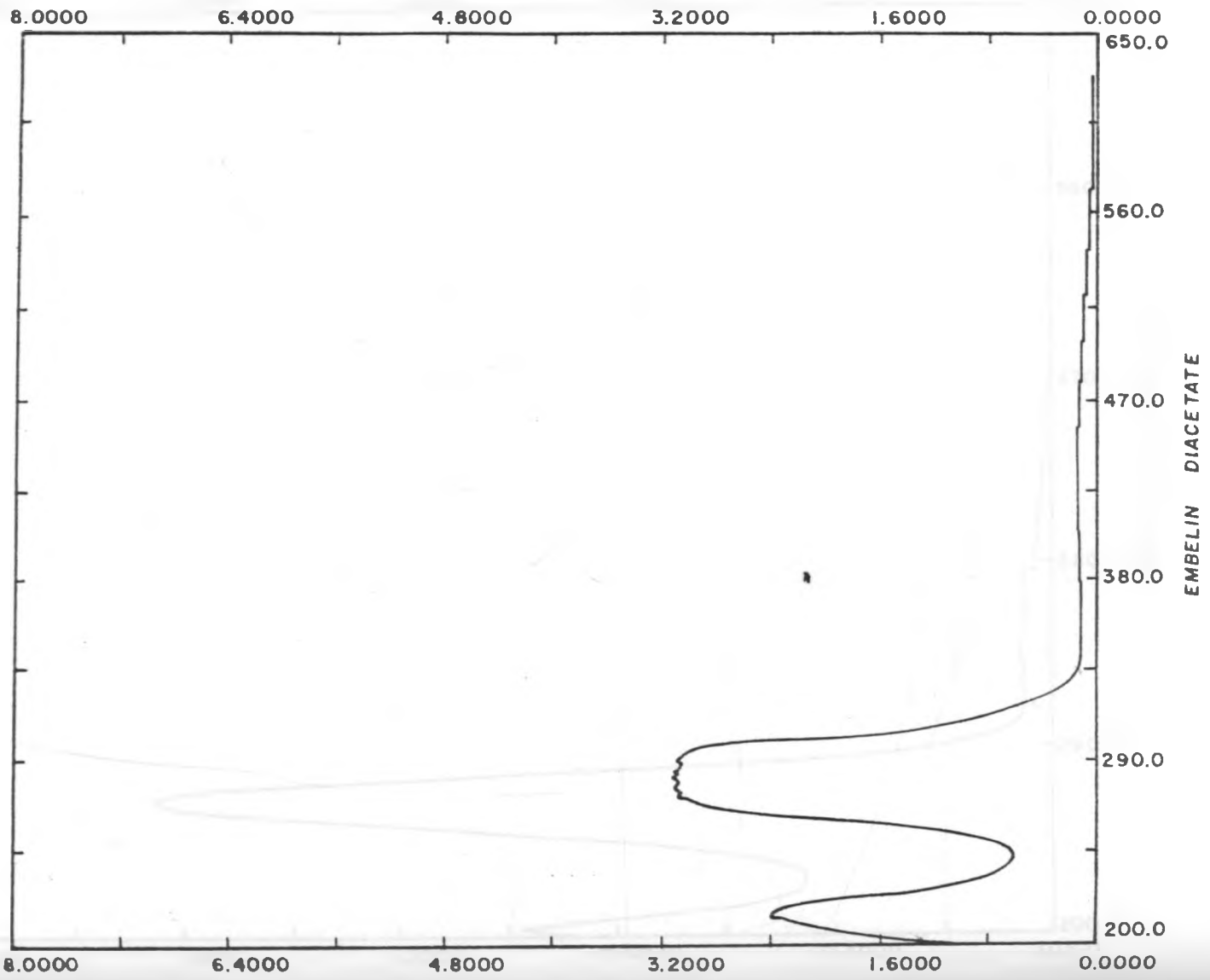
Position of carbon	29	30	31	32	33	34	36	37	38
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-
6	7.05	7.09	7.07	7.06	7.09	7.06	-	-	-
1^{1}	2.44	2.40	2.42	2.42	2.40	2.40	2.40	2.38	2.38
5-OCH_3	-	-	3.84	-	-	3.86	-	-	3.86
5-OAC	2.25	2.25	-	2.25	2.25	-	2.28	2.26	-
Methylene long chain	1.25	1.26	1.26	1.26	1.25	1.25	1.26	1.26	1.26
-CH=CH-	-	-	-	-	-	-	5.35	5.38	5.35
6-CH_3	-	-	-	-	-	-	1.94	1.94	1.94
Vinyl methylene	-	-	-	-	-	-	2.0	2.0	2.0
terminal CH_3	0.88	0.88	0.88	0.89	0.88	0.88	0.89	0.90	0.90

Table 20. Mp, UV/Visible, IR, Ms data for benzoquinone compounds

	5	6	7	12	14	15	35	39	40
Mp (^{o}c)	140-142	140-142	122-124	66-68	25-38	67-69	119-121	104-106	130-132
UV/Visible	292 (4.24)	292 (4.24)	294 (4.40)	280 (4.50)	272 (4.20)	280 (4.20)	280 (4.20)	285 (4.20)	290 (4.20)
λ max nm. (meOH)	425 (2.53)	425 (2.53)	440 (2.40)	420 (2.80)	410 (2.85)	420 (2.70)	420 (2.45)	440 (2.70)	420 (2.40)
IR (cm^{-1})									
C=0	1620	1623	1620	1620 1680 1780	1620 1650	1610	1650	1625	1620
OH	3300	3310	3300	3275	3300	3300	3325	3350	3300
Ms (M^{+})	294	322	418	582	460	362	294	348	350

Table 21. Mp, UV/Visible, IR data for benzoquinone derivatives

	29	30	31	32	33	34	36	37	38
Mp (^{o}c)	52-54	121-123	57-59	63-64	114-116	63-65	72-74	80-82	48-50
UV/Visible	210 (2.6)	208 (2.2)	220 (2.6)	208 (3.2)	208	220 (2.80)	220 (2.45)	220 (3.20)	210 (1.20)
λ max, nm. (meOH)	270 (1.6)	265 (1.2)	280 (1.6)	280 (1.4)	260 (1.2)	290 (1.4)	260 (1.45)	290 (2.40)	280 (1.0)
IR(cm^{-1}) C=0	1685 1780	1780	1680	1680 1780	1780	1680	1670 1780	1780	1680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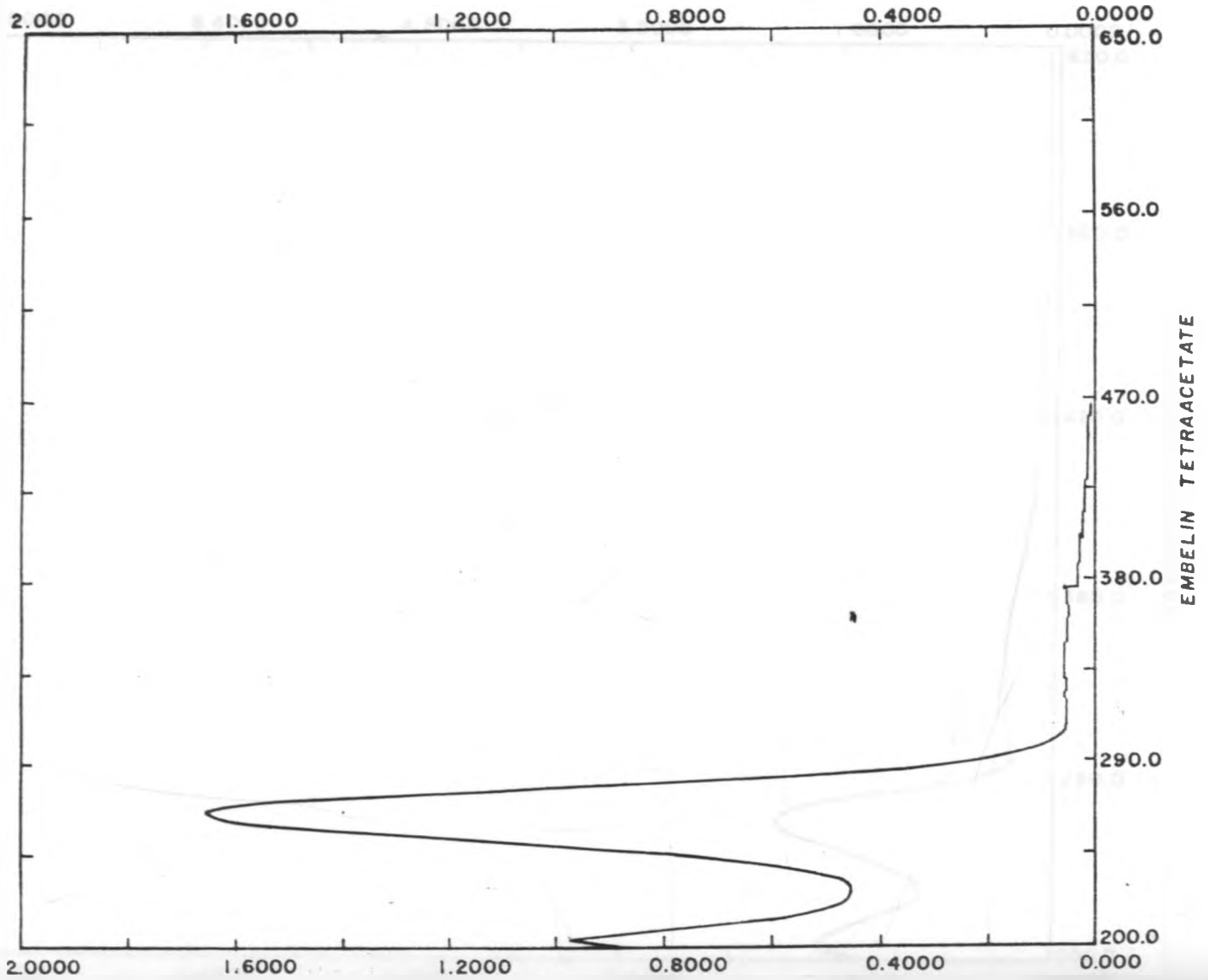
4.8000
3.2000
1.6000
0.0000
650.0
560.0
470.0
380.0
290.0
200.0
EMBELIN DIACETATE


2.000
1.6000
1.2000
0.8000
0.4000
0.0000
650.0
560.0
470.0
380.0
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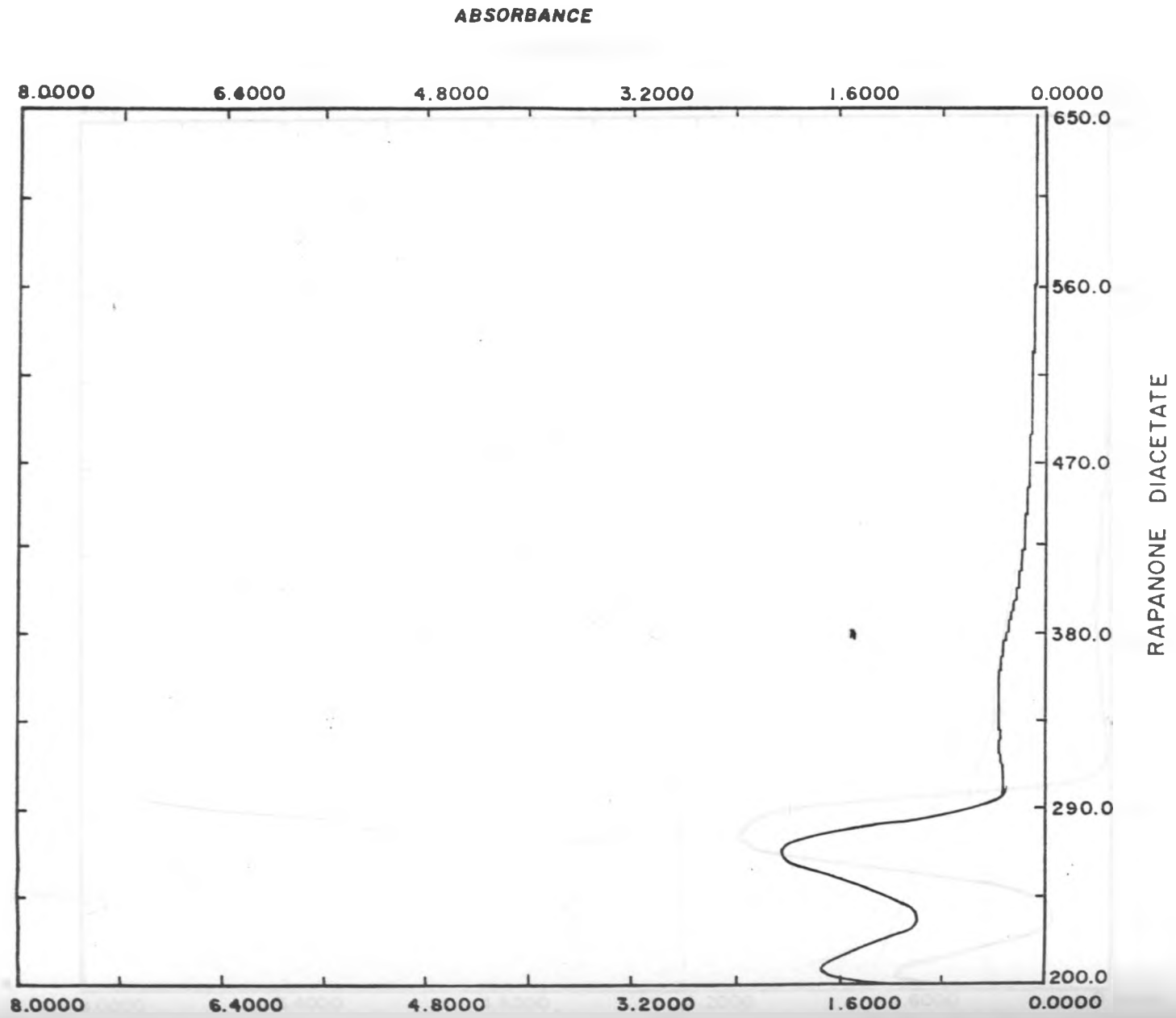
200.0
2.0000
1.6000
1.2000
0.8000
0.4000
0.000
EMBELIN TETRAACETATE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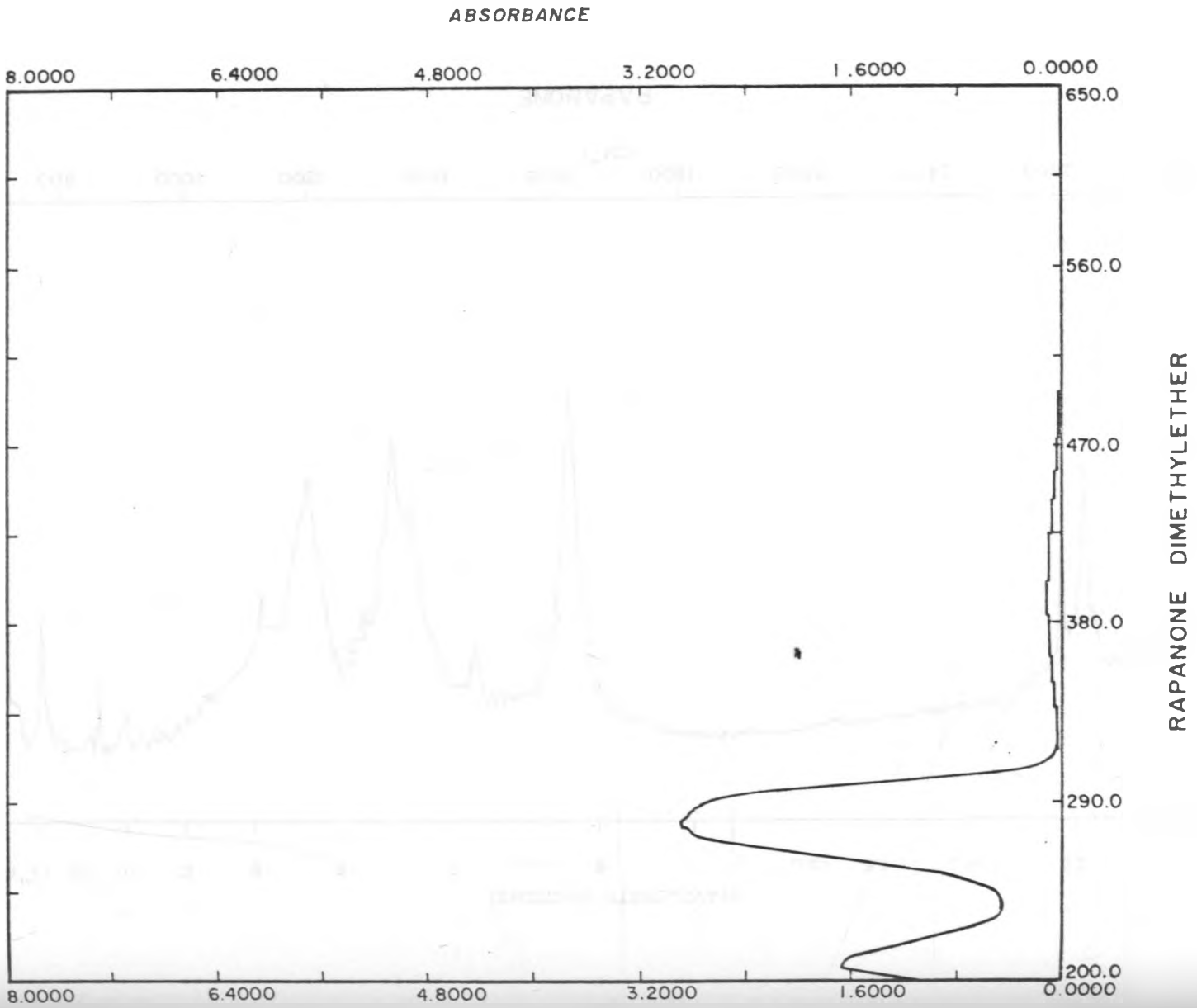
560.0
470.0
380.0
290.0
200.0
RAPANONE DIACETATE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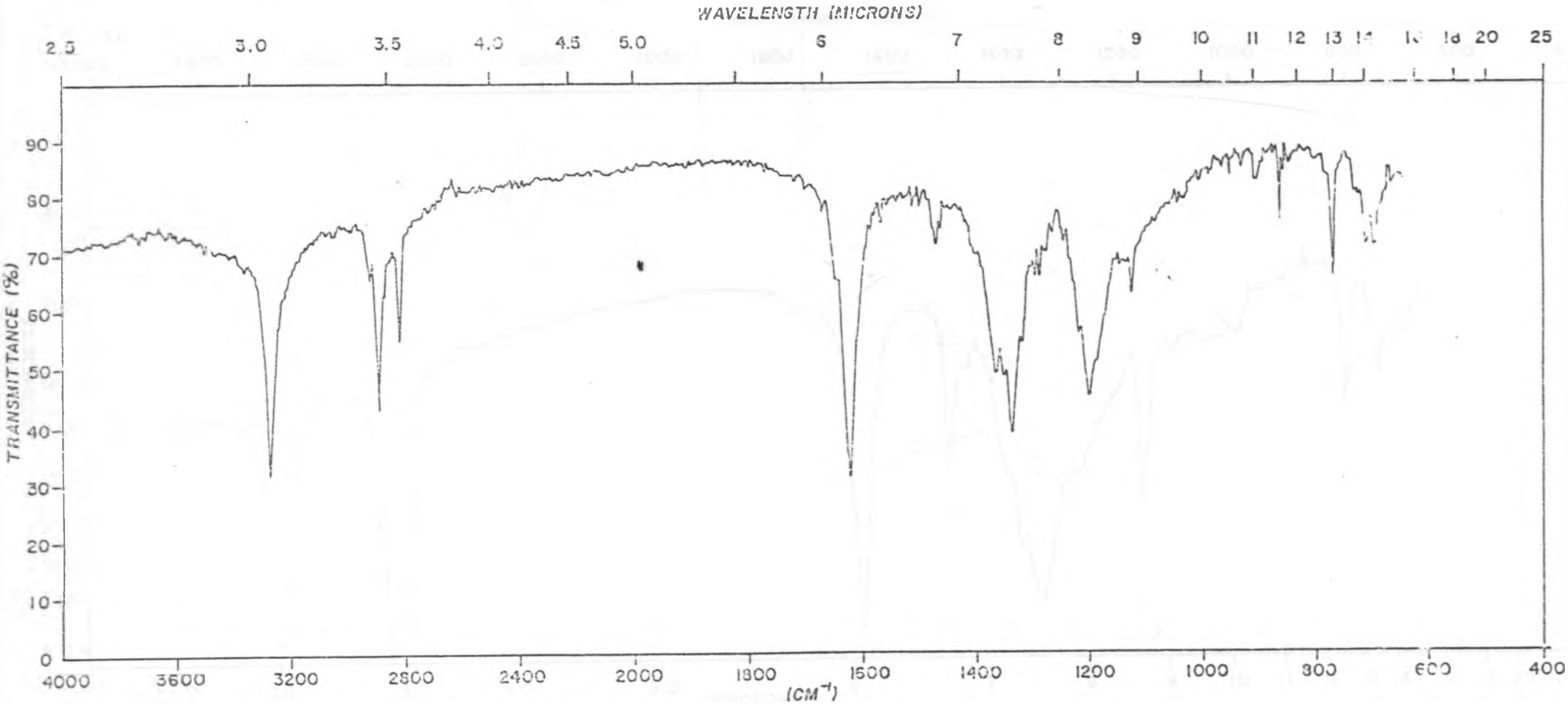
560.0
470.0
380.0
290.0
200.0
RAPANONE DIMETHYLETHER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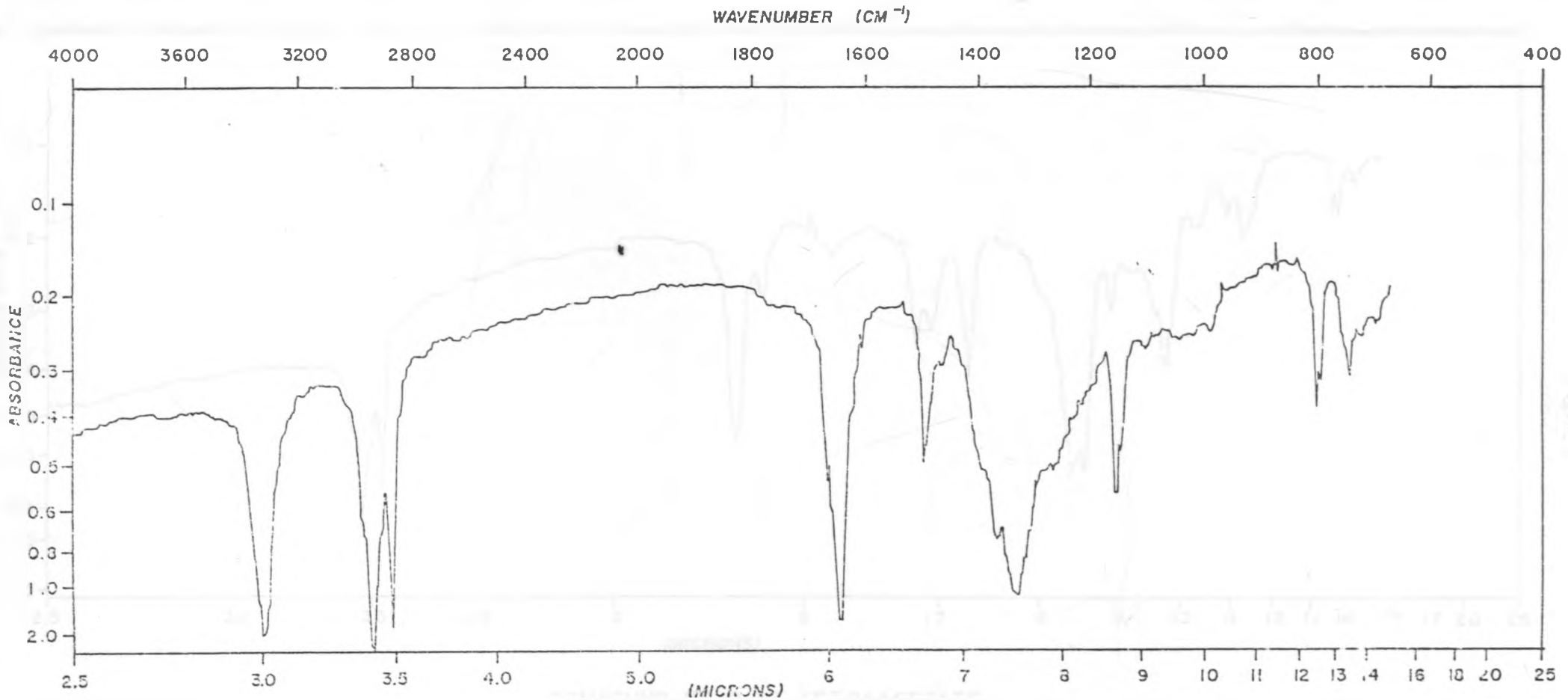
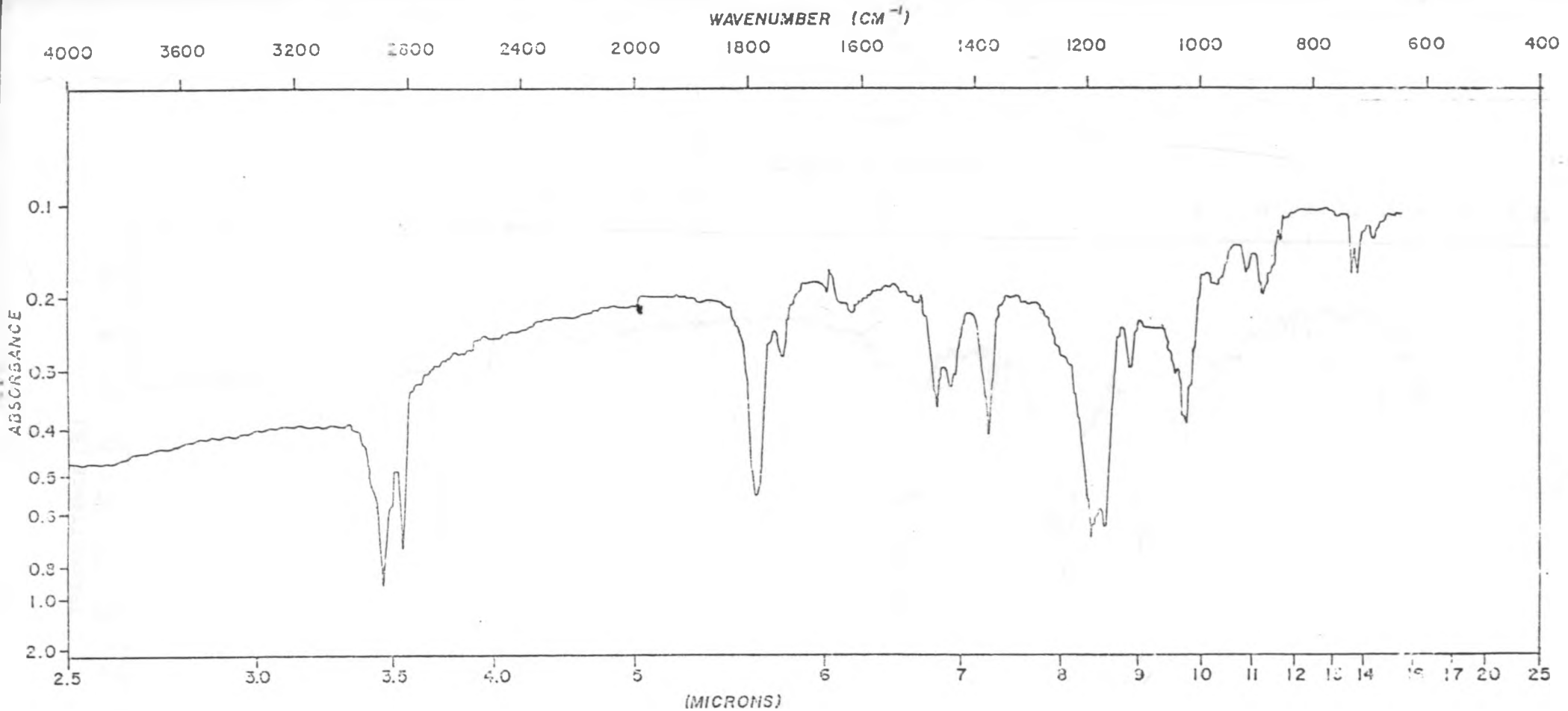
2.5
3.0
3.5
4.0
4.5
5.0
6
7
8
9
10
11
12
13
20
25
TRANSMITTANCE (%)
90
80
70
60
50
40
30
20
10
0
4000
3600
3200
2800
2400
2000
1800
1500
1400
1200
1000
800
400
(CM⁻¹)

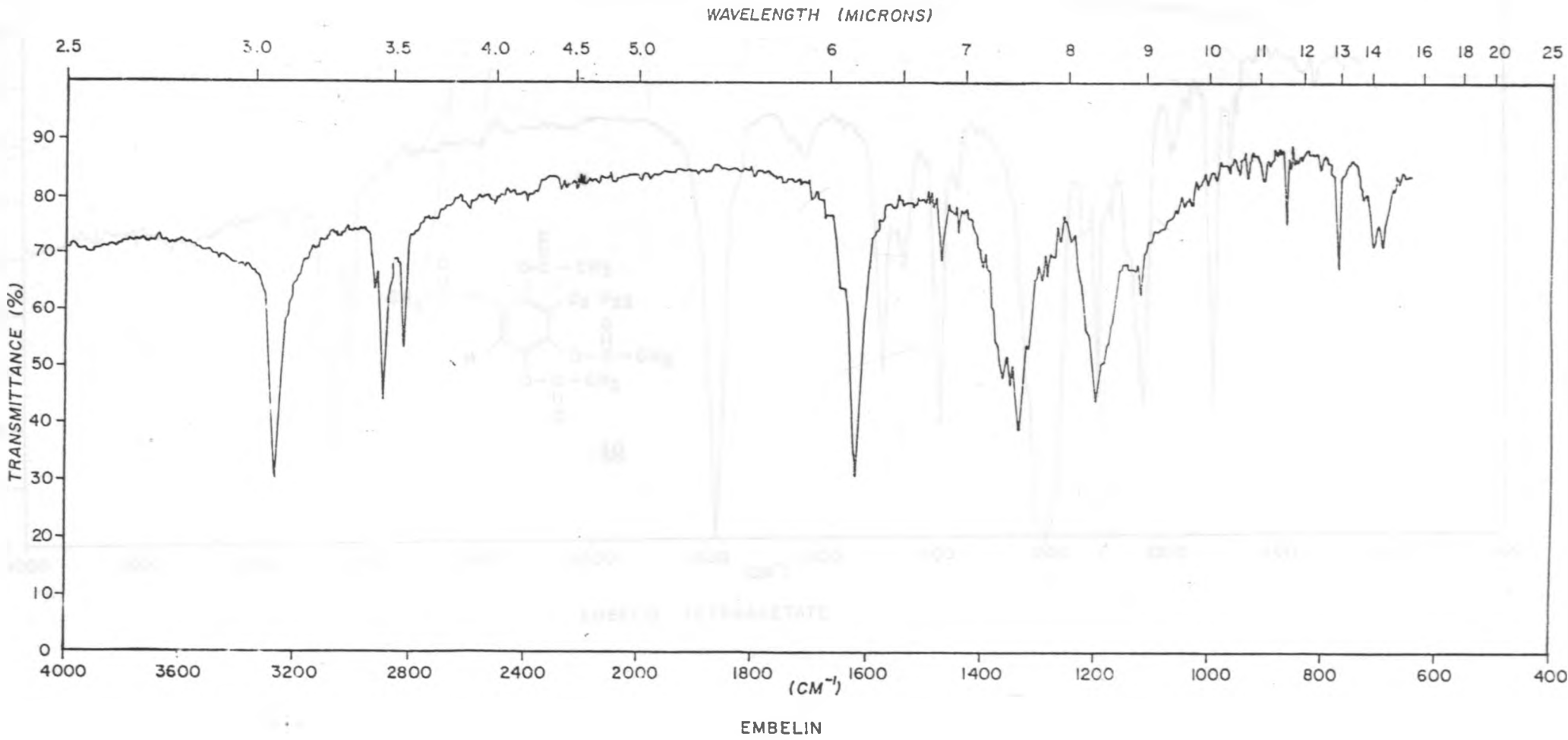
RAPANONE



COMPOUND RF 0.80 35



COMPOUND RF 0.80 TETRAACETATE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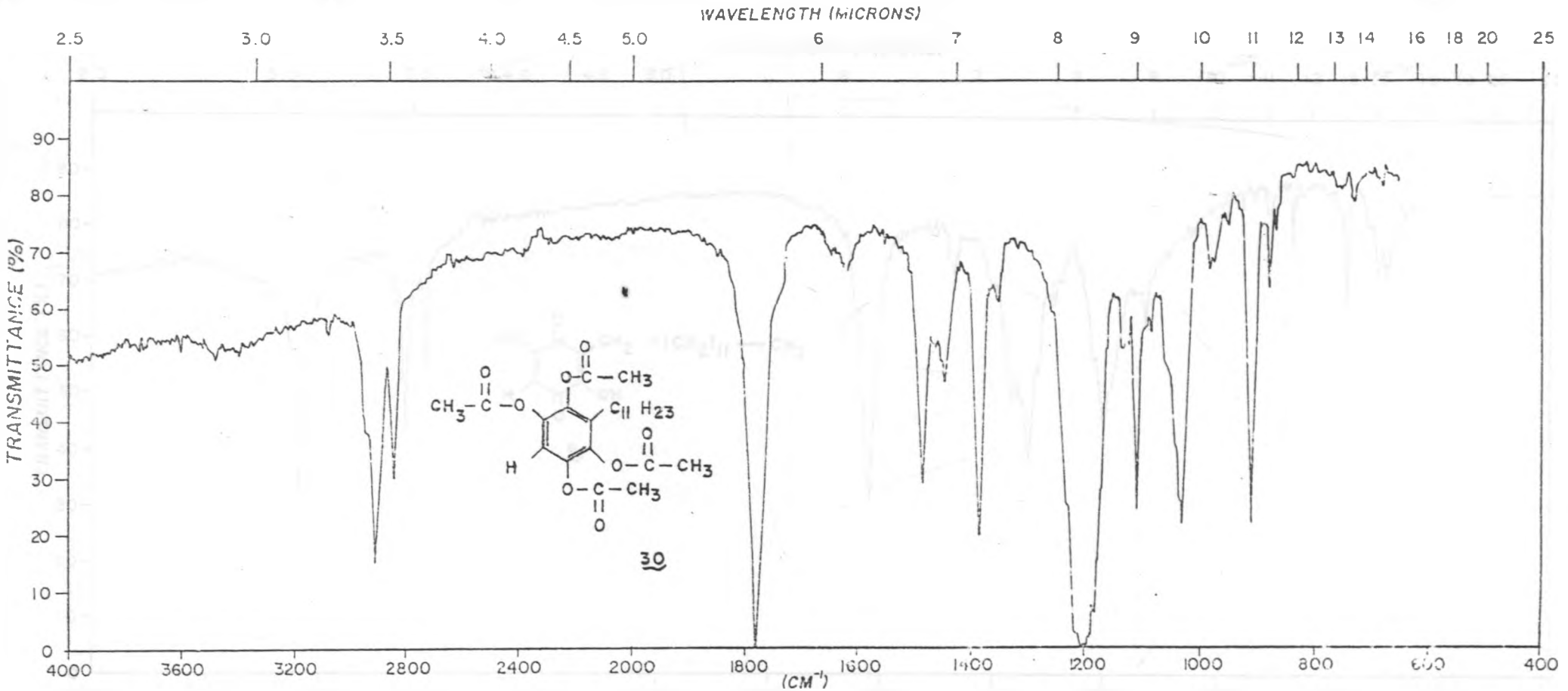
3.5
4.0
4.5
5.0
6
7
8
9
10
11
12
13
14
16
18
20
25
TRANSMITTANCE (%)
90
80
70
60
50
40
30
20
10
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
(CM⁻¹)

EMBELIN



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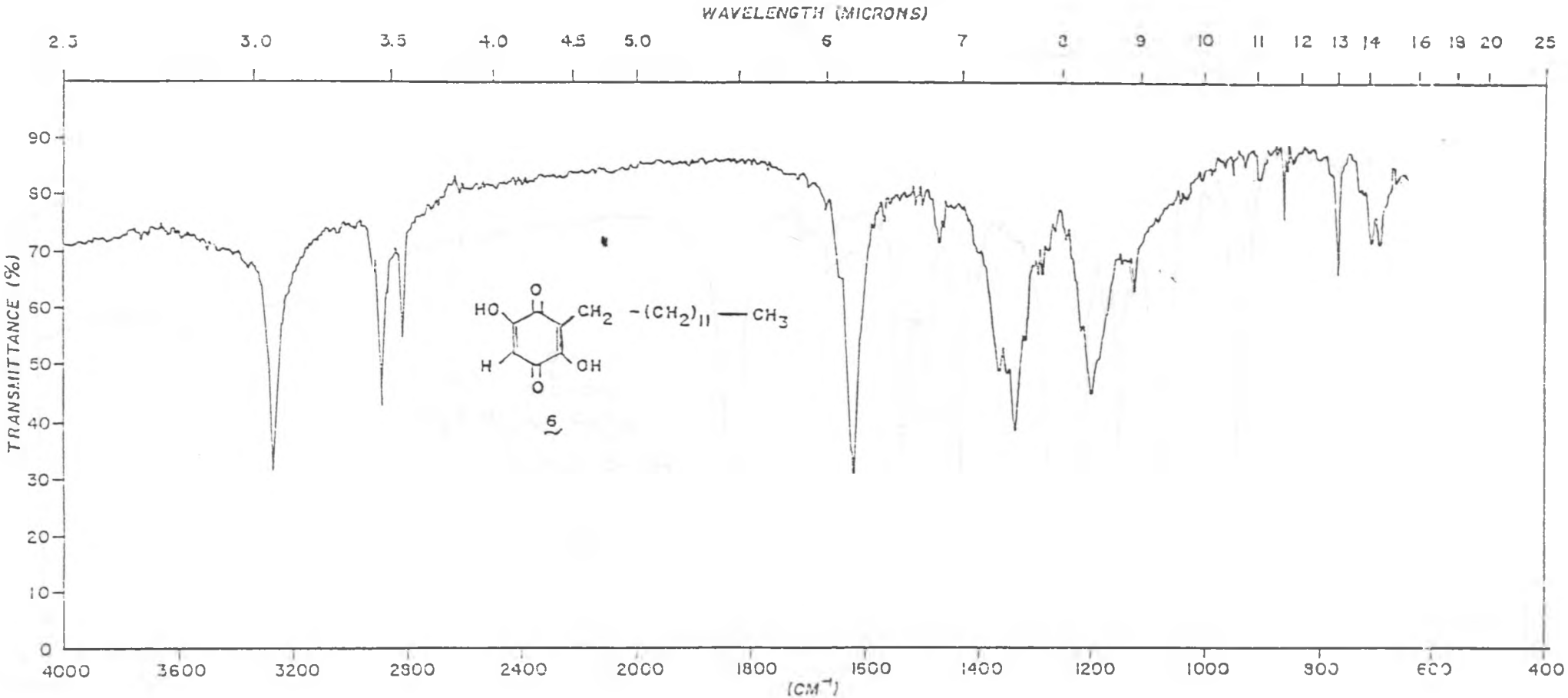
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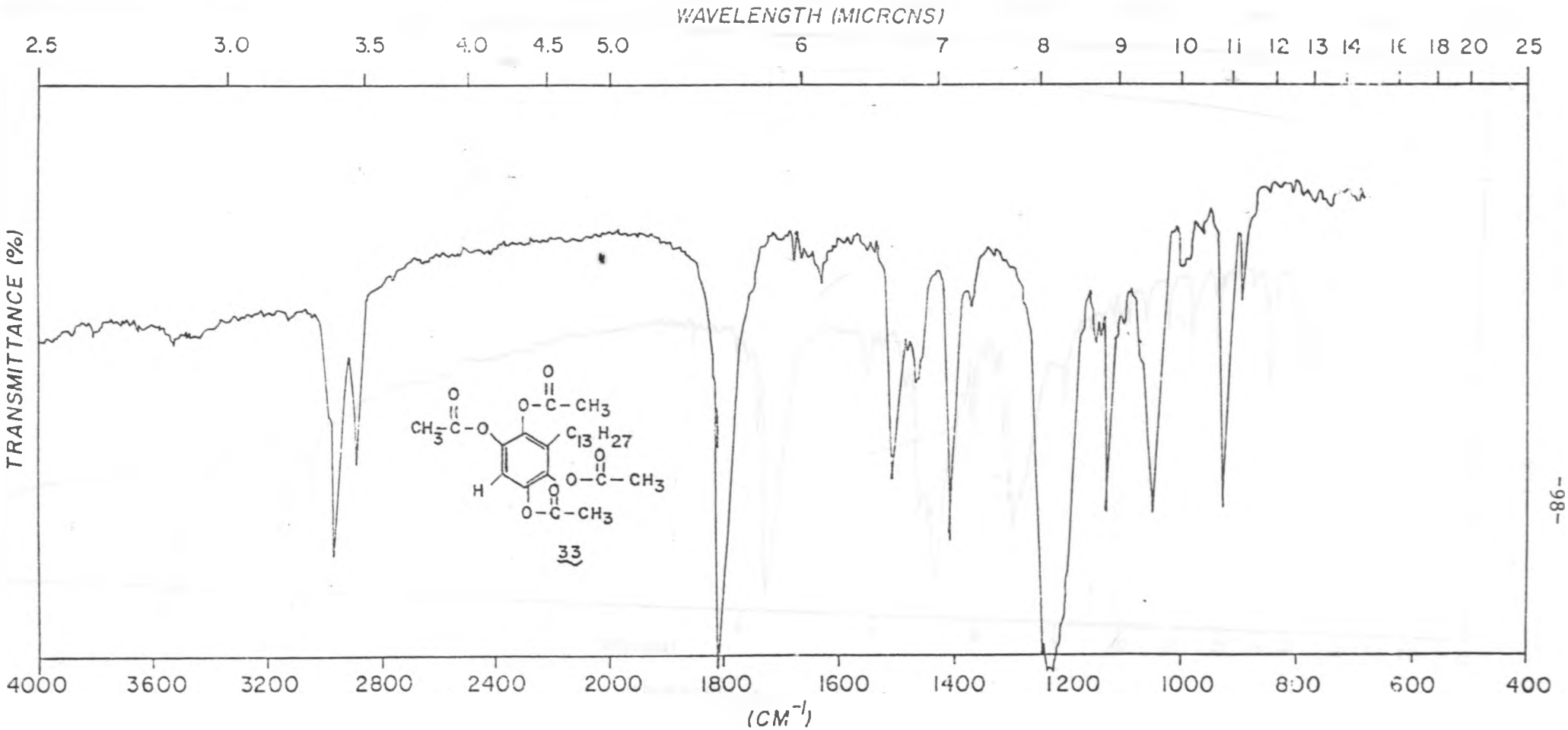
2.5
3.0
3.5
4.0
4.5
5.0
6
7
8
9
10
11
12
13
14
16
18
20
25
TRANSMITTANCE (%)
90
80
70
60
50
40
30
20
10
0
4000
3600
3200
2800
2400
2000
1800
1500
1400
1200
1000
900
600
400
(CM⁻¹)
HO
O
CH2
-(CH2)11—CH3
H
OH
O
6

RAPANONE



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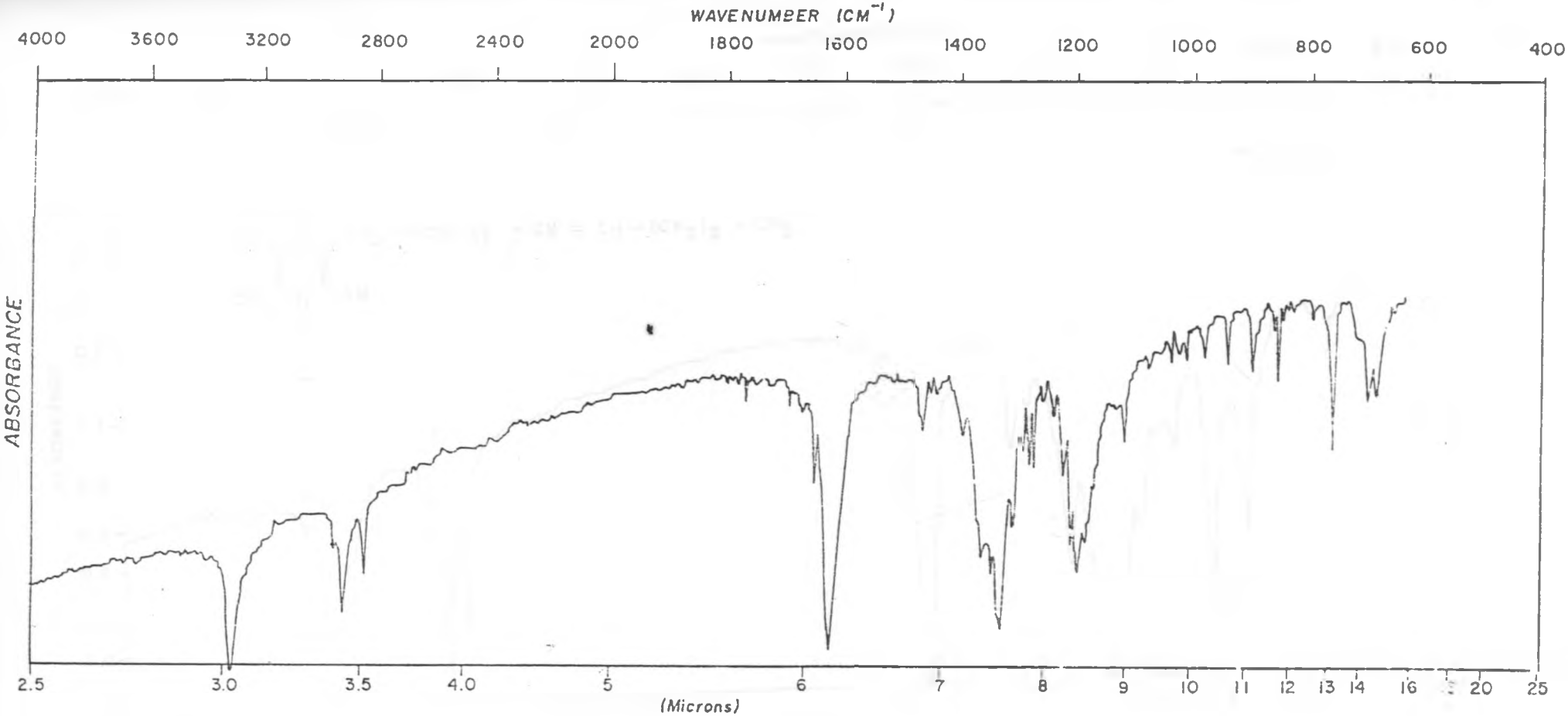
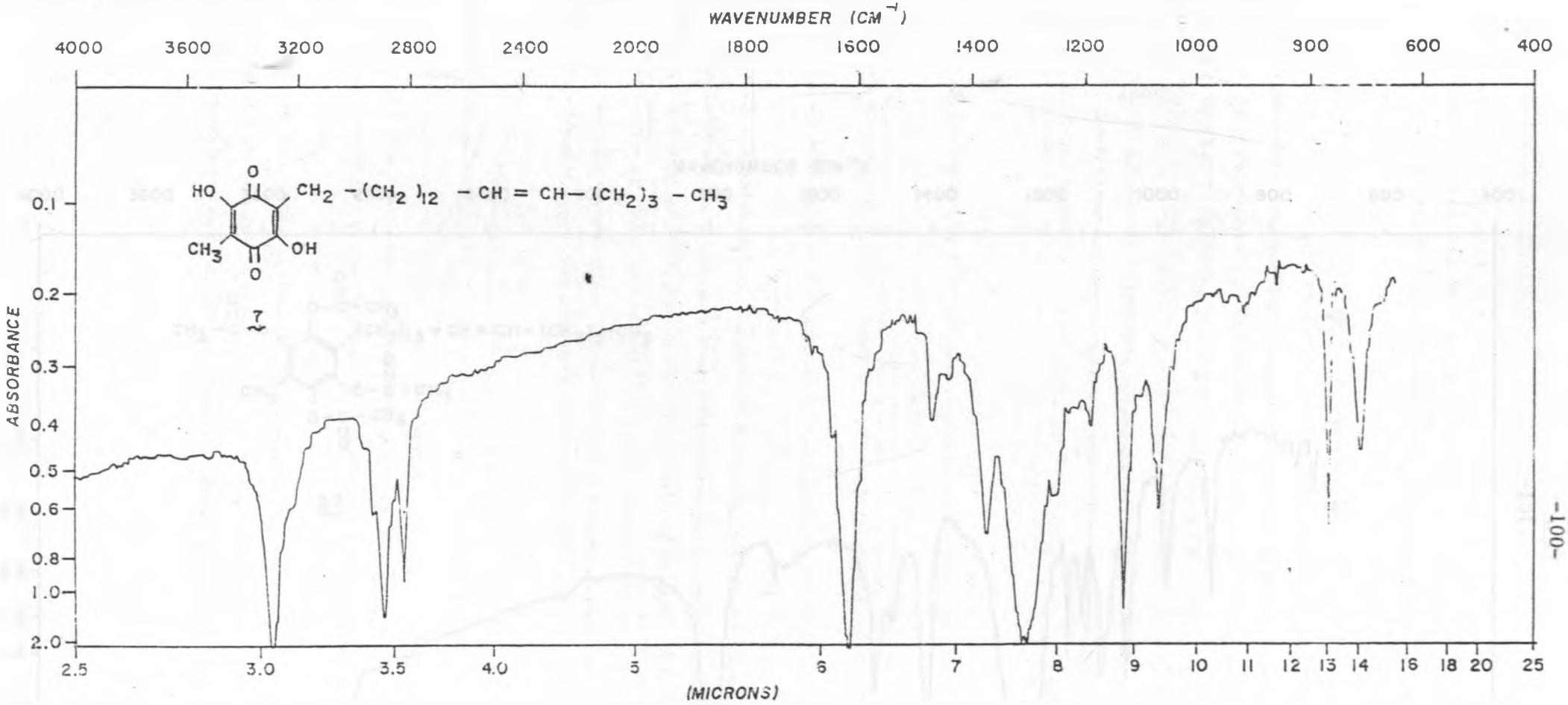
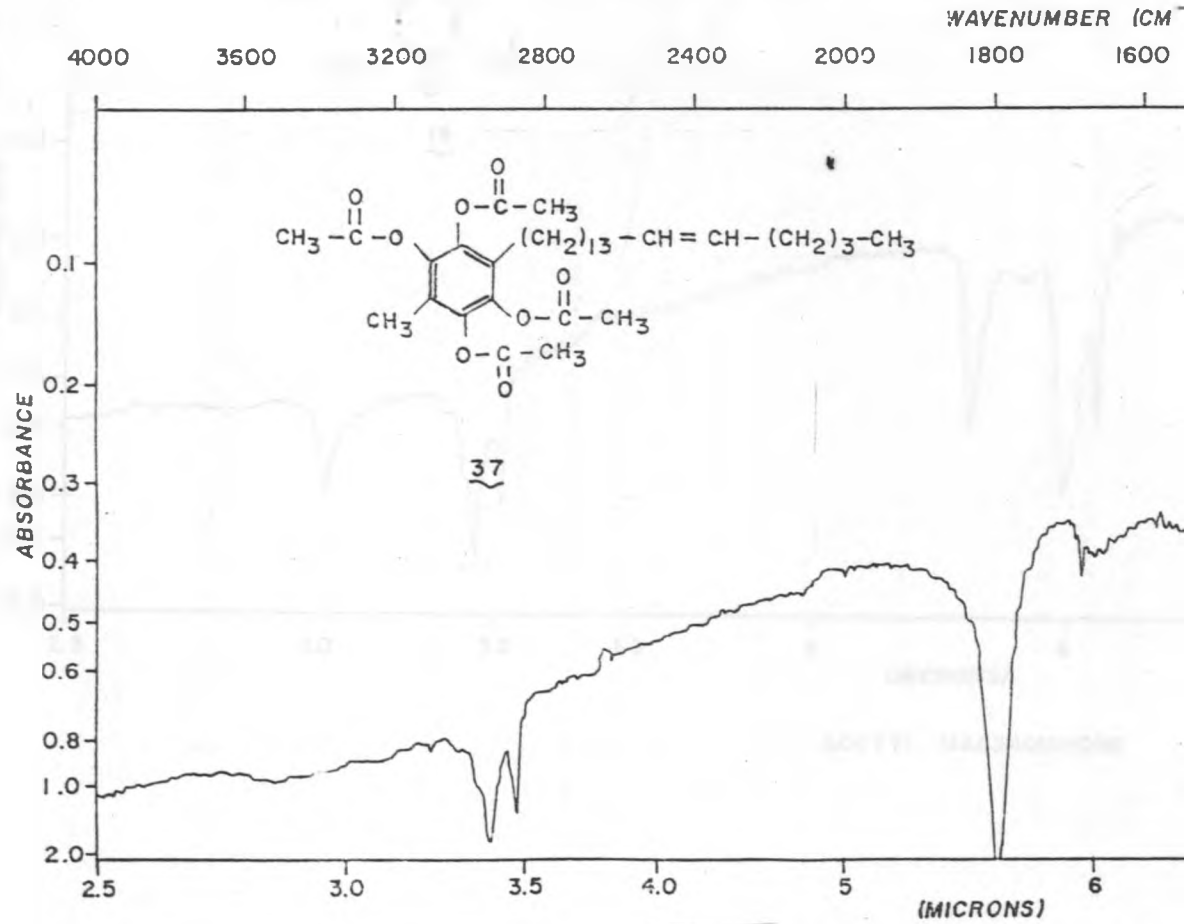


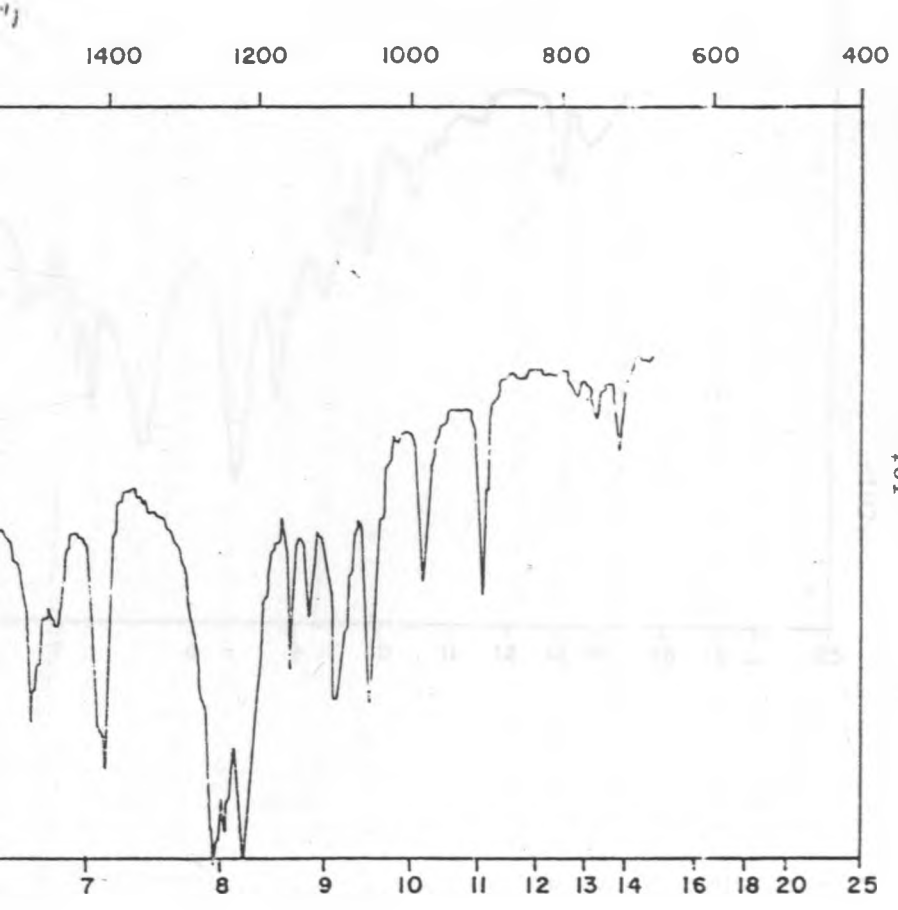
MACROPHYLLIN



MAESAQUINONE



MAESAQUINONE TETRAACETATE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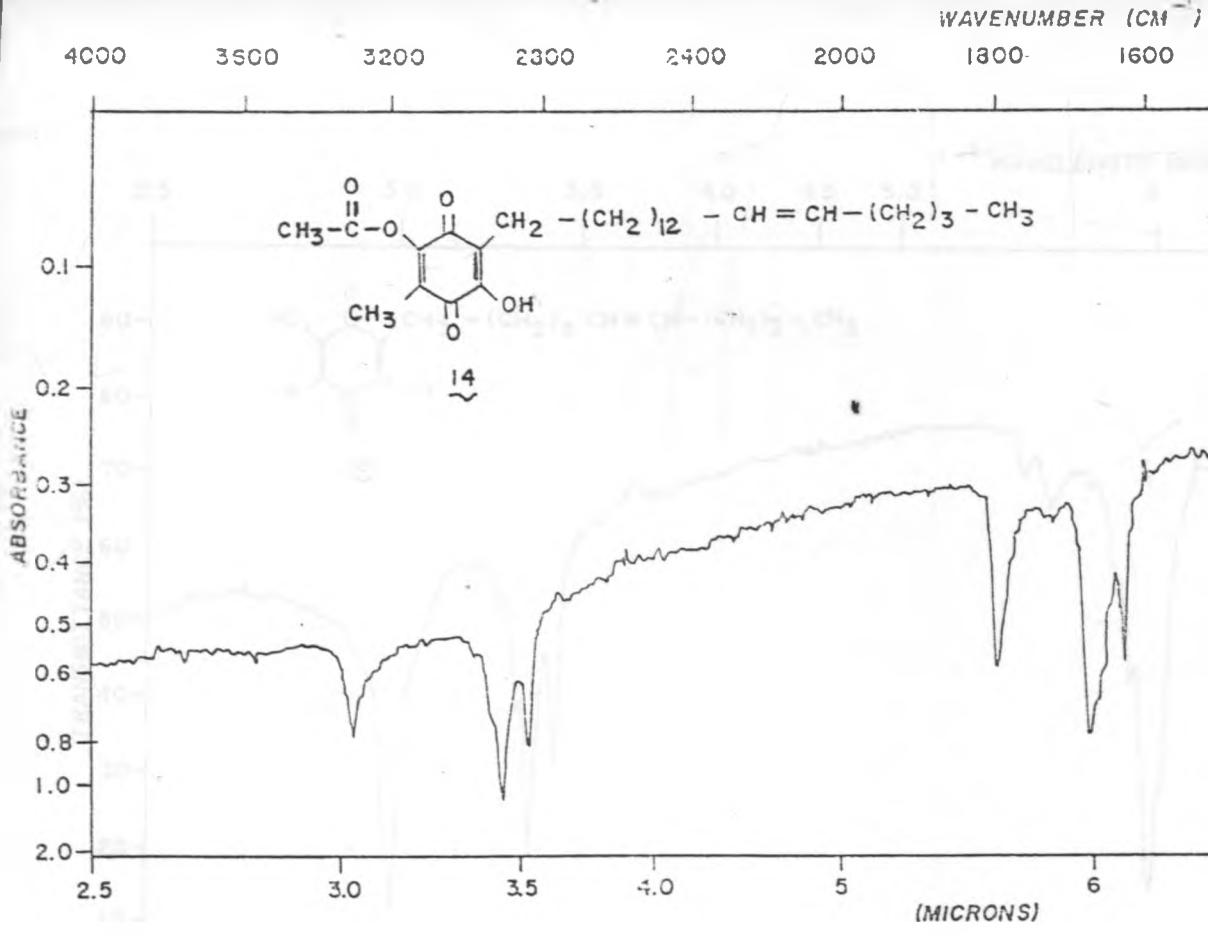
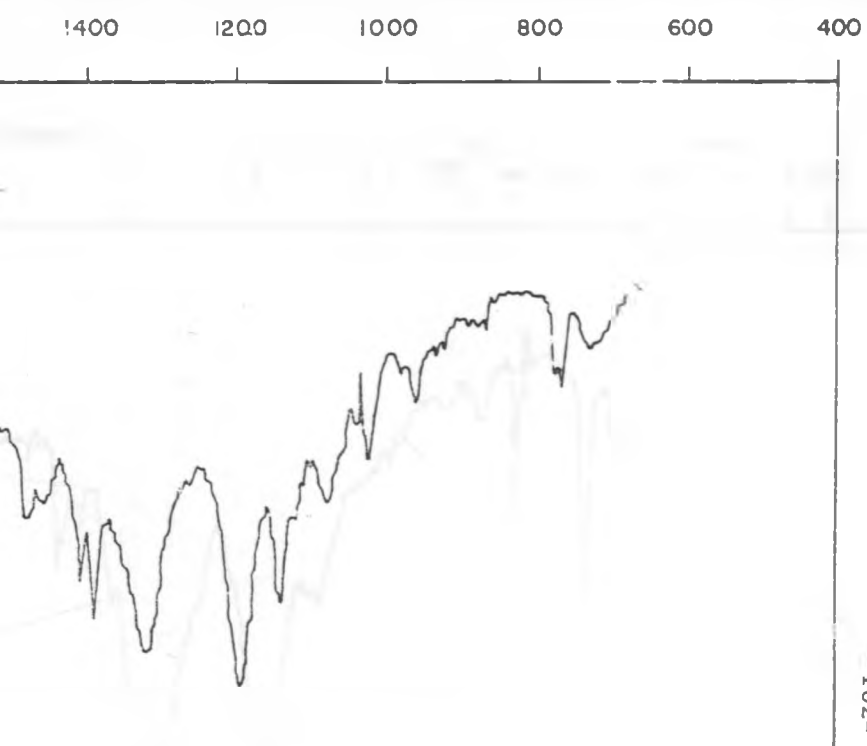
12
13
14
16
18
20
25



14

ACETYL MAESAQUINONE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1200
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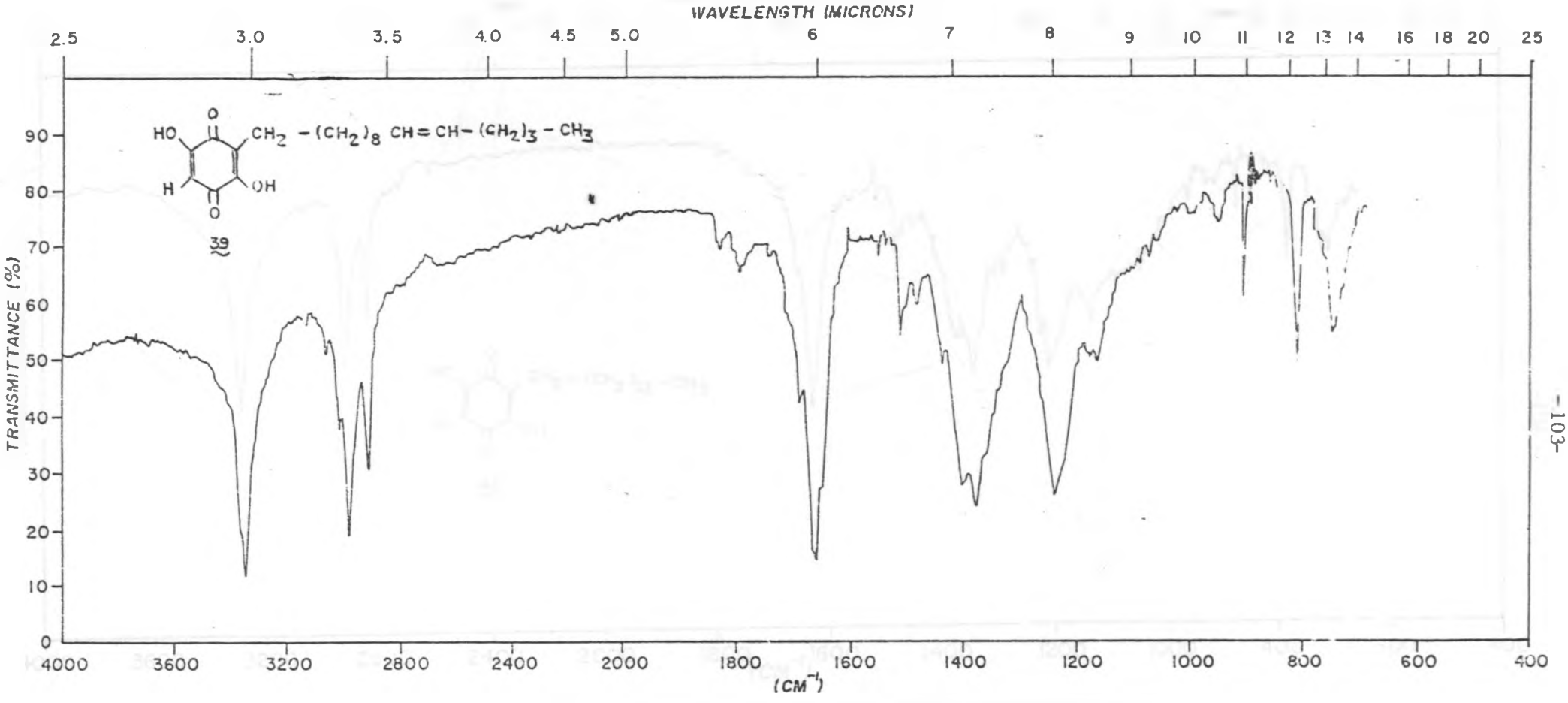
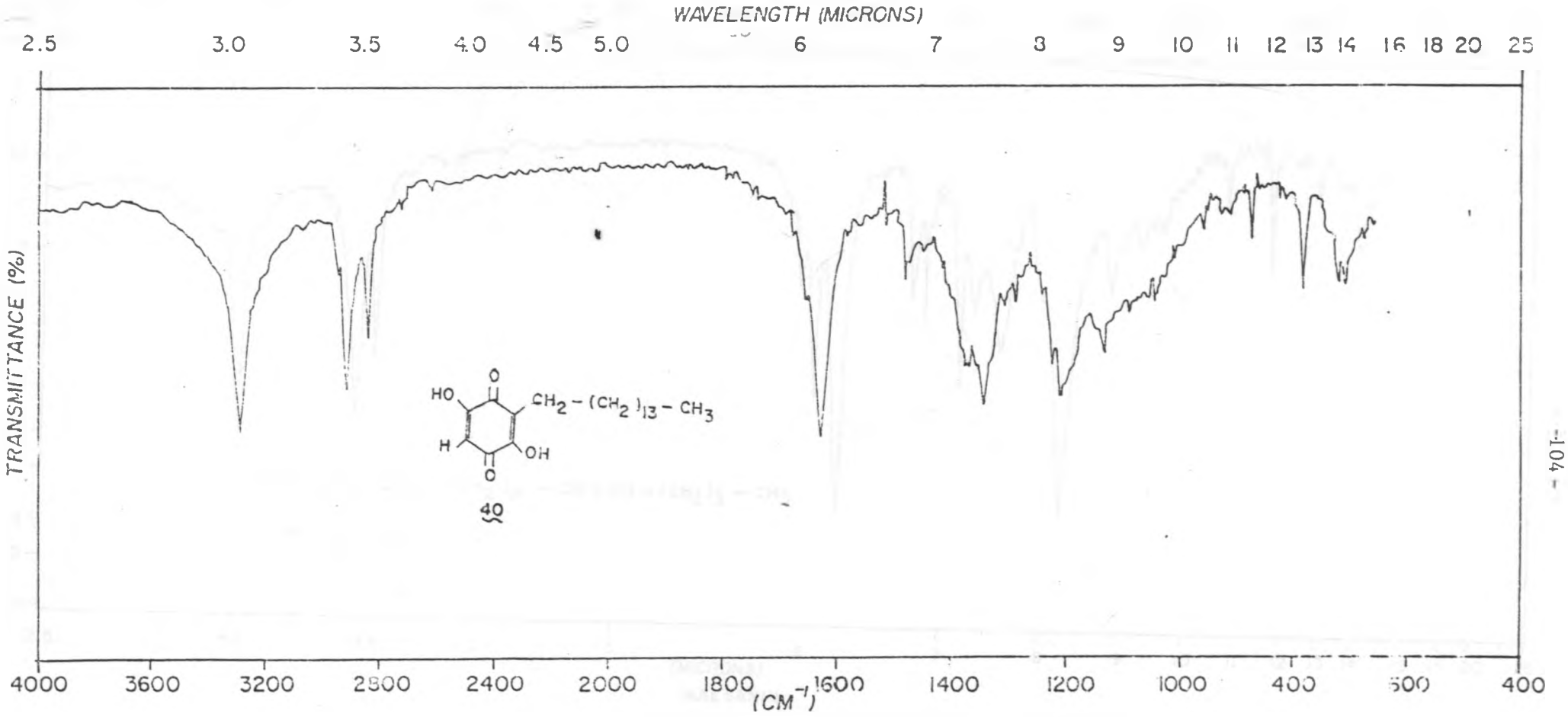
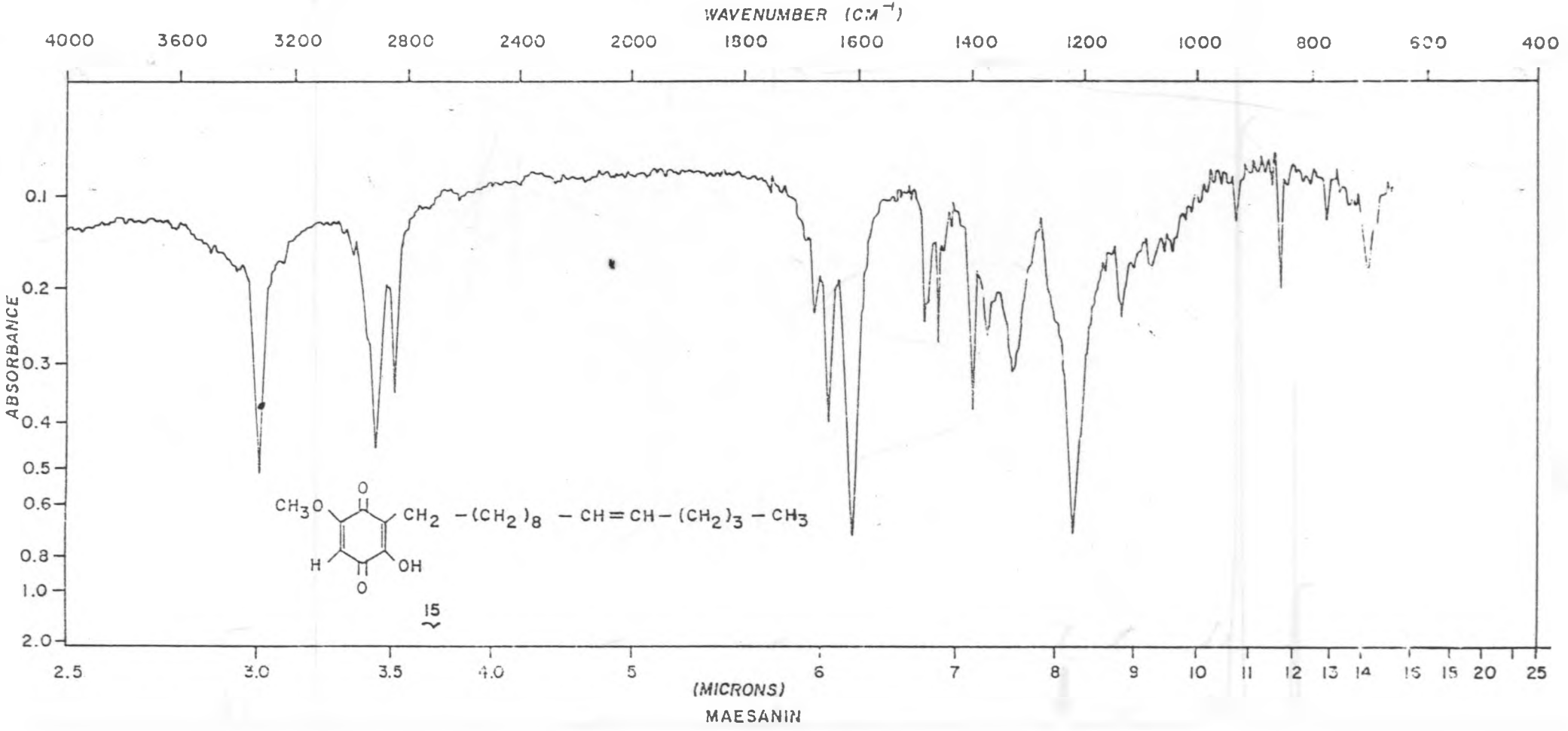
16
25

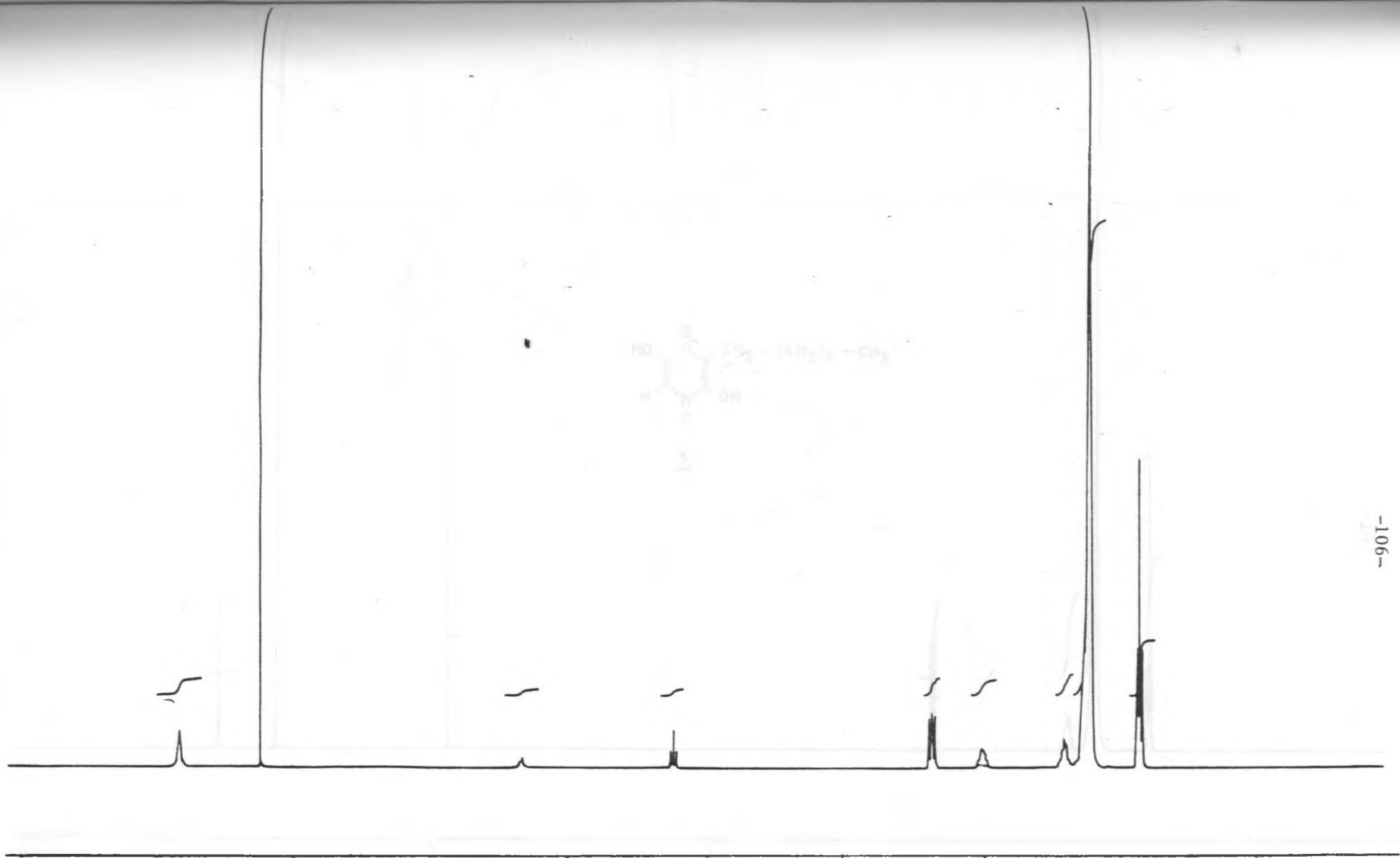


2,5 – DIHYDROXY – 2 – PENTADECENYL BENZOQUINONE

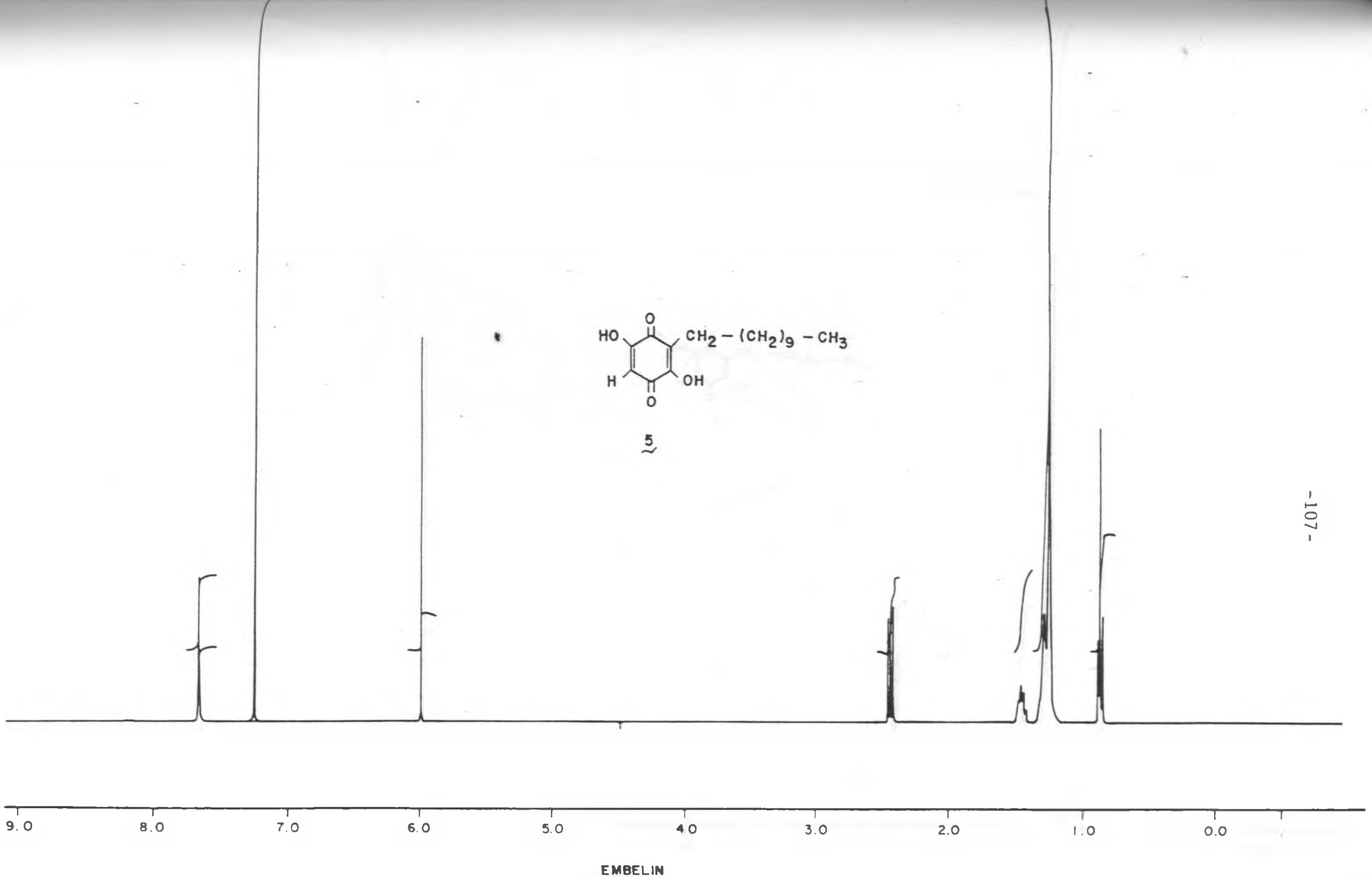


2, 5 — DIHYDROXY — 3 — PENTADECYL BENZOQUINONE


WAVENUMBER (CM⁻¹)
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
ABSORBANCE
0.1
0.2
0.3
0.4
0.5
0.6
0.8
1.0
2.0
2.5
3.0
3.5
4.0
5
6
7
8
9
10
11
12
13
14
15
15
20
25
(MICRONS)
MAESANIN
CH3O
CH2 −(CH2)8 − CH=CH−(CH2)3 − CH3
H
OH
O
O
15



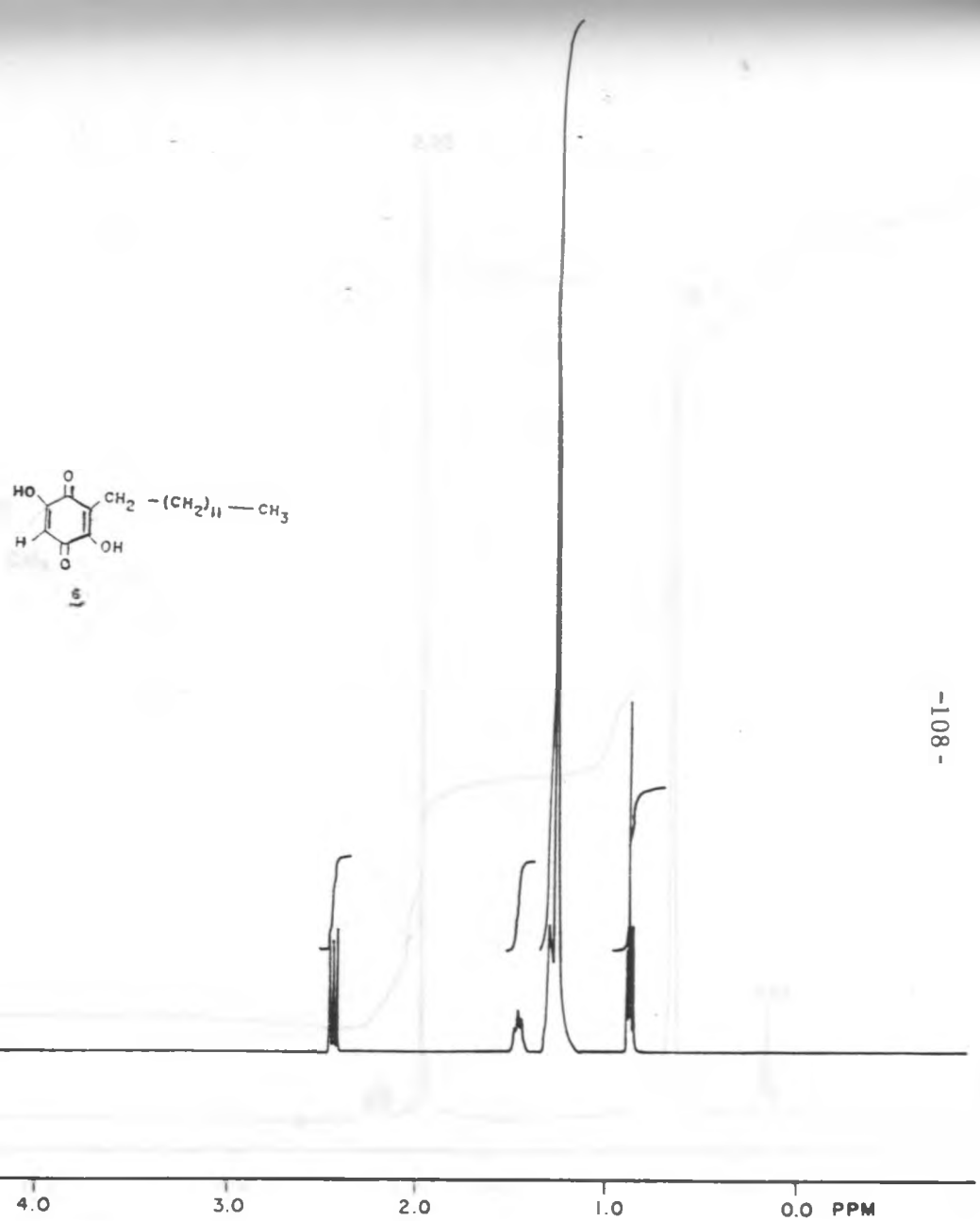
COMPOUND OF RF 0.80 35



5

EMBELIN

RAPANONE



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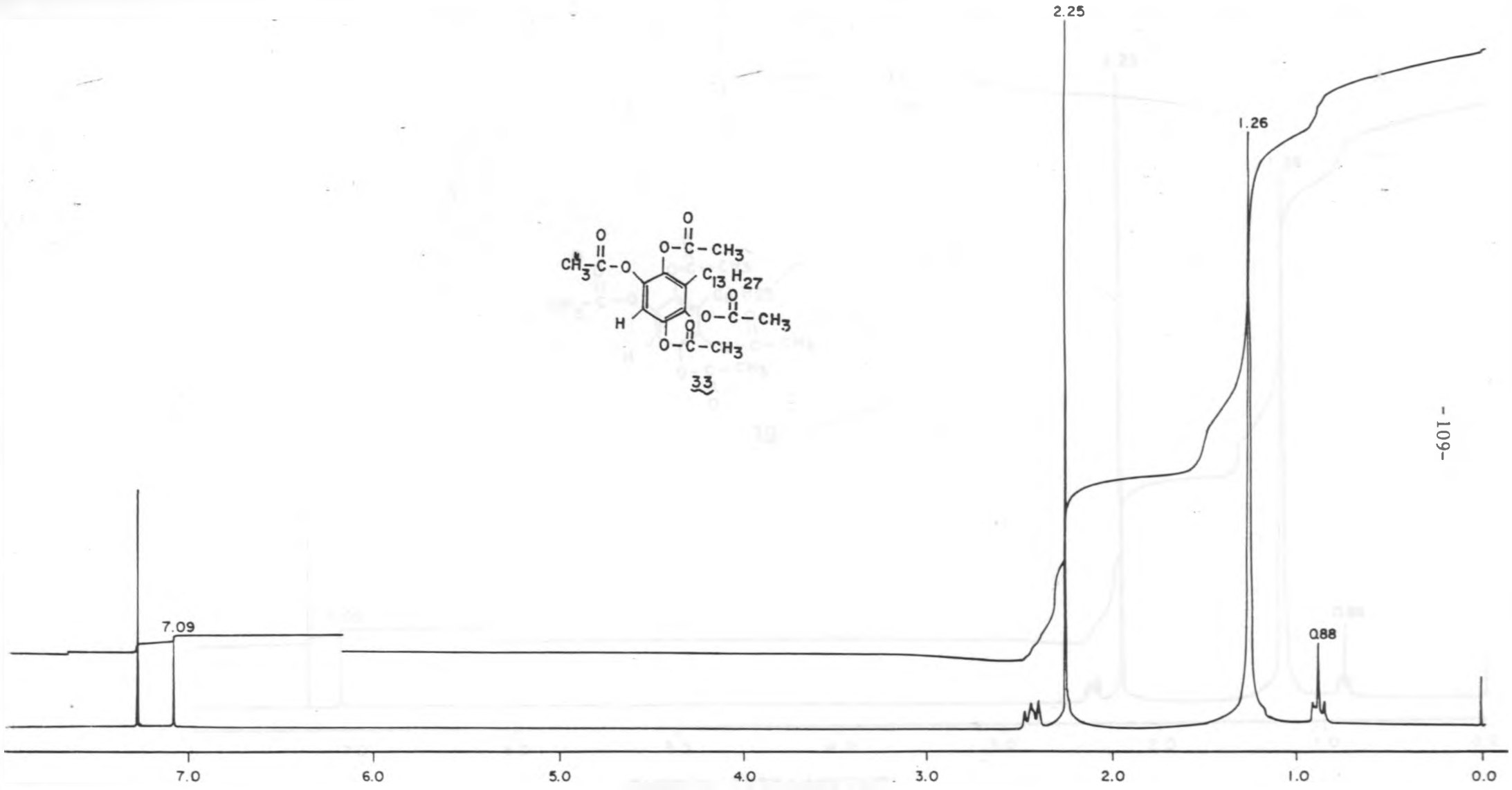
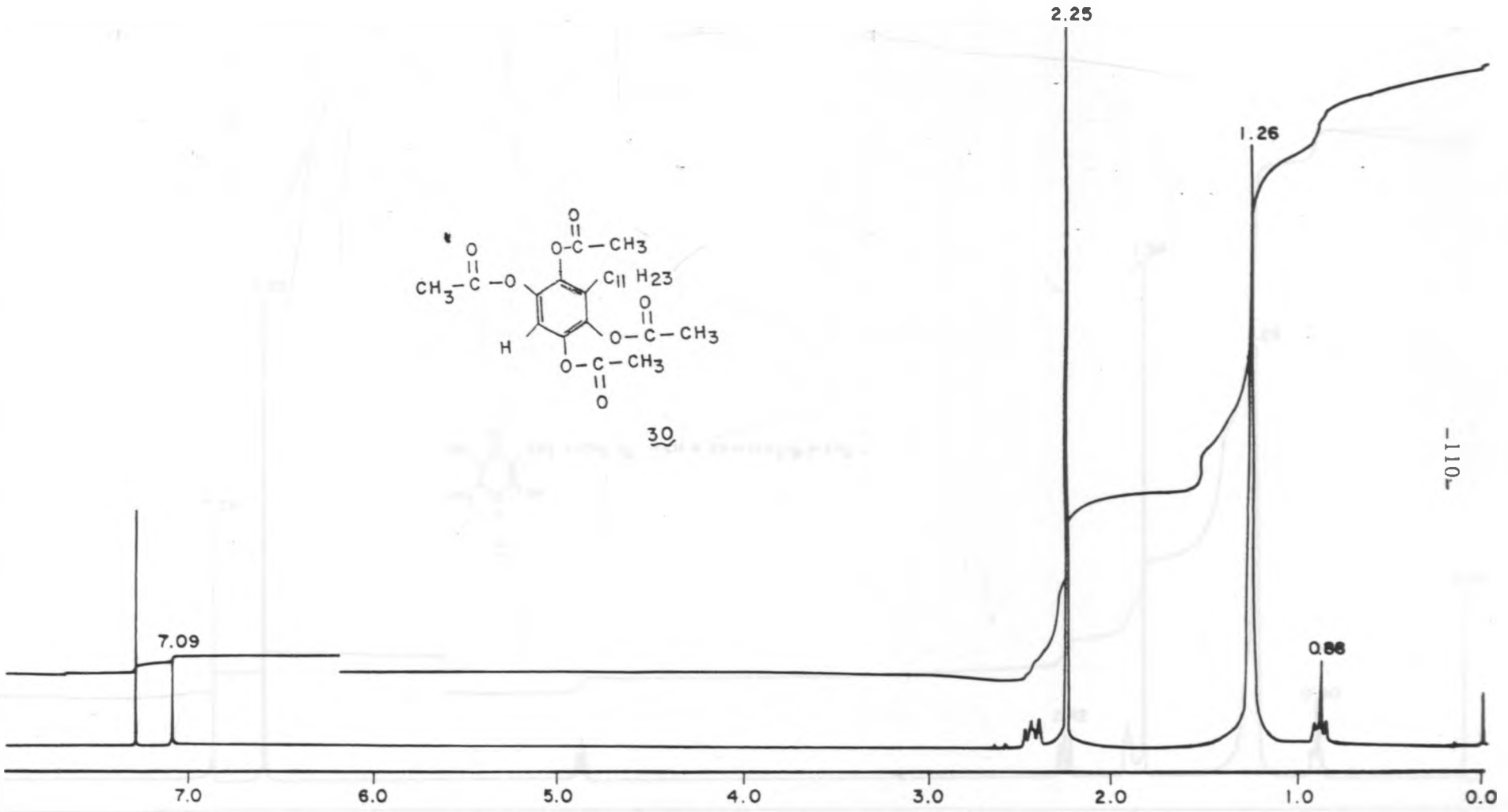


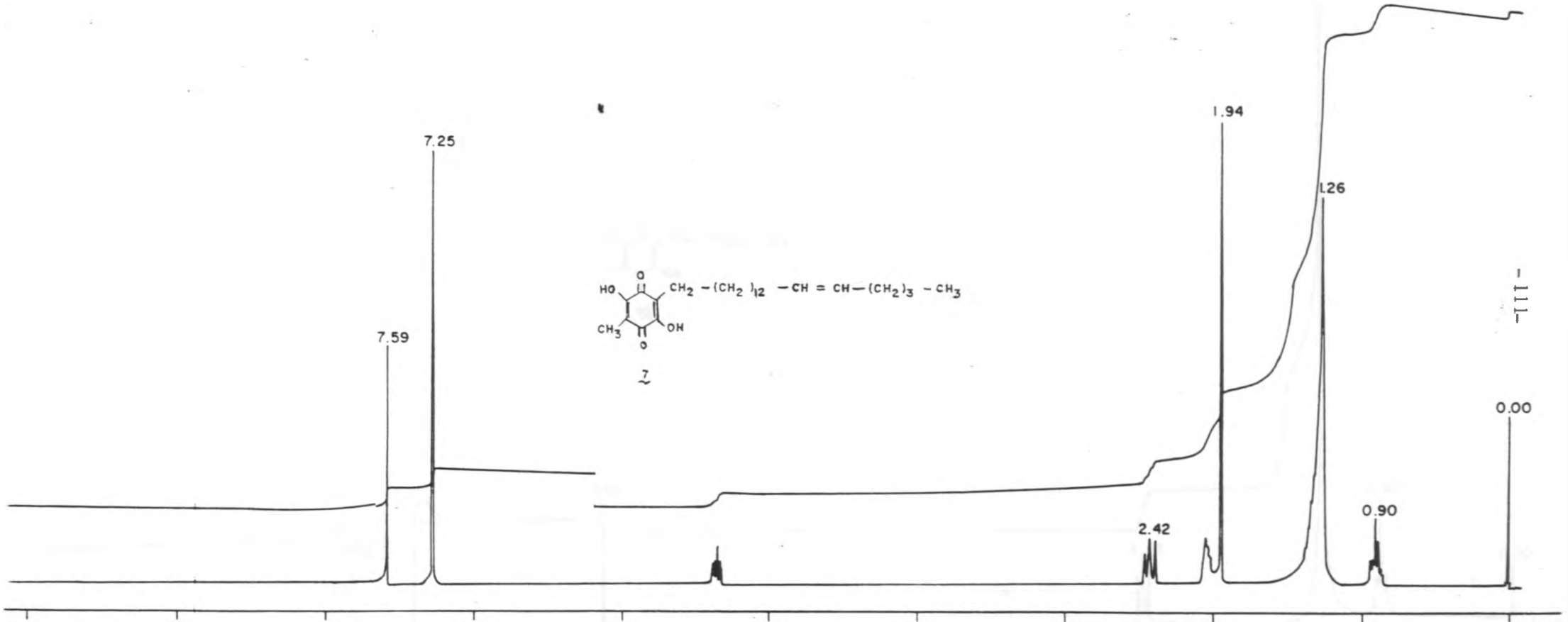
33

RAPANONE TETRAACETATE

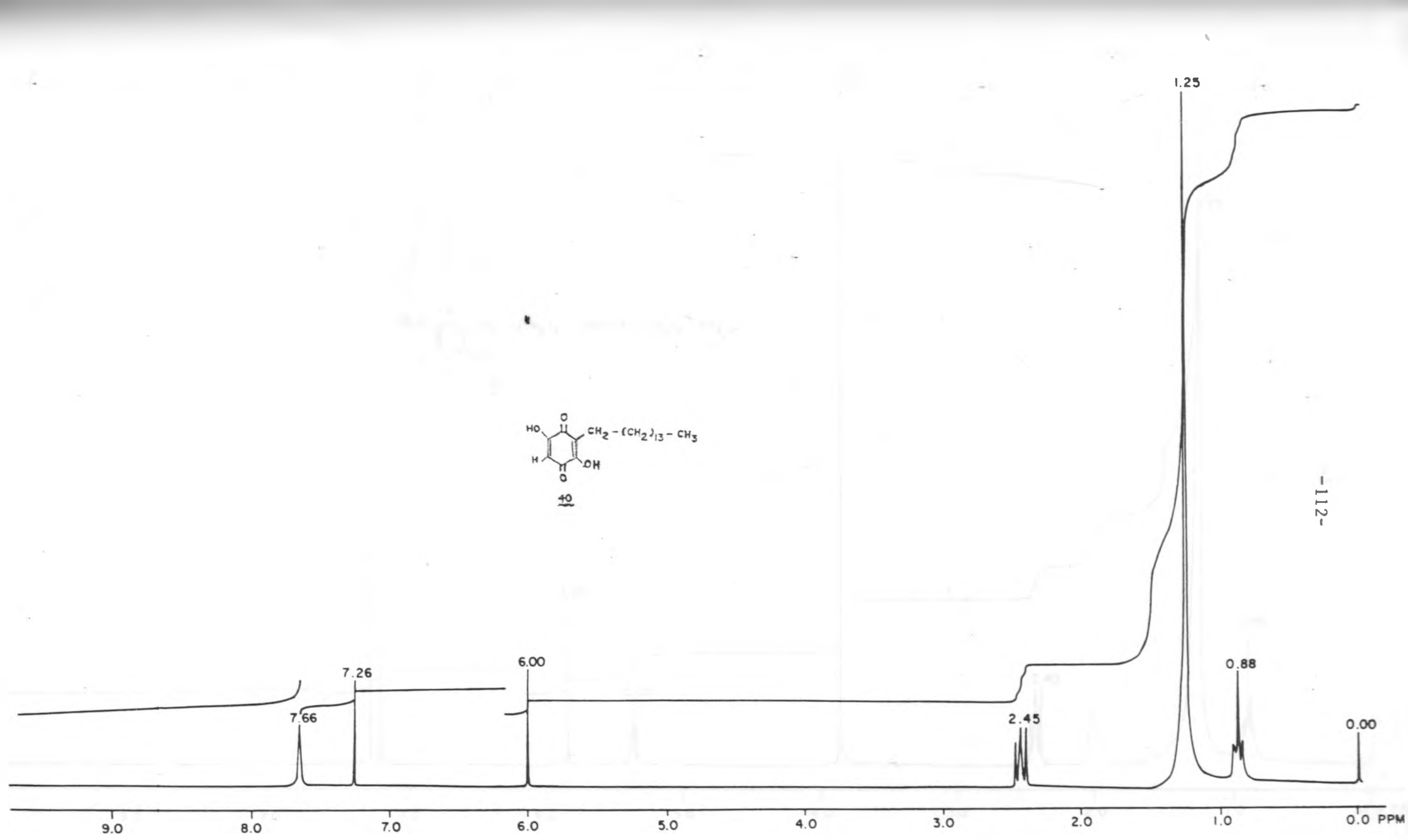


30

EMBELIN TETRAACETATE



MAESAQUINONE



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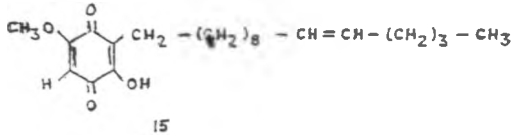
2, 5 - DIHYDROXY - 3 - PENTADECYL BENZOQUINONE



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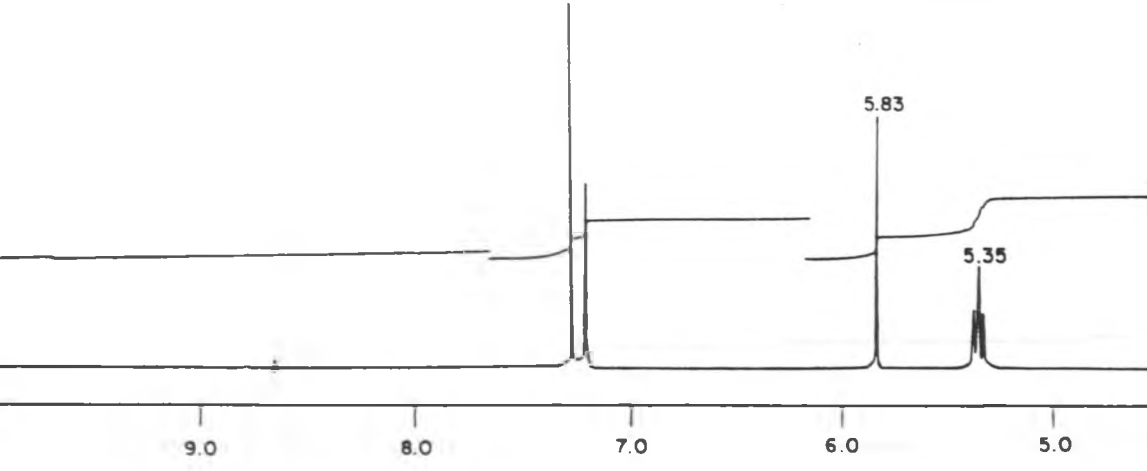
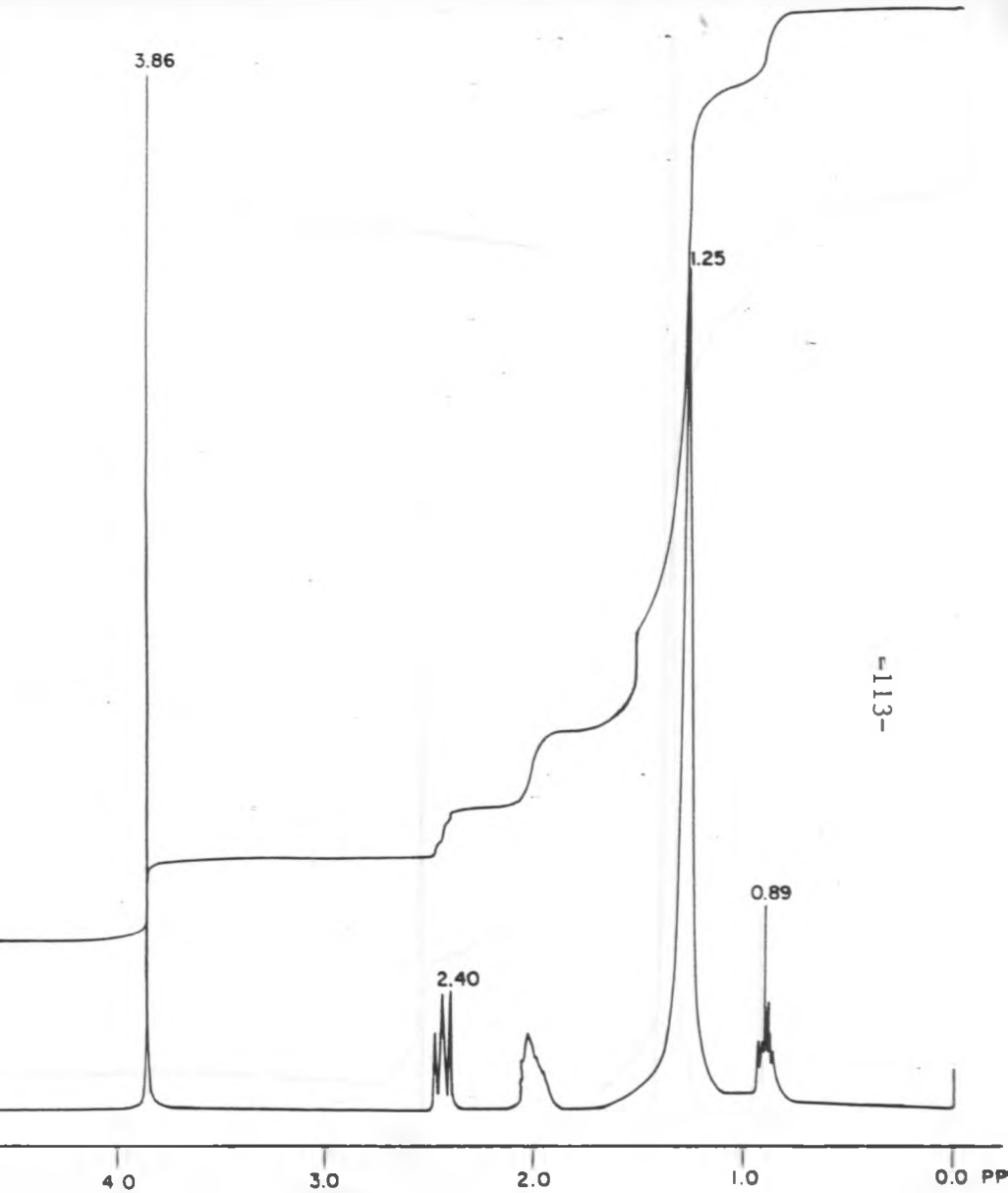


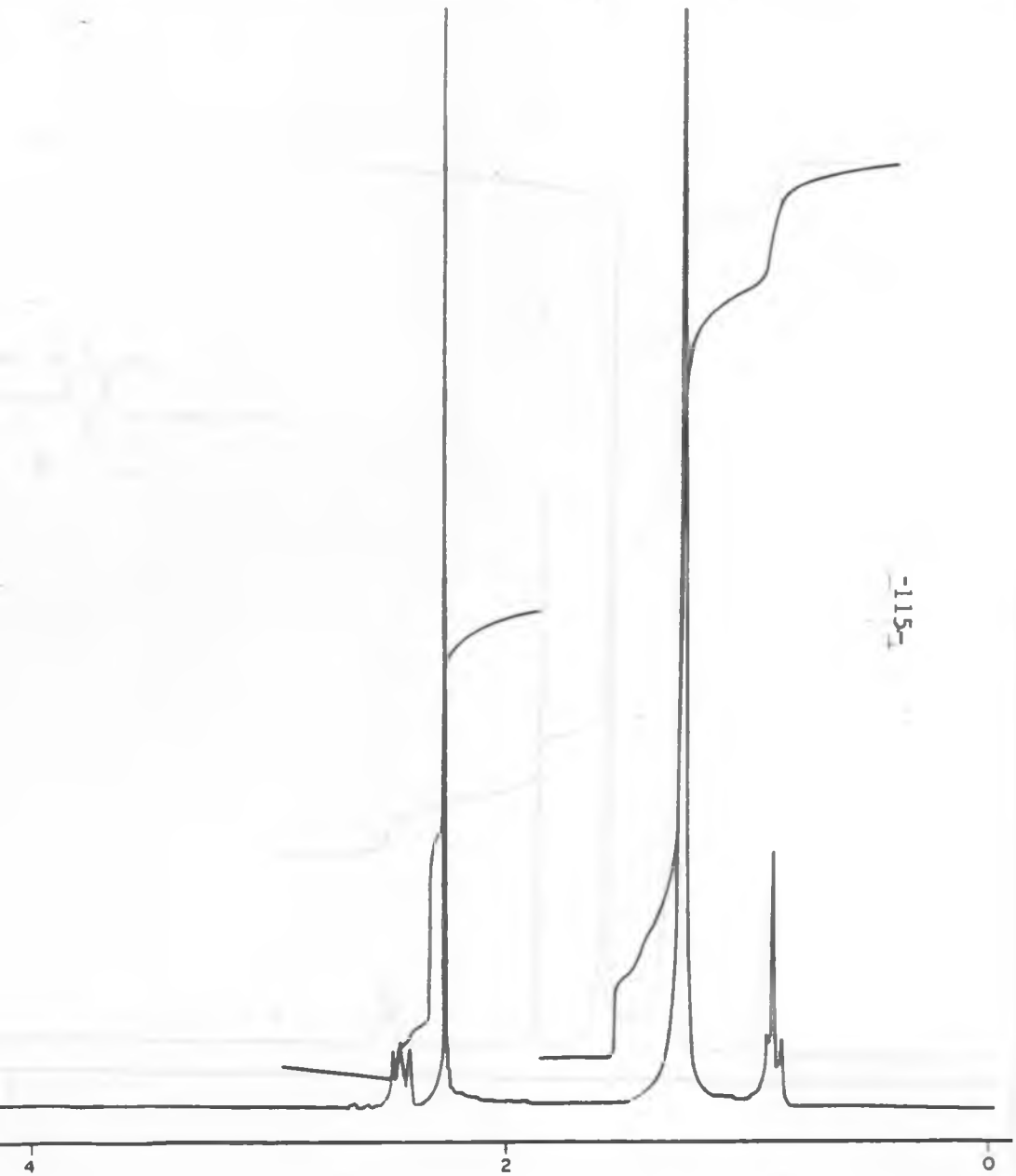
MAESANIN


3.86
1.25
0.89
2.40
4.0
3.0
2.0
1.0
0.0 PP

COMPOUND RF 0.80 TETRAACETATE

33

RAPANONE TETRAACETATE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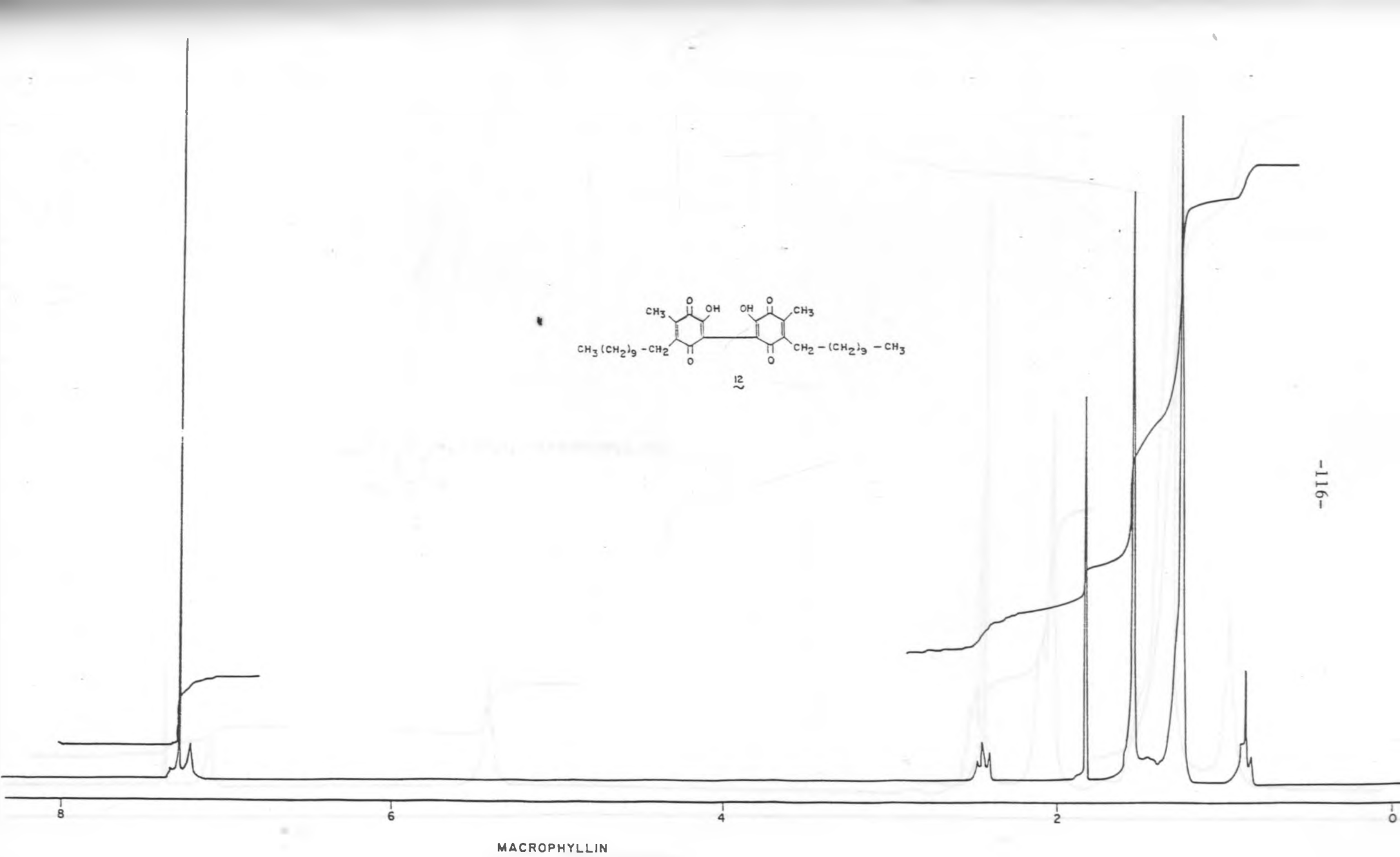
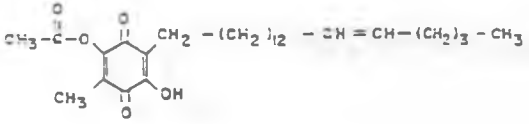
2
0



12

MACROPHYLLIN



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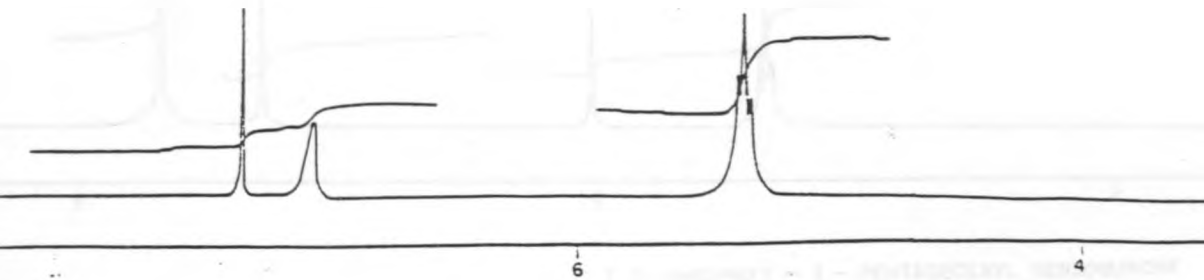
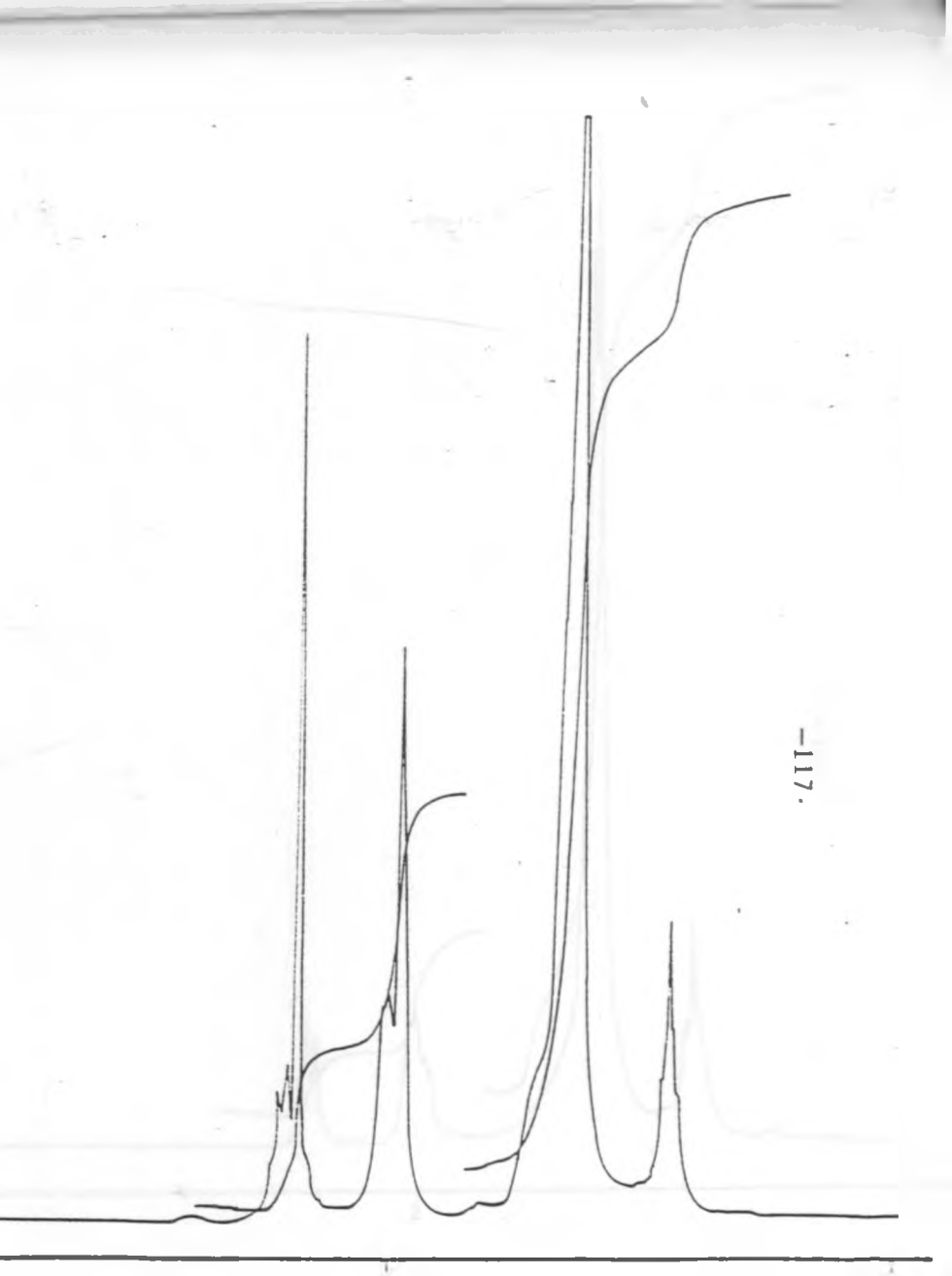


ACETYL maesaquinone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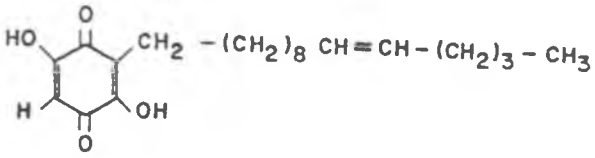
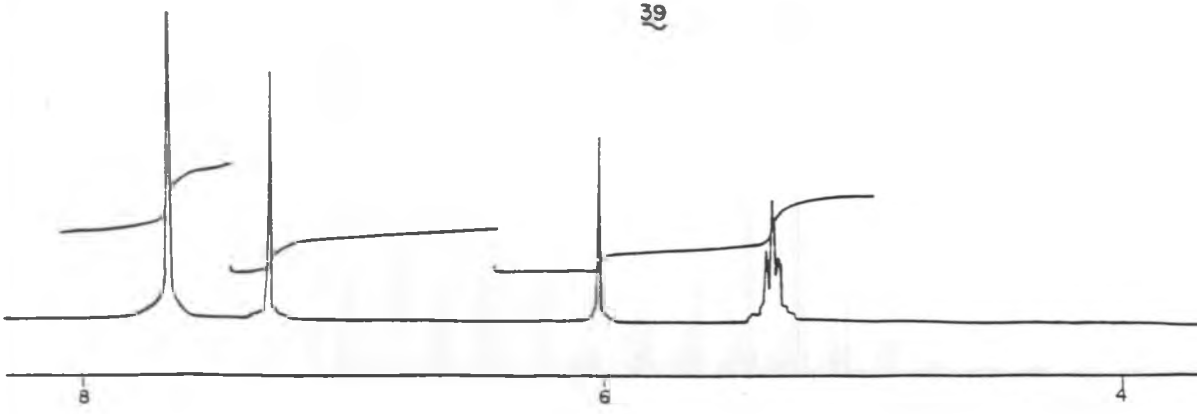
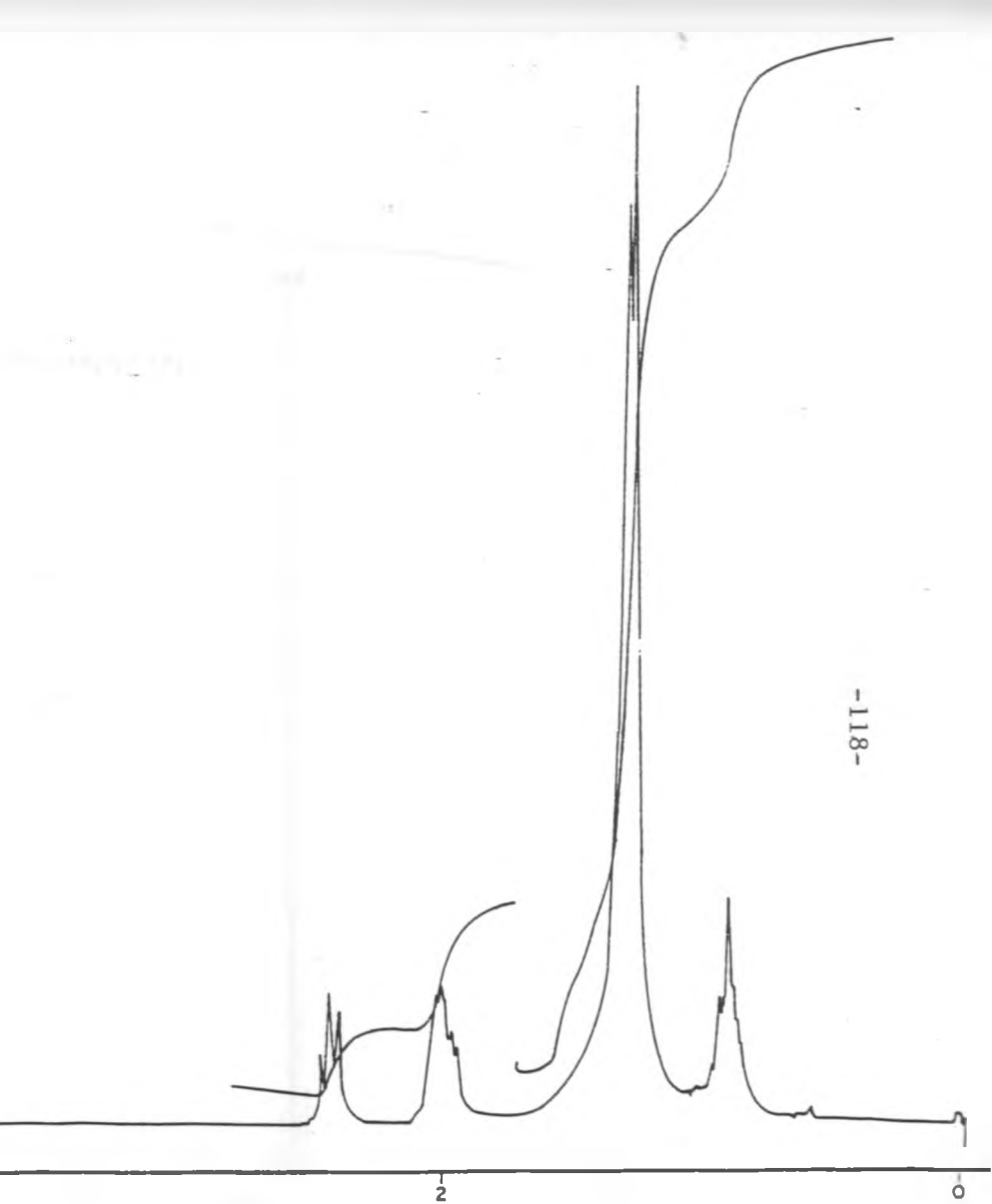
0



39



2, 5 DIHYDROXY – 3 – PENTADECENYL BENZOQUINONE


2
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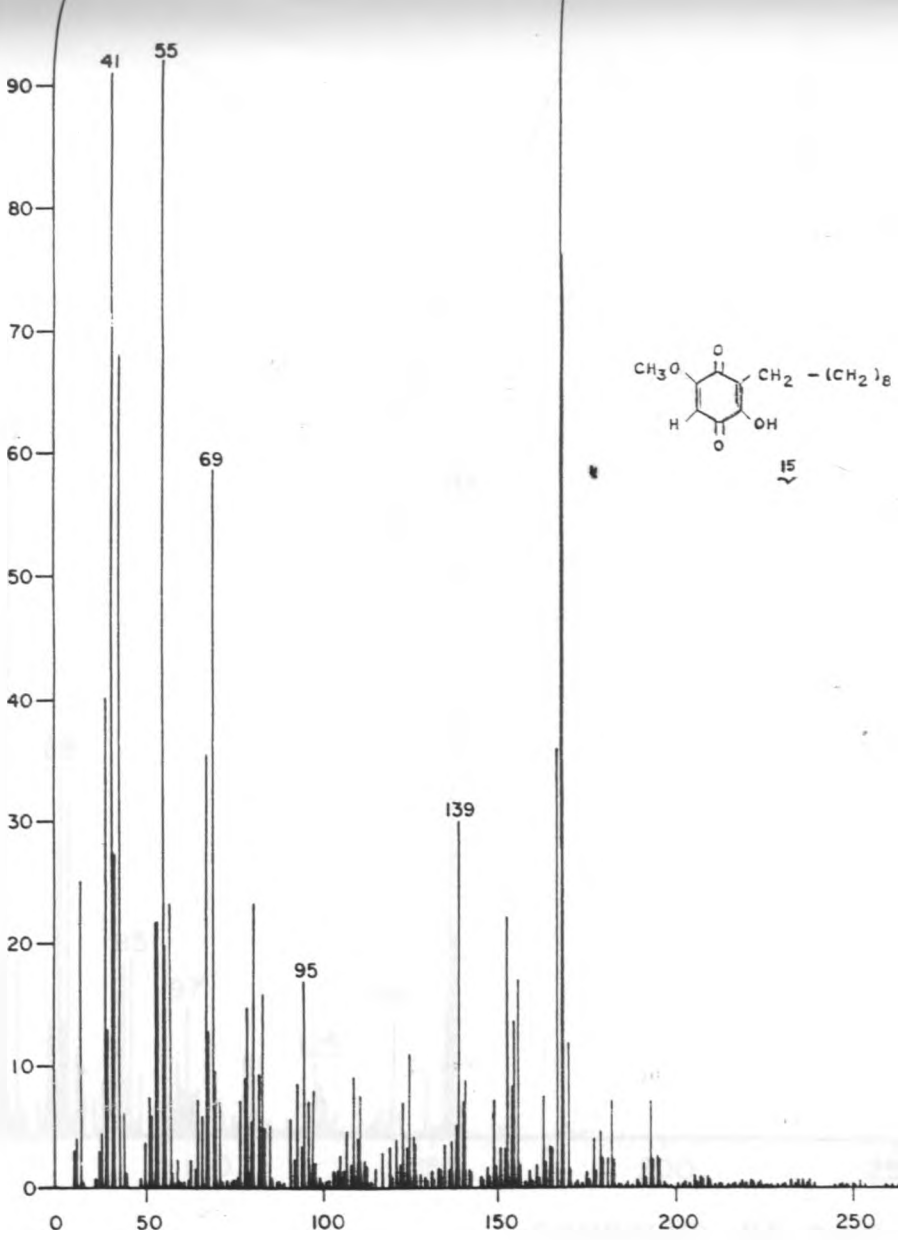
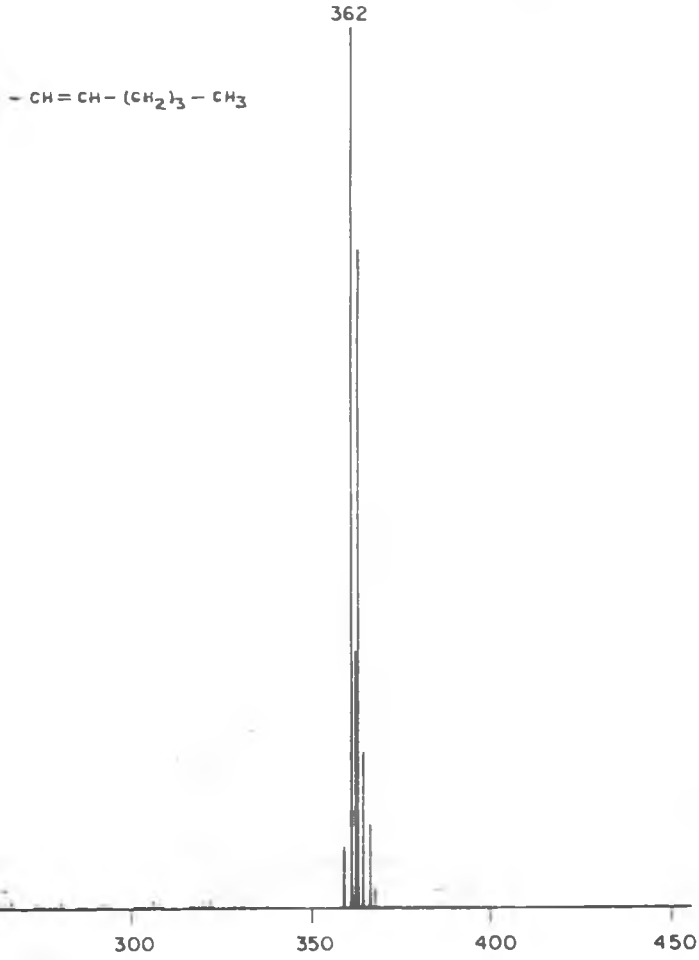
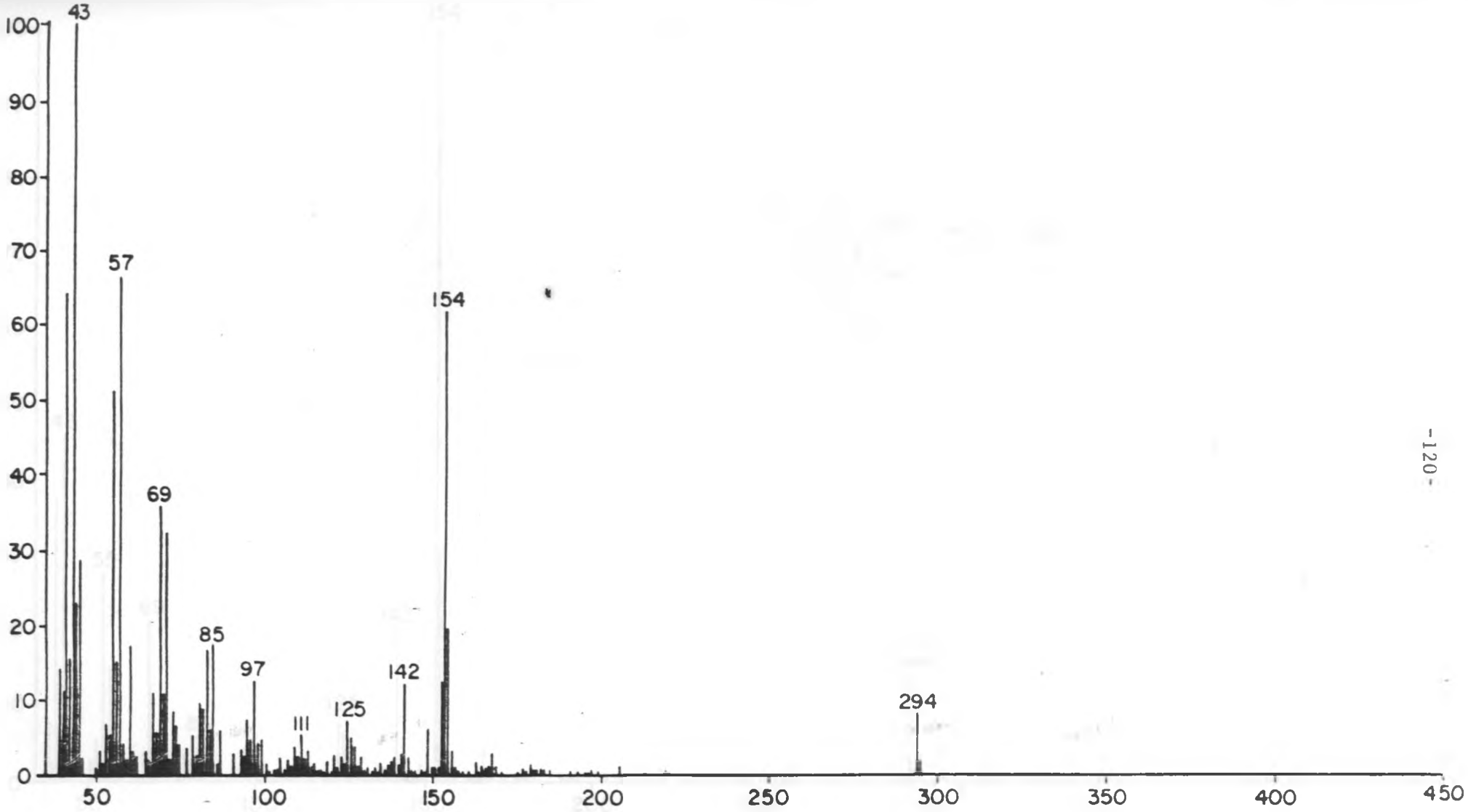
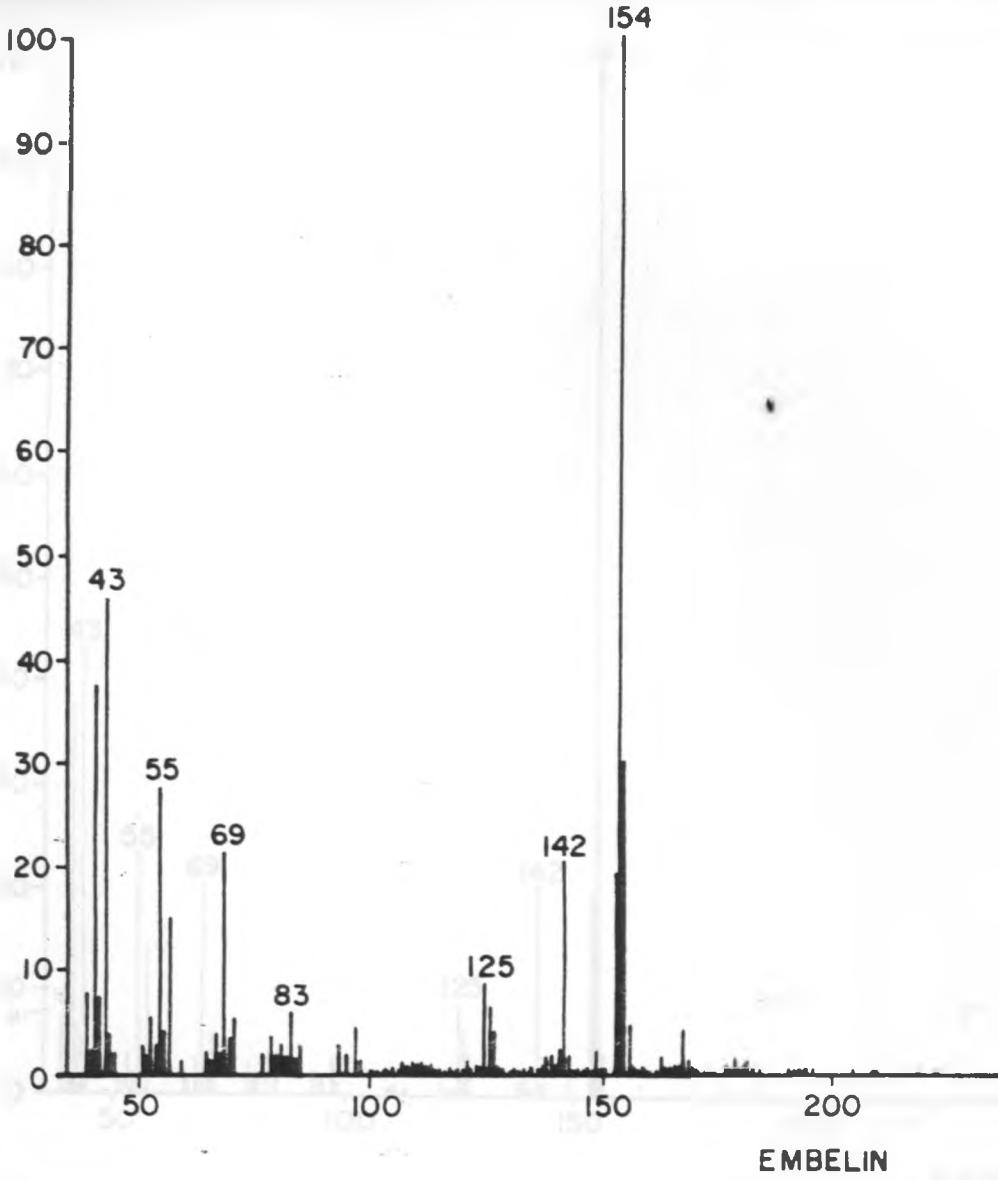

41
55
69
95
139
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
CH3O
CH2 – (CH2)8
H
OH
O
15

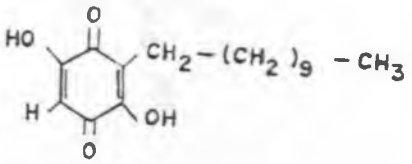
MAESANIN


362
– CH = CH – (CH2)3 – CH3
300
350
400
450



COMPOUND RF 0.80 35


100
90
80
70
60
50
40
30
20
10
0
154
43
55
69
83
125
142
50
100
150
200
EMBELIN



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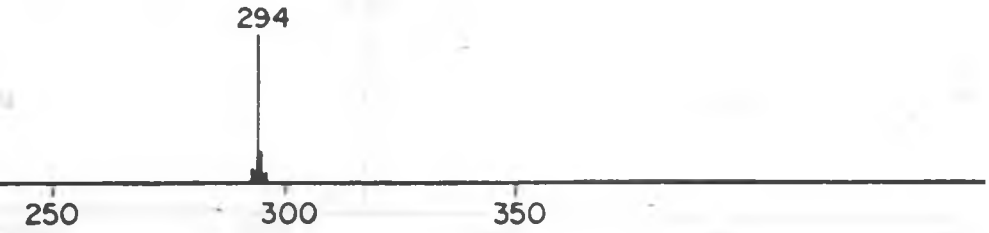

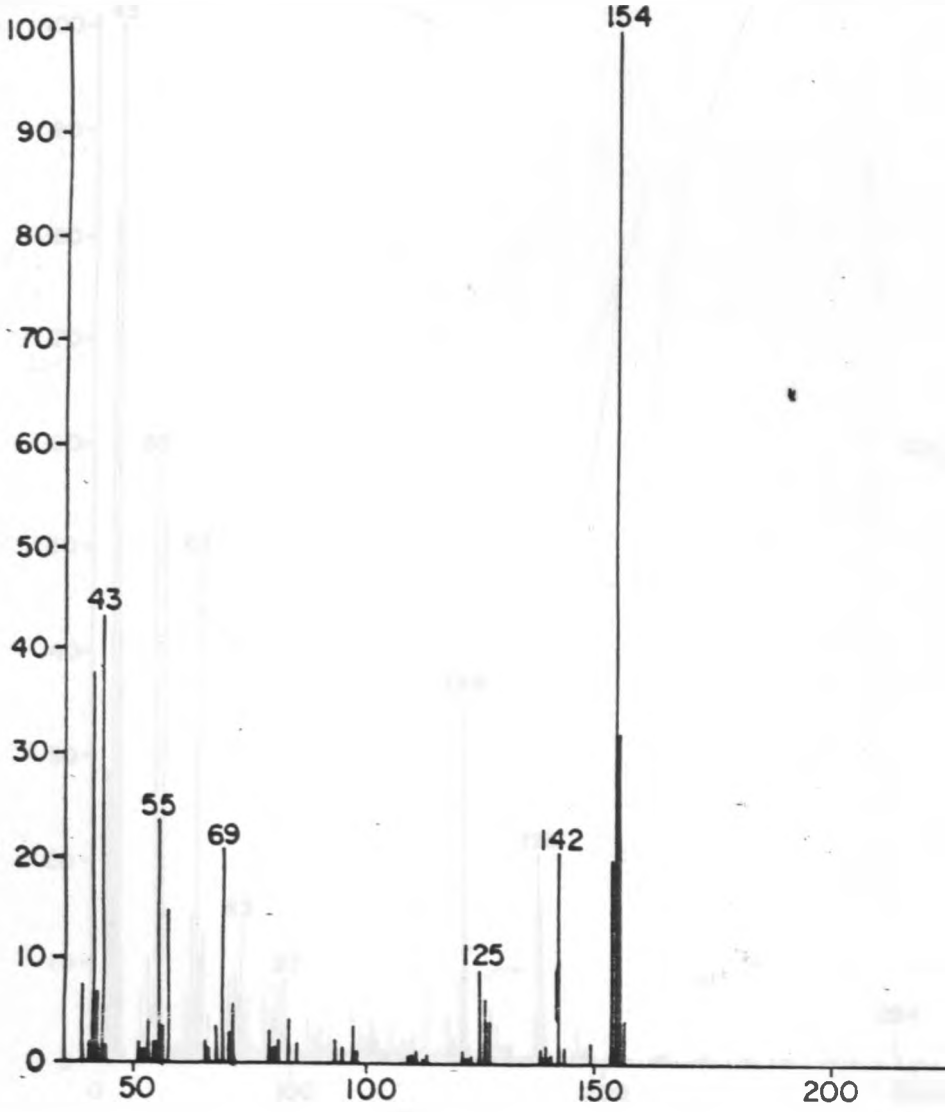
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43
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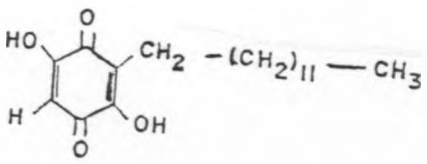
142
125



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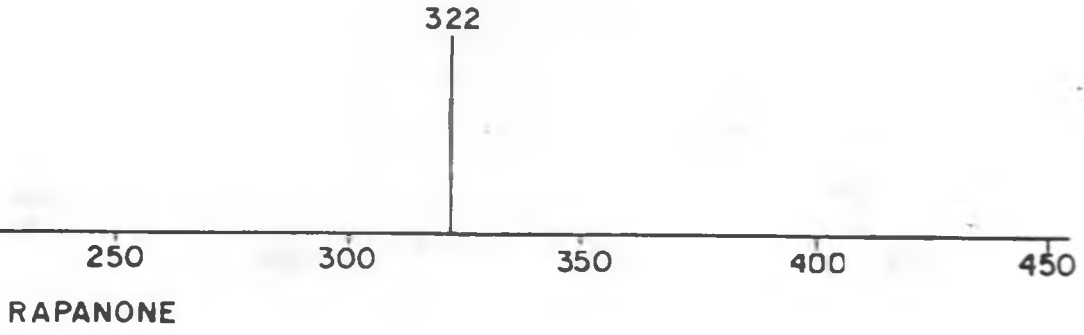
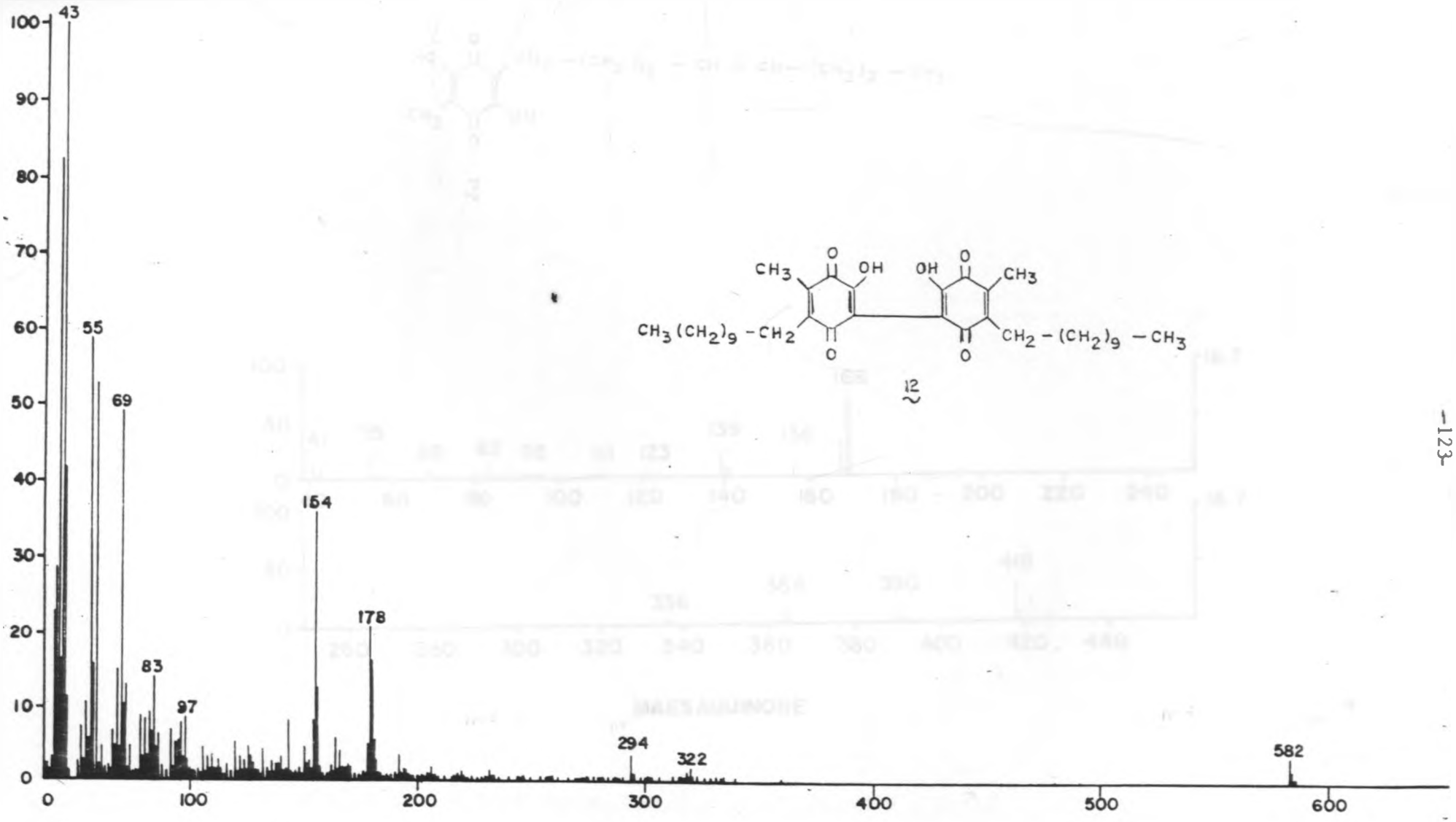
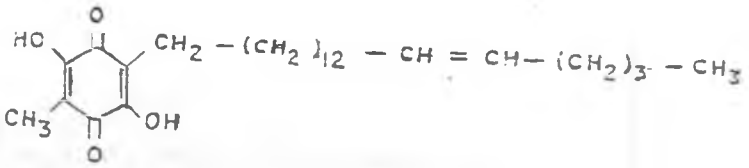


RAPANONE



12

MACROPHYLLIN



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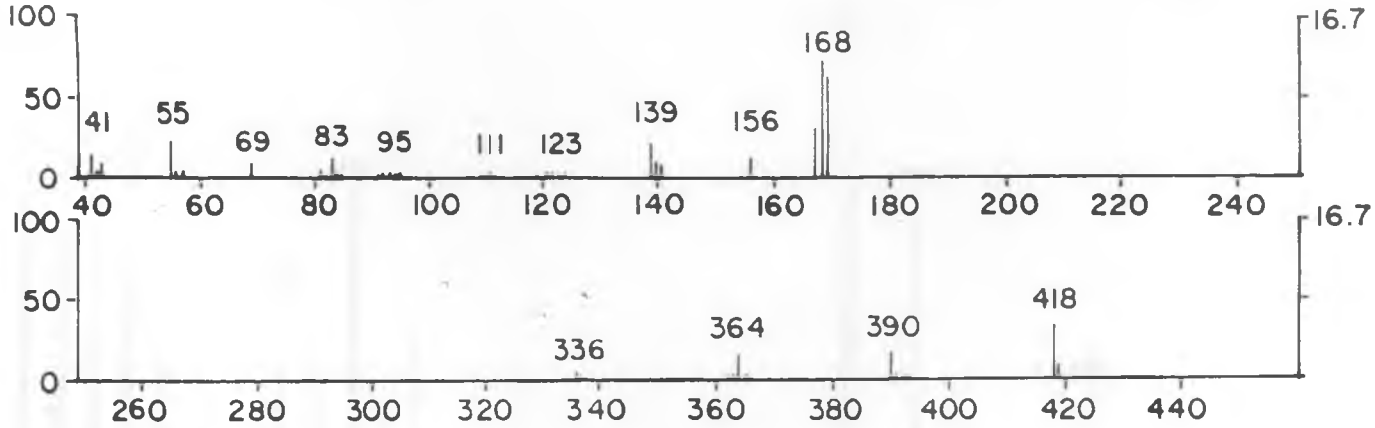
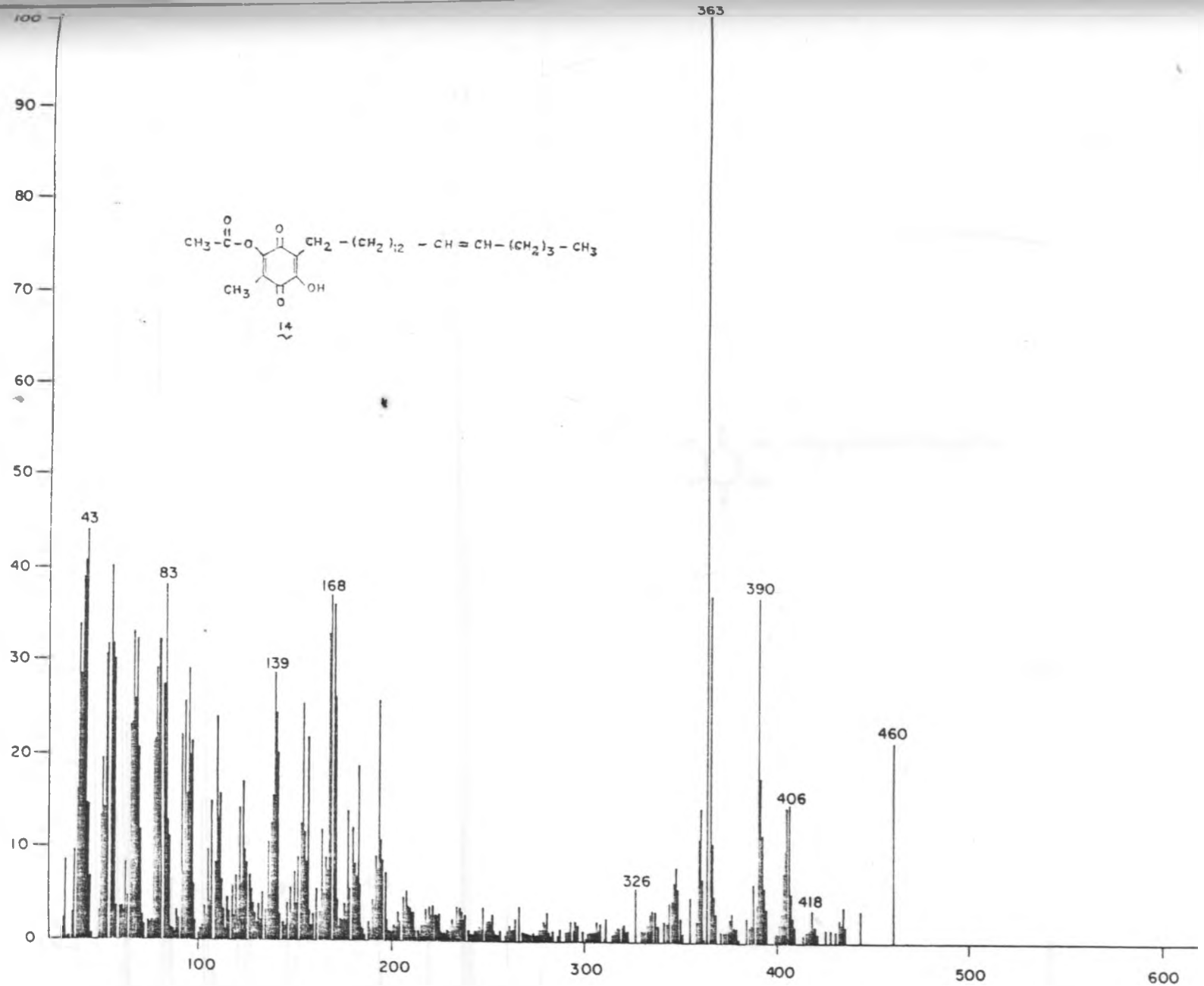
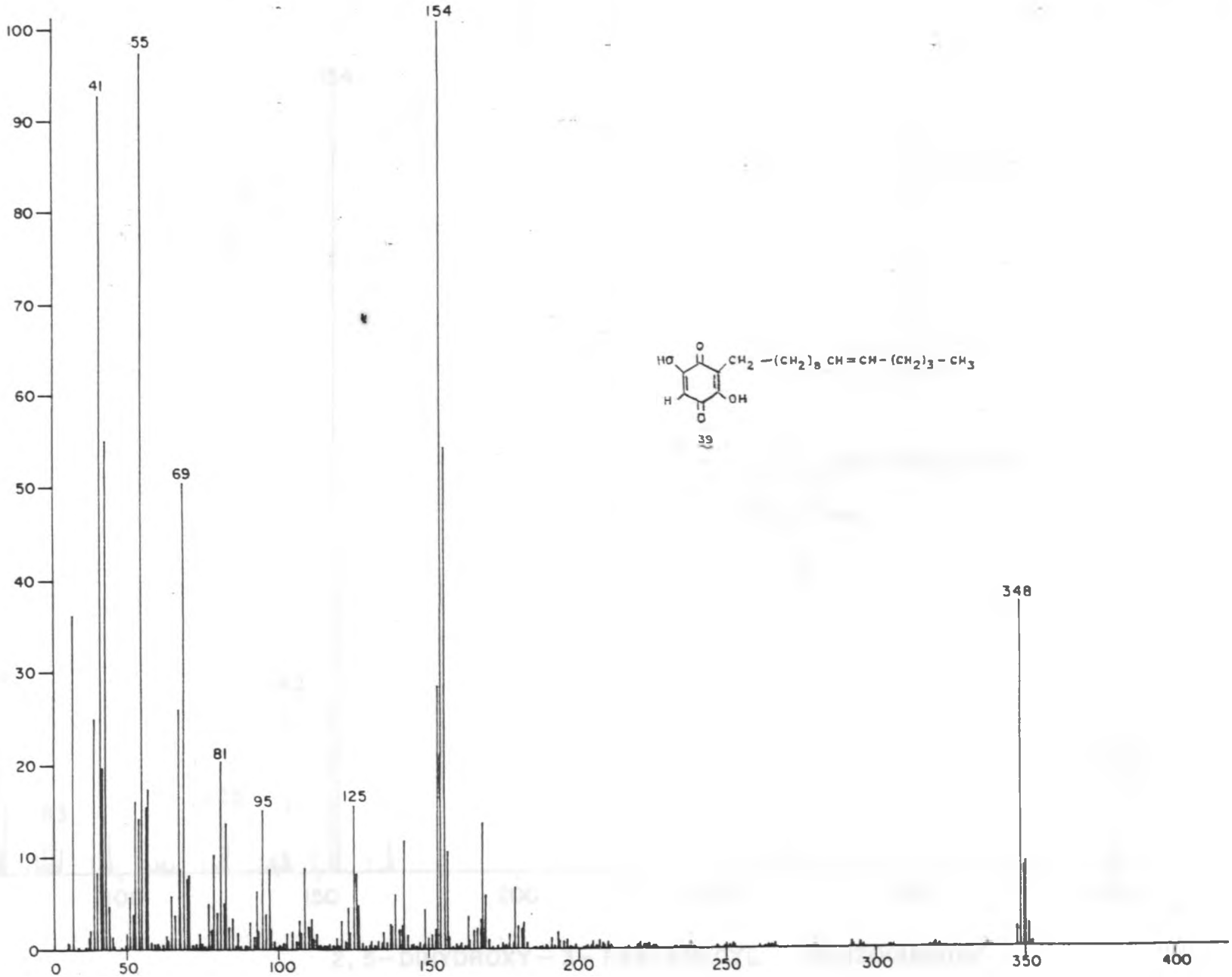


MAESAQUINONE



ACETYLMAESAQUINONE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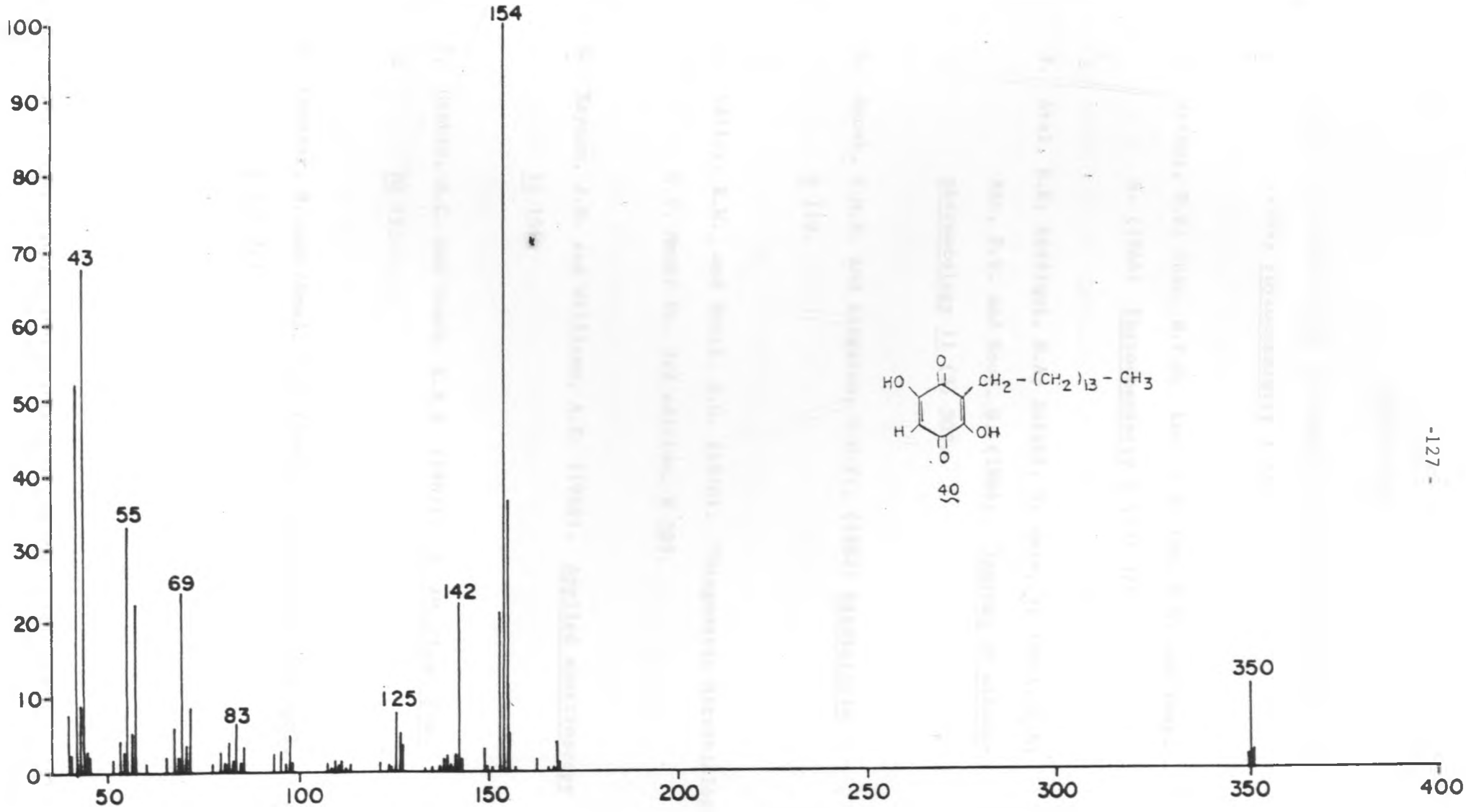
41
55
69
81
95
125
154
348

39



2, 5– DIHYDROXY — 3– PENTADECYL BENZOQUINONE

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