CHEMISTRY AND SOME BIOLOGICAL ACTIVITIES OF BENZOQUINONES OF *EMBELIA SCHIMPERI*.

BY

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A thesis submitted in partial fulfilment for the Degree of Master of Science (Chemistry) of the University of Nairobi.

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

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THIS THESIS IS DEDICATED TO MY BELOVED WIFE, LINNER AND OUR BELOVED DAUGHTERS, CHEBET AND CHEPNG'ETICH.

ACKNOWLEDGEMENT.

I wish to express my sincere gratitude to my university supervisors, Prof J.O. Midiwo and Prof Peter Gitu for their keen and continued interest, guidance and inspiration throughout the course of investigation and compilation of this work.

I also wish to extend my sincere thanks to my wife Linner Chepkwony, my mother Sarah Rugutt, my father Christopher Rugutt, my uncle Simon Rugutt, my cousin Josephine C. Salatt, Mr. John Koech and all my friends who throughout my course as a self-sponsored student, encouraged me morally and supported me financially.

I wish to thank Prof. Berhanu Abegaz of university of Botswana and Mr. Wycliffe Wanyama of ICIPE for generating NMR (^{1}H and ^{13}C) and mass spectral data respectively. I also wish to thank Mr. Wanzala for performing anti-feedant tests.

Lastly, I wish to thank the Department of Chemistry and the University of Nairobi at large for creating a conducive academic environment throughout my stay at the University.

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ABSTRACT

Embelia schimperi and Embelia keniensis are among the five medicinal Myrsinaceae plants found in Kenya which find a wide range of application in ethnopharmacology as anti-microbials and anthelmintics. The berries and root bark of E. schimperi were extracted with cold ethyl acetate and found to contain mostly benzoquinones. The level of benzoquinones was found to be higher in the berries relative to root bark. Chromatographic separation of the bioassay selected fractions on oxalic acid impregnated silica gel (soaking in 3% oxalic acid in methanol) using various solvent systems for E. schimperi led to the isolation and identification of embelin (10), methylvilangin (21), myrsinaquinone (56), decylvilangin (76) and decylanhydrovilangin (77), all of which are benzoquinones. Embelia keniensis was only screened (with aid of TLC analysis) for the presence of the benzoquinone pigments and found to contain trace amounts of embelin (10). All these structures were established using physical and spectroscopic (UV, MS, ^{1}H, ^{13}C and 2-D NMR) data. Two compounds were not characterized.

Biological activity tests such as insect anti-feedant, brine shrimp lethality, larvicidal and anti-microbial were carried out with the pure compounds.

Insect anti-feedant tests were determined with Locusta migratoria using a concentration of 100 μg/ml of each sample which was applied on sucrose treated Whatman No.1 filter paper. In the control experiments, the filter papers had sucrose only. Embelin (10) and 21 were found to have relative anti-feedant percentage (RAP) of 96% and 33% respectively.

The larvicidal tests were done on mosquito larvae, Aedes aegypti. Compound 21 was the only one tested for this activity and was found to be a growth retardant.

Brine shrimp lethality test was done for 21, 76, KCP-02 and KCP-06. The LC_{50} values were found to be 120, 51, 54 and 0 μg/ml respectively.

Stored products pests test was performed using Sitophilus zeamais (maize weevil) and Anthoscelides obtectus (bean weevil) for embelin (10) and it was found that the total number of emerged progeny and reproductibility of the two insects were reduced significantly at all concentrations.

Anti-microbial activity test was performed using Candida albicans, Trichophyton metagrophyte, Microsporium gypsum and Escherichia coli for 10, 21, 56, 76, 77, KCP-02 and KCP-06 and it was found that all the compounds had less than significant activity against the micro-organisms.

CHAPTER ONE.

1.0.0. INTRODUCTION.

In the tropical third world, crop losses either in the field or in storage are tremendous and measures must be taken to effectively protect field and harvested crop. In Kenya it is estimated that 30 % of the crops are lost due to the pest attack (KARI, 1991). Such pests include insects, fungi, bacteria, nematodes and grazing higher animals. For this reason, integrated pest management is desirable. Among the methods used include introduction of natural predators (biological control), pesticides use, crop rotation and introduction of pest resistant varieties.

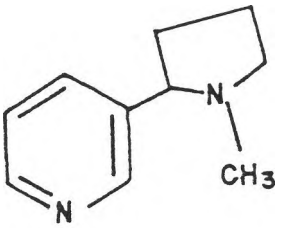
A survey conducted in 1989 indicated that the usage of pesticides based on their cash value is concentrated in the developed world with US accounting for 24%, Western Europe 24%, Far east 9%, Eastern Europe and the then USSR 8% while the whole of Africa and the rest of the third world was only 7% (Cremlyn, 1991). This is an unfortunate situation since these latter countries contain 49% of the world's population and therefore need the greatest protection of crops. On a world scale, pests destroy about half the annual crop but in Africa and most other parts of the third world these losses may be up to 70% of everything produced; despite the need to accelerate food production to cater for the ever increasing population in these countries (Chander and Ahmed.1983).

In Kenya, pesticide cost in 1989 was approximately 42 million Kenya pounds (KARI, 1989). This represented quite a big foreign exchange allocation yet overall this was still not adequate. Nearly all the conventional pesticides for the control of field crops and their respective stored products are manufactured in the developed world. These pesticides are therefore expensive. For this reason the use of pesticides in Kenya and other third world countries is still low. In view of this, self-reliance in agro-chemical development and production is the only way out for Kenya and other third world countries.

Furthermore, petrochemical based pesticides are potential health hazards due to toxic residues. The main petrochemical based pesticides include organochlorides, organoarsenicals, dinitrophenols, organic thiocyanates,

organophosphorus and carbamate compounds. The organo-phosphates and carbamate compounds, even though devoid of persistence, are highly toxic and need to be applied under strict supervision by trained manpower, an aspect which is lacking in the third world.

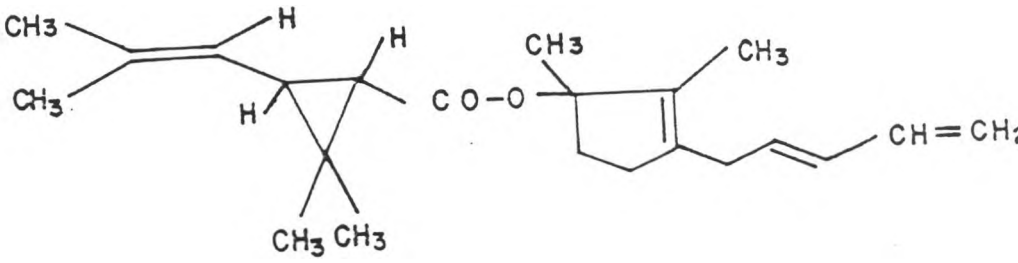
Over the years man has used a large number of plants as insecticides. These plants contain natural deterrent principles which have and can still be utilized to develop environmentally friendly pesticides. Among the botanical insecticides still in use today include pyrethrum, avermectins, derris (rotenone) and nicotine. Nicotine (1), an active principle in tobacco, is an alkaloid which exists naturally as a salt of citric and malic acids (Fuchs and Shroeder, 1983). It is only the levorotatory natural nicotine that is an insecticidal principle. Although this insecticide which is used against aphids, capsids, leaf minor, codling moth and thrips is non persistent contact insecticide, it has high mammalian toxicity and therefore its use is declining.



(1)

Pyrethrin is a contact insecticide obtained from the flower heads of Chrysanthemum cinerariafolium. Pyrethrum is safe for household use because it has low mammalian toxicity and has no persistence in addition to the tremendous knockdown characteristics on flying insects (Woods, 1974). Pyrethrins are the active principles in pyrethrum and are four in number (2 - 5).

Pyrethrin I



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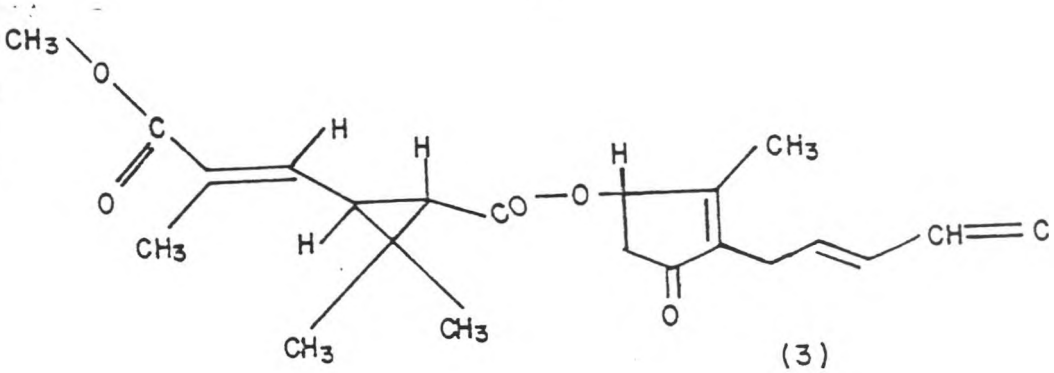
Pyrethrin II



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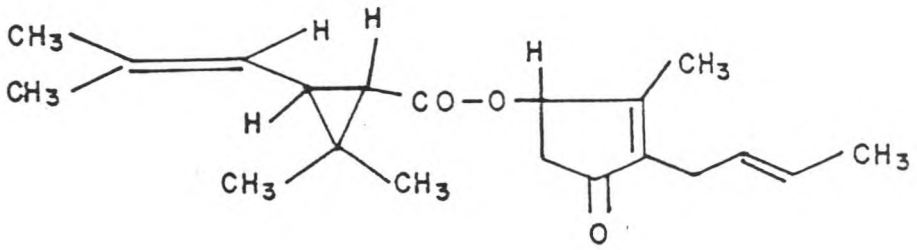
Cinerin I



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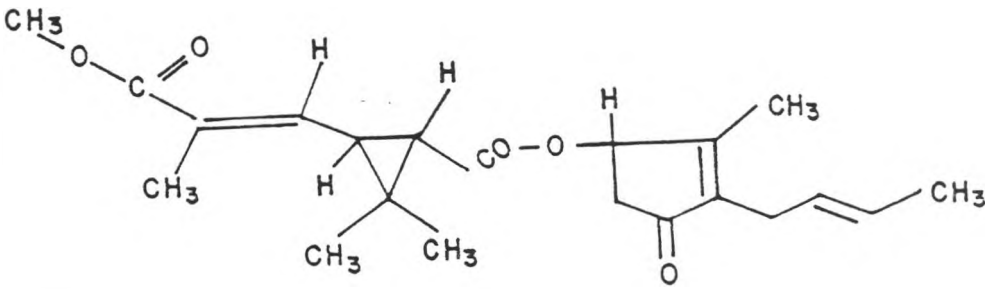
Cinerin II



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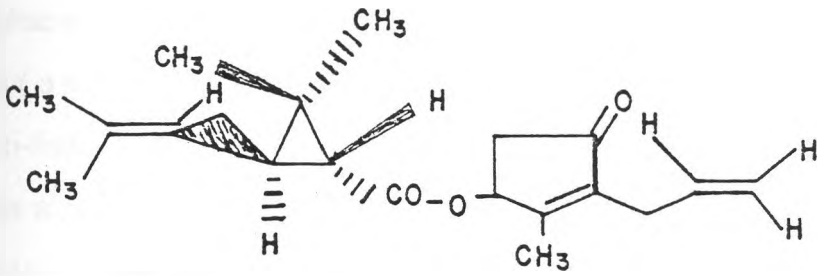
Natural models of pyrethroids have been used to develop synthetic ones. Allethrin (6) and decamethrin (7) which have strong insecticidal properties are among the best examples (Martin and Woodcock, 1983).



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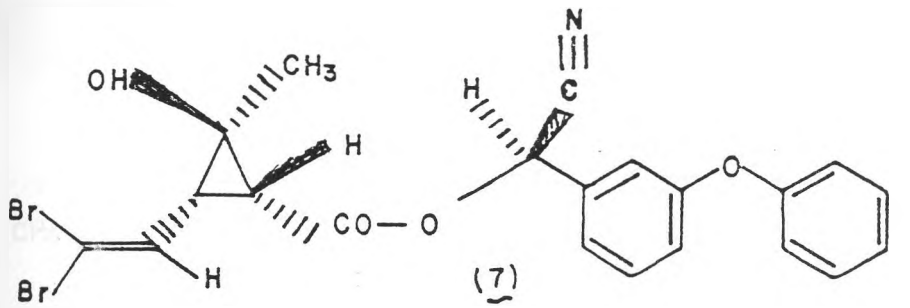


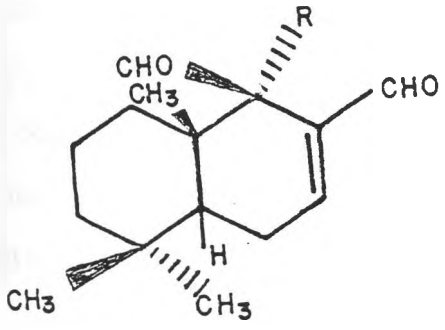
(7)

More plants with efficacious properties are being searched and identified. These properties are anti-feedant or feeding deterrents, repellents and pesticidal effects. Anti-feedants are ecologically appropriate since they only cut down on rather than eliminate insect population and therefore leaving useful non pest insects alone.

The Indian Neem tree. Azadirachta indica which grows in Kenya is a higher plant which is known to be resistant to many varieties of insects. Complex tetranor triterpenoids have been isolated from it (Ley, 1985). These include the

most active anti-feedant against lepidopterous species known, azadirachtin. Azadirachtin is a highly complex molecule and cannot economically be produced by synthetic process. Azadirachta indica produces enough quantities of this compound and its analogues and there are programs to propagate it in several parts of the world.

The Meliaceae triterpenoids are apparently intrinsically anti-feedant. Melia volkensii (a species indigenous to Kenya) and Melia azedarach have also proved to be anti-feedant towards Schistocerca gregaria (Mwangi, 1985). Less complex chemicals also show anti-feedant activity. (-) - Polygodial (8) from Polygonum hydropiper and warbuganal (9) from Warbugia ugandensis are typical simple azadirachtin mimicking substances; they are effective as anti-aphids and anti-African army worm.

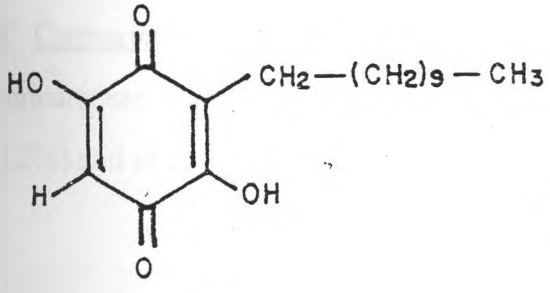


(8) R = H

(9) R = OH

1.0.1. LITERATURE REVIEW

The Myrsinaceae plants are distributed all over the world. The family has received extensive chemical investigations since the beginning of the century. It has been pointed out that the presence of alkylated hydroxybenzoquinone derivatives and a number of triterpenoid based on oleanane/ursen skeleton are a chemotaxonomical characteristics of this family (Ogawa and Natori 1968). Embelin (10), rapanone (11), maesaquinone (12) and bisbenzoquinone, vilangin (13) are among the long chain alkyl-1,4-benzoquinones isolated from some Myrsinaceae species occurring in Japan (Ogawa and Natori, 1968). A chemical study of Embelia ribes was done by Paranjpe and Gokhale (1932). They isolated embelin (10) as the major constituent. Four years later, Krishna and Varma (1936) reported the isolation of the same compound, embelin, from petroleum ether and chloroform extracts of Myrsine africana berries. In a separate study, Kawamura and Hokoku (1937) isolated an orange crystalline substance from the bark and woody portions of Rapanea maximowiczii (Koidz) and named it rapanone (11). Earlier, Stather (1931) screened Myrsinaceae plants of Rapanea and Myrsine genera for their tannin compositions. It was discovered that in the stem bark of Rapanea and /or Myrsine, the percentage of tannin was 8-9%.



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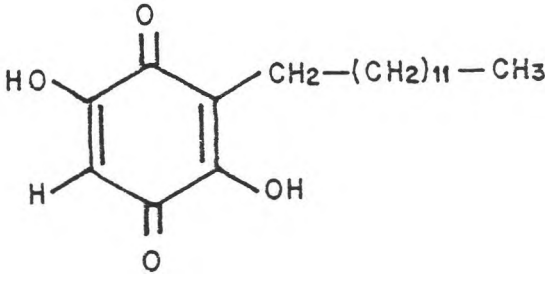

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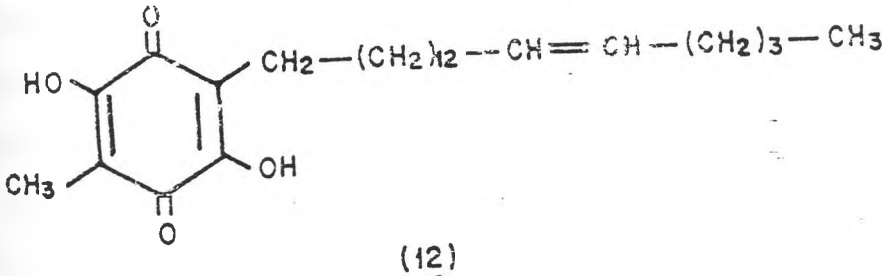

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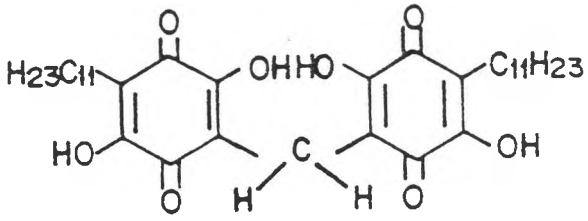

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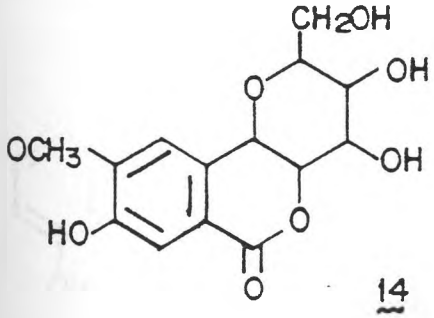
A chemical investigation of Maesa japonica (Moritzii), a plant found in Japan revealed the presence of maesaquinone (12) up to 1.5 % yield (Harimoto, 1939). Similarly from Myrsine africana seeds thought to be originating from British Somaliland (present day Djibouti) but probably originating from Abyssinia (present day Ethiopia) was found to contain 4.8% of embelin. Merian and Schlittler (1948) also reported the isolation of rapanone from Rapanea neurophylla. E. kilimandscharica and M. africana. In a separate report rapanone(11) together with bergenin (14) and leucopelargonidin (15) have been isolated from the roots of Connarus monocarpus, an ever-green shrub belonging to the family Connaraceae. The roots were found to constitute a very good source of rapanone (1.2%) and of bergenin (1.5%).



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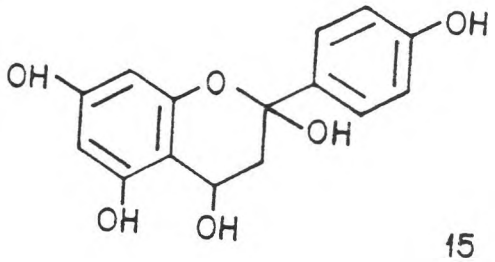

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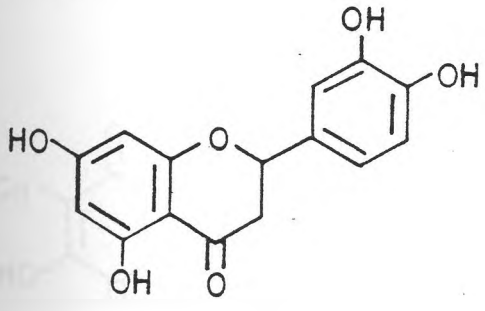
Paris and Rabenoro (1950) carried out a phytochemical evaluation of two Madagascar Myrsinaceae plants namely; E. berbevana and Maesa emirnensis. Their study revealed the presence of sterols, traces of essences, a bitter principle, tannins, saponins and quinones. The fruits were found to contain up to 2% (w/w) embelin. A similar study performed using various parts of R. pulchra showed the existence of embelin up to 0.39% (w/w) in the berries, rapanone 2.8% (w/w) in the root and bark while the stem bark was composed of 1.2% embelin (Wilkinson, 1961).

In India, Embelia ribes has been regarded as one of the most important crude drugs of indigenous medicine since ancient times. The berries of this plant known as "Vidanga" are recommended for relieving headache, rhinitis, hemicrania, epilepsy and insomnia. As part of the study on the chemistry of this plant. a new benzoquinone pigment vilangin (13) (a name taken from the vernacular Telugu name "Viyuvilanga") together with embelin have been isolated from the plant. The new compound constitution was confirmed by synthesis and structural modifications (Rao and Venkateswarlu, 1961). In another study, Cambie and Couch (1967) reported the isolation of vilangin and (+)-quercitol (8) from the flowers of Myrsine australis. a Myrsinaceae plant common to both northern and southern parts of New Zealand. Further chemical investigation of the same plant showed that embelin (10) and two leucoanthocyanins were present in the fruits while from the leaves quercetin (16), (17), rutin(18) and triterpenoid (19) were isolated.



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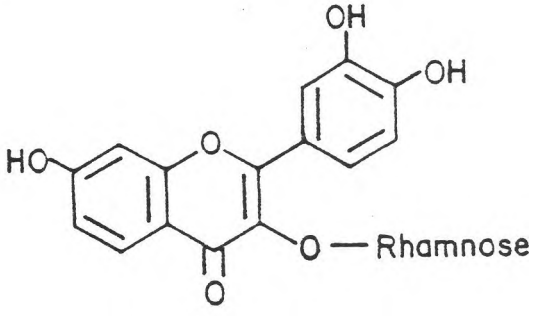

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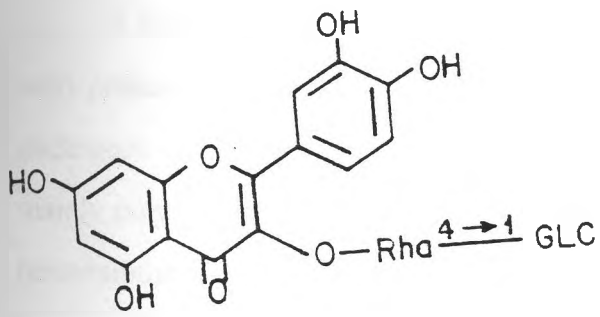

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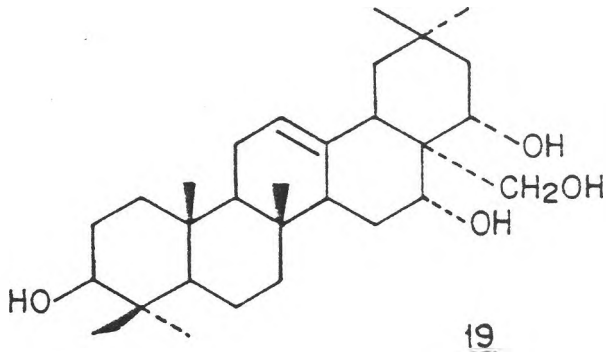

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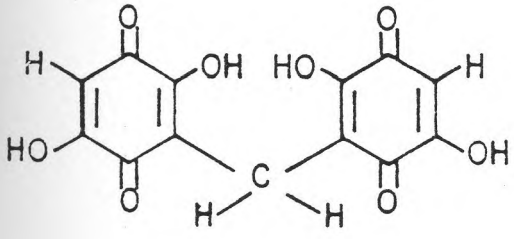
Rao and Venkateswarlu (1962) in a separate publication communicated the synthesis of vilangin by condensing 2,5-dihydroxy-1,4-benzoquinone with formaldehyde to give methylene-bis (2,5-dihydroxy-3,6- benzoquinone) (20) which was subsequently alkylated using dilauroyl peroxide in dioxane solution to give the vilangin. In addition to this, they also revealed that embelin undergoes condensation reactions with various aldehydes to give analogues of vilangin (21) and anhydrovilangin (22). They further noted that with acetic, propionic and benzaldehydes both products are obtained and only the anhydro (22) was achieved with the case of other aromatic aldehydes. In the case of salicylaldehyde, a product of type (23) was also produced.



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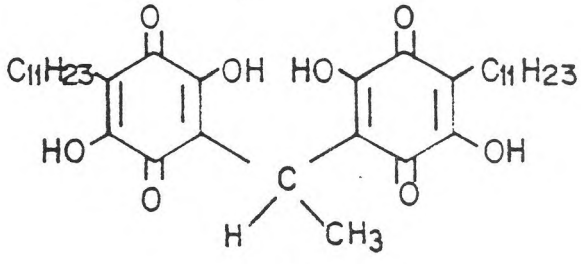

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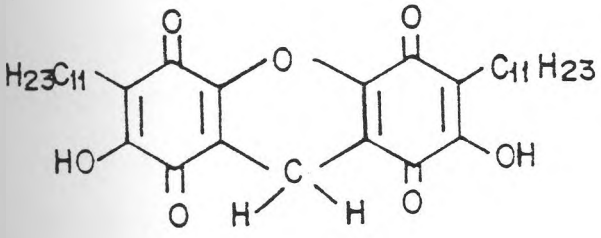

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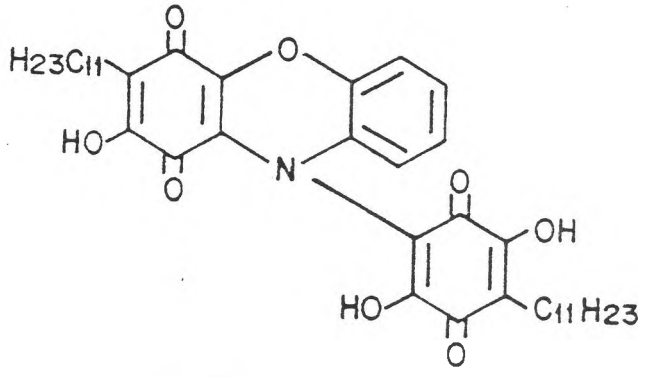

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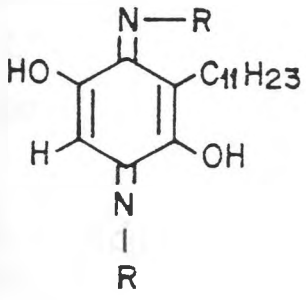
It has also been reported that embelin undergoes condensation reactions with primary amines forming the corresponding quinone di-imine (24) which undergoes decomposition in boiling water or with concentrated HCl, forming mainly polymeric products and affording only traces of embelin. In the case of benzylamine two products are obtained; the normal quinone di-imine (24) and 3-benzylamino (bis desoxy-bis(benzylimino)-embelin (25). Further condensation reactions of embelin (2 moles) with various nitroso compounds (one mole) to give corresponding N-bis (anhydrobenzoquinone) (26) was also communicated (Rao and Venkateswarlu, 1964). In the condensation with p-dimethylamino-m-nitrosobenzaldehyde, a more complicated reaction takes place with the formation of (27) (Jeney and Sohnai, 1955).



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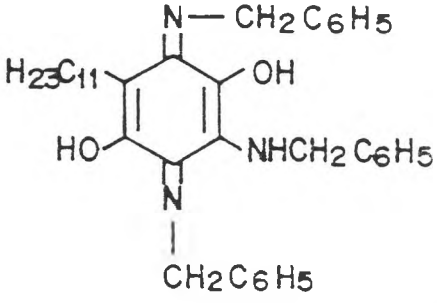

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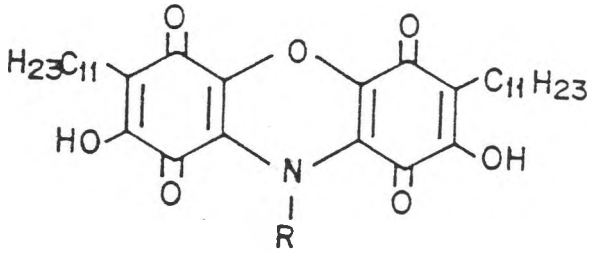

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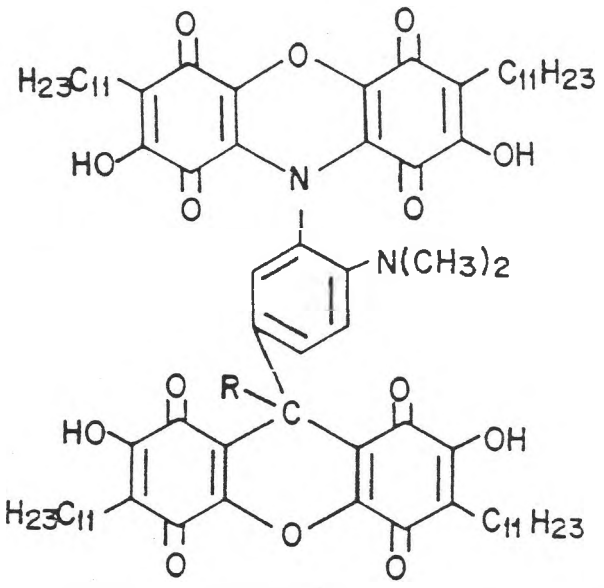
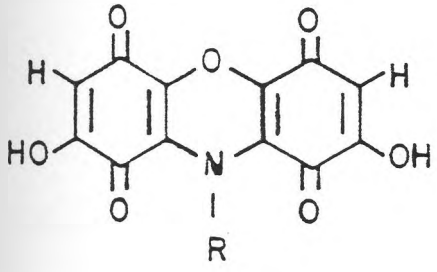


27

While continuing with their study, Rao and Venkateswarlu (1964) extended their work to 2,5-dihydroxy-1,4-benzoquinones which readily underwent condensation with nitroso compounds producing the corresponding N-bis (anhydro-2,5-dihydroxy-3,6-benzoquinone) (28). Similarly with p-dimethyl-m-nitrosobenzaldehyde compound (29) was readily formed.



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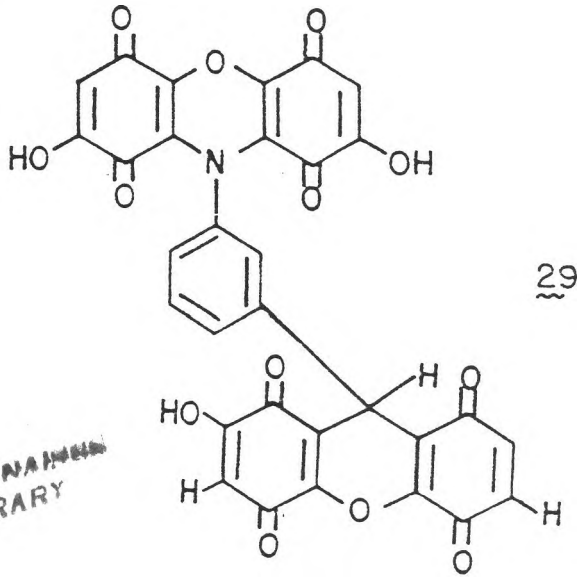
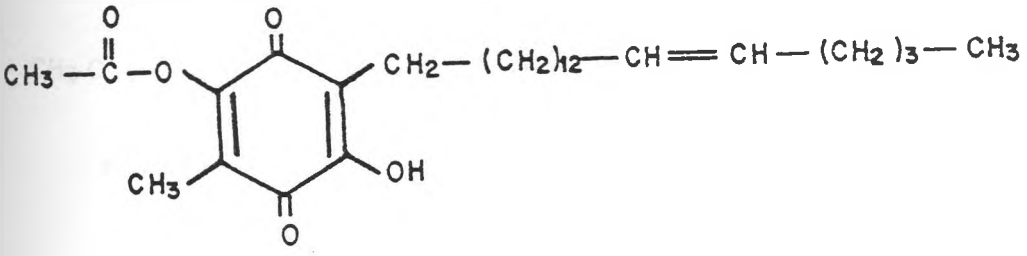


29

In Japan. the eleven species of Myrsinaceae plants were grouped into three genera namely, Ardisia, Myrsine and Maesa. They grow chiefly in Southern Japan (Ohwi, 1964). A discovery of the biological importance of benzoquinone derivatives , mostly associated with the family, (Morton, 1965) led Ozawa et al (1964) and Ogawa and Natori (1968) to work on the biochemical examinations of hydroxybenzoquinone derivatives and the screening of the distribution of these

secondary metabolites among the Japanese Myrsinaceae plants. As a result embelin (10), rapanone (11) maesaquinone (12) acetylmaesaquinone (30), 2-hydroxy-5-methoxy-3-pentadecenyl (tridecenyl, tridecyl)-benzoquinone (31) and bis(benzoquinonyl)-olefines (ardisaquinones) (32), (33) and (34) were isolated. The other methoxy derivatives 35,36,37 and the pigments 32,33, and 34 were found to be restricted to Ardisia species (Ogawa et al, 1968 a,b).



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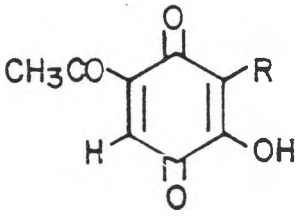
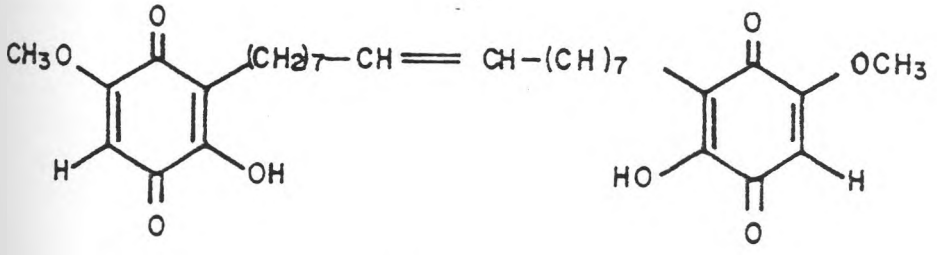


R : $C_{15}H_{29}$
$C_{13}H_{23}$
$C_{13}H_{27}$

31



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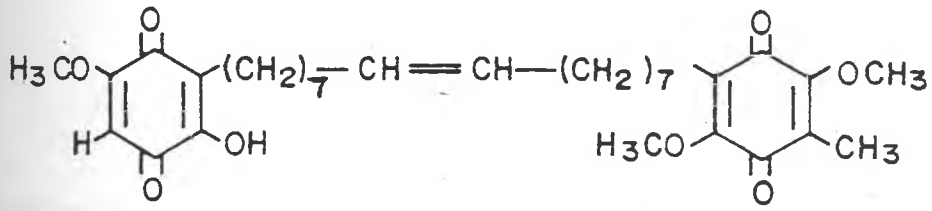

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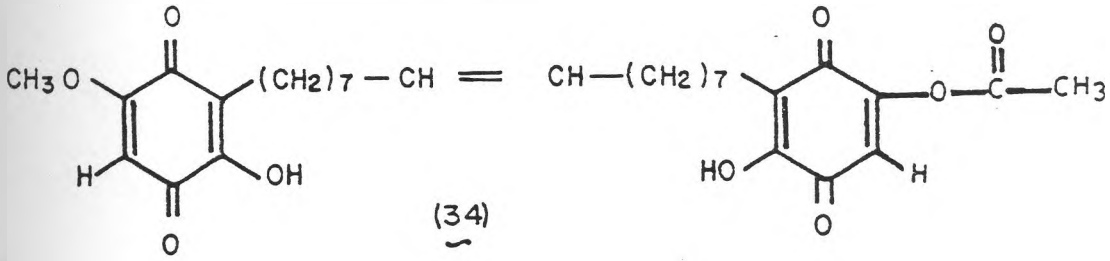

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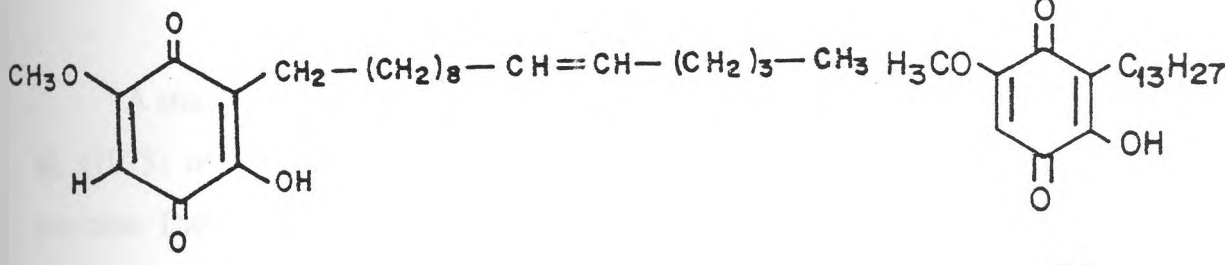

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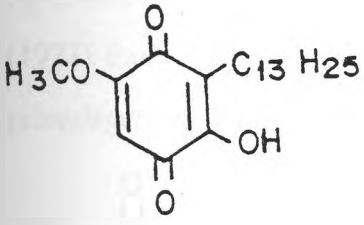

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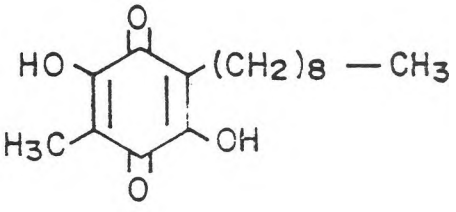
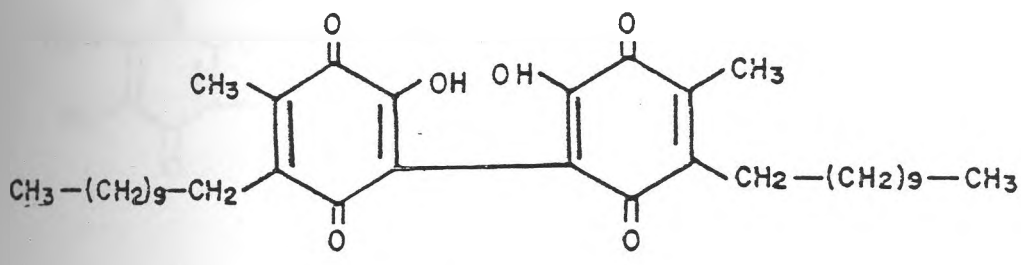


38

A Nepalese Maesa macrophylla. Wall. a plant commonly used in the remedy of fever, cough and ulcers, was found to contain a new benzoquinone pigment, 2,5-dihydroxy-3-methyl-6-nonyl-1,4-benzoquinone (38) up to 2.5 % (w/w) in the leaves (Chandrasekhar et al. 1970). Further chemical analysis by Prabu et al. (1971) showed the presence of another novel benzoquinone, macrophyllin (39) along with (16) and kaempferol (40) in the leaves.



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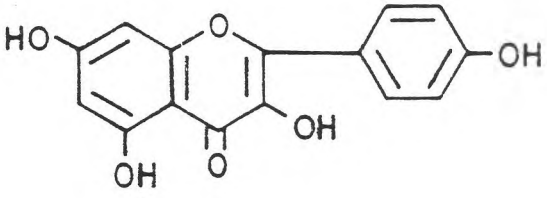

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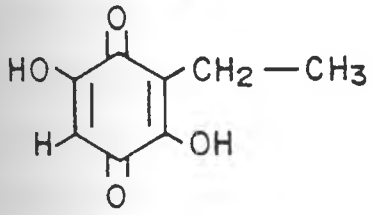
A study on Rapanea umbellata, a Brazilian Myrsinaceae plant, by Bauer et al, (1973) revealed the presence of embelin (10) as the chief benzoquinone pigment. Dallacker and Lohnert (1972) reported the synthesis of a number of benzoquinone pigments and confirmed their structures which included 3,6-dihydroxy-2-ethyl-1,4-benzoquinone (41), dihydromaesaquinone (42), spinulosin (43), oesporein (44), embelin (10), rapanone (11) and vilangin (13). Heltz et al, (1973) found the shoots and leaves of Embelia concinna contain the triterpene primulagenin (45) and embeliagenin (46).



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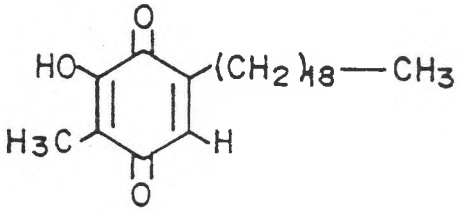

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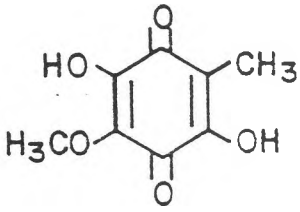

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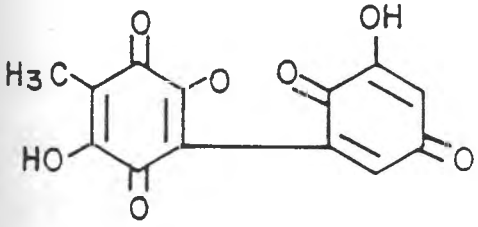

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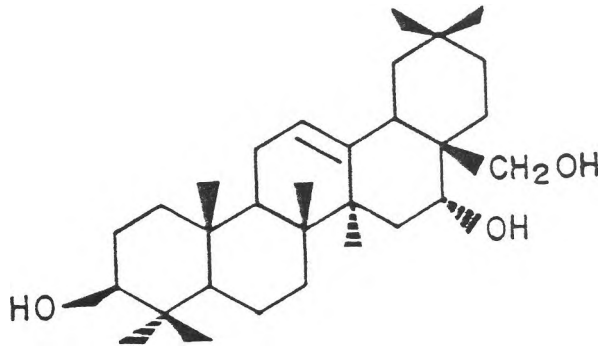

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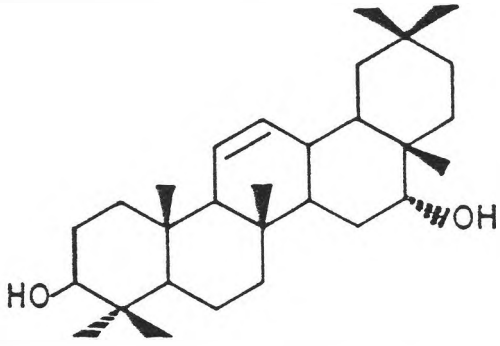
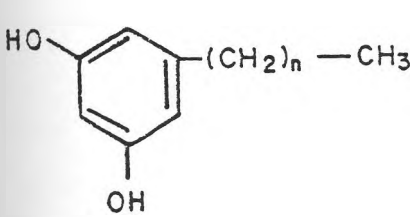


46

In India, Desai et. al (1975) in the course of their chemical investigations of many families of the Indian flora for their biological activities, isolated maesaquinone (12) from Maesa indica, and embelin (10) from Connarus ritchiei.

Another study of a Taiwanese plant, Maesa formosana by Russel et al, (1976) revealed (12) and acetylmaesaquinone (30) as constituents.

Phenolic compounds have also been isolated from fruits of the Myrsinaceae plants. These compounds especially those with side chains are biologically active. Madrigal et al, (1977) reported the isolation and characterization of a series of such phenolic compounds from Rapanea laetevirens. Examples of such compounds are 5-n-alkylresorcinols (47) and 5-n-alkenylresorcinols (48).



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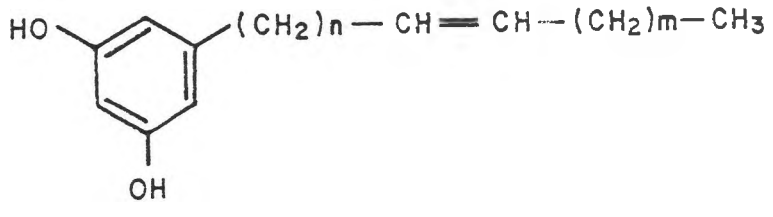


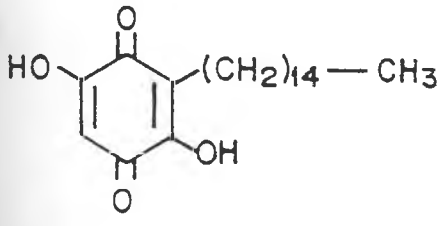
(48)

Midiwo et al (1988) reported the Kenyan Myrsinaceae to show a marked chemical relationship within the group which correlates with their morphological classification. His paper also indicated the existence of a remarkable discontinuity in the distribution of the benzoquinone pigments among the four species found in Kenya namely; Embelia schimperi. Maesa lanceolata. Myrsine africana and Rapanea melanphloes. During the separation of extract from Maesa lanceolata to retrieve compounds for anti-fertility test. Midiwo et al (1990) also isolated two new dihydroxylated benzoquinones which are related to maesanin (35): 2,5-dihydroxy-3-pentadecyl-1,4-benzoquinone(49) and 2,5-dihydroxy-3-(pentadec-10'z-enyl)-1,4-benzoquinone (50).

In their continued analysis of the Kenyan Myrsinaceae, Midiwo and Ghebremeskel (1993) examined Rapanea melanphloes and isolated a bisbenzoquinone- 6,6'-biembelin (72).

While continuing investigation on the Kenyan Myrsinaceae, Midiwo and Arot (1990) reported two new natural benzoquinones, 2,5-dihydroxy-3- methyl-6-undecyl-1,4-benzoquinone (73, muketanin) and 2-O-methyl-muketanin (74) from fruits of Myrsine africana and Maesa lanceolata respectively.

In the process of further careful analysis of M. africana. Midiwo et al (1992) isolated a new dihyroxylated benzoquinone, myrsinone (55) which differs from embelin in the manner of hydroxy group arrangement around the benzoquinone moiety (in that they are 2,3-placed) and 5-O-methyl embelin (75) which was previously reported in Aegiceras corniculatum (Gomez et al 1989)



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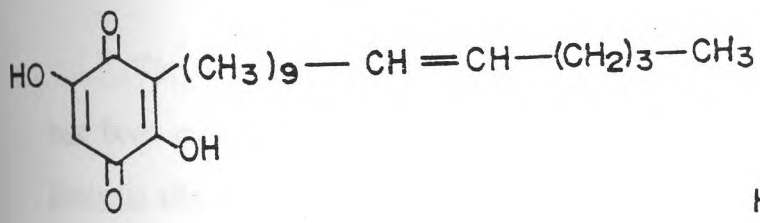

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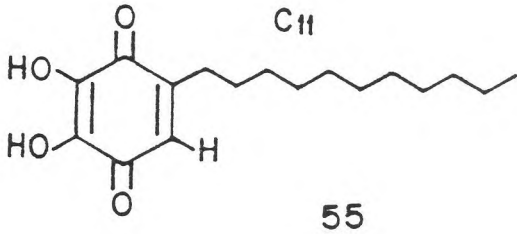

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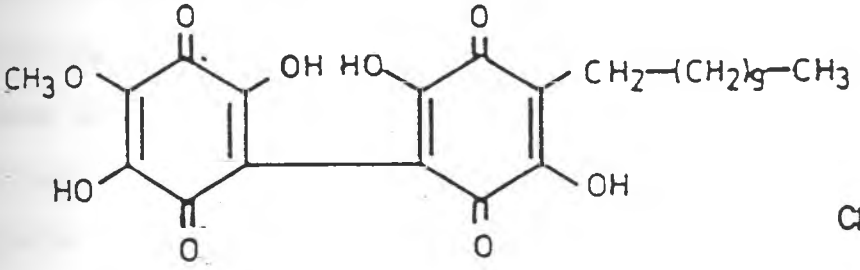

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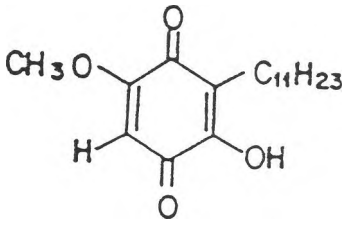

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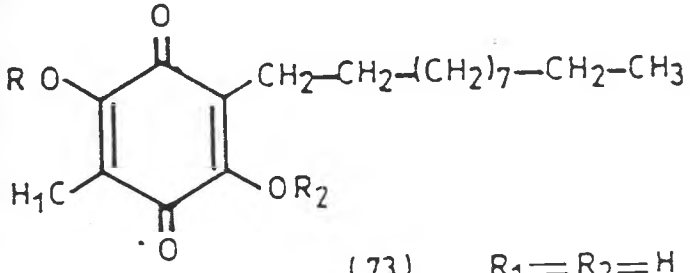
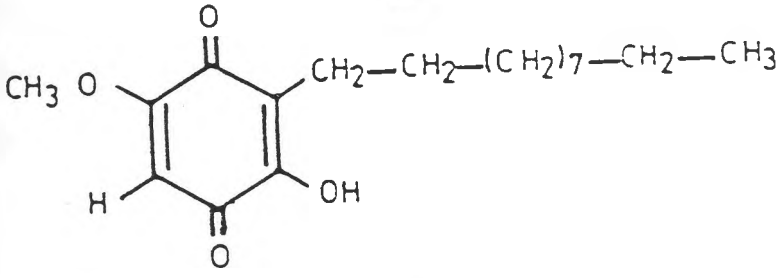


(73) $R_1 = R_2 = H$

(74) $R_1 = CH_3$, $R_2 = H$



(75)

1.0.2. BIOLOGICAL ACTIVITY OF THE MYRCINACEAE PLANT EXTRACTS - REVIEW.

A review of Myrsinaceae plants indicates that quite a number of them and their derived compounds find a wide range of biological uses. These include; anthelmintic, anti-malarial, anti-bacterial, analgesic, anti-allergic, larvicidal in grain storage, anti-cancer and fertility regulation.

The ethno-medical implication of plants from this family as anthelmintic has been extensively researched and documented. A pharmacological study of Embelia ribes (Burm) and Embelia robusta (Roxb) was carried out by Paranjpe and Gokhale (1932). They observed that the gelanic preparation of the plants' dried fruits were effective against tapeworm. Further research evaluated the constituent embelin (10) as the active anthelmintic principle. Doses in the range of 660-800 mg were observed to have no toxicity in man or dogs. In a similar respect, Krishna and Varma (1936) communicated that both petroleum ether and chloroform extracts of Myrsine africana berries exhibited activity against ringworm and other skin related diseases. The efficacy of embelin isolated from the dried berries of E.robusta (Roxb), M semiserrata (wall) and M. capitellata (wall) in this respect was also expressed (Krishna and Varma , 1937). In the same year Kawamura and Hokoku (1937) evaluated rapanone (11) and found it to be a potent anthelmintic principle. Two Myrsinaceae plants from Madagascar, Maesa emirnensis and Embelia berbevana have been used locally as anthelmintic and the active principle was embelin (10) (Paris and Rabenoro, 1950). The berries of E. ribes known in as "Vidanga" in India are formulated into a paste and applied on the skin as a treatment for skin infections. The powder from berries when taken with milk followed by a purgative has been one of the ancient remedies to get rid of tapeworm (Haris , 1987). E. ribes, a Myrsinaceae plant native to India and Hagenia abvssinica (Rosaceae) are commonly used in Eastern Africa where in Ethiopia they are known locally as "Enkoko" and "Kosso" respectively (Gupta et al, 1977; Pankhurst, 1972). They are used as arbortifacients in traditional medicine (Casy, 1960; Farnsworth et al,1975; Seshadri et al, 1978).

A review of past work on Myrsinaceae plants presents information that they have been widely used as anti-bacterial (Duclox and Awschalom, 1926). Harimoto (1939) tested extracts from the fruits of Maesa japonica (Moritzii) against pathogenic Gram-positive and Gram-negative bacteria and found it to be active against both. A pharmacological evaluation of two Madagascan Myrsinaceae plants namely M. emirnensis and E. berbevana revealed that the infusion of

M.emirnensis fruits killed paramecia in dilution of 1:24,000 in one hour while daphnia was killed by 5% infusion in 45 minutes. A sub-cutaneous injection of powdered leaf suspension at a dose of 10 g per Kg killed 100% of mice. The deaths as a result of the injection of stem, root and fruit suspensions were 40%, 70% and 50% respectively. A hemolytic effect of the plant was due to saponins present. It was further reported that the preparations shortened the blood coagulation time and also caused contraction of the isolated guinea pig gut. On the other hand extracts from the roots of E. berbevana killed paramecia at 1:4000 dilution in 5 minutes. The decoction was found to be toxic to epinochus (Gasterosteus aculeatus) but had very little effect on daphnia. The LD_{50} in Mice was 1.g per Kg (Paris and Rabenoro,1950). Joshi and Magar (1952) reported that decoction of dried fruits of E. ribes is used for treating fever and diseases that cause pain in the chest. They further noted that the drug exhibits significant anti-bacterial activity. Earlier, Merian and Schlittler (1948) had reported that an infusion of the decoction was used in the treatment of cough and diarrhoea.The roots of Ardisia solanaceae have been used in folk medicine as febrifuge, antidiarrhoeic and anti-rheumatic (Chopra et al.1956). Huang.et. al (1980), reported the inhibition of growth of Mycobacterium tuberculosis *in vitro* by E. ribes seeds extract. They observed that the active principle was embelin (10). Other active compounds which were noted along with embelin for this activity were barganin and quercetin. Okazaki and Ishikawa (1976) studied the anti-bacterial action of auraptene and rapanone against Gram-positive and Gram-negative bacteria. pathogenic fungi and acid fast bacillus. It was observed that auraptene was fairly active against these micro-organisms while rapanone was found to be extremely antagonistic to the sulphur compounds.

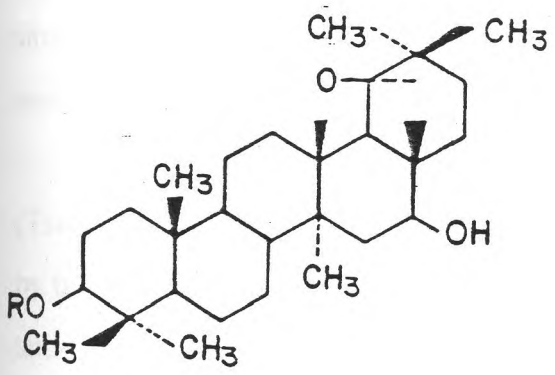
Structural modification of natural embelin was attempted using halogenation and esterification to get different pharmacological profiles (Tukannen et al, 1983). These were found to be active against bacteria and fungi up to 10 µg/ml. It was found that 6-iodoembelin was more effective as an anti-bacterial than embelin while diacetate of haloembelin showed high efficacy against fungi.

Embelin compounds substituted with aromatic amines were also reported to show a good anti-fungal activity (Rao et al,1984).

M.lanceolata is a Myrsinaceae plant native to Eastern Africa and hot water decoction of its fruits is drunk to prevent cholera infection (Kubo,1982). Previous chemotherapeutic evaluation of the plant by Taniguchi. et al (1978) implicated methanol extract of the fresh fruits as anti-bacterial agents. Kubo, et al (1983), further carried out a more detailed bioassay using both crude extract and pure isolates and showed that maesanin (35) is the active principle which involved a non-specific host defence reaction in that mice treated with a single dose 5mg/Kg were significantly protected from normal lethal Escherichia coli infection. Similarly anti-amoebic activity of benzoquinone especially with Entamoeba histolytica showed that rapanone (11) is the active principle in this respect (Shan, 1984). Shan also confirmed the efficacy of rapanone against Trichomonas vaginalis. A screening program in search of biologically active compounds from natural sources for novel anti-parasitic agents such as anti- amoebic and anti-trichomonas was carried out by De Souza (1986). In this case plant selection criteria was based on traditional medicine and ethnotherapeutic reports. The CH_2Cl_2 , MeOH and aqueous extracts of the selected plants were screened for their activity against Entamoeba histolytica and Trichomonas vaginalis *in vitro* and *in vivo* models (Chatterjee et al 1984; et al 1985). Plant extracts that displayed activity were those of Chonimorpha fragans (moon). Apocynaceae and Ardisia oxyphlla (Burm). and Myrsinaceae. Chonemorphine was described as the anti-amoebic principle of C. fragans and rapanone containing a small amount of embelin and its homologue $C_{21}H_{34}O_4$ as the anti-trichomonad principle of A. oxyphlla. The compound rapanone(11) also displayed *in vitro* activity against E. histolytica at 50 µg/ml . Chonemorphine dihydrochloride displayed *in vitro* activity against T. vaginalis (200 µg/ml) and E.histolytica (25 mg/ml) and *in vivo* activity against Hepatic amoebiasis in golden hamster (cured/treated=14/14, 100mg/Kg X 4 doses) and intestinal amoebiasis in weaning instar rats (200mg/Kg X 4 doses).

A phytochemical analysis of Kenyan Myrsinaceae plants by Midiwo and Manguro (1988) afforded benzoquinones, embelin (10), rapanone (11) maesaquinone (12), acetylmaesaquinone (30) and maesanin (35) as the isolates. An anti-bacterial study using the compounds against Gram-positive bacteria Bacillus subtilis, Staphyllococus aureus, Streptococcus mutans and Gram-negative Escheria coli implicated embelin (10) and rapanone (11) as the only active metabolites. They were active against Streptococci (Manguro, 1988). In a separate study Mavi, et al (1993) investigated into the molluscicidal and antifungal properties of R.melanphloes (Mez) and found that methanol extract of the plant leaves showed activity in both assays. The extract exhibited a molluscicidal activity at 50 ppm against the schistosomiasis transmitting snail Biomphalaria glabrata and also displayed anti-fungal activity against the plant pathogenic fungus Cladosporium cucumerinum in a TLC assay (Hostettmann, 1980). Activity guided fractionation of the extract using different chromatographic techniques afforded compounds 51, 52, 53, and 54 in yields of 0.25, 0.25, 0.015 and 0.025% respectively.

Midiwo et al (1996) conducted mosquito larvicidal tests on embelin (10), myrsinone (55) and myrsinaquinone (56) from Rapanea melanphloes using 2nd instar Aedes aegypti larvae. In this test which was done according to methods of Zebitz (1986) and Mwangi and Rembold (1986), ten larvae were introduced and immediately treated with potassium salts of either (10), (55) or (56). The salts were made by adding an equivalent amount of the compounds to potassium hydroxide solution and then transferred into the jars to achieve the required concentrations of the salts. Control jars were not treated with the salt solutions. Larval growth was inhibited and the survivors took longer time to mature than in the controls. The LD_{50} in 48 hours worked out from plots of log of concentration against probits of frequency of embelin, myrsinone and myrsinaquinone were 2.40, 2.54 and 2.69 µg/ml respectively.



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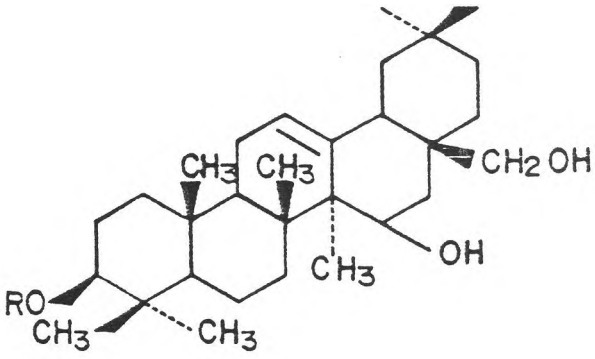

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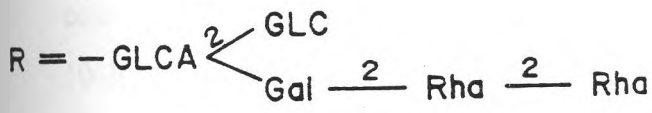

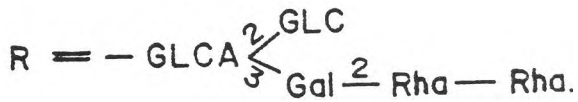

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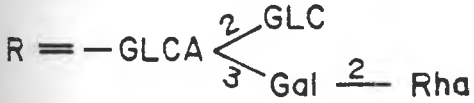

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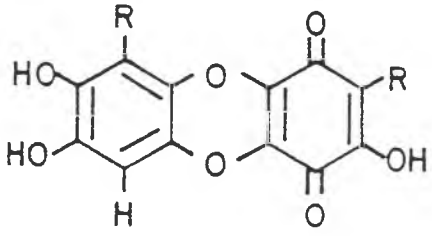


$R \equiv C_{11}H_{23}$

$R \equiv C_{13}H_{27}$

56

While continuing with their work on insect bioactive compounds from Rapanea melanphloes. Midiwo et al (1996) carried out insect antifeedant test using Schistocerca gregaria females done according to Butterworth and Morgan (1971). Each experimental group of animals consisted of eight mid-5th instar females which were starved for 24 hours before the experiment. Whatman # 1 filter papers (2.75x2.75 cm) were then dipped in 100 µg/ml solution of the test compounds while the control papers were not treated further. The filter papers were presented to the test animals after drying and left in the cages for 8 hours in a no-choice

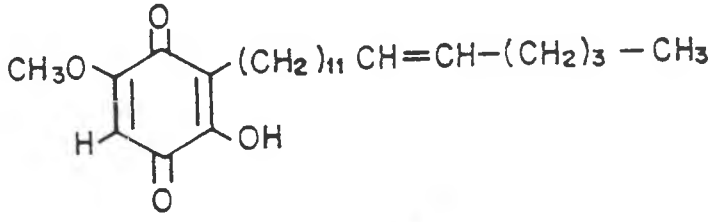
situation. The relative antifeedant percentages (RAP) values were 88.4%, 90.2% and 44.5% for the three compounds, respectively, when tested against S. gregaria.

The effect of natural embelin in prevention of plaque has been discussed (Tsuneo, et al. 1985). In the course of basic studies on the dental carries prevention by traditional medicine, various crude drugs used in Sri-Lanka were screened for anti- plaque action against Streptococcus mutans. It was observed that the methanol extract of the fruits of E. ribes potentially inhibited adherence of viable cells of S. mutans to smooth surfaces with a 50% inhibitor concentration (LC_{50}) of 10-30 µg/ml . They further noted that the extract exhibited anti-bacterial activity against S. mutans and anti-enzymic action against glucosyltranferase . The active principle in this case was embelin, which inhibited the bacterial growth with a minimum inhibitory concentration of 62.5 µg/ml and the glucan synthesis with an LC_{50} of 125 µg/ml. A year later Tsuneo et al (1986) reported that embelin incorporated in tooth paste prevented formation of dental plaque and acid on teeth.

Profile chemical characteristics of benzoquinones in relation to identification of anti- malarial compounds has been extensively studied (Sastry et al 1986). Complexion of anti-malarial drugs such as amodiaquine, chloroquine, primaquine, trimethoprim and pyiremthamine with benzoquinones resulted in formation of colored complex compounds; thus benzoquinones could be used in the detection of anti-malarials. Larvicidal tests using mosquito larvae Aedes aevpti were carried out by Ghebremeskel (1991) and it was observed that embelin (10) and myrsinone (55) had LD_{50} of 2.4 µg/ml and 2.54 µg/ml respectively. Myrsinaquinone (56) on the other hand had LD_{50} of 2.69 µg/ml

Embelin has been studied for analgesic activity on rats and mice . Alat. (1977) in his study on the analgesic property of embelin with rats and mice reported the affective oral administration of the drug and the results obtained were comparable to those of morphine but he pointed out that the action of the drug was different from that of opiates. Gupta, et al (1977) also reported on ten of embelin disalts which showed a remarkable analgesic activities in rats when administered through intraperitoneal injection. A further research by Neth (1977) showed amine

salts of embelin to have similar effects. Dhar (1980) tested potassium salt of embelin on rats for its analgesic activity and found that the drug did not have any effect on serotonin or histamine levels but levels of adrenaline in cerebro-spinal fluid increased while the activity of acetylcholinesterase decreased. In a separate study, embelin was found to be analgesic by oral administration and central routes and the results compared with morphine. Although the drug acts centrally to produce analgesia, its effect is not antagonized by naloxone indicating that a central site of action is different from opiates. Also no precipitation of abstrience syndrome was observed as with morphine. A peripheral site of action of the drug was ruled out as it lacked any demonstrable anti-inflammatory action. However, the high oral efficacy and non-narcotic properties makes embelin more accepted than morphine (Atal, et al 1984). Similarly, Kokai (1985) in his pharmacological studies of benzoquinones, evaluated methanol extracts of Ardisia japonica as arachidonate-5-lipoxy-genase inhibitor. The extract was fractionated by silica gel column chromatography to give the benzoquinones (57) and (58) respectively as the arachidonate-5-lipoxygenase inhibitors and are also effective in treatment of asthma and inflammation.



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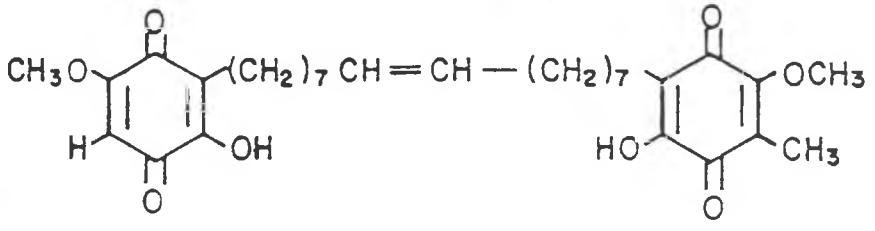
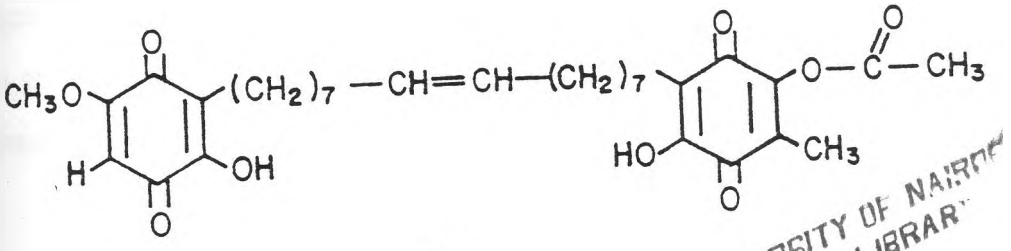


58

There are benzoquinone derivatives with wide range of applications as anti-allergic compounds that have also been reported in the literature. Hideyuki (1984) studied the anti-allergic activities of the derivatives of ardsiaquinones (32), (58) and (59) and found them to be effective and could therefore be used as substitutes for anti-allergy compounds. In the following year, a study on the synthetic benzoquinone derivatives by Iwaki (1985) indicated that the compounds have anti-asthmatic and anti-histamine activities.

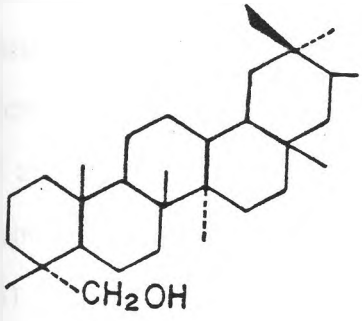


59

A comparative study of fungicidal quinones and natural embelin from E. ribes as protectants of wheat seeds against the stored product insect pests; Sitophilus oryzae (L), Rhyzopertha dominica (F) and Ephestia contella (Walker) was done (Chander and Ahmed, 1989). Embelin and chloranil at concentrations of 0.0125 and 0.025% (w/w) respectively in wheat produced mortalities of 77.8 % and 68.7 % in adult S. oryzae after 14 days of exposure, while dichlone produced 82.8% mortality at 0.01% concentration level. When applied against R. dominica, dichlone even at 0.0125 % concentration level gave 78 % adult mortality after 14 days, whereas the same mortality was observed with embelin and chloranil at 0.025 % and 0.05 % concentration levels respectively. Dichlone did not exhibit any chemosterilant action on the adults of S. oryzae or R. dominica or any inhibitory effect on oviposition as with the controls.

Some Myrsinaceae plants have been screened for anti-cancer activity. Kupchan et al, (1969) tested Myrsine africana (L) leaves for anti-tumor activity against "Walker" intramuscular carcinosarcoma-256 in rats. The chief component of the leaf hydrolysate was lucynoside (60). Information on certain methylated benzoquinone derivatives as having sensitizing activity has been reported (Shultz

et al, 1979). In a similar respect Ardisia cornudentata (Mez), a species used in folk medicine in the south eastern part of China as anti-inflammatory/analgesic medication to improve general blood circulation and also as antidote for snake and insect bites, was subjected to phytochemical investigation by Chang et al, (1987) to identify the active components. In this study, root extracts of the plant were evaluated in several in vitro receptor and enzymatic assays. The methylene chloride extract was found to inhibit the binding of leukotriene, D_4 (LTD_4),to a receptor preparation from a guinea pig lung tissue. Using the LTD_4 binding assay as a guide, two benzoquinones, ardisianone (61) and cornudentanone (62) were isolated as the active components.



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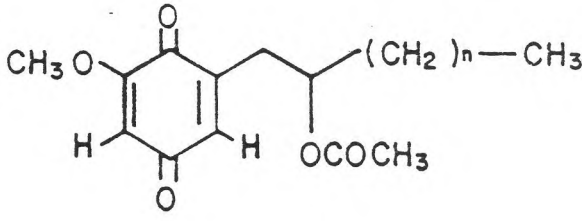


61 n = 12

62 n = 10

In recent years, researchers have been interested in the development of a safe and reversible effective anti-fertility agent of plant origin. Das et al (1976) developed an oral contraceptive by mixing crushed Embelia ribes fruits, Piper longum, asafoetida and purified borax in the ratio 1:1; 1:2 respectively. The drug was administered orally to female rats while not abstaining the test animals from sexual intercourse. This drug was active in preventing implantation of the fertilized ovum. There was no side effect associated with the drug. In continuing with research for anti-fertility agent from plants, embelin isolated from the berries of E. ribes (Burm) has been reported to induce functional sterility in mice, rats and dogs (Munishi and Rao, 1972; Munishi, et al, 1972; Prakash and Mathur 1979; Dixit and Bhargava, 1983). "Garbhanivarana aushadam", an ayurvedic anti-fertility drug agent in India composed of Piper longum, E. ribes and borax, when administered to female rats at a dose of 10% of the food in their diet for 16 days or fed (0.5g per

day for 16 days) to guinea pigs, showed a slight prolongation of the oestrus cycle in both species. Histochemical studies revealed that alkaline phosphatase activity in the uteri of treated animals was enhanced when compared to the control (Geeta et al, 1976).Greep (1976), in his comparative analysis of studies in male and female reproductive physiology noted that the understanding of males' reproductive physiology lags behind that of the females.

While continuing with research on anti-fertility, Kholkute (1978) studied the activity of E. ribes berries and its extract on female rats. The drug was incorporated into their diet and administered at various dose levels. It was found to prolong the diestrus phase of the oestrus cycle and inhibit fertility in 62% of the animals. Serially hot extracted petroleum ether and methanol extracts affected cyclicity and prevented pregnancy in 75% of the rats. Benzene extract showed 51% anti-fertility whereas that of chloroform gave 37% anti-fertility. However, embelin in this case failed to reveal any anti-fertility activity (Kholkute et al, 1978). In male rats, daily administration of 75-300mg/Kg of embelin showed a reduction of testis and prostrate gland weights and indicated an impairment in the metabolic functions of these organs. Acid and alkaline phosphate activities were elevated (Chanhan et al, 1979). Semen analysis and hormonal levels in bonnet monkeys treated with E. ribes berries's extract was studied by Purandare et al, (1979). When the extract was administered orally for 3 months at a dose of 100 mg per day to male monkeys, the quality and quantity of semen was adversely affected. Circulating testosterone levels were also reduced but the luteinizing hormone (LH) levels was however not affected . Testicular biopsy revealed normal spermatogenesis. The reduction in the testosterone level in the circulation may be responsible for the reduced secretory activity of the accessory glands which in turn resulted in a decrease in the volume of semen. Prakash, (1980) studied the effect of embelin on Corpora lutea of cycling guinea pigs and showed that increase in ovarian weight depended on the dosage given to the animals. He also observed that embelin was neither antizygotic nor blastotoxic despite its anti-implantation and abortifacient activities. However, the drug was observed to show an anti-

oestrogenic activity but not anti-progesterone activity (Prakash, 1980). In a previous report Dixit et al.(1978) showed that embelin administered at 80mg/Kg for 100 days to male dogs caused azoospermia and the animals recovered 250 days later. Similarly embelin from the seeds of E. ribes administered at 100 mg/kg and 50mg/Kg to female rats showed 57.9% and 55.5% anti-fertility respectively (Mohana et al. 1981).

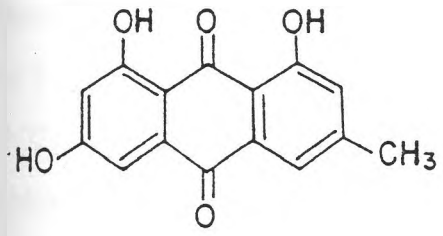
Prakash, (1981) in his continuation with the study of embelin on fertility regulation administered the drug orally to ovariectomized rats and found that the drug altered the activity of various enzymes in the uterine tissue. However, when the drug was given to oestradiol primed rats, a decline of uterine weight was noted. The drug did not bring any reasonable change in the uterine biochemistry of progesterone primed rats. Antispermatogenic effect studies of embelin by Seth, (1983) showed that embelin significantly reduced the sperm count, and the weight of the experimental albino rats. Embelin fed at 50-200mg/Kg body weight for 15 days reduced the weight of the testis significantly and also effectively reduced the sperm count significantly. Studies by Bharghara et al, (1984 and 1985) showed that embelin when given to rats at one to five days of pregnancy at 20-50mg/Kg inhibited implantation of the blastocyst. Agarwal et al. (1986) reported that embelin altered the rat testis histology, glycogen levels, sperm count and accessory sex glands. Effects of embelin on the activity of uterine ß-gluconidase in ovariectomized rats (Prakash and Sukhla, 1987) showed a marginal increase in enzyme activity. In another development, subcutaneous exposure of male albino rats to embelin (20mg/Kg body weight) for 15 to 30 days revealed significant impairment in carbohydrate metabolism in the primary and secondary reproductive tissues. Alterations of the enzyme activities of glycolysis, Krebs's cycle, lipogenesis, NAD and NADP dependent enzymes, transaminases and phosphatases are noted on embelin treatment in testis, epididymis, seminal vesicles and ventral prostate as well as in spermatozoal suspension. Reduction in fertility parameters such as pregnancy attainment and uteri sizes obtained was also noted on embelin treatment. All these changes in embelin treated animals are restored

back to the normal biochemical make up of the control animals, once the drug is withdrawn and the animals are allowed to recover for another 15 to 30 days (Gupta et al, 1989). In a similar study Makawiti and Midiwo (1990) in their preliminary studies have indicated that in white New Zealand male rabbits, embelin dose of 40-80mg/Kg body weight/day for 15 days led to the decrease in testosterone levels. They observed a concomitant correlation in the rise of progesterone and a fall in testosterone level. Luteinizing hormone (LH) showed a 26 % rise in level apparently responding to decreasing testosterone levels. Health parameters such as packed cell volume and weight were monitored and were found to be normal except in animals that were under maesaquinone (56) treatment. It is apparent that the progesteronal activity exhibited by embelin was probably due to its ability to raise the concentrations of progesterone. Further research studies suggested that embelin causes the elevation of progesterone levels by inhibiting the17-hydroxylase enzyme in the testosterone biosynthetic pathway. In their continuation with the study they observed that histological studies showed a major difference between the embelin treated and control rabbits. In the case of treated animals, the epididymis was devoid of spermatozoa and the seminiferous tubules were atrophied. The germ cells in the basal lining appeared normal but the adluminal cells were disorganized, extensively vacuolated and appeared to be sloughing off. It was also observed that some of the meiotic cells and spermatids fell off to the lumen of the seminiferous tubule. The sertoli cells were also found to be highly vacuolated and hyalinised.

The effect of embelin from E. ribes and oleanolic acid from Parthenium hysterophorus on maize and cow pea germination has been investigated (Sinha et al 1981) at 50-520ppm at 22°C . Oleanolic acid markedly reduced the germination percentage 48 hrs after seed treatment. Embelin reduced germination at 100 ppm and 250ppm but stimulated germination at 50 ppm. Data on respiratory rates and catalase activity indicated that both compounds inhibited respiration.

Bioactivity of ethanol extract of the roots of M. africana using brine shrimp lethality test was studied by Mclaughlin and Xia-Hua (1989). From the study,

emodin (63) and 2-hydroxychrysophanol (64) were implicated as the cytotoxic components.



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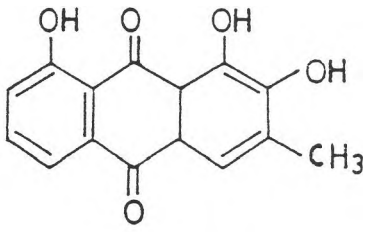
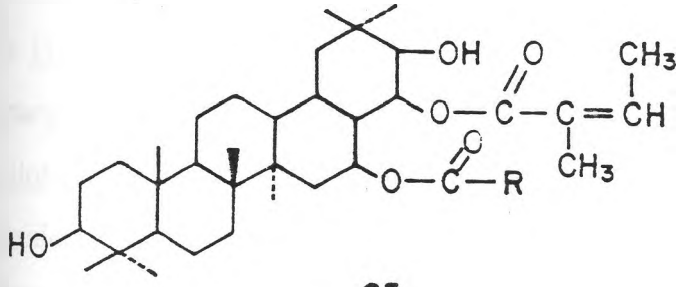


64

A number of medicinal uses have been reported for M. lanceolata, a tree growing in many African countries. In Kenya, the plant is used against cholera (Kubo et al, 1983). Anti-bacterial and anti-viral activity tests using methanol extract of the plant showed that the extract only exhibited a virucidal affect against herpes simplex type 1, vesicular stomatitis and semliki forest A_7 viruses. A bioassay guided fractionation of the extract implicated the compound (65)(16-0-propionyl-22-0-angeloyl-3,21-dihdroxyl-12-oleanen-28-al) as the active principle. Other compounds reported to have been isolated in minor quantities along with (65) are (66-70) (Sindanambiwe et al, 1993).



65

66 R = CH_3

67 R = C_3H_7

68 R = C_4H_9

69 R = C_5H_{11}

70 R = C_6H_{13}

Compounds and their derivatives from Myrsinaceae plants have shown only a few adverse effects. However, the areas sighted have not been fully investigated. E. ribes and Hagenia abyssinica have been reported to cause optic atrophy among Ethiopian population (Low et al, 1985). In their study they investigated the possible toxic effects of ingested E. ribes, H. abyssinica and embelin on visually guided behavior in chicks, followed by histological

examinations of their retina. A high dose of E. ribes was administered for 1 to 5 days while that for H. abyssinica was administered for 1 to 9 days. For the low dose of both E. ribes and H. abyssinica, the dosing regime was for a period of 1, 4 or 9 days. Embelin, the active principle of E. ribes was administered at a dose of 0.02g/Kg per day for 9 days. Control chicks were fed on an equivalent amount of chick feed. From the experiment, they observed that treatment with E. ribes or H. abyssinica significantly reduced the ability of the chicks to detect a moving bead introduced into the peripheral field of vision. They further noted that the degree of constriction of the visual field for detection was dependent upon the total amount of drug administered. Performance on a visual discrimination task which required discrimination of feed grains from pebbles was also impaired in chicks with a total dose of 0.20g and 0.25g of E. ribes or H. abyssinica. Thus, the extent of deficit in visually guided task was found to be dose-dependent. However, the visual deficits observed in E. ribes treated chicks were mimicked by embelin suggesting that embelin may be responsible for the visual defects. Anatomical evidence of degeneration of ganglion cells was found in retinae exposed to high doses of E. ribes (1.25g) and H. abyssinica (2.25g). However,no retinal lesions were detected in chicks following treatment with cumulative doses of less than 0.25g of E. ribes or H. abyssinica. In a separate study the crude extract of twigs and stem of the mangrove plant, Aegiceras corniculatum exhibited toxicity to juvenile fish, Tilapia nilotica at a concentration of 1ppm within a period of 75 minutes. A fractionated guided assay implicated a derivative of embelin, 5-0-methylembelin (71) as the toxic substance in this respect. At the same time, the growth fungus, Pythium ultimum was inhibited by the same compound with an inhibition zone of 8mm diameter with a solution of 1mg of the compound in chloroform (Gomez et al. 1989).

The efficacy of natural embelin (10) against the red flour beetle Tribolium casterenum (Herbst) was studied by Chander and Ahmed (1985). They observed that when the drug was mixed with wheat samples at 0.19% concentration, a high efficacy of action was reported which brings about adult mortality even after 8

months of storage. Reproductivity (progeny/adult day) of the insect was also reduced significantly in the treated samples as compared to the controls at different interval of storage. After 8 months of storage, significant reduction in progeny at the lowest concentration was mainly due to larvicidal action of embelin. Embelin, however, did not inhibit any chemosterilant action or contact toxicity to the adult beetle. A further research on the drug by Chander and Ahmed (1987) revealed that the drug is mutagenic to this strain of insect only when one or two hydroxyl groups on it are methyl substituted. Two years later the proven efficacy of embelin was evaluated as a protectant against a variety of insect pests of wheat storage. The drug showed 50% mortality on both Sitophilus oryzae and Rhysopertha dominica adults at 0.025% concentration (Chander and Ahmed, 1987). Previous studies by Tukannen, (1983) in this respect showed that natural naphthoquinones and benzoquinones were mutagenic to S. typhimunum strain TA 2637 with metabolic activation. Ardisiaquinones (32), (58) & 59 were implicated for this activity.

BROAD OBJECTIVE.

The broad objective of this study was to extensively examine the chemical composition of extracts from berries and root bark of *Embelia schimperi* and *Embelia keniensis.*

Specific Objectives.

a) To isolate bioactive compounds from *Embelia schimperi* and *Embelia keniensis.*
b) Perform toxicity assays of the isolated compounds using brine shrimp.
c) Perform anti-feedant and grain protectant tests on the compounds found to be positive on the brine shrimp assay.

CHAPTER TWO

2.0.0. RESULTS AND DISCUSSION.

Dry ground root bark and fruit of Embelia schimperi and Embelia keniensis were extracted with cold ethyl acetate and the solvent removed in vacuo leaving a dark gummy solid. A preliminary spot-test survey was carried out to determine the quinonoid pigments in two plants using thin layer chromatography (t.l.c). A solvent system consisting of n-hexane, ethyl acetate, acetic acid (80:15:5) revealed that the root bark of Embelia schimperi had components with Rf 0.9, 0.55, 0.53 and 0.47 while the fruit had components with Rf 0.9, 0.64, 0.62, 0.55 and 0.49. All the spots turned permanently pink on exposure to conc. ammonia vapor except the spot with Rf value of 0.47 which absorbed UV radiation. Embelia keniensis had components occurring in very trace amounts and thus was not followed.

The extracts from each of the two parts of Embelia schimperi were pre-absorbed on dry de-activated silica gel and subjected to column chromatography in a column packed under n-hexane with the de-activated silica gel. The column was exhaustively eluted with n-hexane, dichloromethane and ethyl acetate respectively and a fraction of each solvent was collected separately. Each eluate was tested against Artemia salina (brine shrimp) nauplii (larvae). The dichloromethane and ethyl acetate eluate were both found to be active against the organism. However, from t.l.c. analysis, the ethyl acetate eluate was found to contain mainly the more polar components occuring in trace amounts and for this reason, it was not followed. The dichloromethane eluent of the root bark was found to contain the embelin (10), Rf 0.55 (solvent system: n-hexane, ethyl acetate and acetic acid-80:15:5) in larger amounts than others and therefore masked the minor ones on t.l.c plate. This prompted repeated fractional cyrstallization of 10 until very little remained in the mother liquor. The solvent was removed from the mother liquor leaving behind a residue which was pre-adsorbed on de-activated silica gel and subjected to column chromatography using de-activated silica gel. The polarity of the system was increased with increased addition of dichloromethane upto 100%.

The column was finally washed with 5% methanol in dichloromethane. Further purification of the compounds was done using preparative t.l.c, gravity column chromatography and fractional crystallization. The compounds obtained from the root bark were Rf 0.55, embelin (10), Rf 0.9, decylanhydrovilangin (77), Rf 0.53 , myrsinaquinone (56) and KCP-02. The compounds were characterized using their melting points and spectroscopic data. Isolation of components in the dichloromethane eluate of E.schimperi fruit was also done using column chromatography. The eluting solvents were n-hexane, dichloromethane, ethyl acetate or their combinations. Purification of the compounds was done using preparative t.l.c, gravity chromatography and fractional crystallization. The compounds obtained from this part of the plant included Rf 0.55, embelin (10), Rf 0.9, decylanhydrovilangin(77), Rf 0.53, myrsinaquinone (56), Rf 0.62, methylvilangin (21), Rf 0.49, KCP-06 and Rf 0.64, decylvilangin (76). The compounds were characterized using their melting points and spectroscopic data. Each of the pure compounds was tested for the biological activities shown in section 2.0.1

2.0.1. BIOASSAYS.

2.0.2. Brine shrimp lethality test.

This method utilizes brine shrimp (Artemia salina LEACH) and is proposed as a simple bioassay for possible bioactive compounds. The procedure determines LC_{50} values in µg/ml of active compounds and the extracts in the brine medium. Activities of a broad range of known active compounds are manifested as toxicity to the brine shrimp nauplii (Mclauglin et al 1982). The method is rapid, reliable, inexpensive and convenient as an-in-house general bioassay tool.

Methylvilangin (21), decylvilangin (76), KCP-02 and KCP-06 were subjected to this test and their LC_{50} (summarized in Table 1) values are 120, 51, 54 and 0 respectively.These results indicate that 76 and KCP-02 have a moderate activity. Embelin (10) and myrsinaquinone (56) were not subjected to this test

because they had been tested ealier (Ghebremeskel, 1991) while 77 was not tested due to lack sample.

Table 1

Effects of various benzoquinones on *Artemia salina* (shrimp)

Compound	% Deaths at 24 hrs							LC_{50}
Conc.(µg/ml)	5	10	15	20	25	50	100	
KCP-02	10	17	20	27	31	33	62	54
21	10	10	20	23	26	30	36	120
KCP-06	0	0	0	0	0	0	0	0
76	7	13	16	25	32	50	55	51

2.0.3. Anti-feedant test

In the recent years research scientists have focused attention on feeding deterrents for control of insect pests. In 1983, Kraus et al for instance conducted research on anti-feedant effects of Meliaceae extracts. In view of the continued interest focused on feeding deterrents, embelin (10) and methylvilangin (21) were screened for anti- feedant activities using Locusta migratoria migratorioides using "choice" test in which whatman No. 1 filter papers previously soaked in 0.25 M sucrose solution were sprayed with 100 µg/ml of each sample. The relative anti-feedant percentage for each compound was calculated using the formula given below and were found to be 96 and 33 for 10 and 21 respectively.The results (summarized in Table 2) indicate that that embelin (10) offers a significant level of protection to field crops against destruction by locusts. Compounds 56, 76, 77, KCP-02 and KCP-06 were not tested because of lack of samples.

Table 2

Relative Anti-feedant Percentages (RAP) for embelin (10) and methylvilangin (21).

Compound	Average eaten area	Average uneaten area	RAP(%)
10	0.08	6.25	96
21	2.38	3.88	33

$$RAP = \frac{\text{Average eaten areas of control filter paper} - \text{Average eaten areas of sample treated filter paper}}{\text{Average eaten areas of control filter paper} + \text{Average eaten areas of sample treated filter paper}} \times 100$$

2.0.4. Anti-Microbial tests.

An attempt was made to determine anti-microbial activities of 77, 56, 21, 10 and KCP-06. Compound KCP-02 was not available for this test. Concentrations of 25, 50 and 100 µg/ml of each compound were prepared and tested against Candida albicans. Trichophyton metagrophyte. Microsporium gypsum and *Escherichia* coli and all were found to be inactive against the micro-organisms.

2.0.5. Larvicidal test.

In spite of many years of research into malarial drugs, malaria still remains the leading killer disease mainly in Africa. Plasmodium (malaria causing parasite) has been found to be resistant to most drugs in the market today. In view of this

worrying trend , an attempt was made to investigate 21 for larvicidal activity using concentrations ranging from 25 µg/ml to 100 µg/ml in a pond containing twenty (20) 2nd instar larvae of *Aedes aeypti*. Compounds 10 and 56 had been subjected to this test earlier (Ghebremeskel, 1991) while KCP-02, KCP-06, 76 and 77 were not available for testing. Compound (21) was found to disrupt metamorphosis without causing any mortality. In fact, when 100 µg/ml of 21 was applied into a pond containing the 2nd instar mosquito larvae, only a few underwent metamorphosis to the 3rd instar larvae and none attained the adult stage. This biological activity can be used to reduce population of mosquitoes. The results are summarized in Table 3.

Table 3

Larvicidal effect of methylvilangin (21)

Concentration	Population				
	2nd instar	3rd instar	4th instar	Pupae	Adults
100 µg/ml	14	5	1	0	-
75 µg/ml	10	6	3	1	-
50 µg/ml	5	7	6	-	2
25 µg/ml	3	3	2	8	4
10 µg/ml	-	2	3	2	13

2.0.6. Stored Products Pests Test.

In view of tremendous losses due to pest attack in Kenya (*vide supra*), an attempt was made to investigate the level of protection that 10 can offer to stored beans and maize grains. Concentrations of embelin ranging from 0.04% to 0.2% were introduced to jars containing twenty (20) mixed adults of *Anthoscelides*

obtectus and *Sitophilus zeamais*. Each experiment was performed in three replicates.

Results on the effectiveness of embelin against S. zeamais (Figure 1) indicated that even after 5 months of storage, the number of emerged adult progeny and reproductivity reduced significantly at all the concentrations in comparison with the control. After this period of storage, only the highest concentration of 0.1% gave the highest mortality.

Data on the effectiveness of embelin against A. obtectus (figure 2) similarly revealed that the total number of emerged progeny and reproducibility were reduced significantly at all concentrations as compared with controls. The significant reduction in the progeny of S.zeamais and A. obtectus at all concentrations of embelin could possibly be due to the effect of embelin as a larvicide.

It can be concluded from the foregoing discussion that the effectiveness of embelin as a larvicide is retained even after 5 months of storage, indicating stability and slow rate of degradation under the experimental conditions. In general, the effectiveness of embelin was greater against A. obtectus compared to S. zeamais.

Figure 1: Variation of *Sitophilus zeamais* progeny with concentration of embelin after 5 months storage.

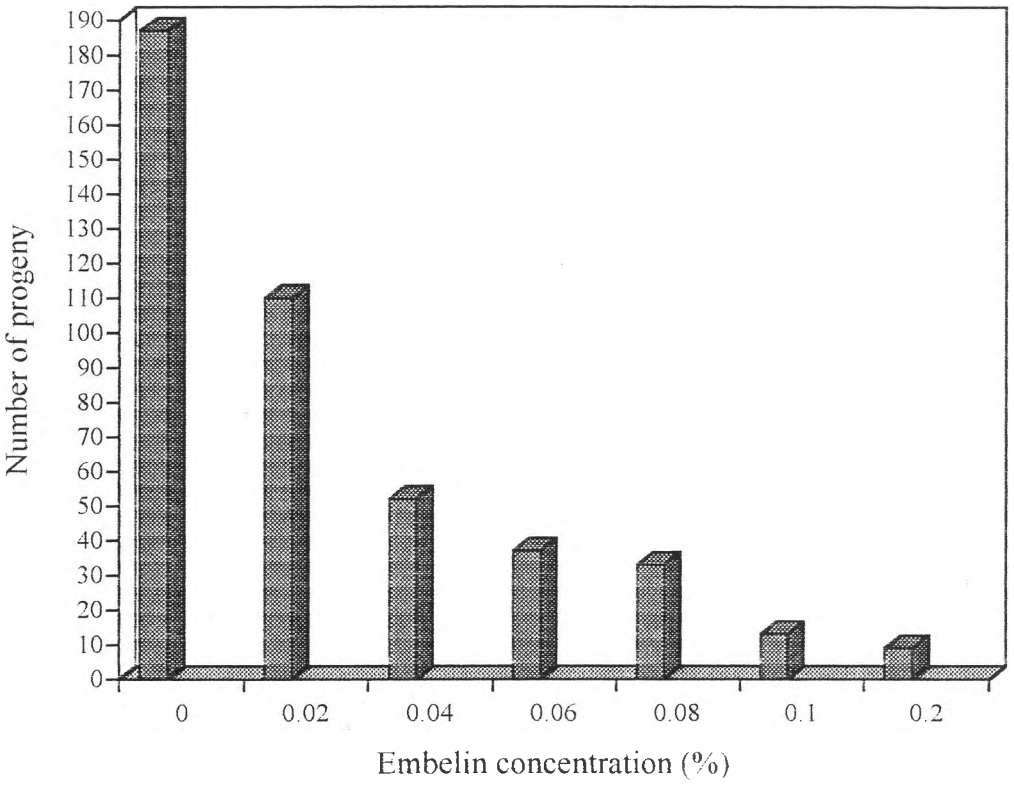
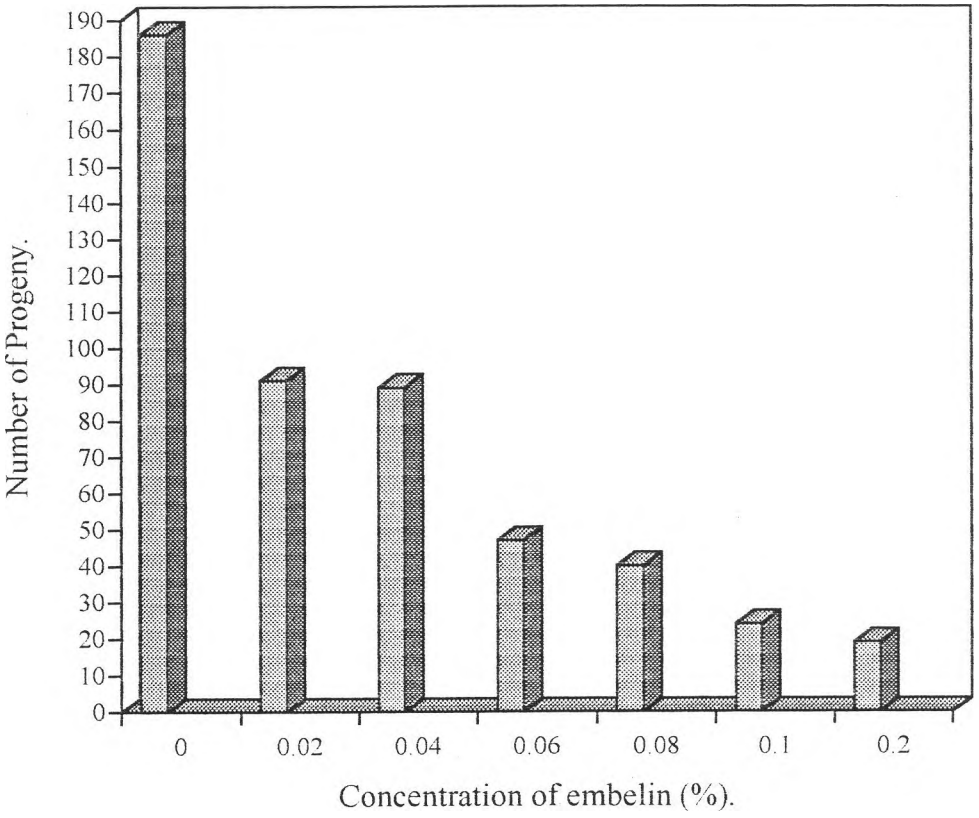


Figure 2: Variation of *Anthoscelides obtectus* progeny with concentration of embelin.



2.1.0. Structural determination of dihydroxyalkyl-1,4-benzoquinones.

During the course of the investigation on the secondary metabolites, embelin (10), which had been characterized from the same plant before, was isolated in high yield from the fruit and root bark relative to other components. It is a golden-yellow compound with Rf 0.55 (eluent: n-hexane - ethyl acetate - acetic acid - 80:15:5) and m.p 141-142°C (same as that reported in the literature by Ogawa and Natori, 1968). The highest concentration of the compound was realized in the fruits which contained 10.2% w/w while the root bark contained 8.1% w/w. Its characteristics matched those of the same compound isolated from the same plant previously in our laboratory.

Chromatographic separation of the dichloromethane eluent of E. schimperi gave an orange compound (21) (solvent system: same as that used for 10) with m.p 129-131°C. This compound turned pink on exposure to conc. ammonia vapor. Its UV-Vis spectrum displayed a peak at 430 nm and 290 nm thus indicating the chromophore of the compound as a 2,5-dihydroxy-1,4-benzoquinone. The compound showed six signals in the ^{1}H NMR spectrum. There was no signal corresponding to the quinonoid proton which usually appears at 6.50-5.0 ppm. This suggests that the quinonoid ring is fully substituted. A singlet peak due to phenolic hydroxyl hydrogens bonded to carbonyl group appeared at 7.82 ppm. The spectrum also contained peaks at 4.39 ppm (H. q, J=7.50 Hz) and 1.59 ppm(3 H, d, J=7.50Hz) which are unusual for the usual alkyl substituted 1,4-benzoquinones from Myrsinaceae. The peak at 4.39 ppm suggests the presence of a proton sitting on the same carbon atom with a methyl group whereas the doublet peak at 1.59 ppm could be due to a methyl group next to a proton. Infact, a methyl group substituting the ring displays a sharp singlet at approximately 1.90 ppm whereas the quinonoid proton appears either as a singlet or a multiplet at 6.5-5.0 ppm (Ogawa and Natori, 1968; Chandrasekhar,1970; Thomson, 1971). Irradiation of the peak at 4.39 ppm collapsed the doublet at 1.59 ppm into a singlet suggesting

that the proton and the methyl group are adjacent to each other and possibly sitting on the same carbon atom. The triplet at 2.40 (J=7.1 Hz) ppm was assigned to the benzylic methylene protons. Integration of this peak relative to the set at 4.39 ppm correspond to four protons calculated for two methylenes. This therefore suggests that the compound under consideration probably consists of two dihydroxyalkyl-1,4-benzoquinones bridged by a carbon holding a proton and a methyl group. A multiplet at 1.43-1.25 ppm on integration relative to the peak at 4.39 ppm corresponded to 36 protons calculated for 18 methylenes whereas the terminal methyl protons which appeared at 0.9 ppm similarly on integration relative to the peak at 4.39 ppm revealed the existence of six protons thus accounting for two methyl groups. The ^{13}C NMR showed a C-6 peak at 115.86 ppm which corresponds to a quartenary carbon bearing the bridging carbon holding a methyl and a proton. This seems to confirm the presence of two alkyldihydroxy-1,4-benzoquinones in (21). The mass specrum showed significant fragments at m/e 320, 294, 180, 154, 139 and 125. The molecular ion did not show. However, the most striking feature of the mass spectrum of this compound was the peak at m/e 294 nm which evidenced the existence of embelin (10) and/or myrsinone (55). The latter was ruled out as additional peaks due to long range coupling between the alkyl side chain and the quinonoid proton were absent in the ^{1}H NMR spectrum. Also evident in the mass spectrum was a base peak at m/e 154 nm and a low abundance peak at m/e 153 nm. Such fragmentation patterns have been observed in embelin (10) and myrsinone (55) both of which have a quinonoid proton in the ring. Having ruled out 55 with the help of ^{1}H NMR data, mass spectral data stongly favoured the existence of embelin moiety as part of the molecule. The spectroscopic data confirm 21 as methylvilangin which according to literature has been synthesized from embelin (10) and acetaldehyde by Rao and Venkateswarlu (1964)

Table 4: ^{1}H and ^{13}C NMR ($CDCl_3$) for 21

Atom	^{1}H (ppm)	^{13}C (ppm)
1,1'	-	-
2,2'	-	-
3,3'	-	116.35
4,4'	-	-
5,5'	-	-
6,6'	-	116.09
H-C-CH_3	4,39 (q, J=7.50 Hz)	28.20
H-C-CH_3	1.59 (d, J=7.50 Hz)	16.75
7,7'	2.40 (t J=7.1Hz)	29.60
8,8',9,9',10,10', 11,11',12,12'13,13'14,14',15,15', 16,16'	1.43 -1.25 (m)	32.13,29.92,29.87, 29.84,29.81,29.77, 29.55,28.18,26.87, 26.87,22.90,22.76
17,17'	0.9 (t, J=13.2 ,6.3 Hz)	14.32
2,5, 2'5'- OH	7.82 (s. D_2O exchangeable)	-

Compound (76) Rf 0.62 (eluent: n-hexane, ethyl acetate and acetic acid-85:10:5) m.p 121-123°C was also isolated from the fruit. It turned permanently pink on exposure to concentrated ammonia vapor on t.l.c. Its UV-Vis spectrum displayed two peaks at 430 nm (2.6) and 290 nm (4.5) thus indicating the chromophore of the compound as a dihydroxy-1,4-benzoquinone. The ^{1}H NMR of the compound exhibited peaks at 7.80 ppm which was assigned to phenolic hydroxyl hydrogen bonded to a carbonyl group. Peaks were also displayed at 4.27 ppm (H, t, J=7.92 Hz), 2.05 ppm (m) and 1.29 to 1.45 ppm (m) which are unusual

for dihydroxy-1,4-benzoquinone. Integration of the peak at 7.80 ppm with respect to that at 4.27 ppm corresponded to four hydroxyl groups. The proton-proton correlation (homonuclear COSY) experiments showed coupling between proton absorbing at 4.27 ppm and the multiplet at 2.05 ppm which in turn coupled with the multiplet at 1.29 to 1.45 ppm. Integration of the peak at 2.05 ppm with respect to that at 4.27 ppm corresponded to two protons. The peak at 4.27 ppm suggested the presence of a proton sitting on the same carbon with a methylene group while the multiplet at 2.05 ppm could possibly be assigned to a methylene group sitting on the same carbon atom with a proton. The multiplet at 1.29 to 1.45 ppm could be due to the remaining methylene groups in the decyl chain. As expected for alkylated dihyroxy-1,4-benzoquinones, there was a triplet at 2.40 ppm (J=7.44 Hz) which was assigned to the benzylic methylene protons. Integration of this peak relative to the set at 4.27 ppm corresponded to four protons calculated for two methylenes. This suggested that the compound probably consisted of two alkyl substituted dihydroxy-1,4-benzoquinones bridged by a carbon holding a decyl group and a proton. Furthermore, irradiating the benzylic peak at 2.40 ppm did not affect the triplet at 4.27 ppm and the multiplet at 2.05 ppm, proving that neither the proton nor the decyl group and the alkyl side chain are vicinal Integration of the multiplet at 1.29 to 1.45 ppm relative to the peak at 4.27 ppm corresponded to 52 protons calculated for 26 methylenes. The terminal methyl groups appeared at 0.88 ppm. Integration of this peak relative to that at 4.27 ppm revealed the existence of nine protons and thus accounting for three methyl groups. On comparing the ^{13}C NMR spectral data of (76) with that of embelin (10), there was only one significant difference for the signal due to C-6. The C-6 peak, which in (10) is centred at 103.80 ppm appeared at 115.1 ppm, corresponding to a quartenary carbon bearing the bridging carbon holding the decyl group and the proton. This seems to confirm the presence of two alkyl dihydroxy-1,4-benzoquinones in (76). The mass spectrum showed significant fragments ions at 320, 294, 180, 139 and 125. The mass spectrum did not show molecular ion peak. However, it showed a base peak at m/e 154 indicating the presence of alkyldihydroxy-1,4-benzoquinone in the

molecule. The peak at m/e 294 evidenced the existence of either embelin (10) or myrsinone (55) with the latter being ruled out as additional peaks due to long range coupling between the alkyl side chain and the quinonoid proton were absent in the ^{1}H NMR spectrum. Having ruled out (55) with the help of ^{1}H NMR data, mass spectral data strongly favoured the existence of embelin unit as part of the molecule. In fact the fragment ion peak at m/e 446 seems to be due to the thermally propagated reaction in the ionization chamber in the manner reported by Rao and Venkateswarlu (1961) which fragments the compound leading to the formation of (10), m/e 294 and (80), m/e 446 as shown in figure 3. The spectral data strongly support 76 as decylvilangin - a compound that is being encountered for the first time as a natural product.

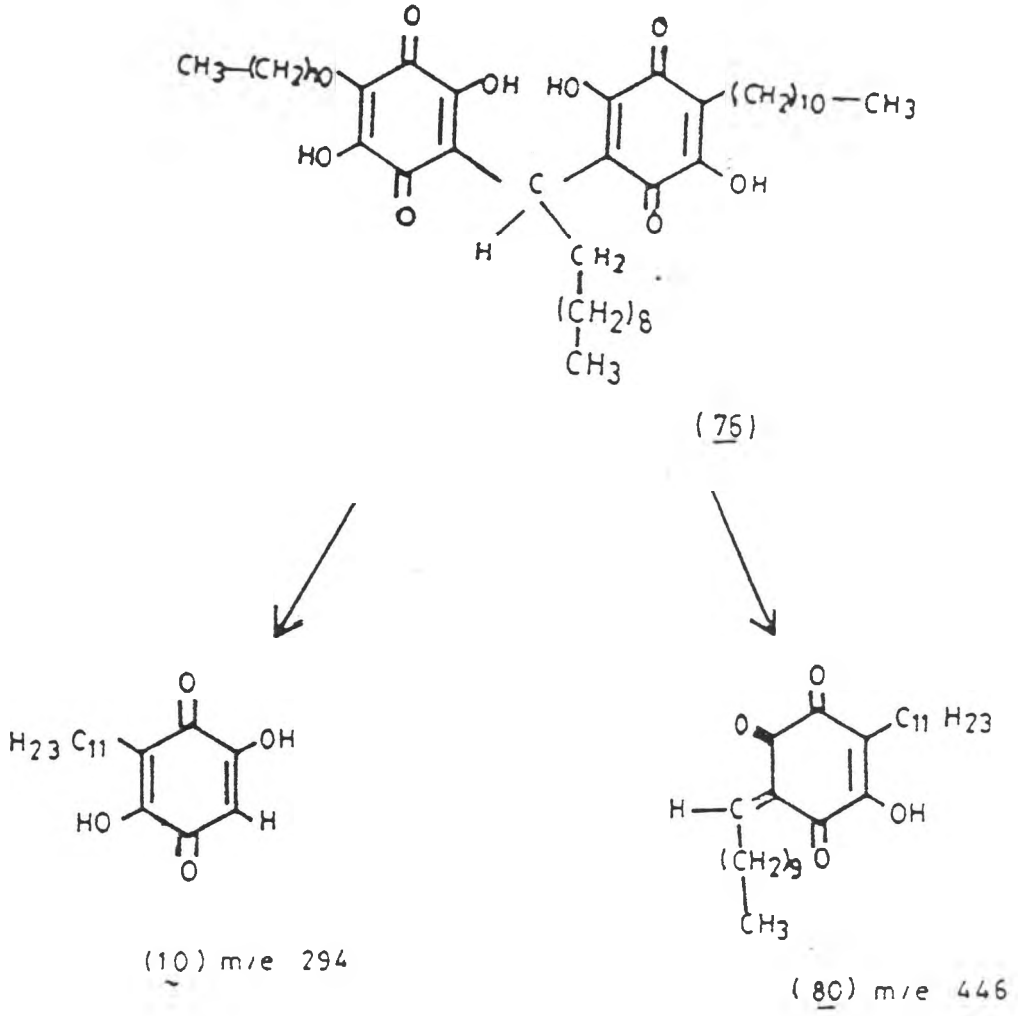


Fig.3. Decylvilangin and its neutral MS fragments.

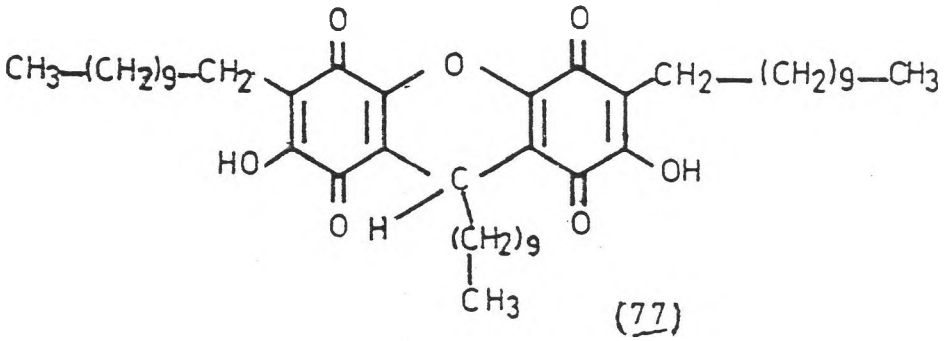
Table 5: ^{1}H and ^{13}C NMR ($CDCl_3$) for 76

Atom	^{1}H (ppm)	^{13}C (ppm)
1,1'	-	-
2,2'	-	-
3,3'	-	116.33
4,4'	-	-
5,5'	-	-
6,6'	-	115.10
H-C-CH_2-$(CH_2)_8$-CH_3	4.27 (t,J=7.92 Hz)	28.63
H-C-CH_2-$(CH_2)_8$-CH_3	2.05 (m)	28.18
H-C-CH_2-$(CH_2)_8$-CH_3	0.9 (t J=6.3 H)	14.33
7,7'	2.40 (t,J=7.44 Hz)	29.60
H-C-CH_2(CH_2)$_8$-CH_3, 8,8',9,9',10,10', 11,11',12,12',13,13', 14,14',15,15',16,16'	1.45 - 1.25 (m)	32.33,32.13,30.43, 29.84,29.77,29.74, 29.55,22.90,22.79
17,17'	0.9 (t J=6.3 Hz)	14.33
2,5, 2',5'- OH	7.84 (s, D_2O exchangeable)	-

The other compound was designated as decylanhydrovilangin, (77) Rf 0.9 (eluent: n-hexane, ethyl acetate and acetic acid-85:10:5) was isolated from the two parts of the plant. It is orange in color with m.p 119-121°C. Like (10), it was realized in highest concentration in fruits 1.9% w/w and a lower concentration in the root bark 0.5% w/w. The compound turned permanently pink on exposure to conc. ammonia vapor which is a positive test for dihydroxy-1,4-benzoquinones. The UV-Vis spectrum displayed absorption bands at 292 nm and 450 nm which is a characteristic feature of a dihydroxy-1,4-benzoquinone moiety (Nakata et al.

Ogawa and Natori, 1968). The ^{1}H NMR of the compound exhibited six signals. There was no signal corresponding to the quinonoid proton which usually appears at 6.50 - 5.0 ppm. This suggests that the quinonoid ring is fully substituted. A singlet peak due to phenolic hydroxyl protons bonded to carbonyl appeared at 7.84 ppm. Peaks were also displayed at 4.27 ppm (H, t, J=7.92 Hz), 2.06 ppm (m) and 1.26 to 1.46 ppm (m) which are unusual for dihydroxy-1,4-benzoquinones. Integration of the peak at 7.84 ppm with respect to that at 4.27 ppm corresponded to two hydroxyl groups. The proton-proton correlation (homonuclear COSY) experiments showed coupling between proton absorbing at 4.27 ppm and the multiplet at 2.06 ppm which in turn coupled with the multiplet at 1.26 to 1.46 ppm. Integration of the peak 2.05 ppm with respect to that at 4.27 ppm corresponded to two protons. The peak at 4.27 ppm suggested the presence of a proton sitting on the same carbon with a methylene group while the multiplet at 2.06 ppm could possibly be assigned to a methylene group sitting on the same carbon atom with a proton. The multiplet at 1.26 to 1.46 ppm could be due to the remaining methylene groups in the decyl chain. Furthermore, irradiating the peak due to benzylic methylene protons at 2.41 ppm did not affect the triplet at 4.27 ppm and the multiplet at 2.06 ppm, proving that neither the proton nor the decyl group and the alkyl side chain are vicinal. As expected for alkylated dihyroxy-1,4-benzoquinones, there was a triplet at 2.41 ppm (J=7.44 Hz) which was assigned to the benzylic methylene protons. Integration of this peak relative to the set at 4.27 ppm corresponded to four protons calculated for two methylenes. This suggested that the compound probably consists of two alkyl substituted dihydroxy-1,4-benzoquinones bridged by a carbon holding a decyl group and a proton. Integration of the multiplet at 1.26 to 1.46 ppm relative to the peak at 4.27 ppm corresponded to 52 protons calculated for 26 methylenes. The terminal methyl protons appeared at 0.89 ppm. Integration of this peak relative to that at 4.27 ppm revealed the existence of nine protons and thus accounting for three methyl groups. On comparing the ^{13}C NMR spectral data of (77) with that of embelin (10), there was only one significant difference for the signal due to C-6. The C-6 peak, which in

(10) is centred at 103.80 ppm appeared at 115.1 ppm, corresponding to a quartenary carbon bearing the bridging carbon holding the decyl group and the proton. This seems to confirm the presence of two alkyl dihydroxy-1,4-benzoquinones in (77). Compound 77 differs from 76 in that it has got two hydroxyl groups less than the latter. It is reasonable to assume that 77 is formed from 76 due to loss of a molecule of water. The mass spectrum showed significant fragments ions at 320, 294, 180, 139 and 125. The peak at m/e 294 evidenced the existence of either embelin (10) or myrsinone (55) at part of the molecule with the latter being ruled out as additional peaks due long range coupling between the alkyl side chain and the quinonoid proton were absent in the ^{1}H NMR spectrum. Also evident in the mass spectrum was the strong fragment peak at m/e 154 and low abundance peak at m/e 153. Such fragmentation patterns are charateristics of embelin (10) and myrsinone (55). Having ruled out (55) with the help of ^{1}H NMR data, mass spectral data strongly favoured the existence of two embelin moeities joined together by a carbon bearing a proton and a decyl group. The spectral data strongly support the compound as decylanydrovilangin (77)



(77)

Table 6: ^{1}H and ^{13}C NMR ($CDCl_3$) for 77

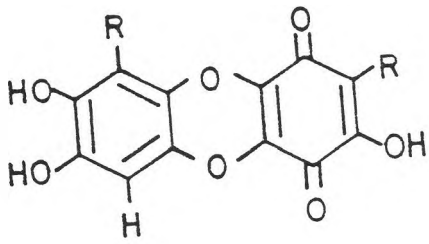
Atom	^{1}H (ppm)	^{13}C (ppm)
1,1'	-	-
2,2'	-	-
3,3'	-	116.32
4,4'	-	-
5,5'	-	-
6,6'	-	115.10
H-C-CH_2-$(CH_2)_8$-CH_3	4.27 (t,J=7.92 Hz)	28.63
H-C-CH_2-$(CH_2)_8$-CH_3	2.05 (m)	28.18
H-C-CH_2-$(CH_2)_8$-CH_3	0.9 (t J=6.3 H)	14.33
7,7'	2.40 (t,J=7.44 Hz)	29.55
H-C-CH_2(CH_2)$_8$-CH_3, 8,8',9,9',10,10', 11,11',12,12',13,13', 14,14',15,15',16,16'	1.45 - 1.25 (m)	32.33,32.15.30.43, 29.84,29.77,29.74, 29.55,22.90,22.79
17,17'	0.9 (t J=6.3 Hz)	14.33
2, 2' - OH	7.84 (s, D_2O exchangeable)	-

The next compound was a red substance (56) Rf 0.53 (solvent system: same as that for 10), m.p 166- 167°C was isolated from the CH_2Cl_2 eluent. It turned pink on exposure to conc. ammonia vapor and therefore could be a dihydroxy-1,4-benzoquinone. Its UV-Vis spectrum displayed a peak at 273.5 nm, 375 nm and 460 nm. The ^{1}H NMR exhibited a triplet for the alkane terminal methyl protons at 0.90 ppm which upon integration corresponded to six protons thus suggesting the presence of two methyl groups. The signal at 1.50-1.20 ppm was assigned to the

chain methylene protons and it corresponded to thirty six protons. There was a triplet at 2.40 (J=7.2 Hz) ppm which could be attributed to the benzylic methylene protons on the quinonoid ring and another triplet at 2.74 (J=7.2) ppm which probably originated from the methylene attached to the aromatic benzenoid system. The compound also exhibited a singlet peak at 7.26 ppm which upon integration corresponded to one proton. The mass spectrum showed the parent molecular ion peak at m/e 570 nm accounting for $C_{34}H_{50}O_7$. The presence of three hydroxyl groups in the molecule was suggested by the characteristic ^1H NMR signals at 7.05, 6.02, 5.84 ppm which were D_2O exchangeable. All these spectroscopic data suggest that (56) is a fused bisalkylbenzoquinone system in which one of the quinonoid rings has been aromatised.

Since the chemical shift of the side chain alkyl group on the quinonoid ring of (56) is quite similar to that of (10), it can be assumed that it has adjacent hydroxyl groups. Considering that there is a single aromatic proton in the ^1H NMR of (56), it can comfortably be concluded that it is not vicinal to the alkyl side chain because such an arrangement leads to long range coupling as in the ^1H NMR spectrum of myrsinone (55). The ^{13}C NMR showed peaks at 146.90 (C-5), 149.30 (C-6), 119.0 (C-11) and 108.0 ppm suggesting a dioxane type of fusion between the benzenoid and quinonoid rings. This was further supported by calculating the carbon chemical shifts around the aromatic ring basing on the fact that the benzenoid and the quinonoid rings are joined to two oxygen atoms as in structure (56) which gave the following results: C1-118.30, C2-146.40, C3-139.10, C4-106.50, C5-142.30 and C6-143.10 ppm. These results could be matched with peaks in the spectrum at 114.32, 149.40, 141.0, 108.34, 146.90 and 114.30 ppm respectively. A heteronuclear COSY experiment showed a three-bond interaction between the ring proton and carbon atoms absorbing at 149.40 and 149.30 ppm - the two carbons C-2 and C-6 which can be interchanged. Complete assignment for all the carbon atoms were made as C7-169.40, C8-148.10, C9-107.90, C10-

173.30, C11-119.00 and C12-108.00 ppm. The spectroscopic data seem to support 56 as myrsinaquinone.



R = $C_{11}H_{23}$

R = $C_{13}H_{27}$

56

Table 7: ^{1}H and ^{13}C NMR data ($CDCl_3$) of 56.

Atom	^{1}H (ppm)	^{13}C (ppm)
1	-	114.33
2	-	149.40
3	-	141.00
4	7.26 (s)	108.34
5	-	146.90
6	-	149.30
7	-	169.40
8	-	148.10
9	-	107.90
10	-	173.30
11	-	111.90
12	-	108.00
13	2.74 (t, J=15.0 Hz)	29.60
13'	2.40 (t,J=7.2 Hz)	29.40
14,14',15,15'.16,16' 17,17',18,18',19,19' 20,20',21,21'	1.49 - 1.25 (m)	31.80, 29.30,29.10, 23.80, 22.60
22, 22'	0.90 (t,J=6.3 Hz)	14.00
2 - OH	6.02 (s, D_2O exchangeable)	-
3 - OH	5.54 (s, D_2O exchangeable)	-
8 - OH	7.05 (s, D_2O exchangeable)	-

CHAPTER THREE.

3.0.0. EXPERIMENTAL.

3.1.0. GENERAL.

3.1.1. Instruments and spectra.

Plant materials were ground into powder using a Willey mill. Gallenkhamp melting point apparatus was used for melting point determination.

The Ultra Violet/Visible spectra were obtained using Beckman DU-50 spectrophotometer. High resolution ^{1}H NMR and ^{13}C NMR spectra were obtained from University of Botswana. Mass spectra were obtained from International Center of Insect Physiology and Ecology (ICIPE).

3.1.2. Plant materials.

Parts of Embelia schimperi were collected from Kericho which is 2400m above mean sea level and 260 Km west of Nairobi while parts of Embelia keniensis were collected from Limuru which is 35 Km west of Nairobi. The plant species were identified at the herbarium in the department of Botany, University of Nairobi. The fruits and roots' bark brought to the laboratory were cut-up dried and ground into powder before solvent extraction.

3.1.3. Qualitative analysis

Thin layer chromatography (TLC) was used analytically to monitor the composition or homogeneity of fractions from columns during column chromatographic separations. The 2,5-dihydroxybenzoquinone compounds are highly unstable on non-deactivated silica gel. For this reason, the commercial analytical TLC plates were de-activated by impregnating with 3% oxalic acid in methanol. This was done by dipping the TLC plates for 2-3 minutes and then drying them for about 5 minutes in an oven at a temperature of 110°C. The plates were developed in one of the following solvent systems; n-hexane, ethyl acetate, acetic acid (85:10:5), n-hexane, ethyl acetate, acetic acid (80:15:5) or n-hexane,

ethyl acetate, acetic acid (75:20:5). The organic compounds were located on the plates by exposing them to either ammonia vapor, U.V radiation and/or iodine vapor. The Rf value for each compound was then determined and recorded.

3.1.4. Quantitative analysis

Column chromatography was employed for the separation of most of the compounds although in some cases preparative thin layer chromatography had to be used to effect separation of compounds whose Rf values were very close.

The Merck silica gel used was normally de-activated using 3% oxalic acid and gravity eluted using n-hexane, dichloromethane and ethyl acetate (or various combinations of these solvents). Solvents were removed from various fractions in vacuo using rotatory evaporator. In cases of incomplete separation, preparative TLC was employed for further purification. A slurry of silica gel in 3% oxalic acid solution was used to prepare plates of 2 mm thickness on a 20 cm x 20 cm glass slab. These were then air dried overnight and transferred to the oven at a temperature of 110°C for about 30 minutes. De-activation of silica gel for use in column chromatography was achieved by soaking in 3% oxalic acid solution. The solution was decanted as much as possible and dried in an oven at a temperature of 110°C for 30 minutes.

3.2.0. PRELIMINARY WORK.

3.2.1. Determination of quinonoid pigments in root bark of *Embelia schimperi* and *Embelia keniensis* using Thin Layer Chromatography.

Dry ground root bark of Embelia schimperi and Embelia keniensis were extracted with cold ethyl acetate and the solvent removed in vacuo leaving dark gummy solids. Qualitative TLC analysis of extract using the solvent mixture n-hexane, ethyl acetate, acetic acid (85:10:5) for development revealed the presence of the following components: Rf 0.9, 0.55, 0.53 and 0.47. The first three turned pink when the plate was exposed to ammonia vapor. The fourth compound was not

ammonia sensitive but absorbed UV radiation . Qualitative TLC analysis of the extract from and Embelia keniensis revealed that the components ocurred in very trace amounts and thus was not followed for large scale extraction.

3.2.2. Thin Laver Chromatographic examination of E. schimperi and E. Keniensis fruits for quinonoid pigments.

Extraction of ground fruits of Embelia schimperi and Embelia keniensis were done as in 3.2.1. TLC analysis of the extract from Embelia schimperi showed presence of the following components: Rf 0.9, 0.64, 0.62, 0.55 and 0.49. All the spots turned permanently pink on exposure to ammonia vapor. TLC analysis of the extract from Embelia keniensis showed that components ocurred in very trace amounts and thus was not followed.

3.3.0. LARGE SCALE EXTRACTION AND ISOLATION OF COMPOUNDS FROM *EMBELIA SCHIMPERI.*

3.3.1. Extraction of root bark.

A quantity of 2 Kgs of dry ground root bark of E.schimperi was soaked in ethyl acetate (3 liters) in the cold for 48 hrs while mechanically stirring. The root bark residue was further extracted for another 24 hours to ensure that the components were exhausively extracted. The extract was then filtered under suction and concentrated in vacuo using rotary evaporator leaving 97.3 g of a sticky dark paste.

3.3.2. Fractionation of the extract.

A sample of the extract (41 g) was pre-adsorbed on dry de-activated silica gel and subjected to column chromatography in a column packed under n-hexane with 500 g of de-activated silica gel. The column was exhaustively eluted with n-hexane, dichloromethane and ethyl acetate respectively and a fraction of each solvent was collected separately. The n-hexane fraction contained mainly fatty acid material; the dichloromethane fraction (with aid of TLC analysis) was found to

contain the quinonoid pigments. The ethyl acetate fraction was not followed because the components occured in trace amounts on TLC.

3.3.3. Isolation of compounds from the dichloromethane extract concentrate.

The concentrate (5.93 g) was found to contain a compound, embelin, (10), Rf 0.55 (solvent system: n-hexane, ethyl acetate and acetic acid - 80:15:5) in large quantities than others and therefore masked the minor ones on t.l.c. This prompted repeated fractional crystallization of the extract in dichloromethane until crystals of embelin were no longer in the mother liquor. A quantity of 3.22 g of the concentrate was pre-adsorbed on de-activated silica gel and subjected to a column packed under n-hexane with de-activated silica gel. The polarity of the system was increased with increased addition of dichlororomethane up to 100% dichloromethane. The column was finally washed with 5% methanol in dichloromethane. A total of 32 fractions were collected and the eluate were monitored by t.l.c. Fractions (1-3) from n-hexane gave mainly fatty acids and their derivatives. These were combined, the solvent recovered and the mixture discarded. Eluate (4-11) yielded a yellowish substance which on t.l.c using n-hexane , ethylacetate and acetic acid (80:15:5) as a solvent system afforded components with Rf values 0.9, 0.55, and 0.47. The latter component was not ammonia sensitive but absorbed UV radiation. The fractions were combined and subjected to column chromatography. Elution of the column with n-hexane and dichloromethane (70:30) afforded four fractions which when monitored on t.l.c with the above solvent system showed components of Rf values 0.53 and 0.49 both of which occurred in very small amounts. Elution of the column with dichloromethane afforded a component with Rf value 0.53 and traces of two of the earlier components whose Rf values are 0.55 and 0.49 (same solvent system).

3.3.4. Purification of dichloromethane concentrate fractions.

Fractions (4-11) were combined and further subjected to column chromatography on de-activated silica gel with n-hexane - dichloromethane (70:30) as eluent. Fractions of 5 ml each were collected and monitored by t.l.c. The

corresponding fractions were combined, solvent evaporated in vacuo and the respective constituents crystallized from dichloromethane - methanol (9:1) to give components with Rf values 0.9 (77, 16 mg), 0.55 (10, 600 mg) and 0.47 (uncharacterized compound , 120 mg). The first two spots turned permanently pink on exposure to concentrated ammonia vapor while the third was UV sensitive. Fractions eluted with n-hexane-dichloromethane (1:1) were concentrated in vacuo to afford a residue (0.39 g) which was subjected to column chromatography over treated silica gel (20 g). Elution was accomplished with the same solvent system. Components with Rf values of 0.55 (10, 64 mg) and 0.53 (56, 19 mg) were obtained. Both spots changed to pink on exposure to concentrated ammonia vapor. Compound (56) was recrystallized from dichloromethane - methanol (9:1). The eluate obtained from dichloromethane were further resolved by column chromatography over treated silica gel before being subjected to preparative TLC to get rid of traces of more polar compounds from (56). The acid treated preparative t.l.c plates used for separation were developed three times. Additional 10 mg of pure (56) was obtained. Eluate from dichloromethane - methanol (95:5) contained mainly the more polar components occurring in very trace amounts.

Compound (10) - embelin, Rf 0.55 (characterized before from the same plant) was an orange compound with m.p 140-142°C (same as in the literature). The ^{1}H , ^{13}C NMR and mass spectra were not obtained.

Compound with Rf 0.9 (77), decylanhydrovilangin is an orange compound with mp 119-121 ^{0}C (unreported in the literature). UVλ max (MeOH) at 292 nm and 450 nm. ^{1}H and ^{13}C NMR data are displayed in table 6. MS (70 eV): m/e (%) M^{+} 722, 294(26) , 155 (46) , 154 (100) , 153 (26), 142 (28) 125(11)

Compound (56) - myrsinaquinone is a red compound with Rf 0.53 and mp 166 - 167^{0}C. UVλ_{max} (MeOH) at 273.5 nm and 430 nm . The 1H and ^{13}C NMR are in table 7. MS (70 eV): m/e (%) M^{+} 570 (100), 598 (3), 552 (1.50), 429 (7), 346 (28), 320 (8), 289 (13), 205 (9).

Compound (KCP -02) is a yellowish oil with Rf 0.47 . UVλ_{max} (MeOH) at 275.5 nm and 428nm. This compound was not characterized due to lack of sample.

3.3.5. Extraction of E. schimperi fruit .

A quantity of 800 g of dry ground E. shimperi fruit was soaked in ethyl acetate (2 liters) in the cold for 48 hrs while mechanically stirring. The fruit residue was further extracted for another 24 hrs. The latter extraction was to ensure that all the components were exhaustivelly extracted. The extract was then filtered under suction and concentrated in vacuo using rotary evaporator leaving 55.78 g of a sticky dark paste .

3.3.6. Fractionation of the extract.

A quantity of 55 g of the extract was pre-adsorbed on dry de-activated silica gel and subjected to column chromatography in a column packed under n-hexane with 550 g of de-activated silica gel. The column was exhaustively eluted with hexane, dichloromethane and ethyl acetate respectively and a fraction of each solvent collected separately. The n-hexane fraction contained mainly fatty acid material; the dichloromethane eluent (with aid of t.l.c analysis) was found to contain the quinonoid pigments. The ethyl acetate fraction was not followed because the components occured in very trace amounts.

3.3.7. Isolation of compounds from dichloromethane eluant.

A sample of 30 g was obtained after concentrating the dichloromethane eluant in vacuo. It was found to contain a compound. embelin, (10), Rf 0.55 (solvent system: ethyl acetate , dichloromethane and ethyl acetate - 80:15:5) in larger amounts than others and therefore masked the minor ones on t.l.c. This prompted repeated crystallization of the extract in dichloromethane until crystals of the compound were no longer in the mother liquor. From the residue (i.e after removal of 10), a quantity of 15 g of which was pre-adsorbed on dry de-activated silica gel and subjected to column chromatography in a column packed under n-hexane with de-activated silica gel. The polarity of the system was increased with

addition of volumes of dichloromethane or ethyl acetate. A total of 29 fractions (200 ml each) were collected and the eluate were monitored with de-activated t.l.c plates.

Fractions 1-5 (eluted with 10% dichloromethane in n-hexane) showed a spot with Rf 0.9 and which turned permanently pink on exposure to conc. ammonia vapor. This was re-purified by crystallizing in dichloromethane - methanol (9:1) to give 26 mg of (77). Fractions 6-12 were mainly composed of components with Rf values 0.9, 0.64 and 0.62. These fractions were combined. solvent evaporated and the residue subjected to repeated column chromatography eluting with n-hexane - dichloromethane (30:70) as a mobile phase to give 3 mg more of the component with Rf value of 0.9 (77) , 0.64 (76, 5 mg) and 0.62 (21. 110 mg) respectively. Fractions 13-17 afforded a spot which turned permanently pink on exposure to conc. ammonia vapor. Upon crystallization , 750 mg of embelin (10) was obtained. Fractions 18-24 (eluted with 50% dichloromethane in n- hexane) afforded 5 mg of the component with Rf value of 0.62 (21) and a pink compound whose Rf value was 0.49 (KCP-06, 30 mg). Fractions 25-29 (eluted with 100% dichloromethane) were combined and subjected to column chromatography over treated silica gel using n-hexane - ethyl acetate (90:10) followed by the same solvent (80:20). Twelve fractions of 100 ml each were collected and t.l.c analysis revealed a mixture of components. Fractions were combined based on their t.l.c profiles and the solvent evaporated. Repeated preparative t.l.c (on de-activated silica gel) with n-hexane - ethyl acetate (90:10) yielded myrsinaquinone (56, 44 mg). Eluate from n-hexane - ethyl acetate(70:30) contained mainly the more polar components occurring in trace amounts.

Compound (KCP-06) is a pink and amorphous compound with Rf 0.49 (solvent system: n-hexane - ethyl acetate - acetic acid - 80:15:5). UVλ_{max} (MeOH) at 280 nm and 425 nm. MS (70 eV): m/e (%) M^+ 530 (47.8), 512 (11.2), 390 (30), 333 (10), 250 (12.4), 249 (13.6), 154 (13.6), 97 (63.6), 83 (64.8), 69 (66), 57 (100), 43 (90), 41 (43). This compound was not characterized because the amount isolated was only enough for biological activity tests.

Compound (76) - decylvilangin, is an orange compound with Rf 0.64 and m.p 121-123°C. Its UVλ_{max} (MeOH) at 290 nm and 430 nm. The ^{1}H and ^{13}C NMR data are displayed in table 5. MS (70 eV): m/e (%) M^{+} 740 (19.6), 334 (7.2), 333 (35.6), 294 (43.6), 168 (7.2), 154 (100), 153 (19.6), 142 (20), 125 (7.2).

3.4.0. BIOASSAY.

3.4.1. Brine shrimp lethality test.

These were performed according to McLaughlin et al which briefly was as follows:Artemia salina (Brine shrimp) were hatched into nauplii (larvae) after 48 hours in artificial "sea water" in the dark and allowed to swim to a lighted area through perforations in the hatching vessel. Pure compounds (kcp-02), 21, kcp-06 and 76 were subjected to this test. The test solutions were prepared at concentrations of 5, 10, 15, 20, 25, 50, and 100 µg/ml (1 ml each) in triplicate in 2 ml dram vials in acetone. The acetone was allowed to evaporate and replaced by "sea water" containing nauplii (10 each). The number of nauplii still alive were monitored after 12 and 24 hours. The control experiments were performed in vials with no samples. The data was processed in a simple program on a personal computer to estimate LC_{50} values with 95 % confidence intervals for statistically significant comparison of potencies. The data collected after 24 hours is summarized in Table 1.

3.4.2. Anti-feedant test.

Anti-feedant activity tests were done on embelin (10), kcp-02 and 21 against Locusta migratoria migratorioides. " Choice" and control experiments were set up with ten mid-5^{th} instar nymphs which were starved for 24 hours before feeding them with the test materials (Morgan et al,1968). A 100 µg/ml (dissolved in dichloromethane)of each sample was sprayed on a 2.75 cm^2 whatman No.1 filter papers previously soaked in 0.25 M sucrose solution. The control filter papers were only treated with 0.25 M sucrose solution. All the papers were dried in an oven at 40 °C. The data collected after 8 hours is summarized in Table 2. section 2.0.3.

3.4.3. Anti-microbial test.

Candida albicans. Trichophyton metagrophyte Microsporium gypsum. and Escherichia coli was each spread on a petri dish smeared with agar as a growth medium. Concetrations of 50, 100 and 400 µg/ml of 77, 56, 21, 10 and KCP-06 were applied into "wells" made in the medium and incubated for a period of between one and three days for any inhibition zones to develop. Results are discussed in section 2.0.4.

3.4.4. Larvicidal Test.

Larvicidal test were done for (21) according to Zebitz (1986) and Mwangi and Rembold (1988). To each jar holding 40 ml of 0.09% sodium chloride, twenty 2nd instar mosquito larvae were introduced and immediately treated with 100, 75, 50, 25 and 10 µg/ml of (21). The results of this test on day 13 are shown on table 3, section 2.0.5.

3.4.5. Stored Products Pests Test.

Natural embelin (10) was tested against Sitophilus zeamais and Anthoscelides obtectus- the two storage pests for maize and beans respectively to evaluate its ability to act as a pesticide. The insects were obtained from standard laboratory cultures maintained in the insectary (Department of zoology). A culture of S.zeamais was maintained on whole maize while A. obtectus was maitained on beans. The solid test compound was incorporated into 1 Kg samples of uninfested beans and maize each in 150 x 150 mm jars at rates of 0.02, 0.04, 0.05, 0.08, 0.1, 0.2 and 0.4% (w/w) and twenty (20) mixed adults of each of the two insects were added. The experiments were performed in three replicates. Data (after five months) on adult emergence and productivity (progeny/adult-days) are summarized in fig.1 (in the case of S. zeamais) and fig.2 (in the case of A. obtectus)

CHAPTER FOUR

.0.0. COMMENTS AND CONCLUSION.

Embelia schimperi has the ability to accumulate different long alkyl side hain benzoquinone compounds.

A number of biological activity tests were performed using these ompounds and a few cases will be highligted.

Clearly embelin (10) can be developed as a commercial insecticide that can e used to protect stored beans and maize grains from attack by their respective veevils over a long period of time. It can also be applied on field crops to deter ocusts from feeding on them. The anti-feedant activity is demonstrated by the arge relative antifeedant percentage (RAP) value of 10.

Another compound that can be developed for commercial use is nethylvilangin (21). This compound was found to disrupt the process of netamorphosis in the mosquito larvae, *Aedes aegypti*. In fact, when 100 µg/ml of 1 was applied into a pond containing 2nd instar mosquito larvae, only a few nderwent metamorphosis to the 3rd instar larvae and none attained the adult stage. his biological activity can be used to reduce population of mosquitoes.

Since the concentration of the benzoquinone compounds is higher in the erries of E. schimperi it is important to encourage the use of the berries to achieve ptimum results and to avoid damage to the shrub.

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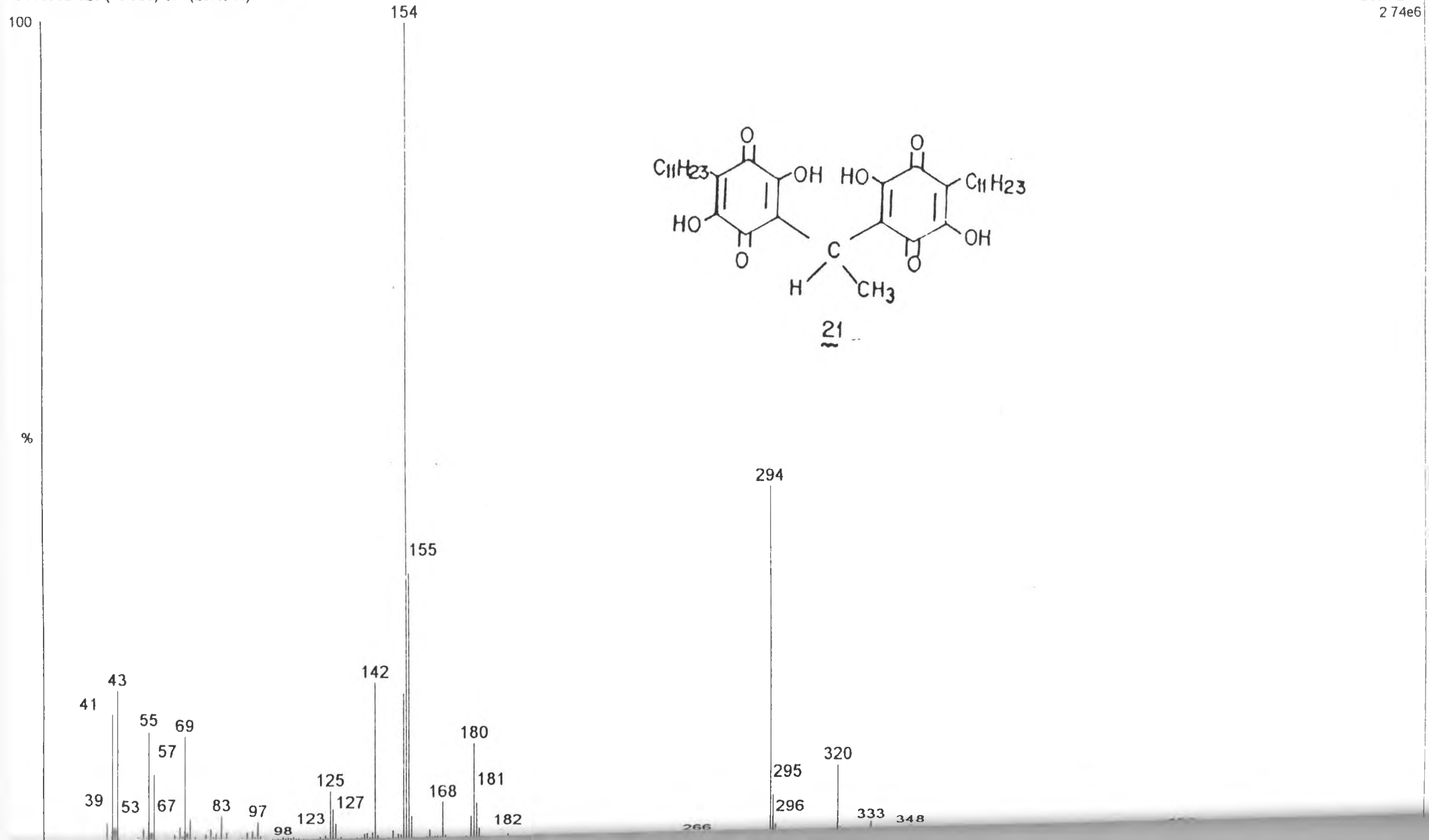
SPECTRA

Mass spectra of isolated compounds.

SAMPLE KC-05 (D.I.P.) Solid Probe program: 25(1)@60-250(6).KIPRONO C.P.01-Oct-199517:48:08NABSA/UONRUNRUN

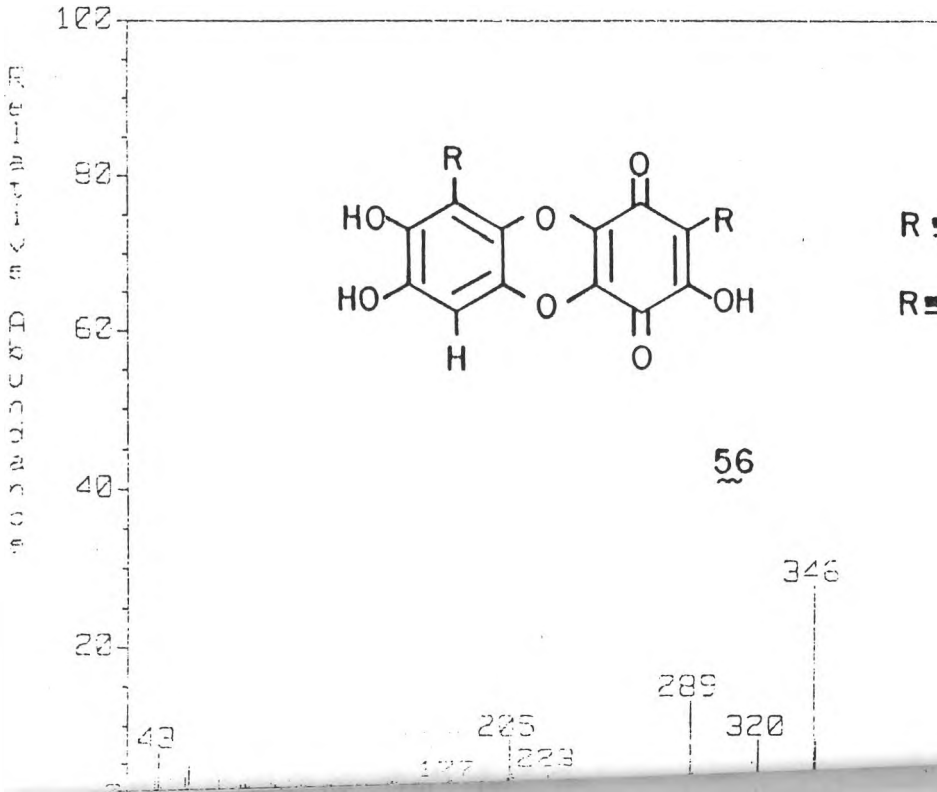
KC11095B 328 (10.959) Cm (207:344)

Scan EI+
2.74e6



21

MASS SPECTRUM Data File: 291
Sample: LMA-25
RT 2'20" EI (Pos.) GC 157.1c BP: m/z 570.
Scan# (36) - (18, 52) [coef. 1.00]



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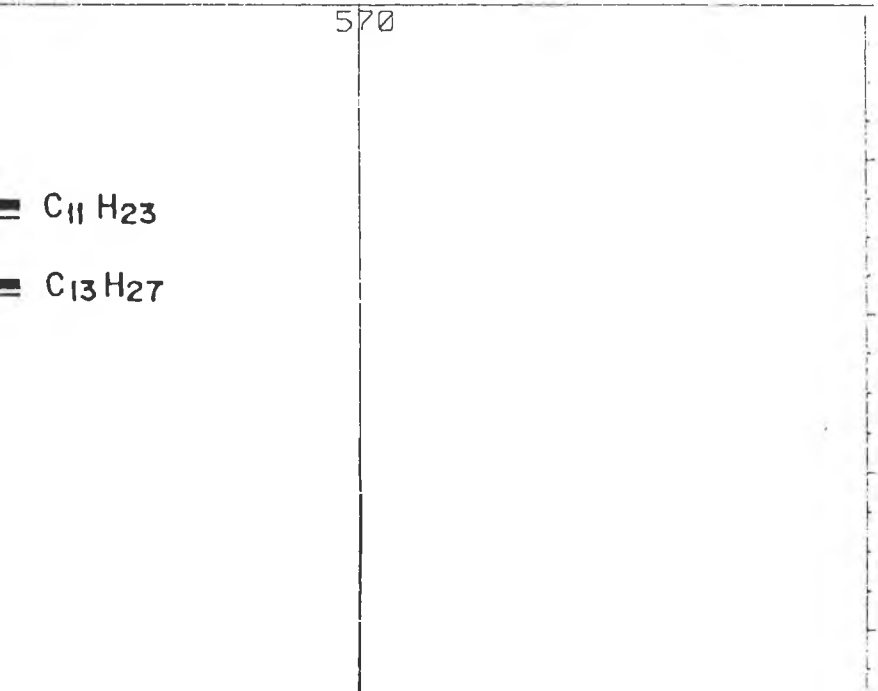

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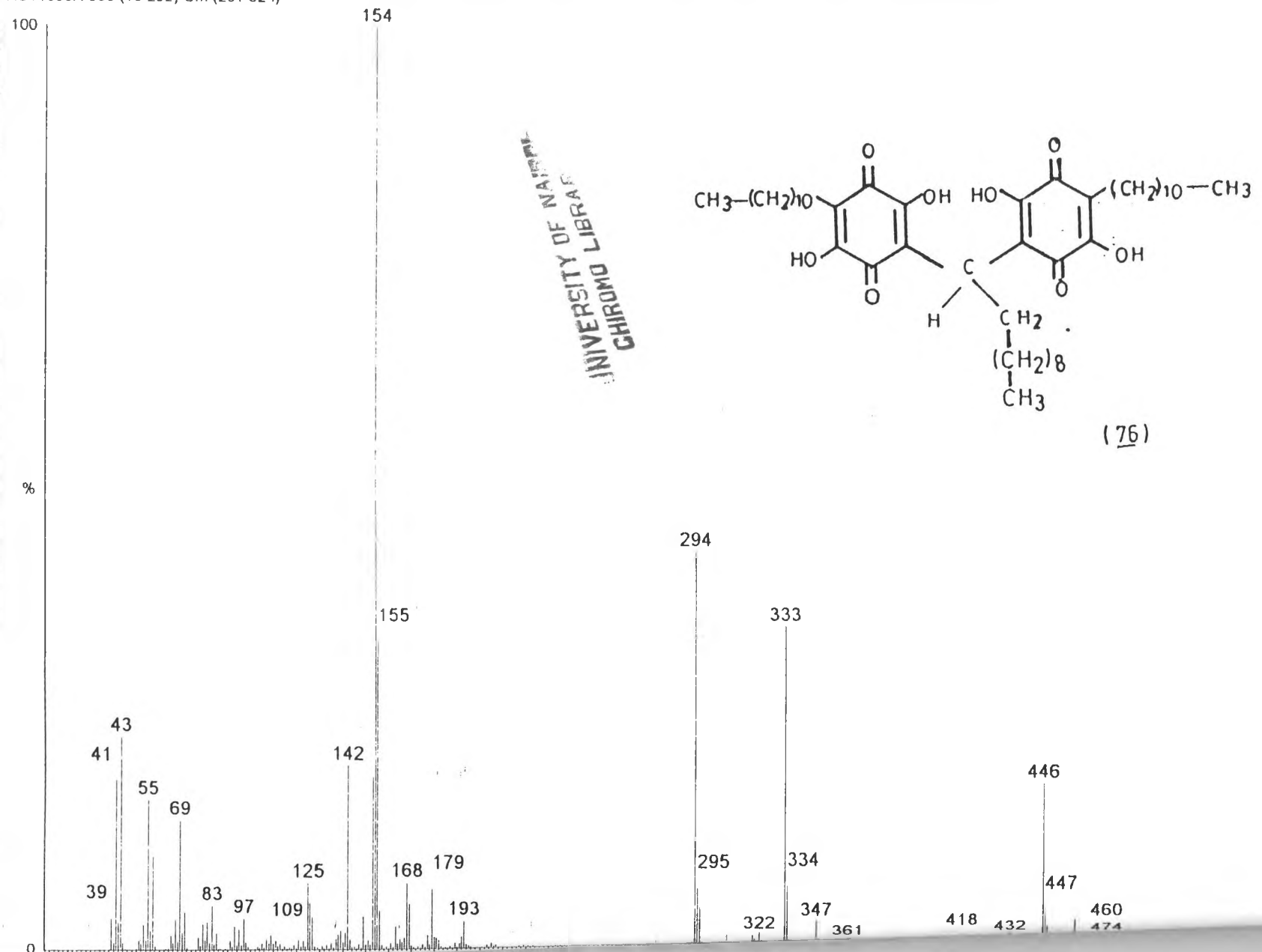
KC11095A 308 (10 292) Cm (201 324)

Scan EI+
2 19e6

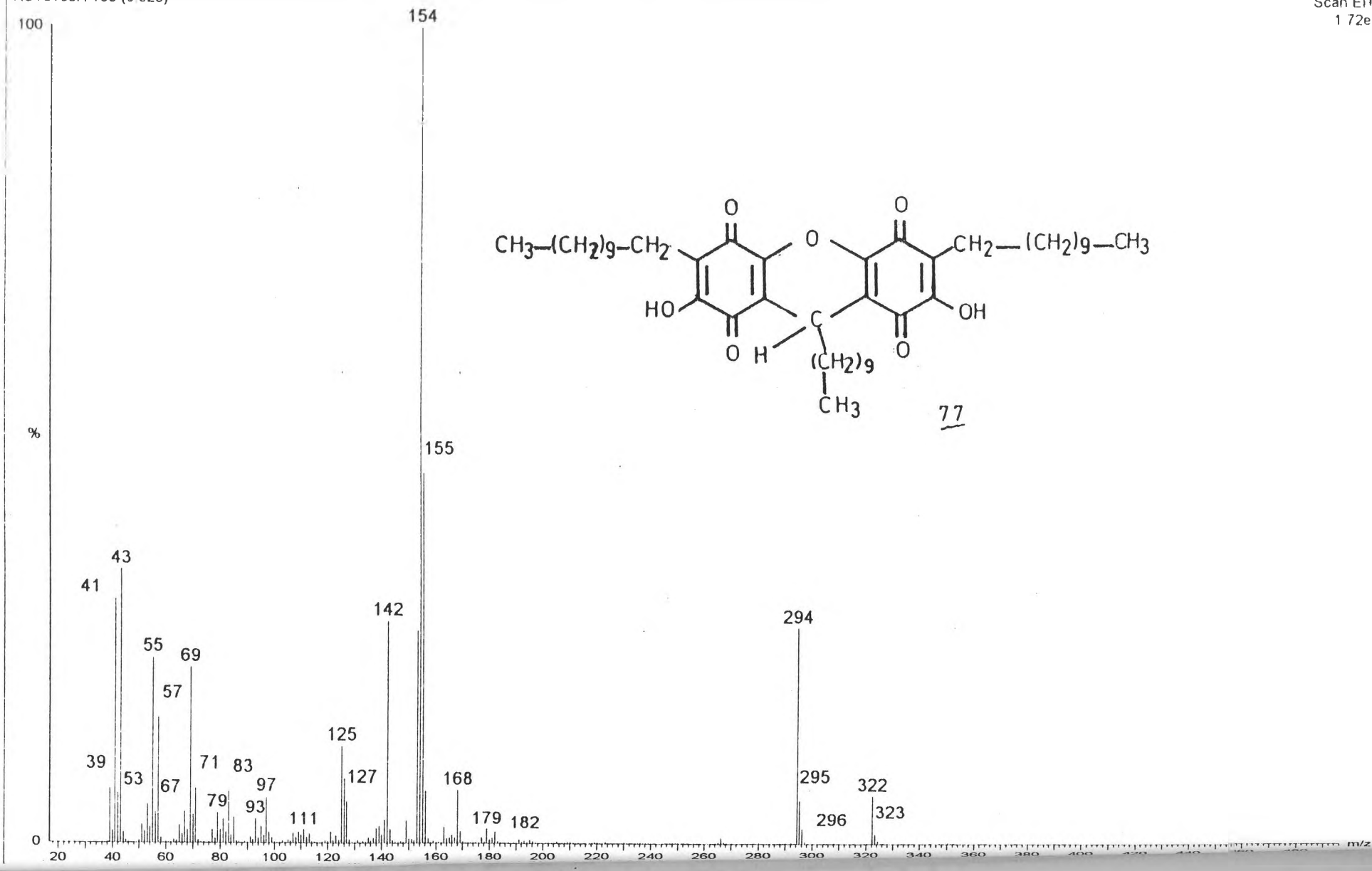


(76)

SAMPLE KCP-1 (SOLID) by Solid Probe. Prog: 25(1)@80-300(5)13-Oct-199509:57:26KIPRONO C. P.

KC13109A 180 (6.025)

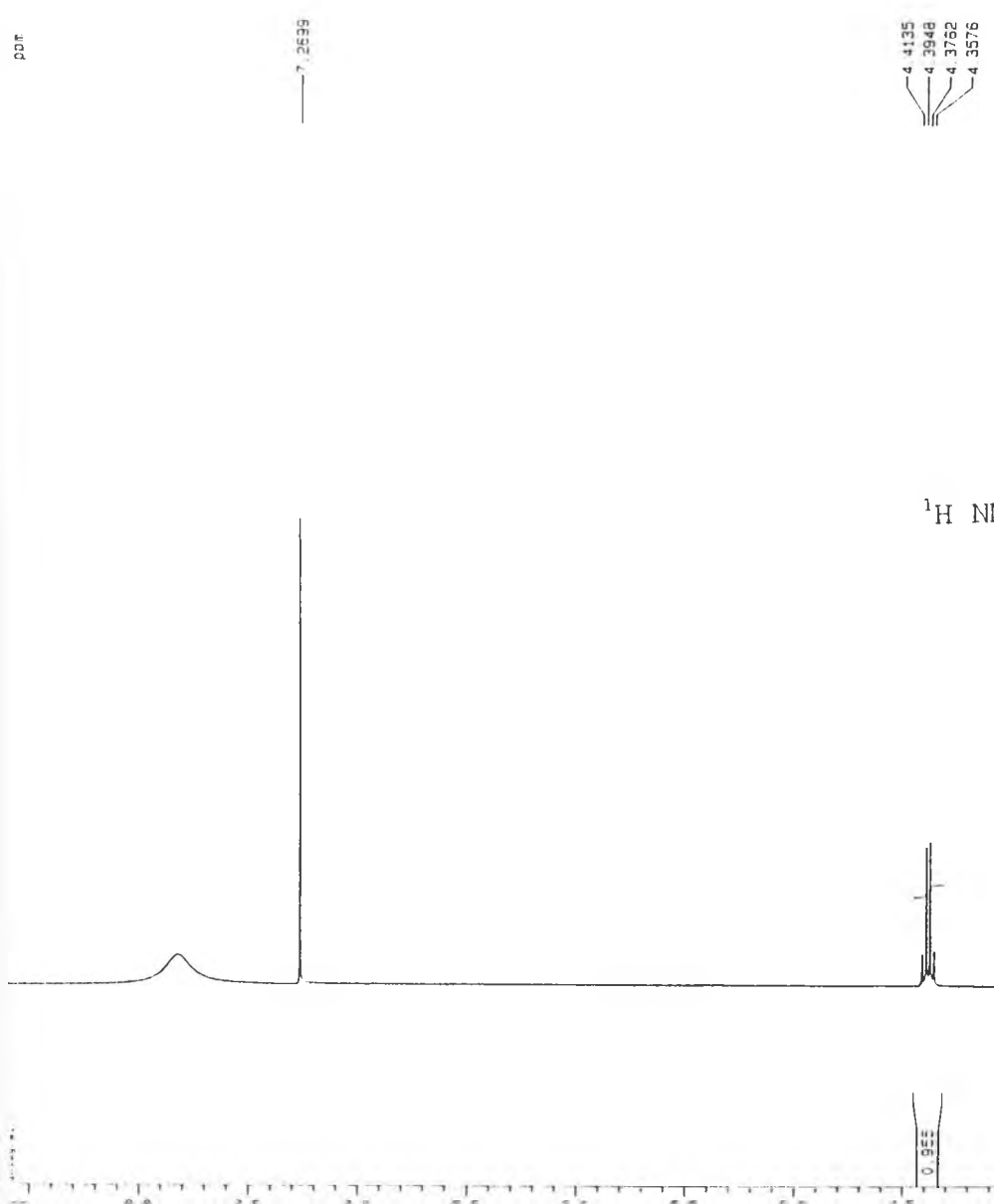
Scan El+
1.72e



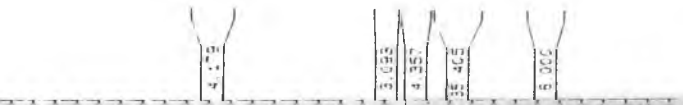
77

^{1}H NMR of isolated compounds.

Jacob
KCP 05 in CDCl3
1H spectrum



^{1}H NMR of 21



Current Data Parameters

NAME	A110138
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

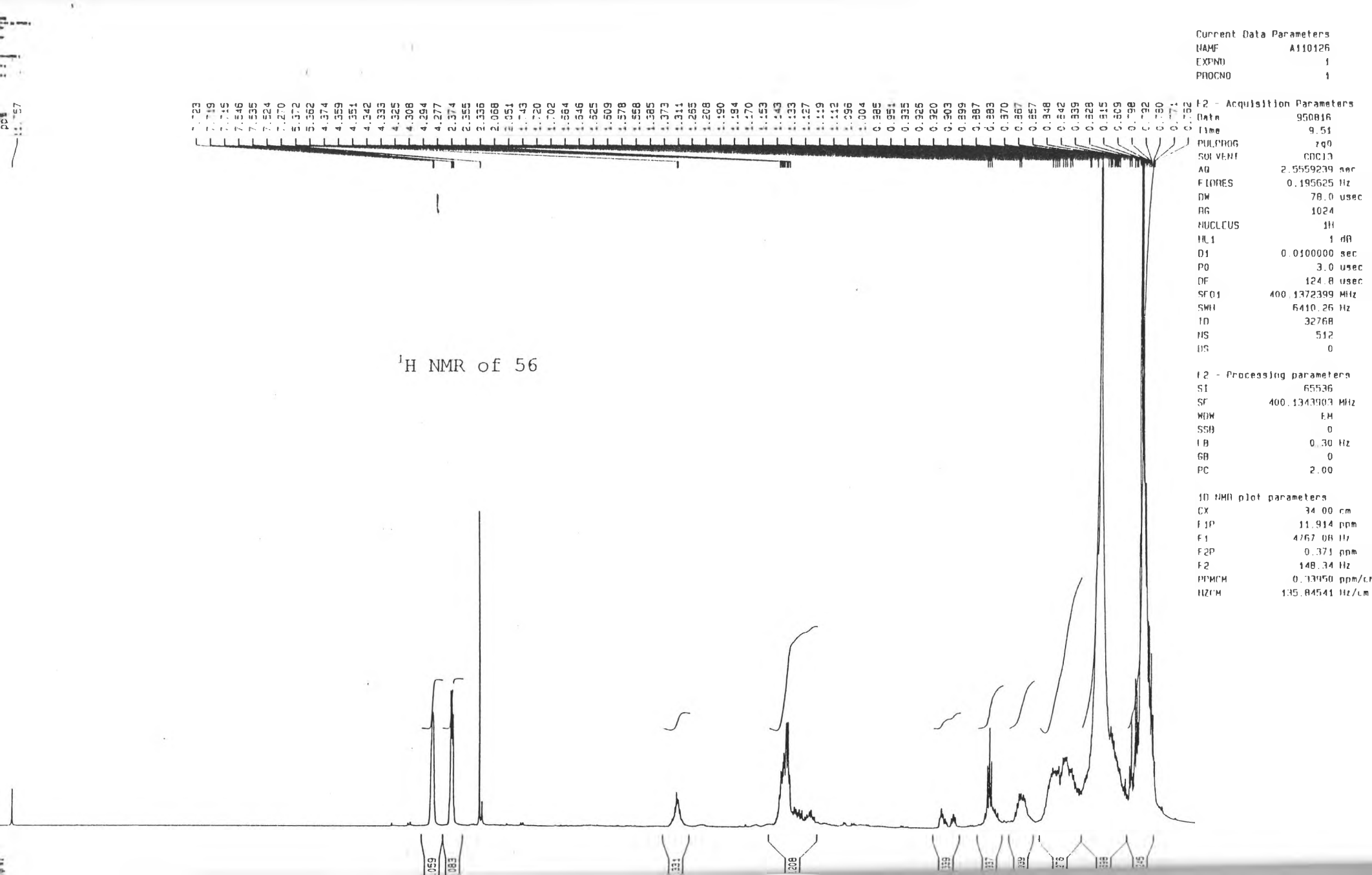
Date	950818
Time	12.19
PULPROG	zg0
SOLVENT	CDCl3
AQ	2.5559239 sec
FIDRES	0.195625 Hz
DW	78.0 usec
RG	1024
NUCLEUS	1H
NL1	1 dB
D1	0.0100000 sec
P0	3.0 usec
DE	124.8 usec
SFO1	400.1372399 MHz
SWH	6410.26 Hz
TD	32768
NS	128
DS	0

F2 - Processing parameters

SI	65536
SF	400.1343904 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	2.00

1D NMR plot parameters

CX	34.00 cm
F1P	8.625 ppm
F1	3451.34 Hz
F2P	0.266 ppm
F2	106.25 Hz
PPMCM	0.24508 ppm/cm
HZCM	98.38501 Hz/cm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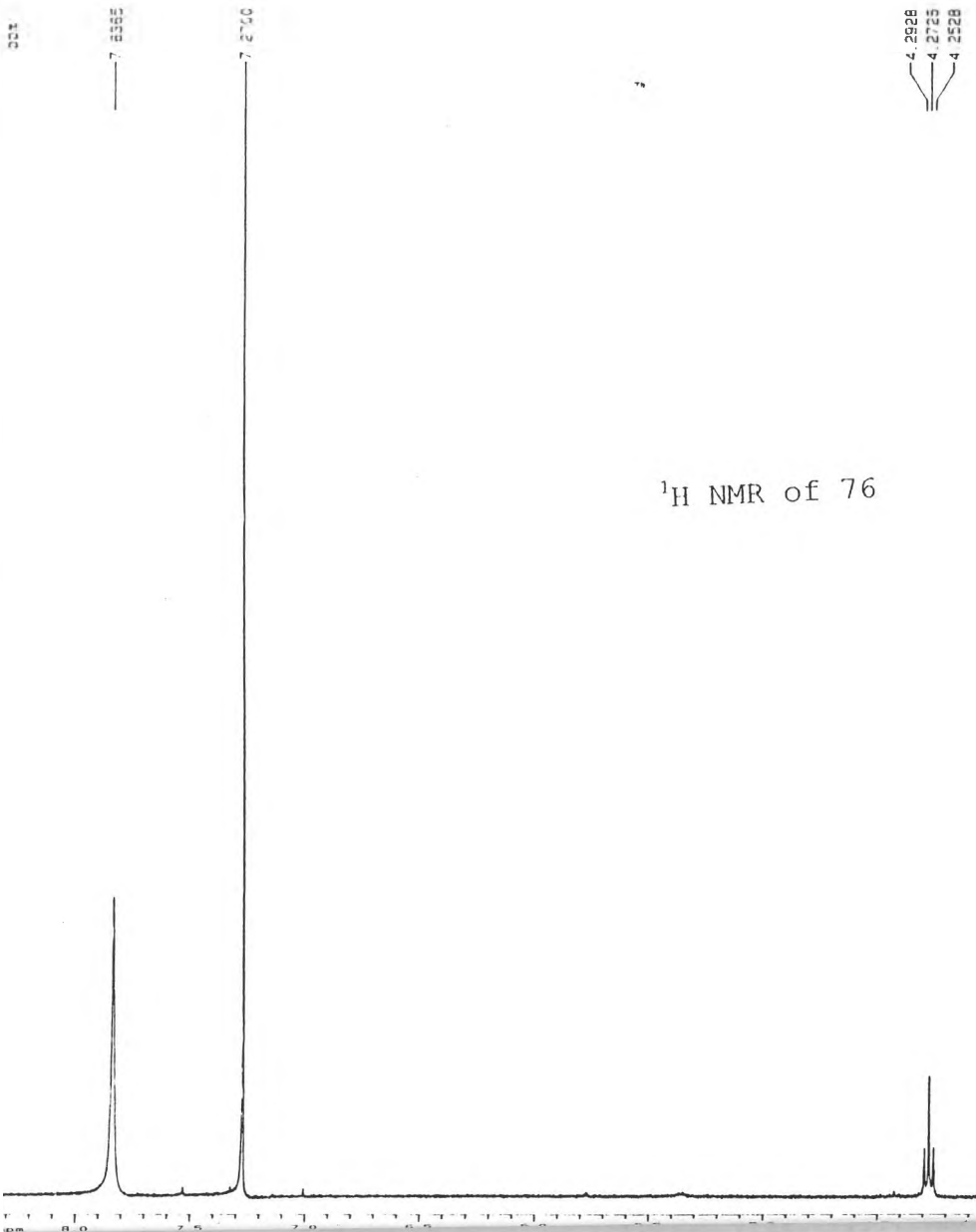
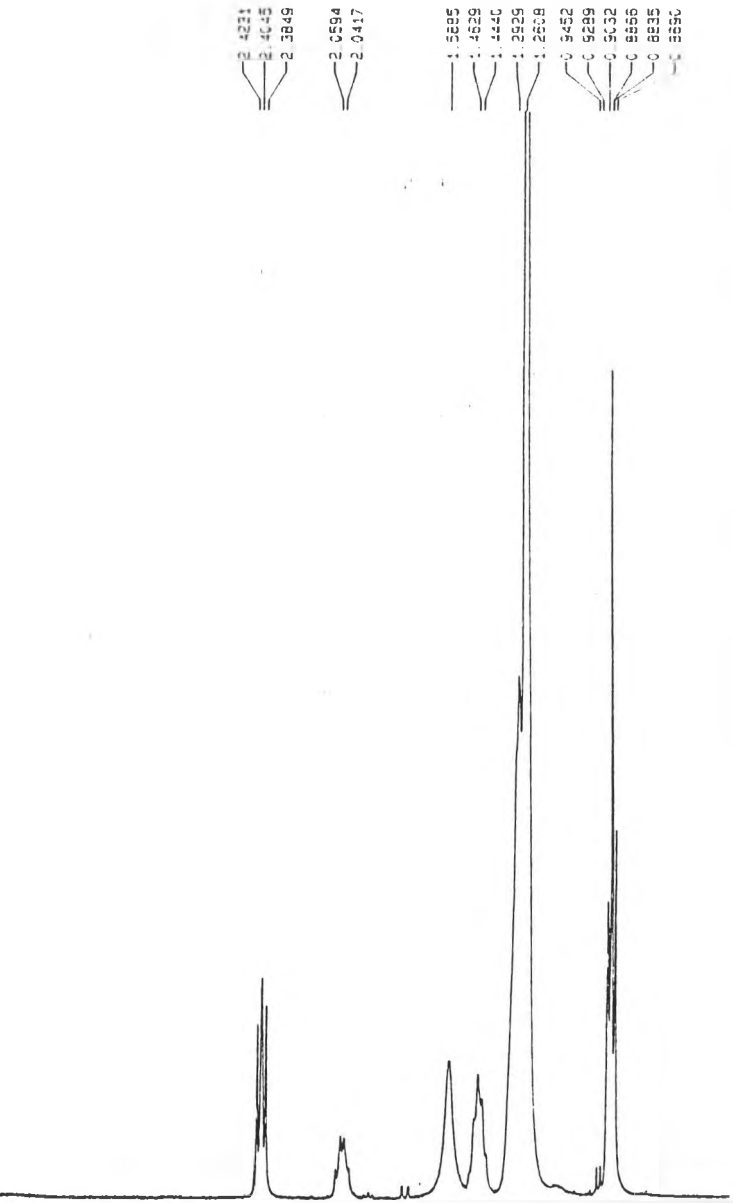
NAME A110126
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date 950816
Time 9.51
PULPROG zg0
SOLVENT CDCl3
AQ 2.5559239 sec
FIDRES 0.195625 Hz
DW 78.0 usec
RG 1024
NUCLEUS 1H
NL1 1 dB
D1 0.0100000 sec
P0 3.0 usec
DE 124.8 usec
SFO1 400.1372399 MHz
SWH 6410.26 Hz
TD 32768
NS 512
DS 0
F2 - Processing parameters
SI 65536
SF 400.1343903 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00
1D NMR plot parameters
CX 34.00 cm
F1P 11.914 ppm
F1 4767.08 Hz
F2P 0.371 ppm
F2 148.34 Hz
PPMCM 0.73950 ppm/cm
HZCM 135.84541 Hz/cm
^{1}H NMR of 56

Jacob
KTP-07 in CDCl3
1H spectrum



^{1}H NMR of 76



Current Data Parameters

NAME	A110296
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date	950919
Time	8.29
PULPROG	zg0
SOLVENT	CDCl3
AQ	2.5559239 sec
SWH	6410.256 Hz
DW	78.0 usec
RG	2048
NUCLEUS	1H
NL1	1 dB
D1	0.0100000 sec
P0	3.0 usec
DF	124.8 usec
SFO1	400.1372399 MHz
SWH	6410.256 Hz
TD	32768
NS	256
DS	0

F2 - Processing parameters

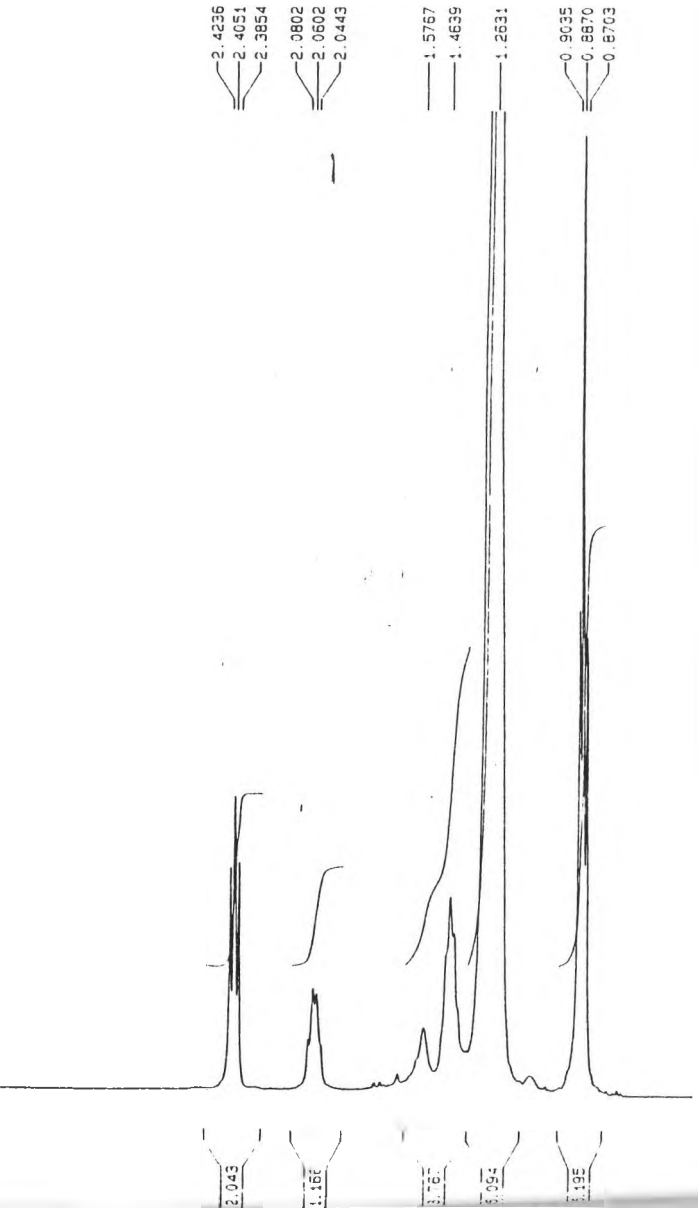
SI	65536
SF	400.1343898 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	2.00

1D NMR plot parameters

CX	34.00 cm
F1P	8.339 ppm
F1	3336.63 Hz
F2P	0.367 ppm
F2	146.75 Hz
PPMCM	0.23447 ppm/cm
HZCM	93.82017 Hz/cm

Jacob
KCP in CDCl3
1H spectrum

^{1}H NMR of 77



Current Data Parameters

NAME	A110291
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date	950917
Time	11.26
PULPROG	zg0
SOLVENT	CDCl3
AQ	2.5559239 sec
FIDRES	0.195625 Hz
DW	78.0 usec
RG	512
NUCLEUS	1H
NL1	1 dB
D1	0.0100000 sec
P0	3.0 usec
DE	124.8 usec
SFO1	400.1372399 MHz
SWH	6410.26 Hz
TD	32768
NS	256
DS	0

F2 - Processing parameters

SI	65536
SF	400.1343901 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	2.00

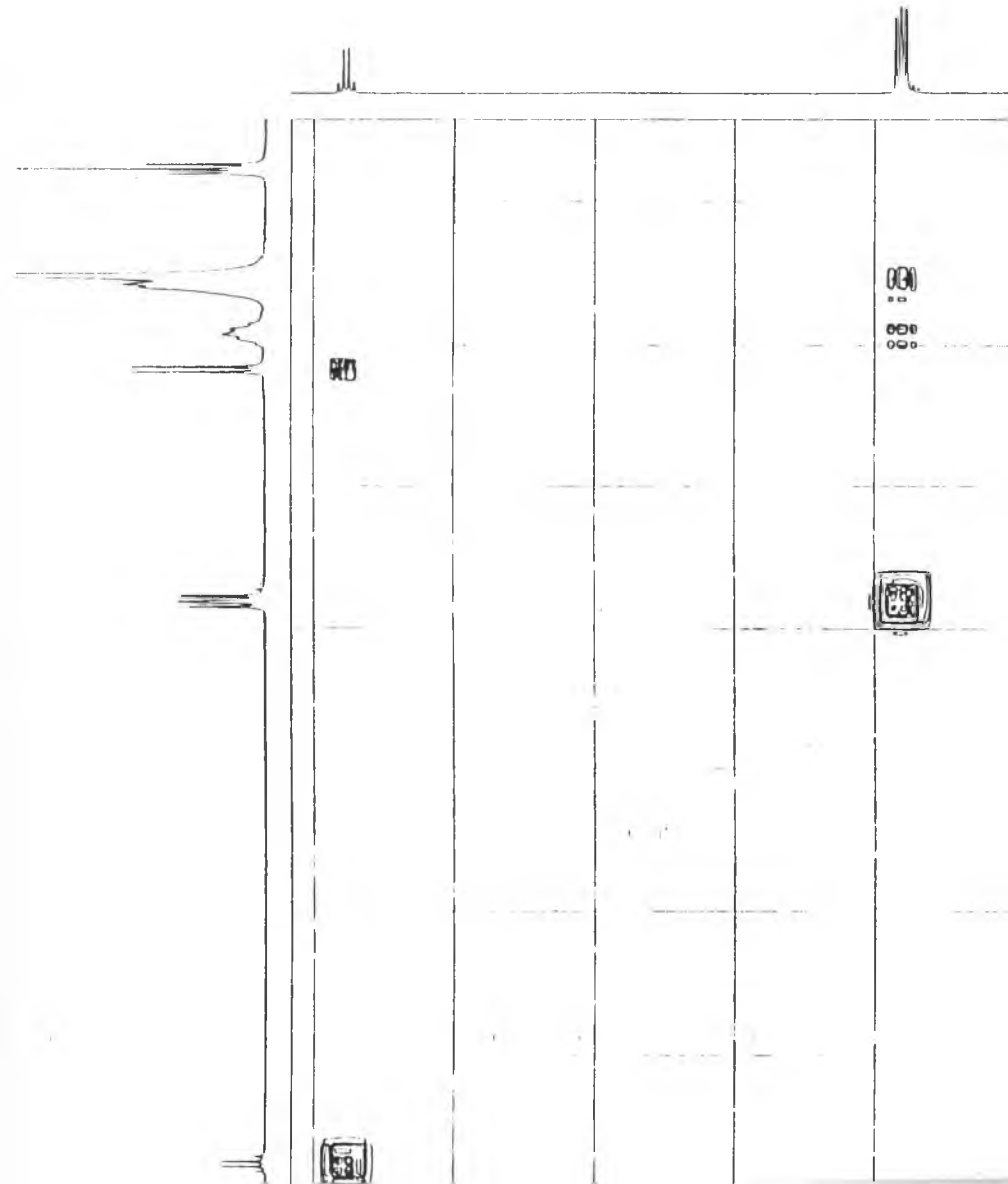
1D NMR plot parameters

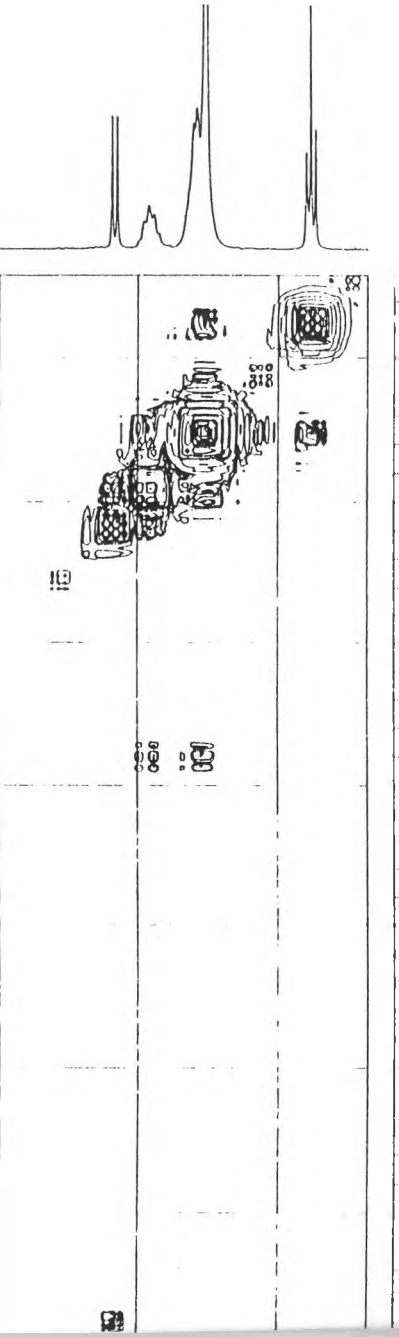
CX	34.00 cm
F1P	8.409 ppm
F1	3364.61 Hz
F2P	0.402 ppm
F2	160.74 Hz
PPMCM	0.23550 ppm/cm
HZCM	94.23165 Hz/cm

Cosy Spectra of some isolated compounds

Jacob
KCP 05 in CDCl3
noesy

COSY Spectrum of 21





Current Data Parameters

NAME	A110138
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

Date	950818
Time	15.32
PULPROG	noesytp
SOLVENT	CDCl3
AQ	0.1054920 sec
FIDRES	4.740595 Hz
DW	103.0 usec
NS	256
NUCLEUS	1H
NL1	1 dB
D1	2.0000000 sec
P1	12.0 usec
D0	0.0000030 sec
D8	0.0000000 sec
DE	164.8 usec
SFO1	400.1366359 MHz
SWH	4854.37 Hz
TD	1024
NS	16
DS	4
INO	0.0001010 sec

F1 - Acquisition parameters

NUO	2
TD	256
SFO1	400.1366 MHz
FIDRES	18.962379 Hz
SW	12.132 ppm

F2 - Processing parameters

SI	1024
SF	400.1343904 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0
PC	2.00

F1 - Processing parameters

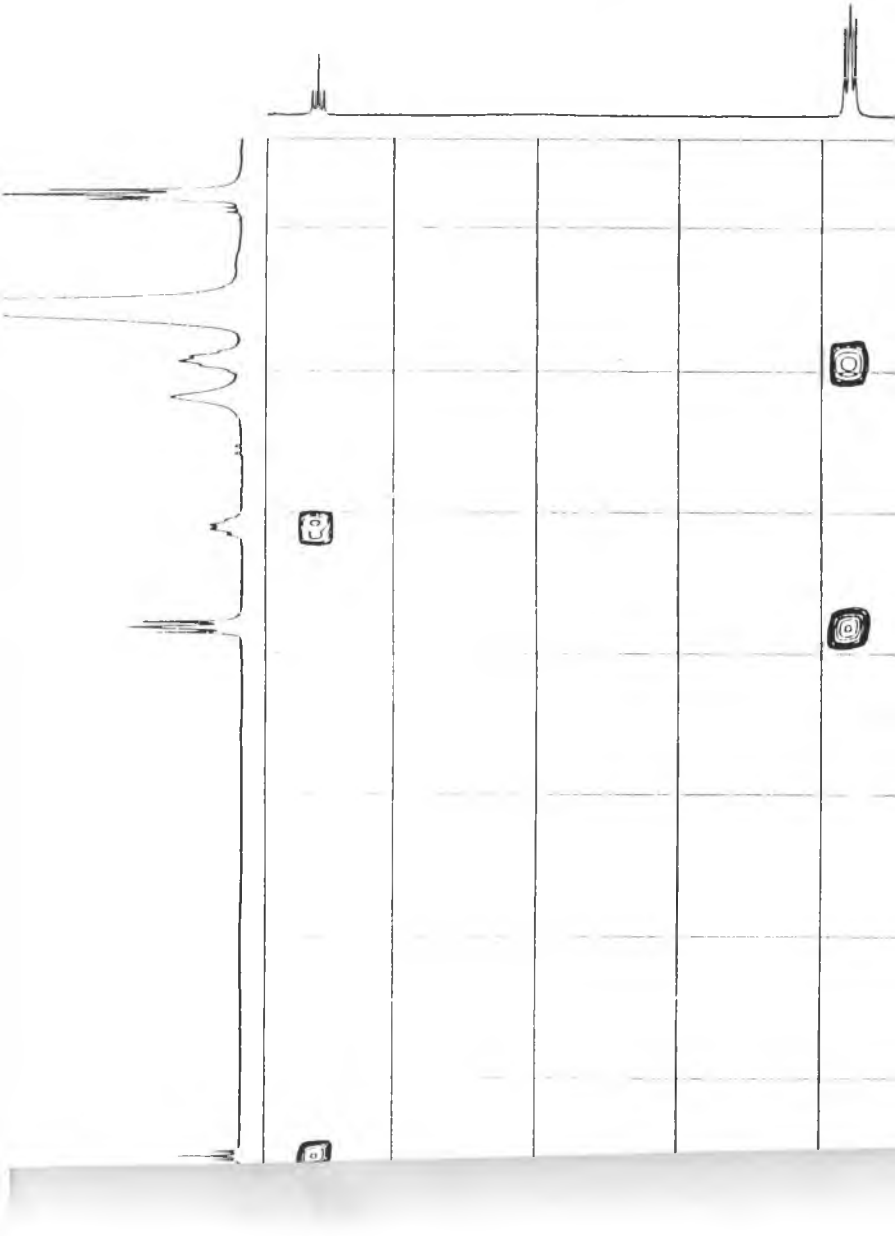
SI	1024
MC2	TPPI
SF	400.1343904 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0

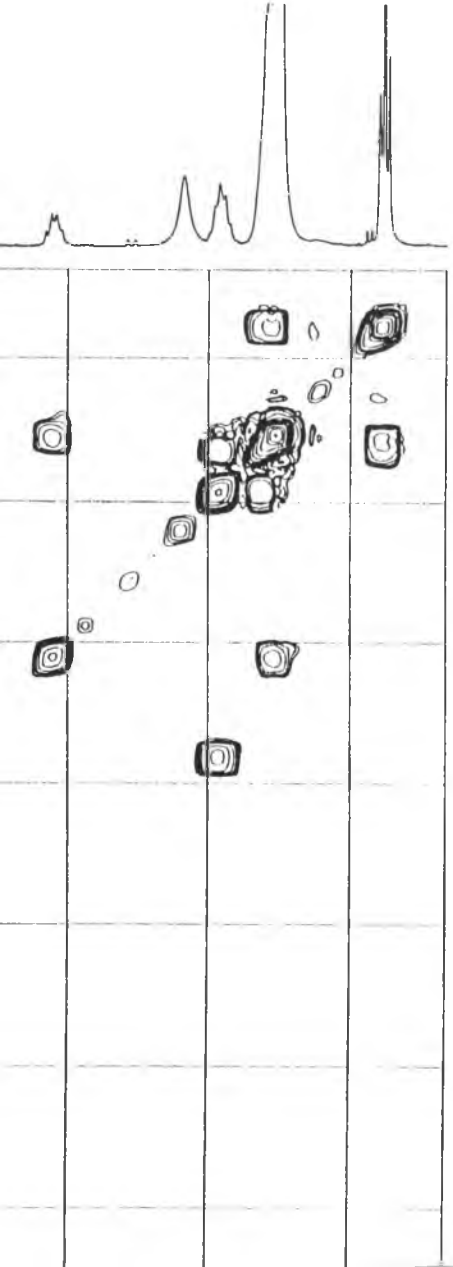
2D NMR plot parameters

CX2	20.00 cm
CX1	20.00 cm
F2PLO	4.581 ppm
F2LO	1833.08 Hz
F2PHI	0.683 ppm
F2HI	273.42 Hz
F1PLO	4.557 ppm
F1LO	1823.60 Hz

Jacob
KCP-07 in CDCl3
cosy45

COSY Spectrum of 76





Current Data Parameters

NAME	A110296
EXPNO	2
PROCNO	1

F2 Acquisition Parameters

Date	950921
Time	10.30
PULPROG	cosy
SOLVENT	CDCl3
AQ	0.1085640 sec
FIDRES	4.606427 Hz
DW	106.0 usec
RG	1024
NUCLEUS	1H
NL1	1 dB
D1	1.0000000 sec
P1	12.0 usec
D0	0.0000[illegible] sec
P0	6.0 usec
DE	169.6 usec
SFO1	400.1363600 MHz
SWH	4716.98 Hz
TD	1024
NS	4
DS	4
IN0	0.0002120 sec

F1 - Acquisition parameters

ND0	1
TD	256
SFO1	400.1364 MHz
FIDRES	18.425707 Hz
SW	11.788 ppm

F2 - Processing parameters

SI	1024
SF	400.1343898 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0
PC	2.00

F1 - Processing parameters

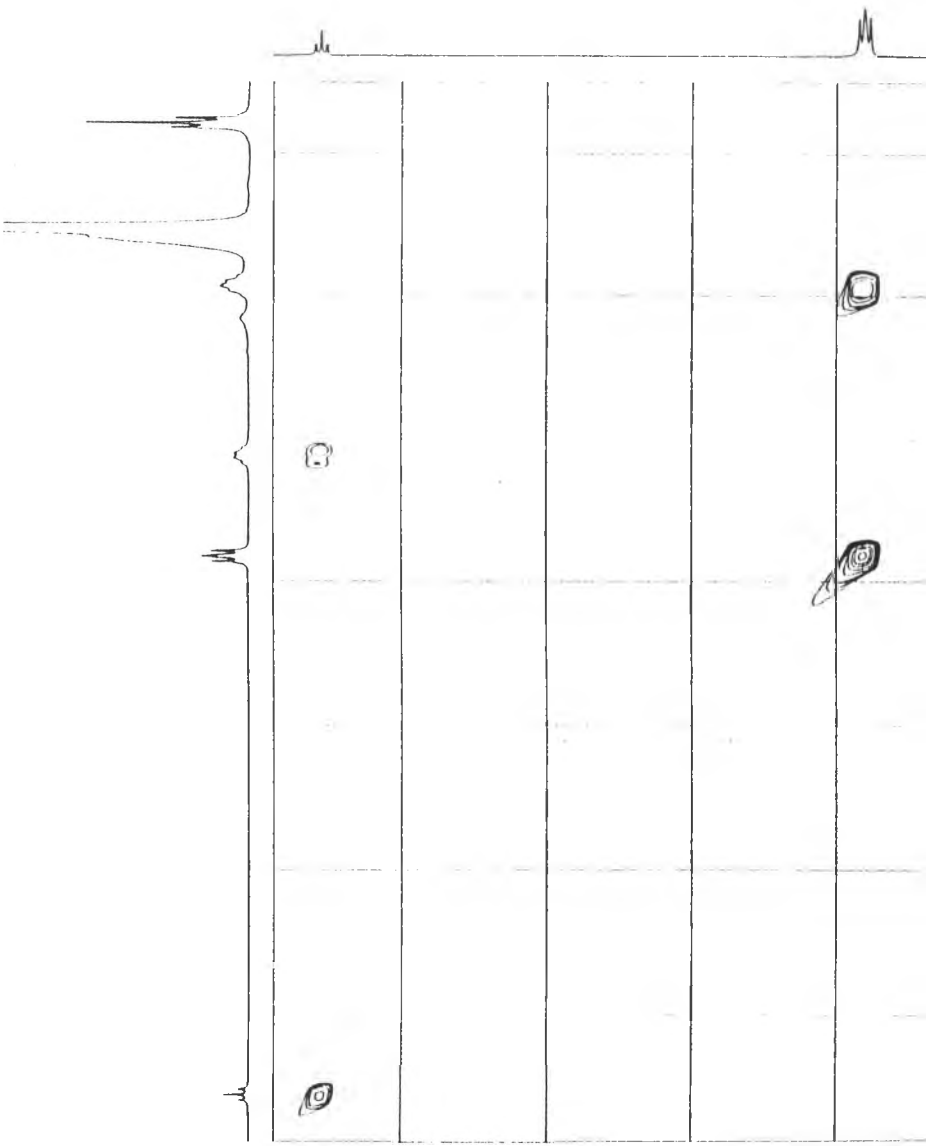
SI	1024
MC2	QF
SF	400.1343898 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0

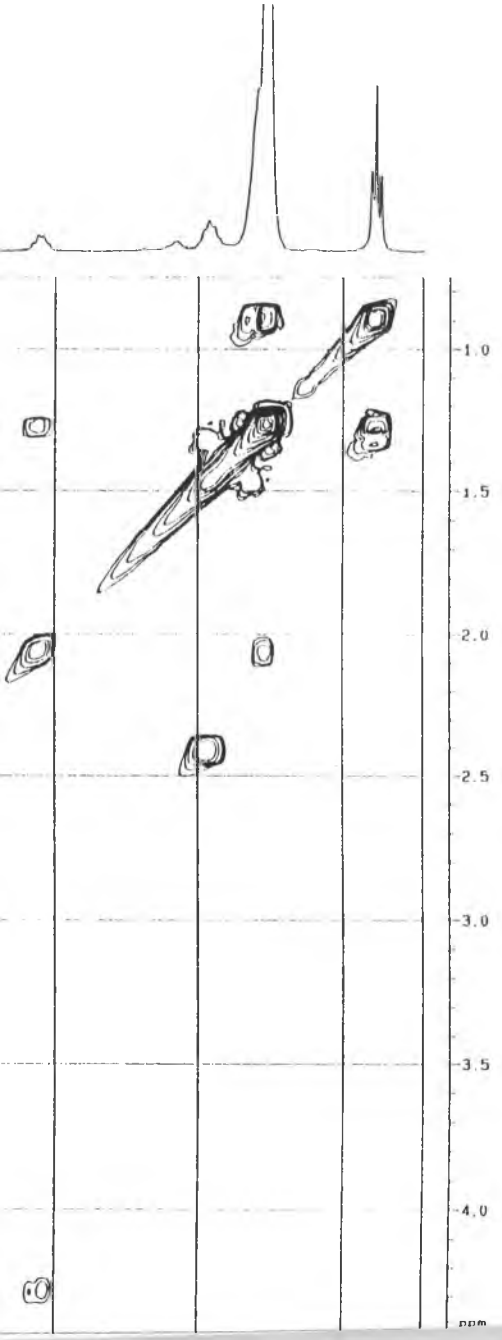
2D NMR plot parameters

CX2	20.00 cm
CX1	20.00 cm
F2PLO	4.452 ppm
F2LO	1781.31 Hz
F2PHI	0.664 ppm
F2HI	265.29 Hz

KCP-10 in CDCl3
cosy45

COSY Spectrum of 77





Current Data Parameters

NAME	A110291
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date	950920
Time	14.42
PULPROG	cosy
SOLVENT	CDCl3
AQ	0.1167560 sec
FIDRES	4.283169 Hz
DW	114.0 usec
RG	128
NUCLEUS	1H
NL1	1 dB
D1	1.0000000 sec
P1	12.0 usec
D0	0.0000030 sec
P0	6.0 usec
DE	182.4 usec
SFO1	400.1363085 MHz
SWH	4385.96 Hz
TD	1024
NS	4
DS	4
IN0	0.0002280 sec

F1 - Acquisition parameters

ND0	1
TD	256
SFO1	400.1363 MHz
FIDRES	17.132675 Hz
SW	10.961 ppm

F2 - Processing parameters

SI	1024
SF	400.1343901 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0
PC	2.00

F1 - Processing parameters

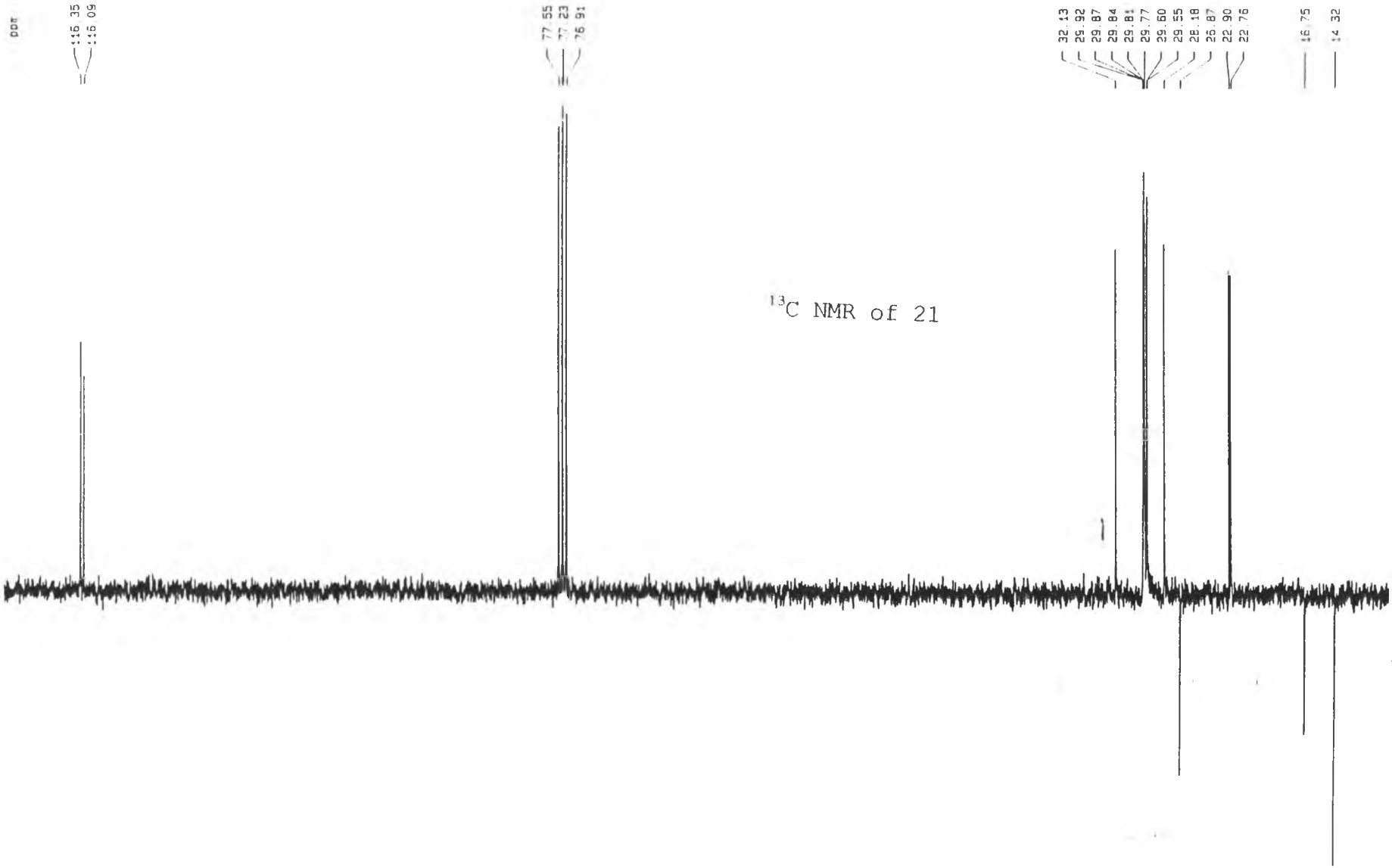
SI	1024
MC2	QF
SF	400.1343901 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	20.00 cm
CX1	20.00 cm
F2PL0	4.441 ppm
F2L0	1777.01 Hz
F2PHI	0.727 ppm
F2HI	290.75 Hz
F1PL0	4.441 ppm
F1L0	1777.01 Hz
F1PHI	0.748 ppm
F1HI	299.31 Hz

^{13}C NMR of isolated compounds.

Jacob
KCP-05 in CDCl3
Jmod; C, CH2 up; CH, CH3 down



^{13}C NMR of 21

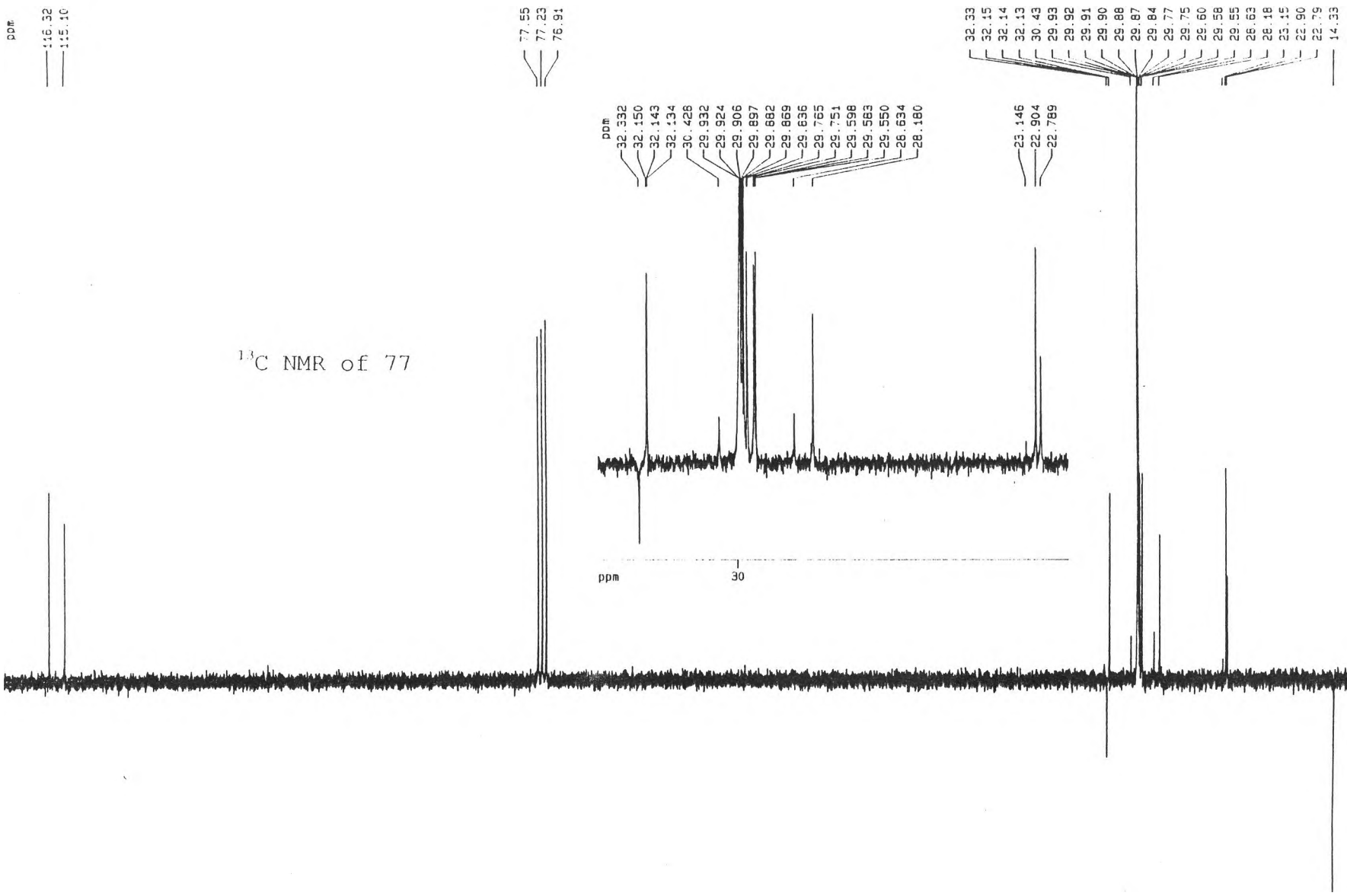
Current Data Parameters
NAME A110138
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date 950818
Time 14 40
PULPROG Jmod
SOLVENT CDCl3
AQ 1.3107400 sec
FIDRES 0.381470 Hz
DW 20.0 usec
RG 32768
NUCLEUS 13C
D20 0.0067000 sec
S2 24 dB
PL1 90 dB
D1 4.0000000 sec
D13 0.0000030 sec
P1 9.4 usec
P2 18.8 usec
DE 32.0 usec
SFO1 100.6250000 MHz
SWH 25000.00 Hz
TD 65536
NS 400
DS 2

F2 - Processing parameters
SI 65536
SF 100.6138486 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 34.00 cm
F1P 122.576 ppm
F1 12332.08 Hz
F2P 9.913 ppm
F2 997.35 Hz
PPMCM 3.31364 ppm/cm
HZCM 333.39807 Hz/cm

Jacob
KCP-01 in CDCl3
jmod: C, CH2 up; CH, CH3 down



Current Data Parameters

NAME	A110291
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

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Time	7.09
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SOLVENT	CDCl3
AQ	1.3107400 sec
FIDRES	0.381470 Hz
DW	20.0 usec
RG	32768
NUCLEUS	13C
D20	0.0067000 sec
S2	24 dB
HL1	90 dB
D1	4.0000000 sec
D13	0.0000030 sec
P1	9.4 usec
P2	18.8 usec
DE	32.0 usec
SFO1	100.6250000 MHz
SWH	25000.00 Hz
TD	65536
NS	1500
DS	2

F2 - Processing parameters

SI	65536
SF	100.6138494 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	34.00 cm
F1P	119.956 ppm
F1	12069.27 Hz
F2P	9.389 ppm
F2	944.63 Hz
PPMCM	3.25199 ppm/cm
HZCM	327.19531 Hz/cm