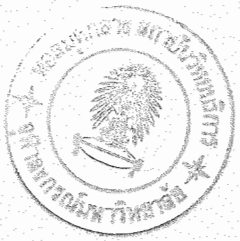
สารออกฤทธิ์ทางชีวภาพจากต้นจันทน์ชะมด *Mansonia gagei* Drumm.



นางสาว พัฒทรา แซ่เตียว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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BIOACTIVE COMPOUNDS FROM *Mansonia gagei* Drumm.

Ms. Pattara Tiew

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctoral of Philosophy in Chemistry
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Thesis Title	Bioactive compounds from *Mansonia gag*ei Drumm.
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พัฒทรา แซ่เตียว : สารออกฤทธิ์ทางชีวภาพจากต้นจันทน์ชะมด *Mansonia gagei* Drumm. (Bioactive compounds from *Mansonia gagei* Drumm.) อ.ที่ปรึกษา : ศ.ดร. อุดม ก๊กผล, อ.ที่ปรึกษาร่วม : ผศ.ดร. วรินทร ชวศิริ และ ศ.ดร. ดี ฮาเวิร์ด ไมล์, 178 หน้า
ISBN 974-17-0882-3

ในการศึกษาองค์ประกอบทางเคมีจากต้นจันทน์ชะมด สามารถแยกสารได้ 16 ชนิด จากสิ่งสกัดไดคลอโรมีเทน เอทิลอะซีเตต และเมทานอลของเนื้อไม้ โดยอาศัยสมบัติทางกายภาพ และเทคนิคทางสเปกโทรสโกปี สามารถพิสูจน์ทราบสูตรโครงสร้างของสารเหล่านี้ คือ mansorin A (**1**), mansorin B (**2**), mansorin C (**3**), mansonone N (**4**), mansonone O (**5**), mansonone P (**6**), mansonone Q (**7**), mansonone C (**8**), mansonone E (**9**), mansonone G (**10**), mansonone H (**11**), dehydrooxoperezinone (**12**), 3-methoxy-4,5-dihydroxybenzaldehyde (**13**), mansoxetane (**14**), mansonone R (**15**) และ mansonone S (**16**) mansonones N, O, P, Q, R, S และ mansoxetane เป็นสารใหม่ที่พบในธรรมชาติ เมื่อทำการทดสอบฤทธิ์ชีวภาพของสารที่แยกได้ พบว่า mansonone C แสดงฤทธิ์ทางชีวภาพได้ดีที่สุด คือ มีความเป็นพิษในระดับสูงต่อไรสีน้ำตาล *Artemia salina* Linn. ลูกน้ำยุงที่ก่อให้เกิดโรคไข้เหลือง *Aedes egypti* และเซลล์มะเร็งหลายชนิด อีกทั้งแสดงฤทธิ์ต้านเชื้อรา *Cladosporium cucumerinum* และ *Candida albicans* สำหรับ mansonone E แสดงฤทธิ์เช่นเดียวกับ mansonone C ยกเว้นไม่แสดงฤทธิ์ฆ่าลูกน้ำยุงที่ก่อให้เกิดโรคไข้เหลือง นอกจากนี้ mansorins A และ B แสดงฤทธิ์ต้านเชื้อรา *C. cucumerinum* mansonone N และ 3-methoxy-4,5-dihydroxybenzaldehyde แสดงฤทธิ์ต้านอนุมูลอิสระ DPPH ส่วน mansonones G, H และ dehydrooxoperezinone แสดงฤทธิ์ต้านการแข็งตัวของเลือดในระดับที่น่าสนใจ

ภาควิชาเคมี.....เคมี.....ลายมือชื่อนิสิต.....

สาขาวิชาเคมี.....เคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2545.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4173814823 : MAJOR CHEMISTRY
KEY WORD: *Mansonia gagei* / MANSONONE / COUMARIN / BIOACTIVE COMPOUND
PATTARA TIEW : BIOACTIVE COMPOUNDS FROM *Mansonia gagei* DRUMM. THESIS ADVISOR : PROF. UDOM KOKPOL, Ph.D., THESIS COADVISOR : ASSIST. PROF. WARINTHORN CHAVASIRI, Ph.D. AND PROF. D. HOWARD MILES, Ph.D., 178 pp. ISBN 974-17-0882-3.

The investigation of chemical constituents from *Mansonia gagei* Drumm., the heartwood extracts; dichloromethane, ethyl acetate and methanolic extracts, were separated to furnish sixteen compounds. By means of their physical properties and spectroscopic data, these compounds were firmly elucidated their strutures as mansorin A (**1**), mansorin B (**2**), mansorin C (**3**), mansonone N (**4**), mansonone O (**5**), mansonone P (**6**), mansonone Q (**7**), mansonone C (**8**), mansonone E (**9**), mansonone G (**10**), mansonone H (**11**), dehydrooxoperezinone (**12**), 3-methoxy-4,5-dihydroxybenzaldehyde (**13**), mansoxetane (**14**), mansonone R (**15**) and mansonone S (**16**). Among them, mansonones N, O, P, Q, R and S together with mansoxetane were characterized as new naturally occurring compounds. Moreover, those compounds were tested for their biologically activities. Mansonone C displayed the most potency activities. It showed high toxicity against brine shrimp *Artemia salina* Linn., larvae *Aedes egypti* and a variety of cancer cell lines. In addition, it revealed antifungal activities against *Cladosporium cucumerinum* and *Candida albicans*. Mansonone E exhibited the same activities as mansonone C except for larvicidal activity. Mansorins A and B were found to be active against *C. cucumerinum*. Mansonone N and 3-methoxy-4,5-dihydroxybenzaldehyde possessed radical scavenging properties toward DPPH. Furthermore, mansonones G, H and dehydrooxoperezinone displayed significant activity in antithrombin assay.

Department...Chemistry.....Student's signature.....Pattara Tiew.....
Field of study.....Chemistry.....Advisor's signature.....U. Kokpol.....
Academic year.....2002.....Co-advisor's signature.....W. Chavasiri.....
.....D. Howard Miles.....

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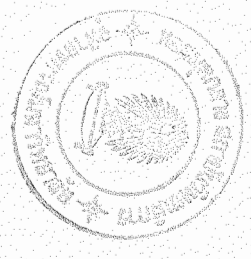
LIST OF ABBREVATIONS

δ	=	chemical shift
°C	=	degree celsius
$CDCl_3$	=	deuterated chloroform
CD_3OD	=	deuterated methanol
EIMS	=	electron impact mass spectrometry
d	=	doublet
dd	=	doublet of doublet
DEPT	=	distortionless enhancement by polarization transfer
DMSO	=	dimethylsulfoxide
DPPH	=	2,2-diphenyl-1-picrylhydrazyl radical
FT	=	fourier transform
g	=	gram
IR	=	infrared
J	=	coupling constant
HPLC	=	High Performance Liquid Chromatography
Hz	=	hertz
Kg	=	kilogram
LC_{50}	=	concentration that caused 50% lethality
LPLC	=	Low Pressure Liquid Chromatography
m/z	=	mass per charge
m.p.	=	melting point
μg	=	microgram
m	=	multiplet
mg	=	milligram
mL	=	millilitre
MW	=	molecular weight
ppm	=	parts per million (or μg/mL)
nm	=	nanometre
NMR	=	nuclear magnetic resonance
OH	=	hydroxy
OMe	=	methoxy

LIST OF ABBREVATIONS (Cont.)

q	=	quartet
R_f	=	retardation factor
R_t	=	Retention time
s	=	singlet
t	=	triplet
TLC	=	thin layer chromatography
UV	=	ultra-violet
ν_{max}	=	wave number causing maximum absorption
w/w	=	weight by weight

CHAPTER I



INTRODUCTION

Natural products, in recently, have attracted the attention of the agrichemical and pharmaceutical industries as potentially rich sources of pesticides, medicines, and other bioactive products for use on or by humans, plants, and animals. Bioactive natural products include compounds extracted from orgainsms, usually plants and microbes, which exhibit beneficial responses. These may include anticancer, antifungal, antiviral, insecticidal, nematocidal, herbicidal, or otherwise useful reactions affecting harmful organisms and/or their hosts. Natural products are not universally, simply by their nature, safe relative to the environment or the organism targeted for benefit, but their occurrence in nature is a strong indication that they have existed for a long period without harmful biological or environmental effects.

Thailand is located in a tropical region of the world where a vast biodiversity exists and which possesses a large number of medicinal plants that have been in used traditional treatment in the primary health care system. Therefore natural products research in Thailand is targeted toward the goal of studying the biological activity and chemical constituents of medicinal plants for both to obtain new drug leads and to contribute to the economic development of Thailand by developing them into new pharmaceuticals that provide new industry.

Mansonia gagei Drumm. is a Thai medicinal plant that belongs to the Sterculiaceae family. As herbal medicine, the heartwoods of this plant have been used as a cardiac stimulant and refreshment agent.[1] According to a preliminary study involving collaborative research between Natural Products Research Unit, Department of Chemistry and Department of Biology, Chulalongkorn University with the aim of screening for bioactive compounds possessing cytotoxicity against brine shrimp (*Artemia salina* Linnaeus), the ethanolic extract of the heartwoods of *M. gagei* gave attractive results. This plant was selected for exploration in 1995 for

the chemical constituents of nonpolar extracts. This work yielded three novel compounds along with three known mansonones.[2]

As part of the continuing study on the constituents of *M. gagei*, this dissertation deals with the isolation and bioactivities of the heartwood extracts and preliminary screening test of roots, barks and leaves of *M. gagei*.

1.1 Botanical Aspect and Distribution

Mansonia gagei Drumm. is the only species belonging to *Mansonia* genus in Sterculiaceae family found in Thailand.[3] This plant is commonly known as *chan-cha-mod*, *chan-hom*, *chan-khao* or *chan-pha*-ma which is used as herbal medicine. Following folklore beliefs, the heartwoods of this plant were utilized as cardiac stimulant, antiemetic, antidepressant and refreshment agents.[1]

The general characteristics of the plants in *Mansonia* genus are cited as follows: "Large tree; scattered in dry evergreen forests on the slopes of limestone hills, leave simple, toothed, flower several, in a short infloresence. Naturally dry wood scented, used in cremation ceremony".[4] Flowers, leaves and heartwoods of *M.gagei* are presented as shown in Fig. 1.1.

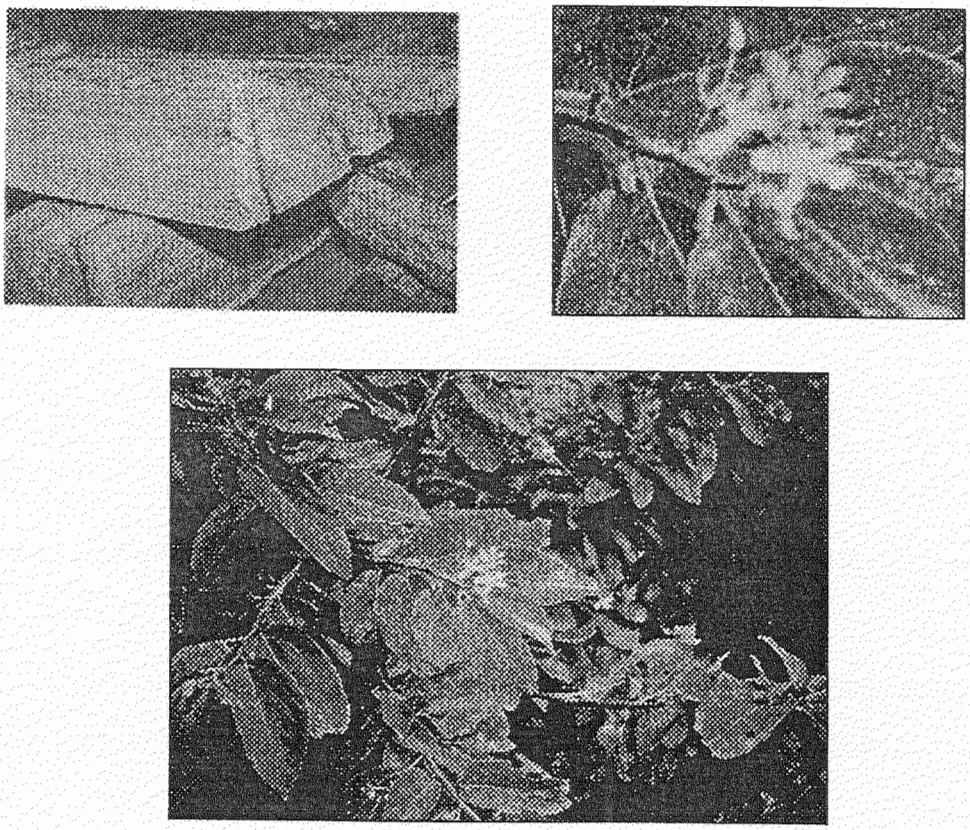
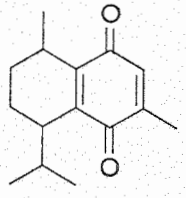


Fig 1.1 Flowers, leaves and heartwoods of *M. gagei* Drumm.

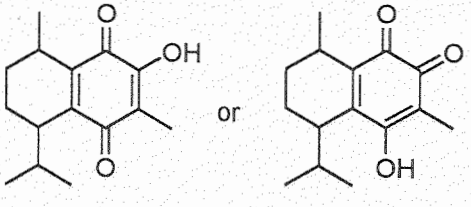
1.2 Chemical Constituents of Plants in *Mansonia* genus

The Literature survey on chemical constituents of plants belonging to *Mansonia* genus revealed that only two species, *Mansonia altissima* Chev. and *Mansonia gagei* Drumm., were investigated.

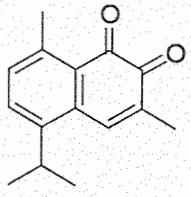
Mansonia altissima Chev., from tropical West Africa, was investigated for its chemical constituents since 1965 by Marini Bettolo *et al.*[5] The examination arosed from the observation that its heartwood sawdust caused several irritative symtoms and heart trouble for workers engaged in the regional furniture industry. Moreover, its bark extracts are used by natives for poisoning darts. M. Bettolo and his co-workers separated the active principles from the chloroform extract of *M. altissima* yielding six compounds designated as mansonones A-F, which had two main characteristics; the C15 empirical formula with a cadinane sesquiterpenoid skeleton and a quinonic character. Mansonones C-F were elucidated their structures as 1,2-naphthoquinone derivatives whereas mansonone A was characterized as 1,4-naphthoquinone derivative. However, for mansonone B, its structure was ambiguous and proposed for two alternative formula as 1,2- or 1,4-naphthoquinone derivative.



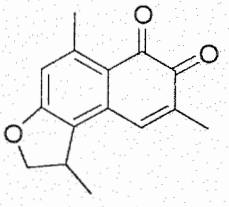
MANSONONE A



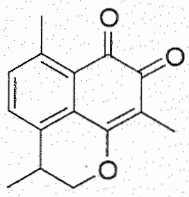
MANSONONE B



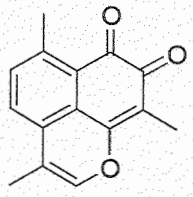
MANSONONE C



MANSONONE D



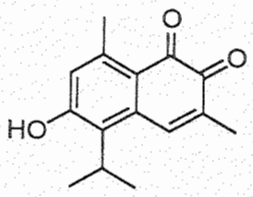
MANSONONE E



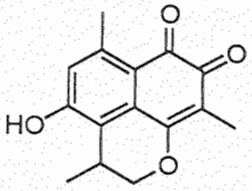
MANSONONE F

Mansonones A, C, E, F and two additional mansonones, mansonones G and H, together with β-sitosterol and β-sitosteryl palmitate were obtained from the acetone extract of the heartwood of *M. altissima* reproted by Tanaka *et al.* in 1966.[6] In addition, the structure of mansonone A was redressed as 1,2-naphthoquinone, in

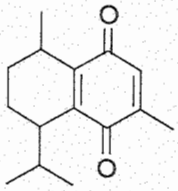
stead of 1,4-naphthoquinone that proposed by Marini Bettolo and coworkers in 1965.[5] The proposed structure of this compound was endorsed by the absorption spectrum λ_{max}(MeOH) at 432 nm which is the characteristic absorption of *o*-quinone structure. Moreover, the dehydrogenation of this compound with chloranil under mild conditions gave mansonone C.



MANSONONE G

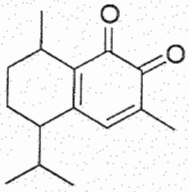


MANSONONE H



MANSONONE A

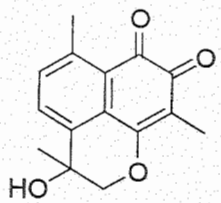
a) proposed by Marini Bettolo[5]



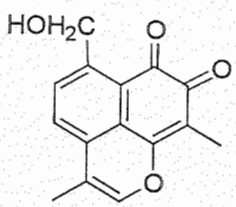
MANSONONE A

b) proposed by Tanaka[6]

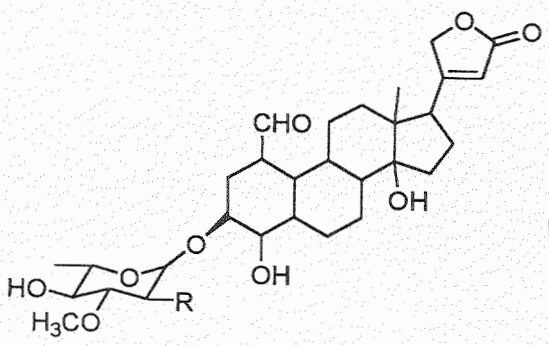
Continuous investigation on chemical constituents from the heartwood extract of *M. altissima* resulted in the isolation of mansonones I and L in 1967 by Shimada *et al.*[7] and 1969 by Galeffi *et al*,[8] respectively. Not only were the constituents of the heartwoods reported but also those of the seeds of *M. altissima* were examined. In 1967 Allgeier *et al.* isolated and elucidated the structures of two cardiac glycosides as strophanthidin 2,3-di-*O*-methyl-6-deoxy-β-D-glucopyranoside (mansonin) and strophanthidin 3-*O*-methyl-6-deoxy-β-D-glucopyranoside (strophothevoside).[9,10]



MANSONONE I



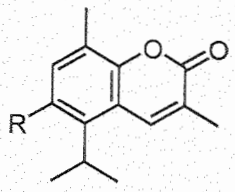
MANSONONE L



R = OCH_3 : MANSONIN

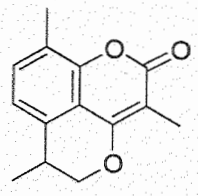
R = OH : STROPHOTHEVOSIDE

There is only one report concerning the chemical constituents of the heartwoods of *Mansonia gagei* Drumm. addressed by Puntumchai in 1997.[2,11] Three new coumarins, mansorins A-C, together with three known mansonones, mansonones C, G and H were isolated as the constituents.



R = OCH_3 : MANSORIN A

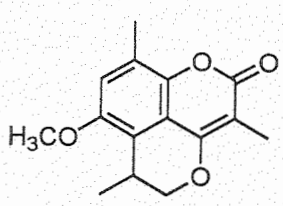
R = OH : MANSORIN B



MANSORIN C

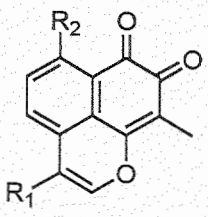
1.3 Mansonones in Other Plants

Mansonones have been found in other genus in Sterculiaceae family. For example, mansonones E, F, H and a new mansonone M were isolated from the root bark of *Helicteres angustifolia* in 1990.[12] This plant was known as one of tumour inhibitory plants.



MANSONONE M

Mansonones and other *ortho*-naphthoquinones were also discovered in other families. The isolation of three davidianones A, B and C, as well as four known compounds, mansonones E, F, H and I, were acheived from the root bark of *Ulmus davidiana* Planch in Ulmaceae family.[13] This plant is a deciduous tree which is widely distributed in Korea. The stem and root bark of this species have been used in oriental traditional medicine for treatment of oedema, mastitis, gastric cancer and inflammation.

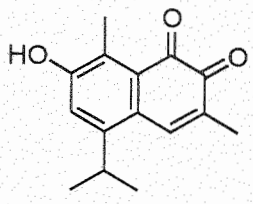


$R_1 = CH_2OH$ $R_2 = CH_3$: DAVIDIANONE A

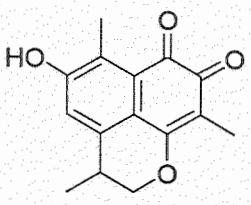
$R_1 = CH_3$ $R_2 = COOCH_3$: DAVIDIANONE B

$R_1 = CH_3$ $R_2 = CH(OCH_3)_2$: DAVIDIANONE C

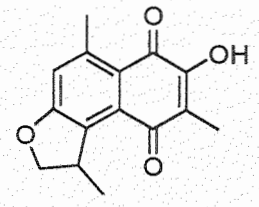
Malvaceae, which is taxonomically very closely related to Sterculiaceae, had as well been reported to compose of *ortho*-naphthoquinones. For example, the heartwood of *Azanza garckeana*, or known as *Thespesia garckeana*, yielded six *o*-naphthoquinones; mansonones E-H, azanzones A-B.[14] In addition, the mansonones C-F as well as thespesone, thespone and a new mansonone, 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[1,8-b,c]pyran-4,8-dione were identified from *Thespesia populnea*.[15-16] The root of *Aristolochia liukiuensis* Hatsusima in Aristolochiaceae family was explored for the constituents and mansonone G and dehydrooxoperizinone were isolated.[17]



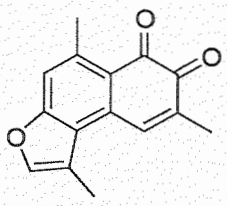
AZANZONE A



AZANZONE B



THESPESONE



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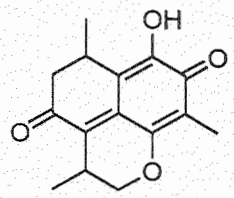
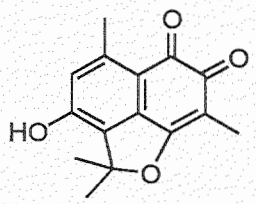


7-HYDROXY-2,3,5,6-TETRAHYDRO-3,6,9-TRIMETHYLNAPHTHO[1,8-b,c]PYRAN-4,8-DIONE



DEHYDROOXOPEREZINONE

1.4 Biological Activities of Mansonones

In addition to the natural occurring mansonones derived from plants, the reports of the occurrence of mansonones in infected plants were addressed. In 1970, Elgersma and Overeem first found that mansonones E and F accumulated in *Ulmus hollandica* Belgia as phytoalexins (antifungal antibiotics synthesized by plants in response to microbial infections).[18] The alcoholic extracts of the xylem of healthy young branches of *U. hollandica* and branches infected with *Ceratocystis ulmi* were comparatively examined. TLC of the extracts of the infected branches revealed three spots which were not observed on chromatograms of extracts of the healthy branches. Moreover, the orange and violet spots, which were proved to be mansonones E and F, respectively, possessed fungitoxicity against *Cladosporium cucumerinum*. In addition, the mansonones show a striking similarity with the naphthols which have been found in the heartwood of *Ulmus rubra*, *U. glaba* and *U. carpinifolia*. Especially the structural relationship with 7-hydroxycadalene is noteworthy. In 1985-6 accumulation of mansonones A, C, D, E, F and G in infected *U. americana* L. by *C. ulmi* were isolated and identified by Dumas *et al.*[19] Further studies, in 1983 and 1986, showed that different isolates of *Ophiostoma ulmi* (Buism.) Nannf. can induce different quantities of mansonones in elms and that a close relationship exists between mansonone accumulation and DED (Dutch elm disease) resistance.[20,21] The studies of mansonones as phytoalexins have been continuing up till now.

The effects of mansonones A, C, E and F on lipid peroxidation, P450 monooxygenase activity, and superoxide anion generation by rat liver microsomes were studied in 1990 by Villamil *et al.*.[22] The results revealed that mansonone C had a greater effect than mansonones E and F on NADPH-dependent lipid peroxidaion, O_2^- production and ascorbate oxidation, whereas mansonone E was more effective than mansonones C and F on aniline 4-hydroxylase activity. Mansonone A was in all respects relatively less effectively less effective than mansonones C, E and F. In 1996, three new sesquiterpene *o*-naphthoquinones, davidianones A, B and C, together with four mansonones, mansonones E, F, H and I isolated from the root bark of *U. davidiana* were tested for their antioxidative activities by a thiobarbituric acid method using rat liver microsomes.[13] Davidianones A and C as well as mansonones E and F were active with IC_{50} 0.12, 0.80, 0.03 and 0.04 μg/mL, respectively.

As the literature mentioned above, *M. gagei* was interesting for further studying the chemical constituents, not only the heartwoods but also in other parts, along with the biological activities, for examples, antifungal, anticancer activities *etc.*

The goal of this research can be summarized as follows:

1. To extract and isolate the chemical constituents from the heartwood extract of *M. gagei* which exhibited the significant bioactivities.
2. To elucidate the structures of the isolated substances.
3. To search for biologically active principles.
4. To test for preliminary screening bioactivities of other parts, including root, bark and leaf of *M. gagei*.

CHAPTER II

EXPERIMENTAL

2.1 Plant Material

The dried heartwoods, roots, barks and leaves of *Mansonia gagei* Drumm. were collected from Saraburi province, Thailand. The identity of this plant has been compared with the voucher specimen no. 43281 at the herbarium of the Royal Forestry Department of Thailand.

2.2 Equipments

Melting points were determined with a Fishers-Johns melting point apparatus (uncorrected) and a Mettler-FP 80/82 hot stage apparatus (uncorrected). The ^{1}H NMR and ^{13}C NMR spectra including 2D-NMR were obtained with a Bruker model ACF 200 spectrometer, a Jeol, model JNM-A500 and a Varian Unity Inova. EI-MS and D/CI-MS were obtained on FISONS MS8000 and Finnigan MAT TSQ-700 triple stage quadrupole instruments. Optical rotation was determined by a Perkin-Elmer 241 polarimeter. Low Pressure Liquid Chromatography (LPLC) was performed on Lobar LiChroprep RP-18 (15-25 μm, 460×36 mm i.d., Merck). Medium Pressure Liquid Chromatography (MPLC) was performed on silica gel prepacked column Kusano CPS-221-5. Purity of the compounds was checked by HPLC with a Nova-Pak RP-18 column (4 μm; 150×3.9 mm i.d.; Waters) using an MeCN-H_2O gradient (20:80 → 100:0) in 30 minutes. The detector was set at 210 and 254 nm. UV spectra were recorded with a Varian DMS 100S UV-VIS spectrophotometer using MeOH as solvent. CHNS/O quantity was analysed by Perkin Elmer PE2400 series II: option CHN.

2.3 Chemicals

Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 PF_{254}) and glass plates precoated with RP-18 WF_{254s}. Adsorbents used for open column chromatography were Silica gel

(Kieselgel 60, Merck) and Sephadex LH-20 (Pharmacia). All solvents used in this research were distilled prior to use except those being HPLC grade for HPLC analysis.

2.4 Bioassay Procedures

2.4.1 Brine Shrimp Cytotoxic Lethality Test

Microwell cytotoxicity assay using *Artemia salina*[23] is admired as the preliminary screening test for plant extracts in order to isolate the active compounds since it is a simple, inexpensive and rapid technique. Moreover, this bioassay can be employed as an indication of antitumour activity.[24] General procedures were described as follows.

a) Hatching the Shrimp

Brine shrimp eggs (*Artemia salina* Linn.) were hatched in an open shallow rectangular plastic box filled with artificial seawater (38 g of NaCl dissolved in 1 L of deionized or distilled water). The box was divided into two unequal compartments linked with 2 mm i.d. holes. The eggs were sprinkled into the larger compartment which was darkened with aluminum foil while the smaller was illuminated with the 20-watt lamp, and the box was kept at 22-29°C. After 24 hours, nauplii were collected by disposable pipette from the smaller compartment.

b) Sample Preparation

Four milligrams of tested compound or plant extract were dissolved in a small amount (80 μL) of the most soluble solvent. Seawater was then added to the test solution until the total volume was 4000 μL. Dissolution could be assisted by vigorous stirring with shaker to afford solution I (1000 ppm). Serial dilution of this stock solution was made to obtain solution II (100 ppm) and solution III (10 ppm), respectively. Finally, control solution was also prepared.

c) Bioassay

Five nauplli were transferred to each well of 24-well microplates by the disposable pipette, and tried to keep 100 μL of seawater. Six replications were made for each concentration. The covered plates were kept in the same condition as hatching. After 24 hours, numbers of dead nauplii in each well were counted under binocular microscope.

d) LC_{50} Determinations

$LC_{50's}$ and 95% confidence intervals were calculated by probit analysis program. In some cases where data were insufficient for this program, $LC_{50's}$ were estimated using logit transformation which did not provide confidence intervals.

2.4.2 Antifungal Test

Diseases caused by fungi bring phenomenal losses in agriculture. At the same time, the growing occurrence of systemic mycoses, in particular candidiasis, cryptoceosis and aspergillosis, as a consequence of the spread of AIDS and the increasing use of immunosuppressive drugs is a major concern of public health. In the past, efforts in the study of antifungal plant constituents have been mainly directed towards compounds active against plant pathogenic fungi. That plant-derived constituents may offer potential leads for novel agents against systemic mycoses has been demonstrated by Hufford and Clark.[25] Bioautographic assays on TLC plates are ideally suited for the screening of plant extracts and subsequent activity-guided fractionation to me detection of antimicrobial compounds, since they permit a direct localization of active compounds within a complex matrix. Bioautographic assays can be divided in three groups:[26]

(a) direct bioautography -: the microorganisms grow directly on the thin layer chromatographic (TLC) plate.

(b) contact bioautography -: the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact.

(c) agar overlay or immersion bioautography -: a seed agar medium is applied onto the TLC plate. This technique can be considered as a hybrid of direct and contact bioautography.

Two bioautographic assays against *Cladosporium cucumerinum* and *Candida albicans*, respectively, were performed in Prof. Kurt Hostettmann's laboratory (Institute of Pharmacognosy and Phytochemistry, University of Lausanne, Switzerland). *C. cucumerinum* is a spore producing fungus which has been employed for more than 25 years for the detection of antifungal substance.[27] This fungus infests plants of the family Cucurbitaceae and is well suited for the detection of substances active against plant pathogenic fungi using direct bioautography. This bioassay is quick, simple and safe since the fungus is not pathogenic to humans.

Direct bioautography is not possible with yeasts such as *C. albicans*. To overcome this problem, a bioautographic agar overlay assay has been developed.[28] Even though the agar overlay test is somewhat more labor intensive compared with the straightforward procedure with *C. cucumerinum* and requires a minimum of microbiological expertise for maintenance of yeast cultures and *ad hoc* preparation of the inoculum, the assay can be readily set up in a chemistry department. As yeasts do not produce spores, working with weakly pathogenic *C. albicans* strains requires only minimal precautionary measures.

General procedures for antifungal test against *C. cucumerinum* and *C. albicans*[29] were summaried as described as follows.

a) Sample preparation for bioautographic assays

Geometric dilutions were obtained from freshly prepared stock solutions of extracts, isolated and reference compounds at the concentrations of 10, 1 and 1 mg/mL, respectively, in an appropriate solvent for preliminary screening test. 10 µL of these dilutions were applied on the TLC plates using graduated capillaries. Dilution test was further performed at the concentrations of 0.50, 0.25, 0.12 and 0.06 mg/mL if the isolated compounds showed positive results.

b) Bioautographic assays

Direct bioautography with *Cladosporium cucumerinum*: after application of the samples on a silica gel 60 F_{254} Aluminium sheet (Merck), the TLC plates were developed in an appropriate solvent system and thoroughly dried for complete removal of solvents. The plate was then sprayed with a suspension of *C. cucumerinum* in a nutritive medium and incubated for 2-3 days in polystyrene boxed with a moist atmosphere. Clear inhibition zones appeared against a dark grey background. Nystatin (Sigma) was used as a reference compound.

Direct bioautography with *Candida albicans*: after application of the samples on a silica gel 60 F_{254} glass sheet (Merck), this was developed using an appropriate solvent system and thoroughly dried for complete removal of solvents. An inoculum of yeast ($\approx 10^7$ cells/mL) in molten malt agar (Biokar Diagnostics) was distributed over the plates. The medium solidified as a thin layer ($\approx$ 1 mm) and the plates were then incubated overnight at 30°C in polystyrene boxes with a moist atmosphere. Inhibition zones were visible after spraying with an aqueous solution of methylthiazolyltetrazolium bromide (MTT) (2.5 mg/mL). Active compounds

appeared as clear spots against a purple background. Nystatin (Sigma) was used as a reference compound.

2.4.3 Larvicidal Test

Mosquitoes, in particular species of *Anopheles*, *Aedes* and *Culex*, are important vectors of tropical diseases: *Anopheles* spp. are responsible for the transmission of malaria; *Aedes* spp., and most notably *A. aegypti*, transmit diseases caused by arboviruses (arthorpod *bo*rne *virus*) such as yellow fever and dengue fever. The ideal control method is the systematic treatment of their breeding places with larvicidal agents. Plants can provide lead compounds for the development of new larvicidal agents. At the same time, plant-derived preparations can represent an alternative to the use of synthetic pesticides, cheap and readily available to the affected population. Crude plant extracts and pure compounds were systematically tested for larvicidal properties using a simple bench-top assay.[30] General procedures were summarized as described as follows.

Preliminary test for extracts: Extracts (5.5 mg) were dissolved in a small amount (100 μL) of DMSO using 10 mL tubes. The solutions were then added approximately 20 instar II larvae of *Aedes aegypti* in tap water and the final volume was adjust to 10 mL. The concentration of solutions was 500 ppm. The tubes were incubated in darkness at 26-28°C for 24 hrs. Larvae lethality was observed under lab light. All samples were measured in duplicate.

Dilution test for isolated compounds:

a) Prepared stock solution

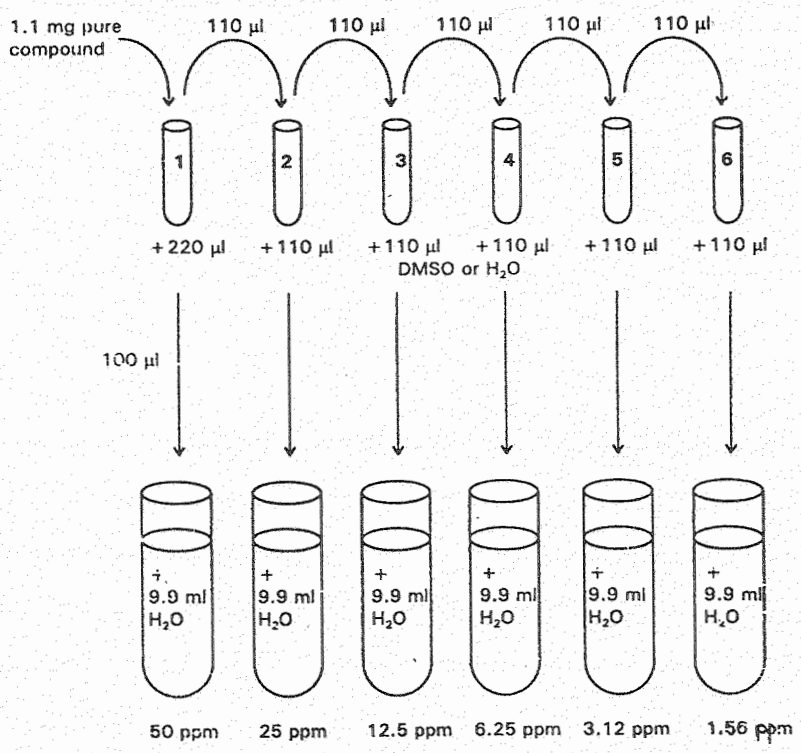
The isolated compounds (1.1 mg) were dissolved with DMSO 200 μL in a small tube No.1. Tubes No.2-6 were filled with 110 μL of DMSO. Then 110 μL of solution in tube No.1 was added to tube No.2. Following the same procedure, six concentrations of stock solution were prepared in tubes No.1-6.

b) Bioassay

Aliquots of stock solutions 100 μL were added to a tube containing approximately 20 larvae of *A. aegypti* in tap water and the final volume was adjusted to 10 μL. Six concentrations, 50, 25, 12.5, 6.25, 3.12 and 1.56 ppm, were prepared for testing. The tubes were incubated in darkness at 26-28°C for 24 hrs. Larvae lethality

was observed under lab light. All samples were measured in duplicate. Plumbagin (Roth) was used as a reference compound.

The overall procedure of dilution test for isolated compounds can be shown in Scheme 2.1.



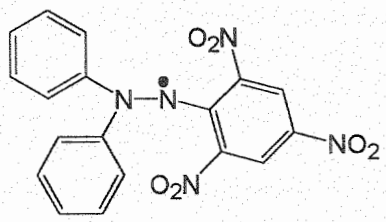
Scheme 2.1 The procedure of dilution test

2.4.4 Antioxidant Assay

There are a wide variety of methods that have been used to measure the free radical scavenging and antioxidative activity of different types of compounds. The rate of autoxidation of methyl linoleate under mild or accelerated conditions has been used to evaluate the antioxidative activity of phenolic compounds. Also a number of radical scavenging assays including measurement of hydroxyl, superoxide anion and 2,2-dipenyl-1-picrylhydrazyl (DPPH) radical scavenging activity have been used. DPPH is considered to be very stable and to be destroyed only by antioxidants or radical scavenger (AH) which can donate hydrogen atom as equation (1.1). That is, the more effective the compound in destroying DPPH, the better the compound as antioxidant.

$$DPPH\bullet + AH \longrightarrow DPPH\text{-}H + A\bullet \quad \text{.....(1.1)}$$

A TLC autographic assay being able to determine the most active components directly after chromatographic separation would thus provide a fast, simple and efficient method for the analysis and isolation of potential antioxidative compounds in natural extracts.



DPPH (2,2-diphenyl-1-picrylhydrazyl) radical

Radical Scavenging Assay

This test was performed on thin-layer chromatography.[31] Plant extracts and pure compounds (10 μL) were applied on aluminium-backed silica gel 60 F_{254} plates (Merck). The plates were developed in appropriate solvent systems before being dried out. For free radical scavenging assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (2 mg/mL in MeOH) was used as a spray reagent. The active compounds were seen as clear spots against a purple background.

For preliminary test, extracts and pure compounds were prepared at 1 mg/mL in MeOH. In case of dilution test, pure compounds were prepared at 1, 0.5. 0.25, 0.13, 0.06 mg/mL in MeOH.

2.4.5 Antithrombin and Anticancer Tests

It is believed that hypercoagulability in cancer is related to an increase of TF (Tissue Factor = the major physiologic activator of the extrinsic systems of blood coagulation) in the patients. As the cancer increases and spreads, it affects the normal tissues, exposes TF and activates platelets. The cancerous cells can also produce TF and therefore thrombin can form directly on the cancer cell surface. TF is also produced as part of an imflammatory response of the body, which is activated by the

presence of a tumor, as it would be by a strange organism. It can be said that the lower the activity of thrombin, the lower the coagulability, and therefore, the lower the possiblility tumor cells have of adhering to any tissue or of spreading.

Antithrombin Assay[32]

Antithrombin is a potent inhibitor of the reactions of the coagulation cascade. Although the name, antithrombin, implies that it works only on thrombin, it actually serves to inhibit virtually all of the coagulation enzymes to at least some extent. General procedures of antithrombin test were described as follows:

Samples were prepared at a concentration of 1 mg/mL and diluted 1/10 in buffer in eppendorf tubes. The buffer 50 μM Tris-NaCl (pH 8.0) was prepared by using 0.05 M Tris(hydroxymethyl) aminomethane, 0.138 M NaCl and 0.0027 M KCl. The Eppendorf tubes were then capped and shaken in order to throughly mix the solution. The pure methanol (50 μL) was placed into the first well of a Corning 96 flat bottom plate (well diameter 6.4 mm) as the reference. The sample solutions (50 μL each) were placed into the first two rows of the plate. Then 50 μL of the thrombin solution (which has been prepared by reconstituting 500 units of Bovine Plasma Lyophilized powder from Sigma Chemical Company) was added in each well. The plate was then incubated at 37°C for 5 minutes. Subsequently, 50 μL of the chromogenic reagent (D-PheE-L-Pipecoyl-Arg *p*-nitroanilide) from Sigma Chemical Company (2mmol/L) was added in each well. The 96-well plate was then placed in a Molecular Devices kinetic microplate reader. The absorbance at 405 nm was measured continuously over a 5 minute period. A plot of this continuous measurement was obtained as well as the absorbance at the end of 5 minutes. The percent inhibition (I%) was calculated using the following equation (1.2)

$$I\,\% = \frac{v_{sample}}{v_{blank}} \times 100\,\% \qquad \text{(1.2)}$$

v is a slope of absorbance curve at 405 nm.

Anticancer Assay

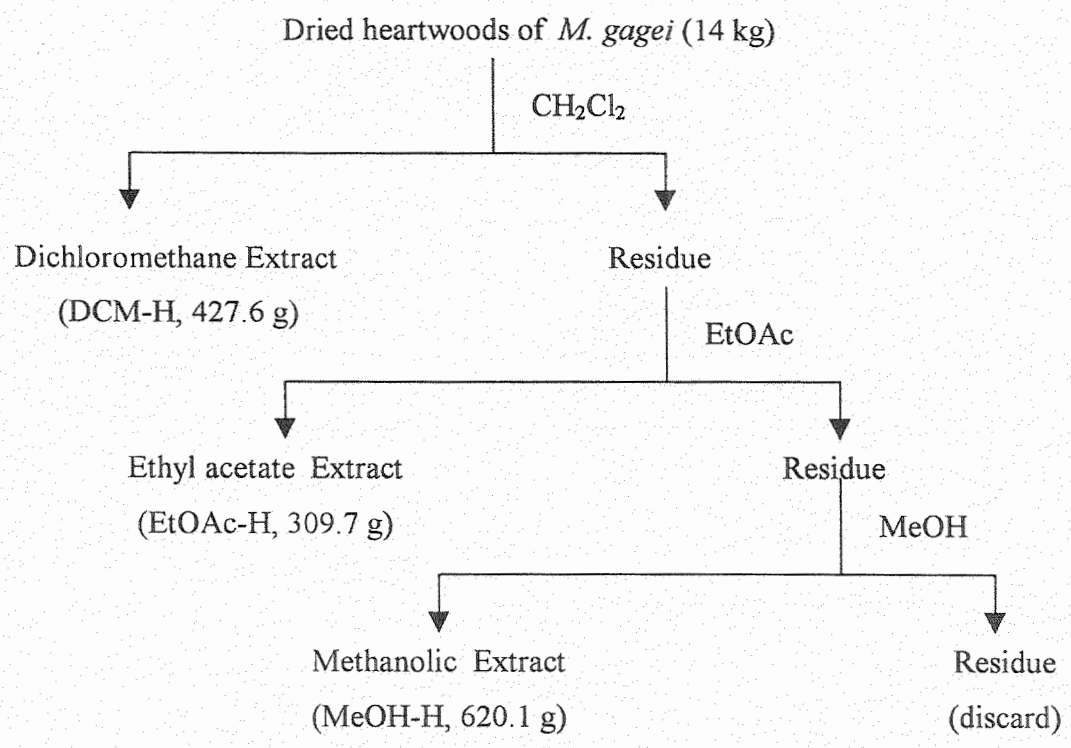
This cytotoxicity assay was used to determine the inhibitory effect of test samples on the growth of Mouse Leukemia (L1210), Human Myeloma (CCL155), Human Colon Adenocarcinoma (CCL220), Human Leukemia (Jurkat Cells) and Human Breast Carcinoma (HTB123). Cells were grown in RPMI 1640 media + 10% horse serum + 1% antibiotic/antimycotic for approximately 48 hours at 37 °C/5% CO_2 in the presence of the test plant extract. Growth/non-growth of the cells (e.g., cell density) was determined using Promega's MTS/PMS assay system. Methotrexate was used as the positive control. The negative control also contained the same volume of matrix to offset any deleterious effects it may have on growth of cells. Samples were run in duplicate or quadruplicate.[33]

2.5 General Extraction

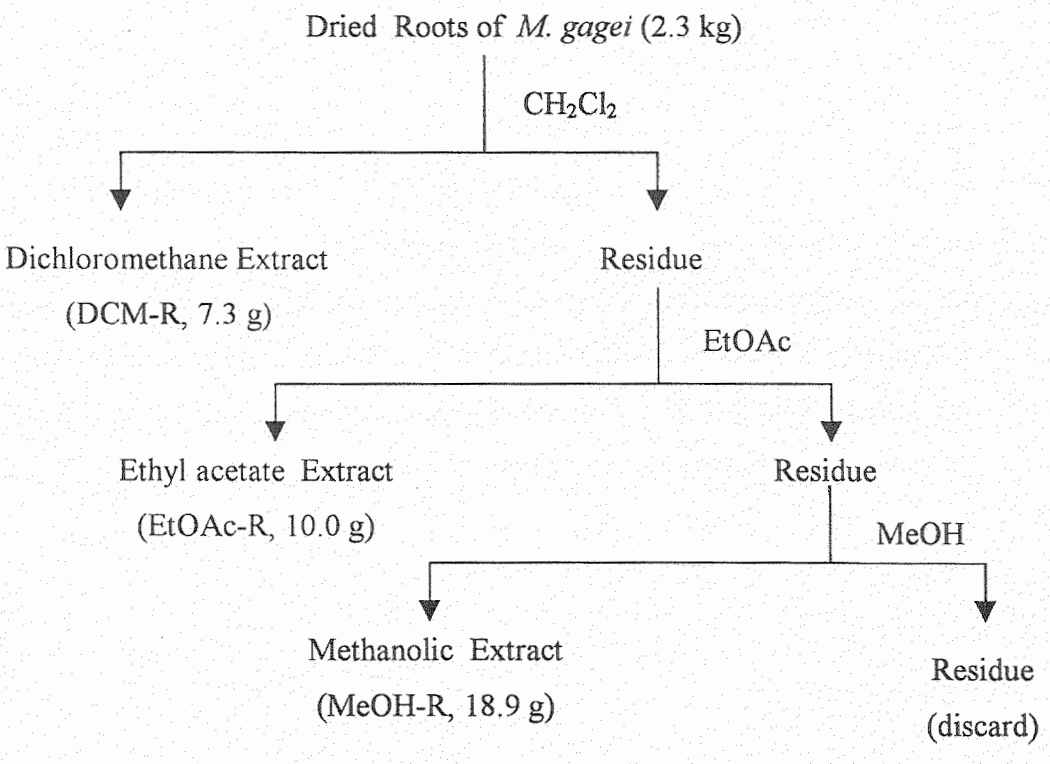
2.5.1 Extraction Procedure of Heartwoods, Roots and Barks of *M. gagei*

The dried heartwoods of *Mansonia gagei* Drumm. (14 kg) were milled and extracted with dichloromethane three times at room temperature. The dichloromethane extract was evaporated under vacuum to yield a black extract, DCM-H (427.6 g, 3.08 % yield of the dried heartwood). The residue was successively extracted with ethyl acetate and methanol as the same above mentioned procedure to give the ethyl acetate extract, EtOAc-H (309.7 g, 2.23 % yield of the dried heartwood) and methanolic extract, MeOH-H (620.1 g, 4.46 % yield of the dried heartwood), respectively. The extraction procedure is summarized in Scheme 2.2.

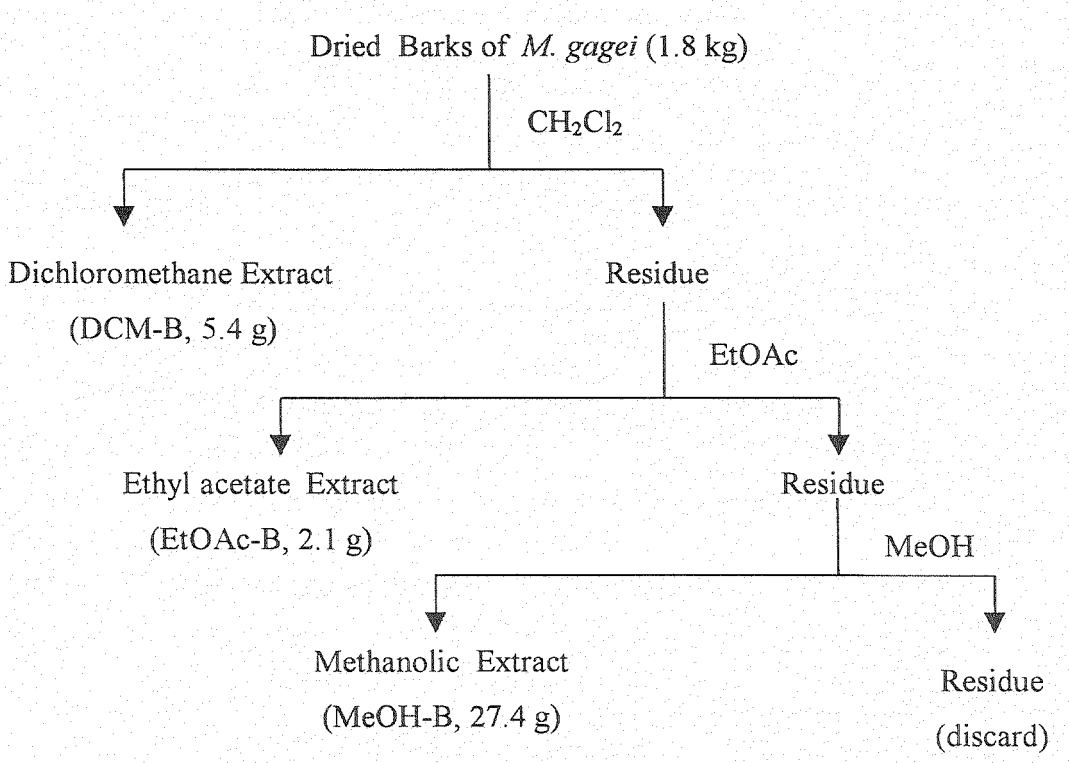
The roots and barks were extracted with the same procedure as that employed for the heartwoods. Their procedures are presented as in Schemes 2.3-2.4.



Scheme 2.2 The extraction procedure of the heartwoods of *M. gagei*



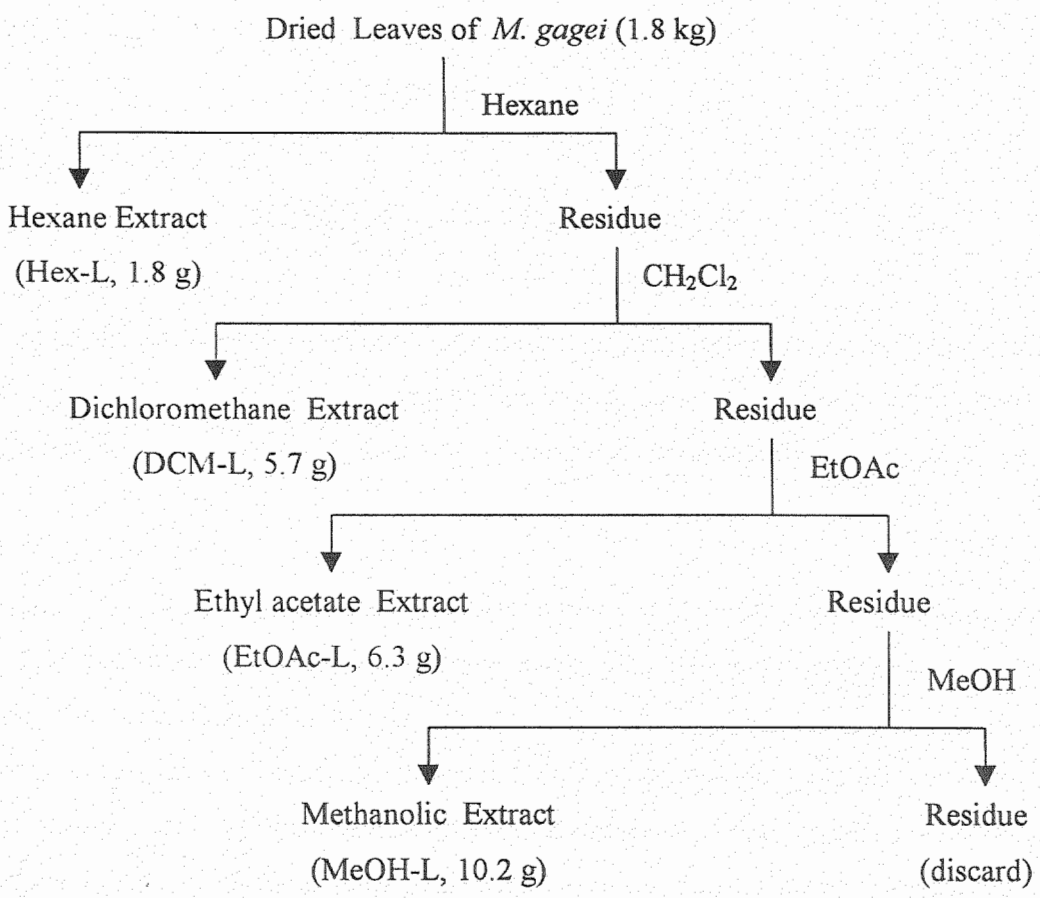
Scheme 2.3 The extraction procedure of the roots of *M. gagei*



Scheme 2.4 The extraction procedure of the barks of *M. gagei*

2.6.2 Extraction Procedure of Leaves of *M. gagei*

The sun dried leaves of *M. gagei* (3.8 kg) were extracted with hexane for three times at room temperature. Evaporation of the solvent under vacuum yielded 1.8 g of hexane extract. Following the same procedure as described earlier, the extraction residue was successively extracted with dichloromethane, ethyl acetate and methanol to give 5.7 g of the dichloromethane extract, 6.3 g of the ethyl acetate extract and 10.2 g of the methanolic extract. The extraction procedure is shown in Scheme 2.5.



Scheme 2.5 The extraction procedure of the leaves of *M. gagei*

CHAPTER III

RESULTS AND DISCUSSION

According to the impressive preliminary cytotoxic activity against brine shrimp *Artemia salina* Linn., the heartwoods of *Mansonia gagei* Drumm. were chosen for investigation the chemical constituents and searching for biologically active principles.

PART I

3.1 Preliminary Bioassay Screening Results of the Extracts from the Heartwoods

The heartwoods of *Mansonia gagei* were extracted with appropriate solvents following the procedure described in Chapter 2. All extracts, DCM-H, EtOAc-H and MeOH-H, were then preliminarily screened for their cytotoxicity against brine shrimp *Artemia salina* Linn., as well as for their antifungal activities against *Cladosporium cucumerinum* and *Candida albicans*, larvicidal activity against *Aedes aegypti*, radical scavenging properties in the DPPH assay, antithrombin and anticancer activities. The results derived from those activities could be utilized as a guidance to determine the fractions of interest for further isolating the lead compounds. The results are shown in Tables 3.1-3.3 and Figures 3.1-3.2.

Table 3.1 Brine shrimp cytotoxicity test of the extracts from the heartwoods of *M.gagei*

Extract	LC_{50}	Activity
DCM-H	3.53	High
EtOAc-H	12.56	Medium
MeOH-H	120.80	Low

Note High activity ($LC_{50} < 10$ μg/mL)
Medium activity ($10 \leq LC_{50} \leq 100$ μg/mL)
Low activity ($LC_{50} > 100$ μg/mL)

Table 3.2 Antifungal, larvicidal and radical scavenging activities of the extracts from the heartwoods of *M.gagei*.

Extract	*Cladosporium cucumerinum*[a]	*Candida albicans*[a]	*Aedes aegypti*[b]	DPPH[a]
DCM-H	+	+	-	+
EtOAc-H	+	+	-	+
MeOH-H	-	-	-	+

[a] : tested amount: 100 μg of crude extract
[b] : tested amount: 500 ppm of crude extract
+ : active
- : not active

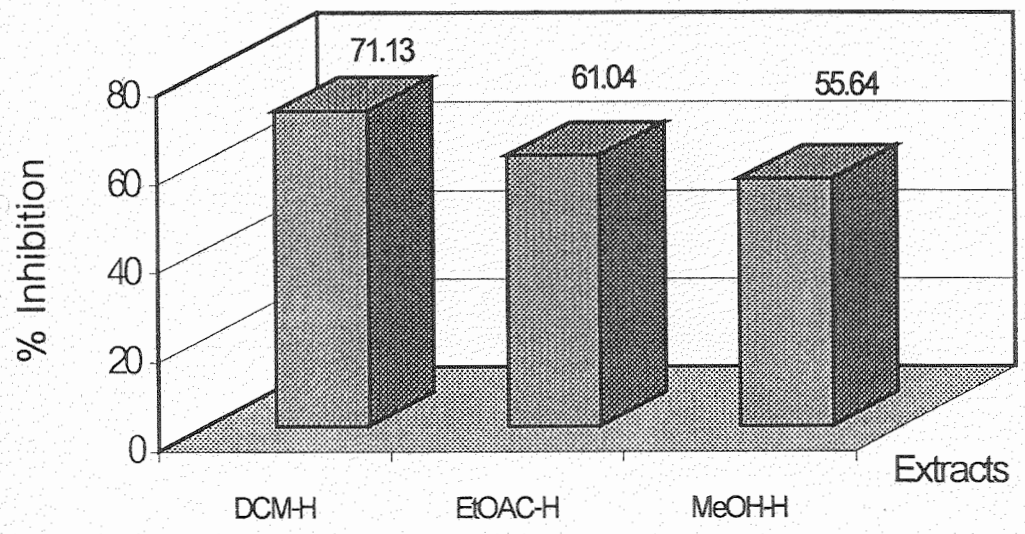


Fig. 3.1 The antithrombin activity of the extracts from the heartwoods of *M. gagei*

Table 3.3 The anticancer activity of the extracts from the heartwoods of *M.gagei*

	Mouse Leukemia	Human Myeloma	Human Colon Adenocarcinoma	Human Leukemia	Human Breast Carcinoma
DCM-H	98.7	90.2	86.8	94.5	80
EtOAc-H	99.5	85.8	82.6	97.5	89.5
MeOH-H	100	96.4	84.8	93.9	91.8
Methotraxate	97.6	89.1	61.8	65.1	17.5

Test dose: 10 μg/well

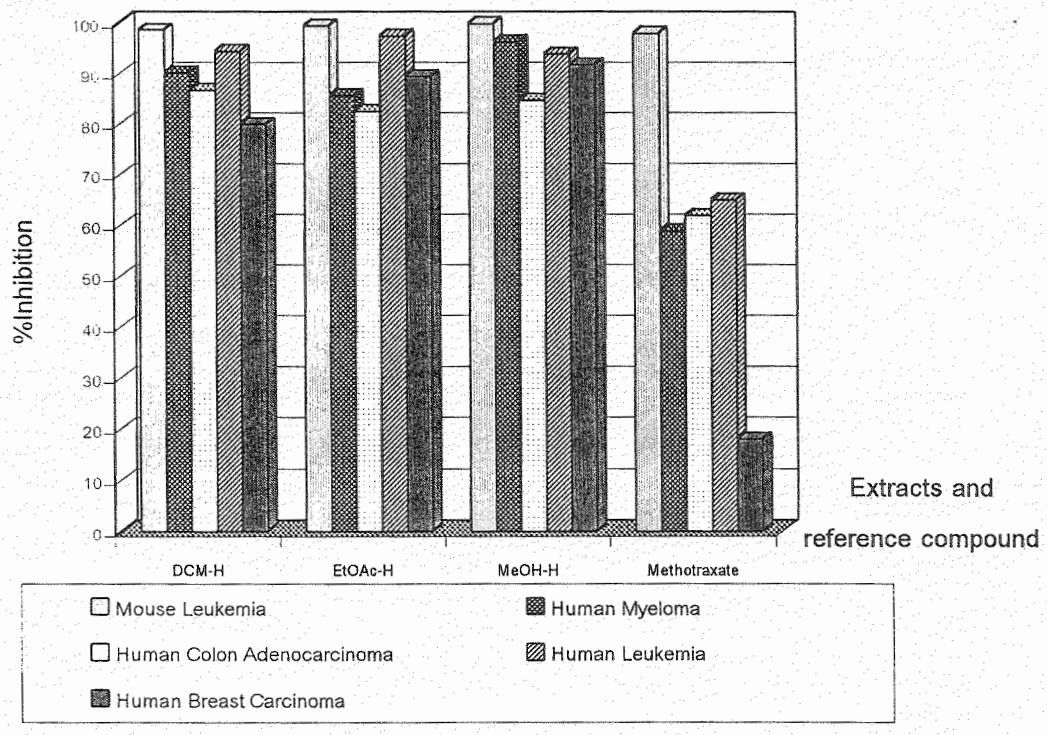


Fig 3.2 The anticancer activity of the extracts from the heartwoods of *M. gagei*

The bioactivity screening results revealed that the most promising extract for further investigation was the dichloromethane extract (DCM-H). It exhibited high cytotoxicity against brine shrimp, gave positive tests for antifungal and radical scavenging assays, and showed high activity in antithrombin and anticancer tests. Based upon these activities, the dichloromethane extract was selected as the priority extract for further work in regard to biological active constituents.

3.2 Separation of the Dichloromethane Extract of the Heartwoods

3.2.1 Fractionation

A part of the crude dichloromethane extract (102.2 g) was subjected to silica gel quick column chromatography using step gradient of hexane-CH_2Cl_2, CH_2Cl_2-EtOAc and EtOAc-MeOH as solvent systems. The fractions were collected and combined according to TLC results. Ten fractions, QC1-QC10, were obtained. Each fraction was further assayed for their brine shrimp cytotoxicity. The results of fractionation and their activities are shown in Table 3.4.

Table 3.4 The results of fractionation by quick column chromatography of the dichloromethane extract from the heartwoods of *M. gagei* and their brine shrimp cytotoxicity activity.

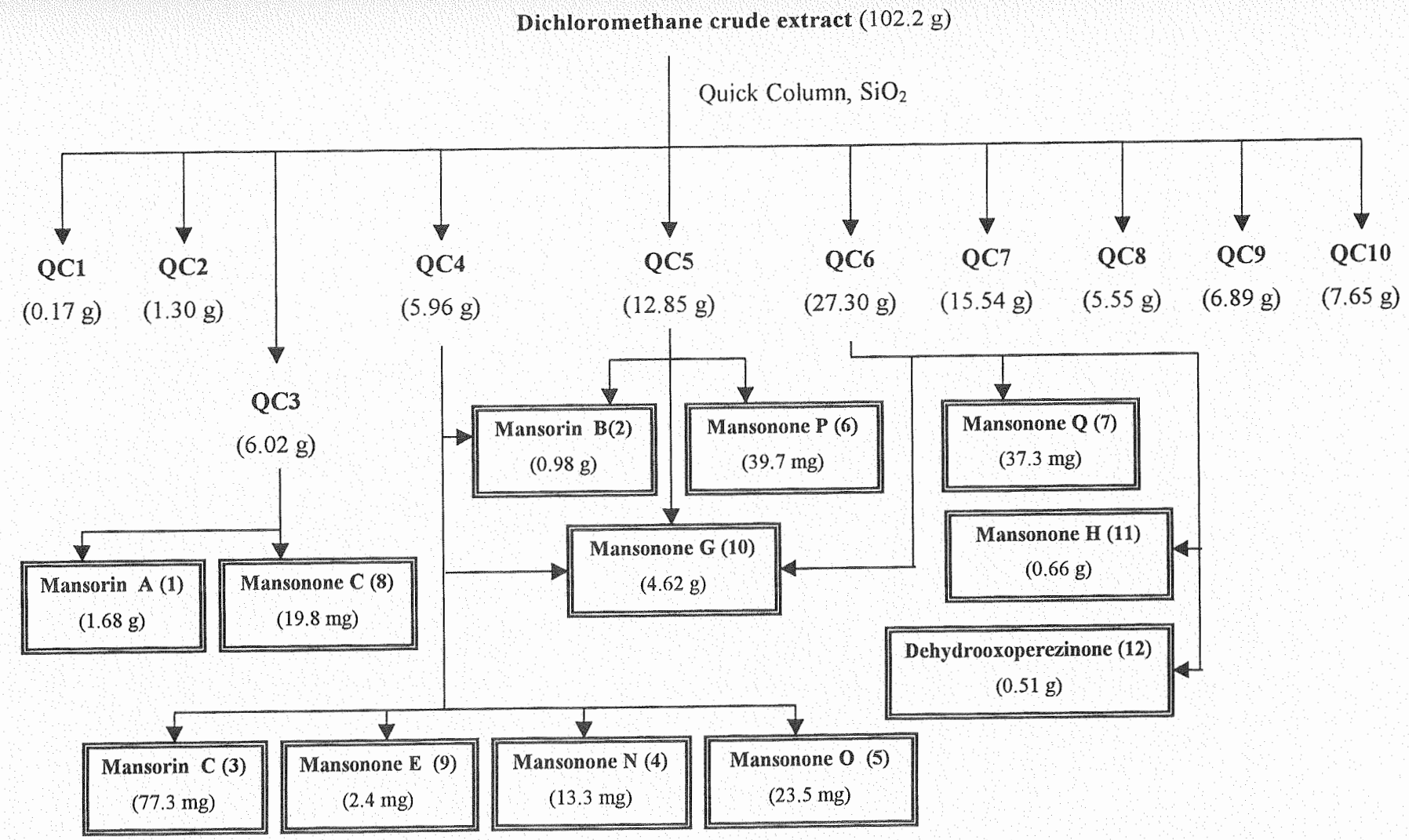
Fraction No./ Eluent (% vol. by vol.)	Weight (g)	LC_{50} (μg/mL)	Activities
QC1: 100% Hexane	0.17	45.37	Medium
QC2: 25% CH_2Cl_2 in Hexane	1.30	45.21	Medium
QC3: 50% CH_2Cl_2 in Hexane	6.02	37.52	Medium
QC4: 75% CH_2Cl_2 in Hexane	5.96	21.91	Medium
QC5: 100% CH_2Cl_2	12.85	14.88	Medium
QC6: 25% EtOAc in CH_2Cl_2	27.30	26.26	Medium
QC7: 50% EtOAc in CH_2Cl_2	15.54	77.24	Medium
QC8: 75% EtOAc in CH_2Cl_2	5.55	55.63	Medium
QC9: 100% EtOAc	6.89	311.54	Low
QC10: 5% MeOH in EtOAc	7.65	298.78	Low

Note : High Activity ($LC_{50} < 10$ μg/mL)
Medium Activity ($10 \leq LC_{50} \leq 100$ μg/mL)
Low Activity ($LC_{50} > 100$ μg/mL)

From the results presented in Table 3.4, QC1-QC8 displayed moderate cytotoxic activity. Among them, QC5 was the most active fraction. These fractions were therefore carried out for further investigation for active principles.

3.2.2 Separation

Each fraction, QC1-QC8, was subjected to silica gel column chromatography using mixtures of hexane-CH_2Cl_2, CH_2Cl_2-EtOAc and EtOAc-MeOH of increasing polarity as eluents. Twelve pure compounds were obtained after further separation of fractions on silica gel column chromatography. Mansorin A (1.68 g), mansonone C (19.8 mg) were isolated from QC3. Mansorin C (77.3 mg), mansonones E (2.4 mg), N (13.3 mg) and O (23.5 mg) were obtained from QC4. Mansonone P (39.7 mg) could be separated from QC5. Mansonones H (0.66 g), Q (37.3 mg) and dehydrooxoperezinone (0.51 g) were derived from QC6. Mansorin B (0.98 g) was fruitfully isolated from both QC4 and QC5. Mansonone G (4.62 g), the major compound of this extract, was obtained from QC3-QC6. Among these twelve compounds, mansonones N, O, P and Q have been characterized as new naturally occurring compounds. The isolation procedures are summarized in Scheme 3.1.

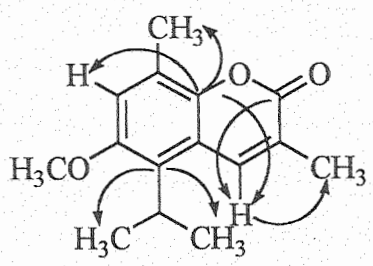


Scheme 3.1 Isolation diagram of the dichloromethane extract from the heartwoods of *M. gagei*

3.3 Structural Elucidation

3.3.1 *Compound 1:* Mansorin A

Compound **1** was isolated as a yellow crystal, m.p. 134-135 °C. This compound showed a single spot on TLC with an R_f value of 0.40 (silica gel/hexane:ethyl acetate = 8:2). The IR absorption of Compound **1** (Fig 3.3) showed the presence of an α,β-unsaturated lactone at 1711 cm^{-1} and an aromatic moiety at 1600 cm^{-1}. Its molecular formula was established as $C_{15}H_{18}O_3$ by EIMS ($[M]^+$ at m/z 246) (Fig 3.4) and elemental analysis (Found %C 72.98 and %H 7.59; calcd. for $C_{15}H_{18}O_3$ MW. 246.30; %C 73.17 and %H 7.31. The ^{13}C NMR and DEPT spectra (Fig 3.6-3.7) indicated that Compound **1** possessed a coumarin skeleton based on a total of 15 carbons, comprising a carbonyl lactone carbon at δ_C 162.1, eight olefinic and aromatic carbons between δ_C 116.7 and 153.7, four methyl carbons at δ_C 15.7, 17.6 (2C) and 21.4, a methoxy carbon at δ_C 56.2, and a methine carbon at δ_C 26.6. In the ^1H NMR spectrum of Compound **1** (Fig 3.5), an isopropyl (δ_H 1.38, d, 6H, J = 7.3 Hz; 3.56, m, 1H), two methyl (δ_H 2.23, s, 3H; 2.42, s, 3H) and one methoxy (δ_H 3.83, s, 3H) groups were observed. The complete assignments of these protons were established from the analysis of the HMBC and ^1H-^1H NOESY spectral data. In the HMBC spectrum (Fig 3.9), the carbonyl lactone carbon at δ_C 162.1 (C-2) showed correlation with H-4, whereas the quaternary carbon at δ_C 146.6 (C-8a) showed correlations with H-4, H-7 and 8-CH_3. The quaternary carbon at δ_C 129.6 (C-5) revealed $^3J_{C\text{-}H}$ interactions with 9-$(CH_3)_2$ and H-7. The HMBC correlations of Compound **1** are shown below.



Moreover, the ^1H-^1H NOESY spectrum (Fig 3.11) supplied important data to prove the actual structure of this compound. Significant correlations between H-4, 3-CH_3, 9-$(CH_3)_2$ and H-9, as well as between H-7, 8-CH_3 and 6-OCH_3, were observed. The above evidence led to establish the structure of Compound **1** as 3,8-dimethyl-5-isopropyl-6-methoxy coumarin, first isolated from the heartwoods of *Mansonia gagei* in

1995[2] and confirmed its structure by X–ray crystallography (Fig 3.12). Herein, Compound **1** was designated as mansorin A. The ^{1}H and ^{13}C-NMR spectral assignments are tabulated in Table 3.5.

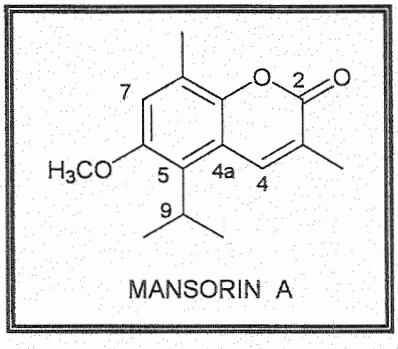
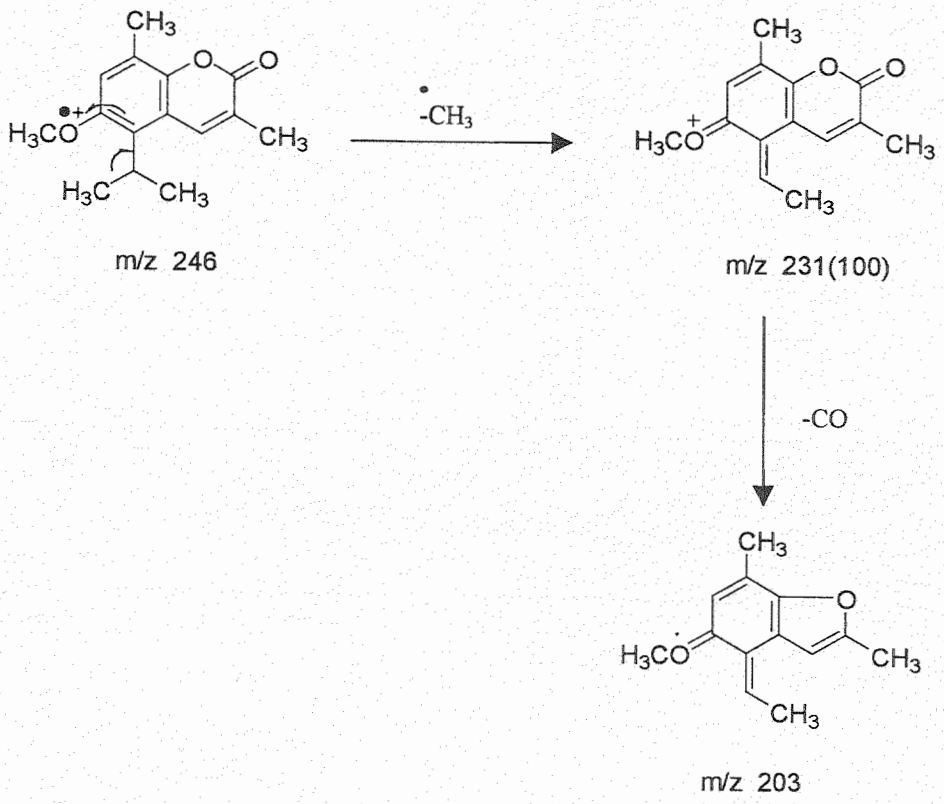


Table 3.5 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **1**

Position	Chemical shift (ppm)[a]	
	δ_H (J in Hz)	δ_C
2	-	162.1
3	-	124.6
4	7.90, s, 1H	136.7
4a	-	117.8
5	-	129.6
6	-	153.7
7	6.90, s, 1H	116.7
8	-	123.8
8a	-	146.6
9	3.56, m, 1H	26.6
3- CH_3	2.23, s, 3H	21.4
6-OCH_3	3.83, s, 3H	56.2
8- CH_3	2.42, s, 3H	15.7
9-$(CH_3)_2$	1.38, d, 6H (7.3)	17.6 (2C)

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively

The mass spectrum (Fig 3.4) displayed the molecular ion peak at m/z 246 ($[M]^+$), and other fragmentations at m/z 231 (M^+-CH_3) and 203 (M^+-CH_3-CO). The proposed fragmentation pattern of Compound **1** is shown in Scheme 3.2.



Scheme 3.2 The proposed fragmentation pattern of Compound **1**

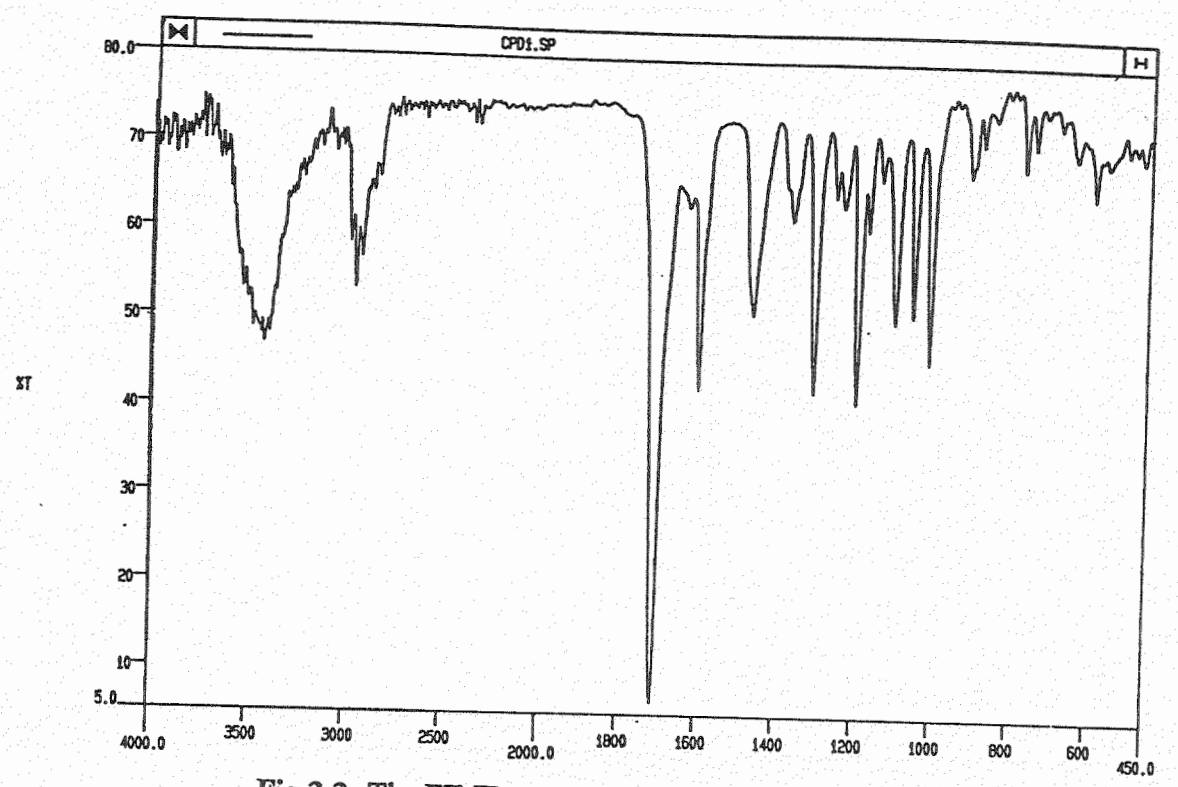


Fig 3.3 The FT-IR spectrum of Compound 1

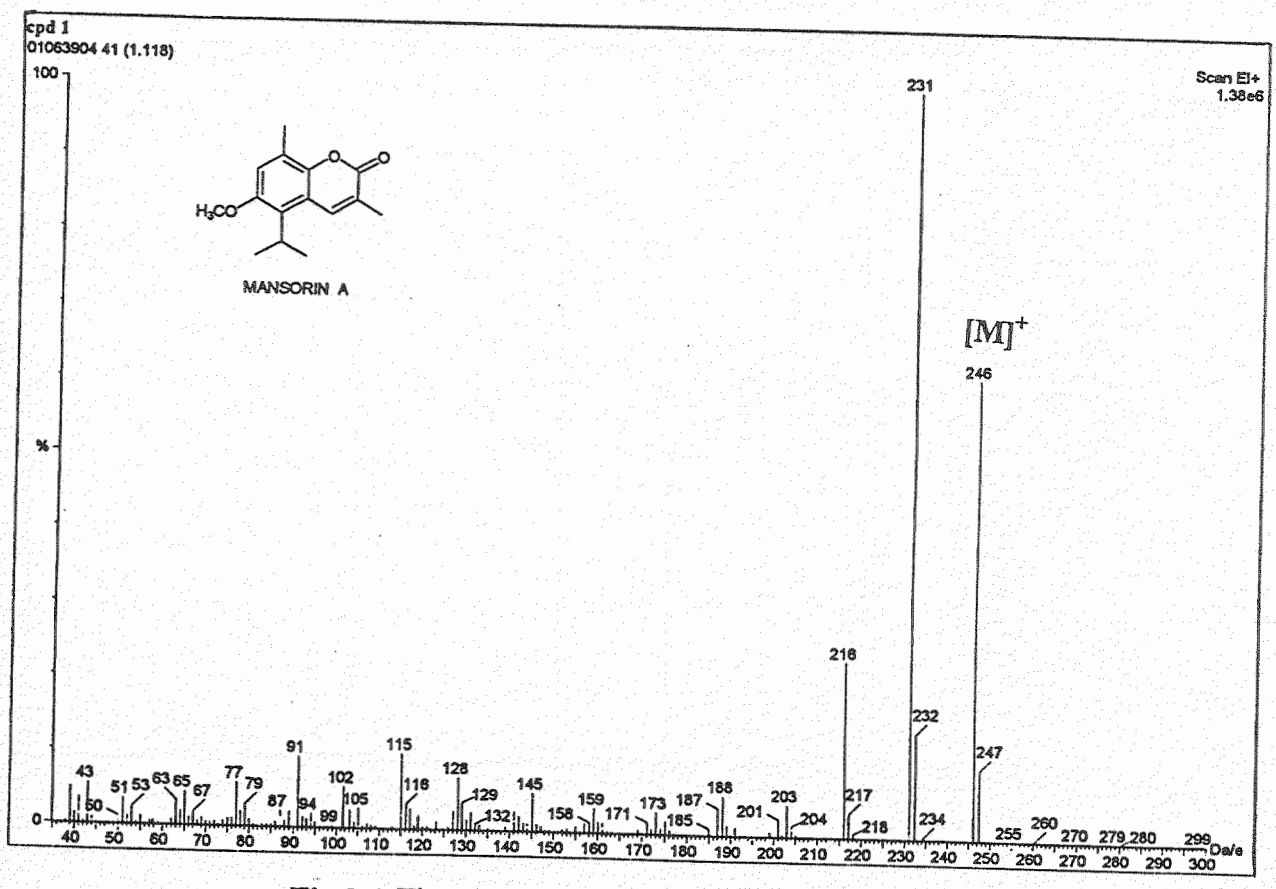


Fig 3.4 The mass spectrum of Compound 1

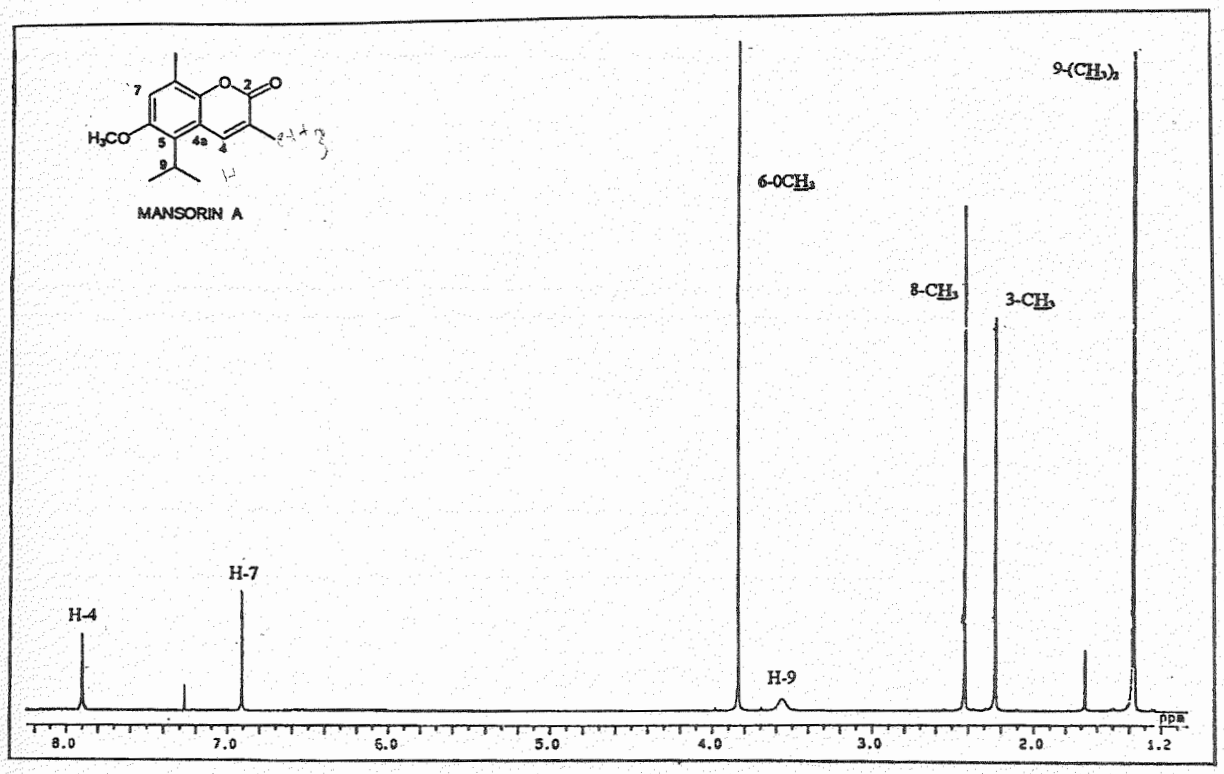


Fig 3.5 The ^{1}H-NMR spectrum of Compound 1

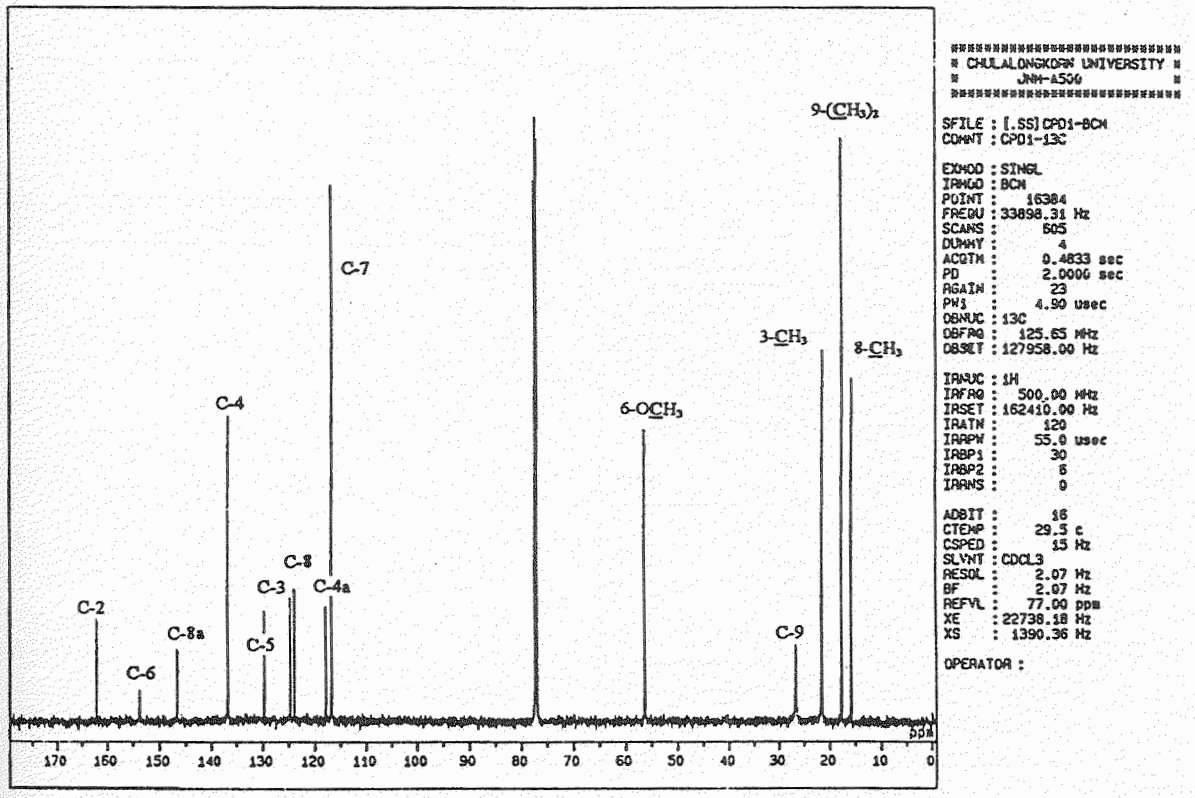


Fig 3.6 The ^{13}C-NMR spectrum of Compound 1

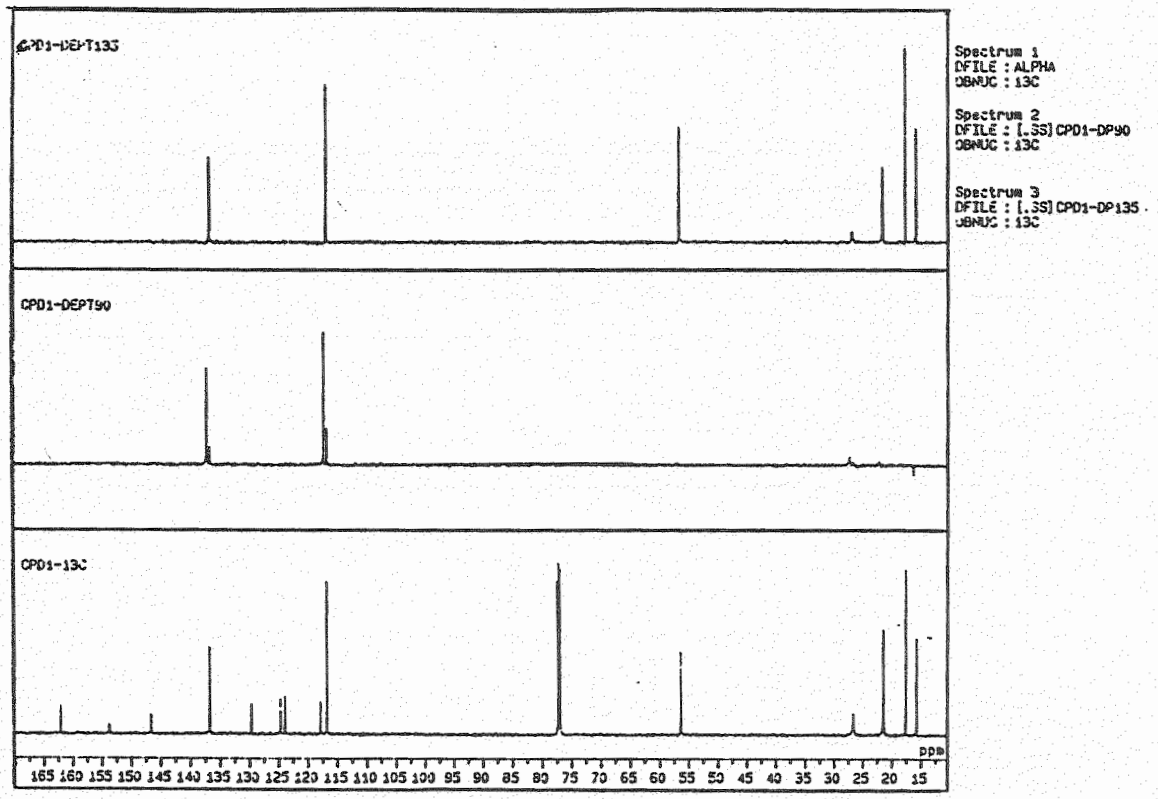
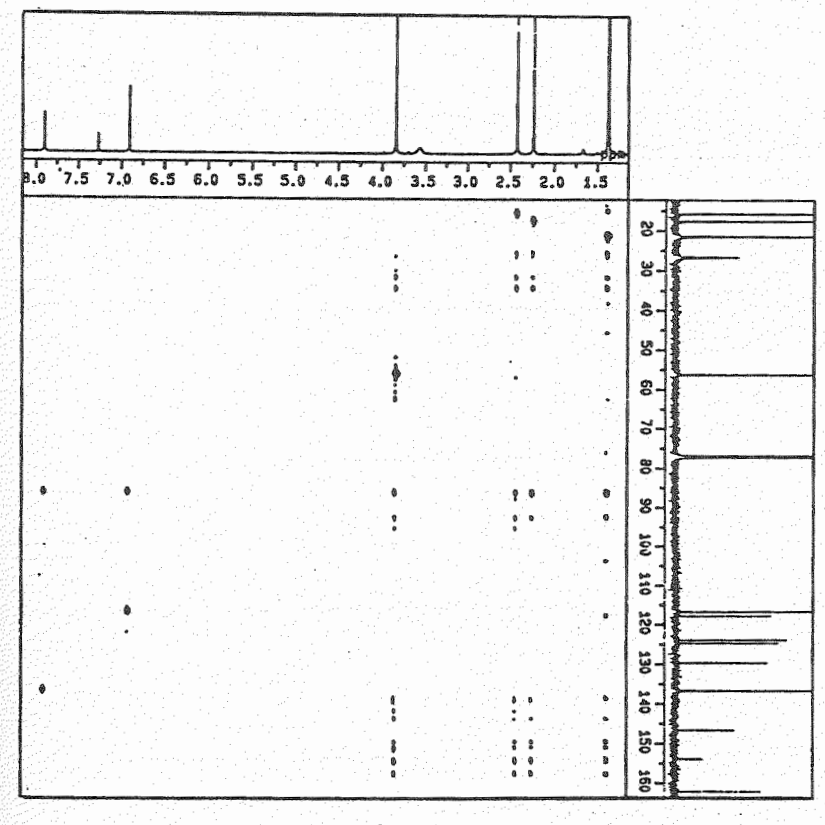


Fig 3.7 The DEPT 90 and 135 spectra of Compound 1



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JNM-A500

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PD : 1.5000 sec
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PW1 : 25.00 usec
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OBFRQ : 500.00 MHz
OBSET : 161930.26 Hz

IAMUC : 13C
IAFRQ : 125.65 MHz
IASET : 126157.90 Hz
IATM : 120
IAPW : 1.8 usec
IABP1 : 100
IABP2 : 2
IANS : 4

ACBIT : 16
CTEMP : 29.5 c
CSPED : 0 Hz
SLVNT : CDCL3
RESOL : 5.08 Hz
BF : 0.31 Hz
REFVL : 0.00 ppm
XE : 3516.03 Hz
XS : -313.02 Hz

OPERATOR :

Fig 3.8 The HMQC spectrum of Compound 1

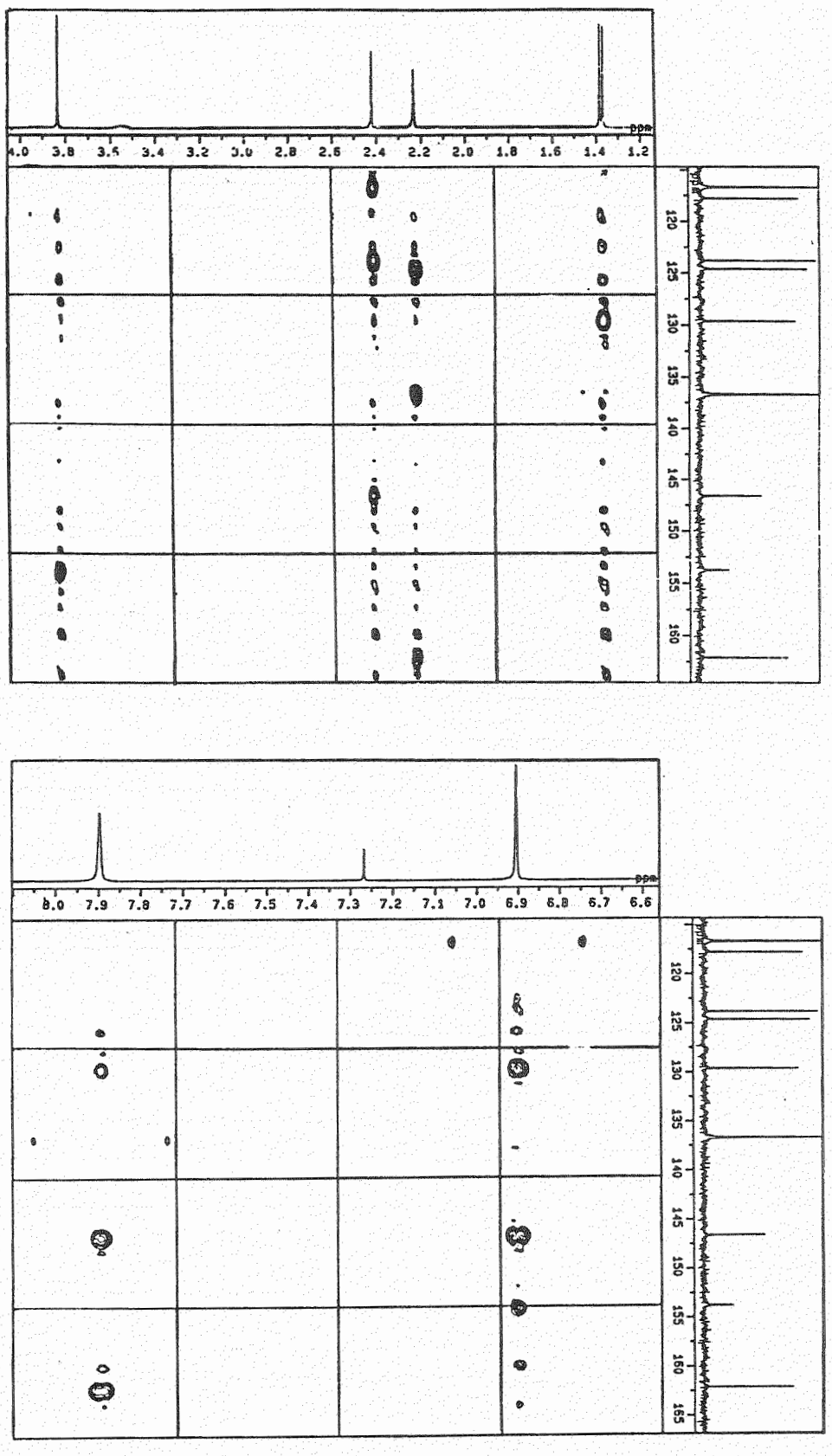
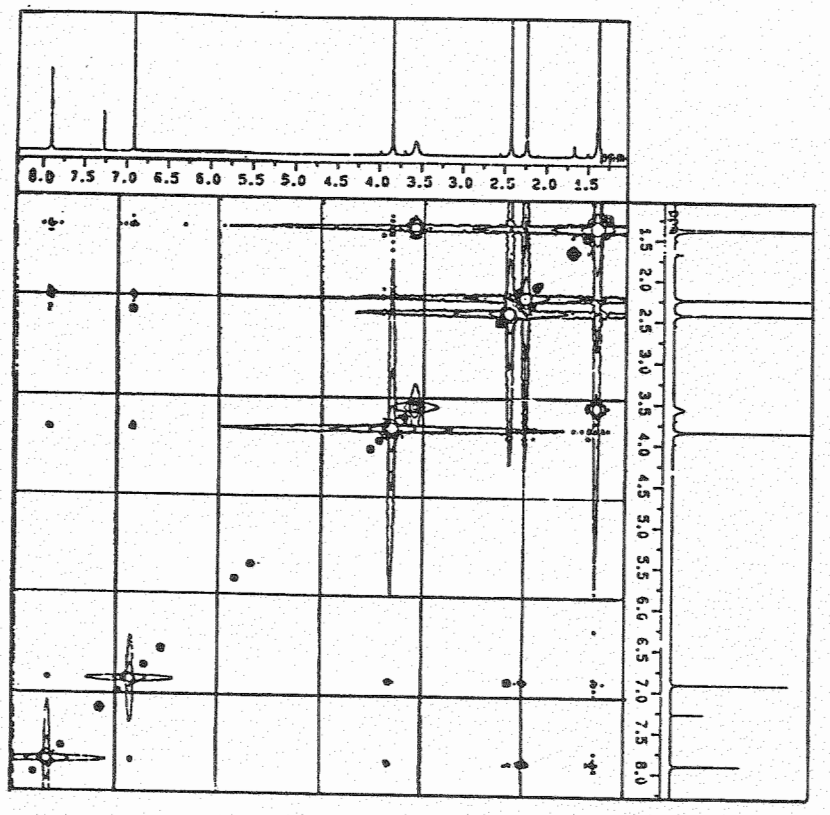


Fig 3.9 The HMBC spectra of Compound 1

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PD    :   1.5000 sec
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PW1   :     6.80 usec
OBSNC : 1H
OBSFRQ :   500.00 MHz
OBSET : 161893.95 Hz

IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :      120
IRAPW :     30.0 usec
IRBP1 :       25
IRBP2 :        2
IRAMS :        0

ACQIT :       16
CTEMP :     29.6 c
CSPEL :       11 Hz
SLVNT : CDCL3
RESOL :    10.48 Hz
BF    :     0.31 Hz
REFVL :     8.71 ppm
XE    :  3635.91 Hz
XS    :  -351.02 Hz

OPERATOR :
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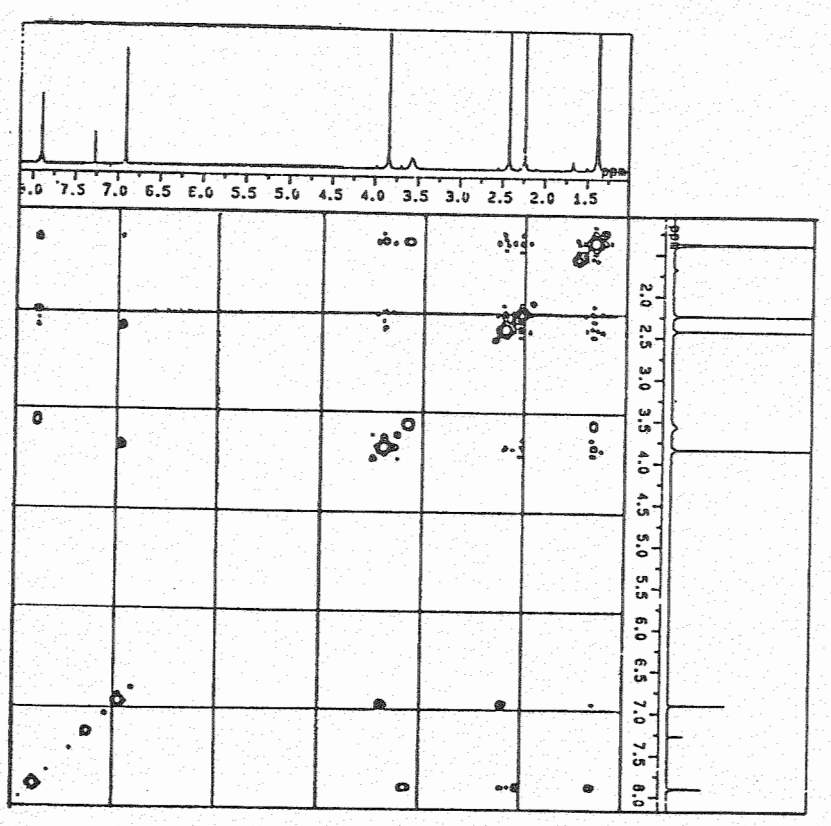
Fig 3.10 The COSY spectrum of Compound 1

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IRMOD : NON
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SCANS :       16
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PD    :    2.0000 sec
RGAIN :       18
PW1   :     6.80 usec
OBSNC : 1H
OBSFRQ :   500.00 MHz
OBSET : 161923.25 Hz

IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :      120
IRAPW :     30.0 usec
IRBP1 :       25
IRBP2 :        2
IRAMS :        0

ACQIT :       16
CTEMP :     29.6 c
CSPEL :        0 Hz
SLVNT : CDCL3
RESOL :    10.10 Hz
BF    :     0.31 Hz
REFVL :     8.71 ppm
XE    :  3576.86 Hz
XS    :  -282.92 Hz

OPERATOR :
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Fig 3.11 The ^{1}H-^{1}H NOESY spectrum of Compound 1

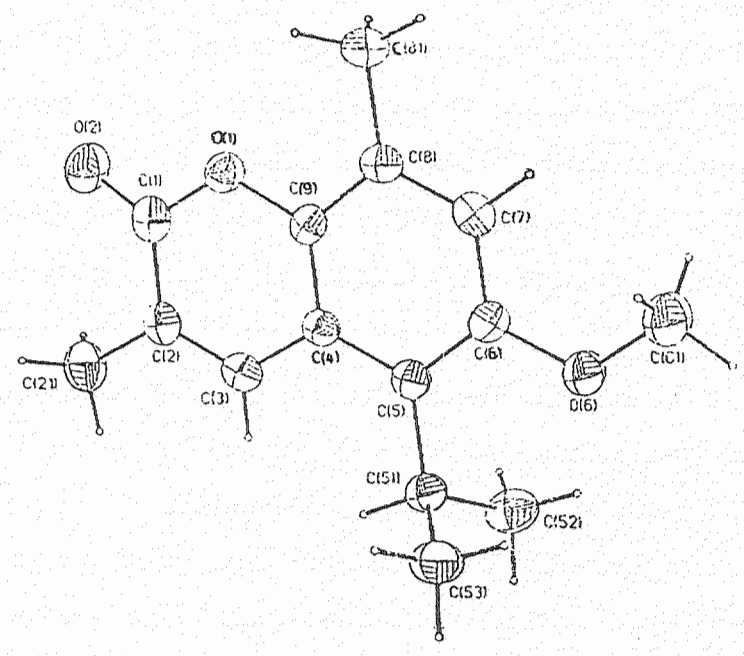
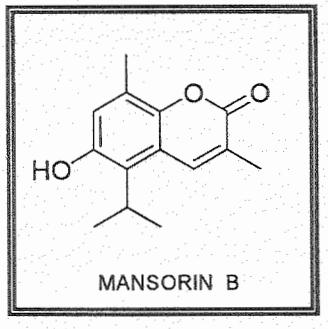


Fig 3.12 ORTEP of Compound 1

3.3.2 *Compound 2:* Mansorin B

Compound **2** was obtained as a pale yellow powder, m.p. 202-204 °C. This compound showed a single spot on TLC with R_f value 0.40 (silica gel; hexane : ethyl acetate = 7:3). The molecular formula of this compound was proposed to be $C_{14}H_{16}O_3$ by elemental analysis (Found %C 72.66 and %H 7.10; calcd. for $C_{14}H_{16}O_3$ MW 232.28: %C 72.41 and %H 6.89). The IR spectrum of Compound **2** (Fig 3.13) exhibited bands typical of a coumarin skeleton (α,β-unsaturated lactone at 1695 cm^{-1}) and a hydroxy group (a broad band at 3300-3400 cm^{-1}). The ^1H NMR spectrum of Compound **2** (Fig 3.15) indicated the presence of an isopropyl group at δ_H 3.51 (1H) and 1.40 (6H), two methyl groups at δ_H 2.22 (3H) and 2.31 (3H), an aromatic proton at δ_H 6.79 (1H), an olefinic proton at δ_H 7.90 (1H) and a hydroxy group at δ_H 5.67 (1H). The ^{13}C NMR spectrum of Compound **2** (Fig 3.16) clearly showed the signal of an ester carbonyl at δ_C 162.6. Comparison of the ^1H and ^{13}C NMR spectra of Compound **2** with those of Compound **1** (Table 3.6) clearly indicated that the methoxy group at C-6 of Compound **1** was replaced by a hydroxy group in Compound **2**. This observation was in accordance with correlation between H-7 and 6-OH in the ^1H-^1H NOESY spectrum (Fig 3.21). Thus, the structure of Compound **2** could be deduced to be 3,8-dimethyl-5-isopropyl-6-hydroxy coumarin, previously reported in the literature.[2] Herein, Compound **2** was named as mansorin B.



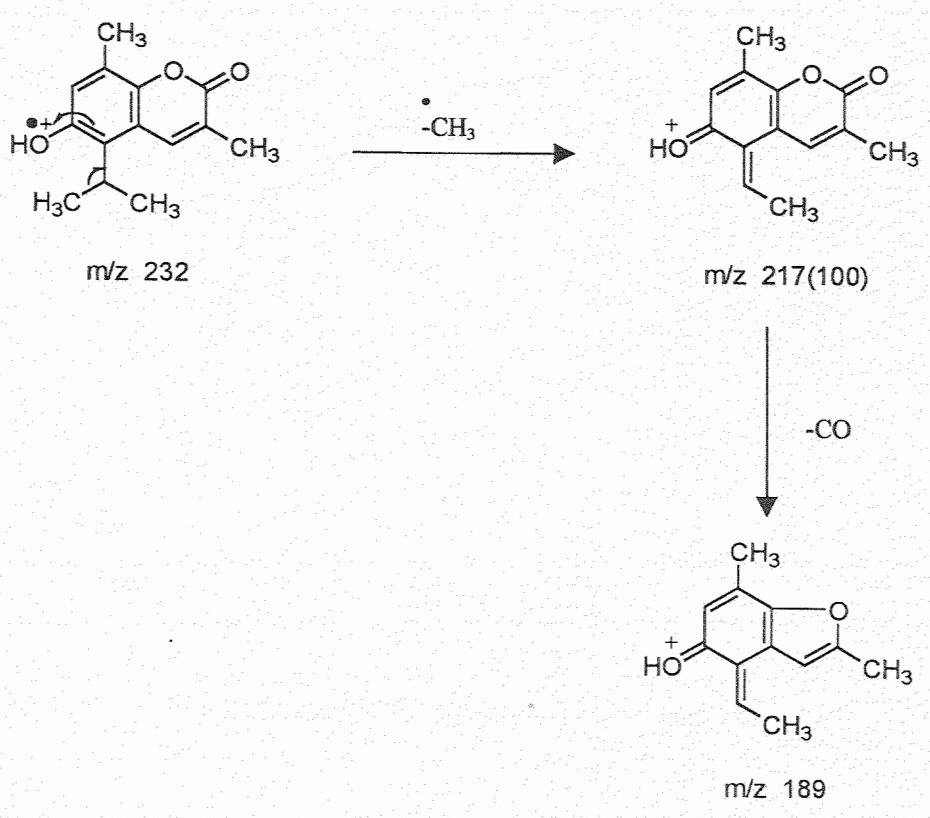
MANSORIN B

Table 3.6 ^{1}H-NMR and ^{13}C-NMR spectral data of Compounds **1** and **2**

Position	Chemical Shift (ppm)			
	1[a]		**2**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2	-	162.1	-	162.6
3	-	124.6	-	124.3
4	7.90, s, 1H	136.7	7.90, s, 1H	137.1
4a	-	117.8	-	117.9
5	-	129.6	-	126.7
6	-	153.7	-	150.0
7	6.90, s, 1H	116.7	6.79, s, 1H	120.9
8	-	123.8	-	124.1
8a	-	146.6	-	147.6
9	3.56, m, 1H	26.6	3.51, m, 1H	26.6
3- CH_3	2.23, s, 3H	21.4	2.22, d, 3H (1.2)	17.6
6-OCH_3	3.83, s, 3H	56.2	-	-
6-OH	-	-	5.67, s, 1H	-
8- CH_3	2.42, s, 3H	15.7	2.31, s, 3H	15.3
9-$(CH_3)_2$	1.38, d, 6H (7.3)	17.6 (2C)	1.40, d, 6H (8.0)	21.9 (2C)

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively
[b] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 200 and 50 MHz, respectively

The mass spectrum (Fig 3.14) displayed the molecular ion peak at m/z 232 ($[M]^+$), and other fragmentations at m/z 217 (M^+-CH_3) and 189 (M^+-CH_3-CO). The proposed fragmentation pattern of Compound **2** is shown in Scheme 3.3.



Scheme 3.3 The proposed fragmentation pattern of Compound **2**

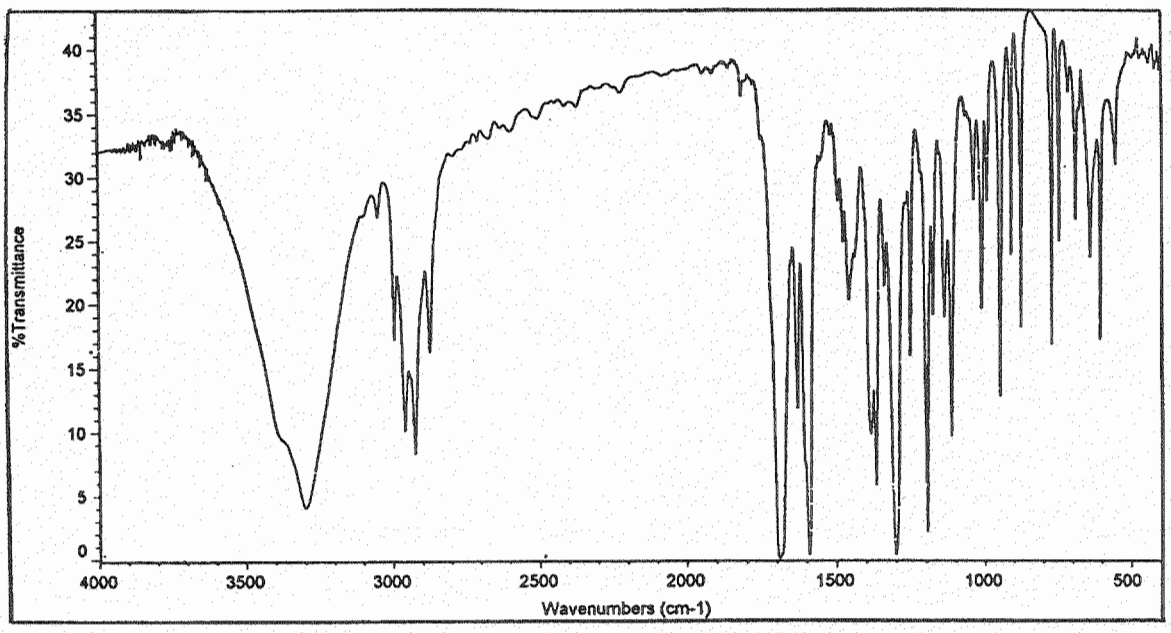


Fig 3.13 The FT-IR spectrum of Compound 2

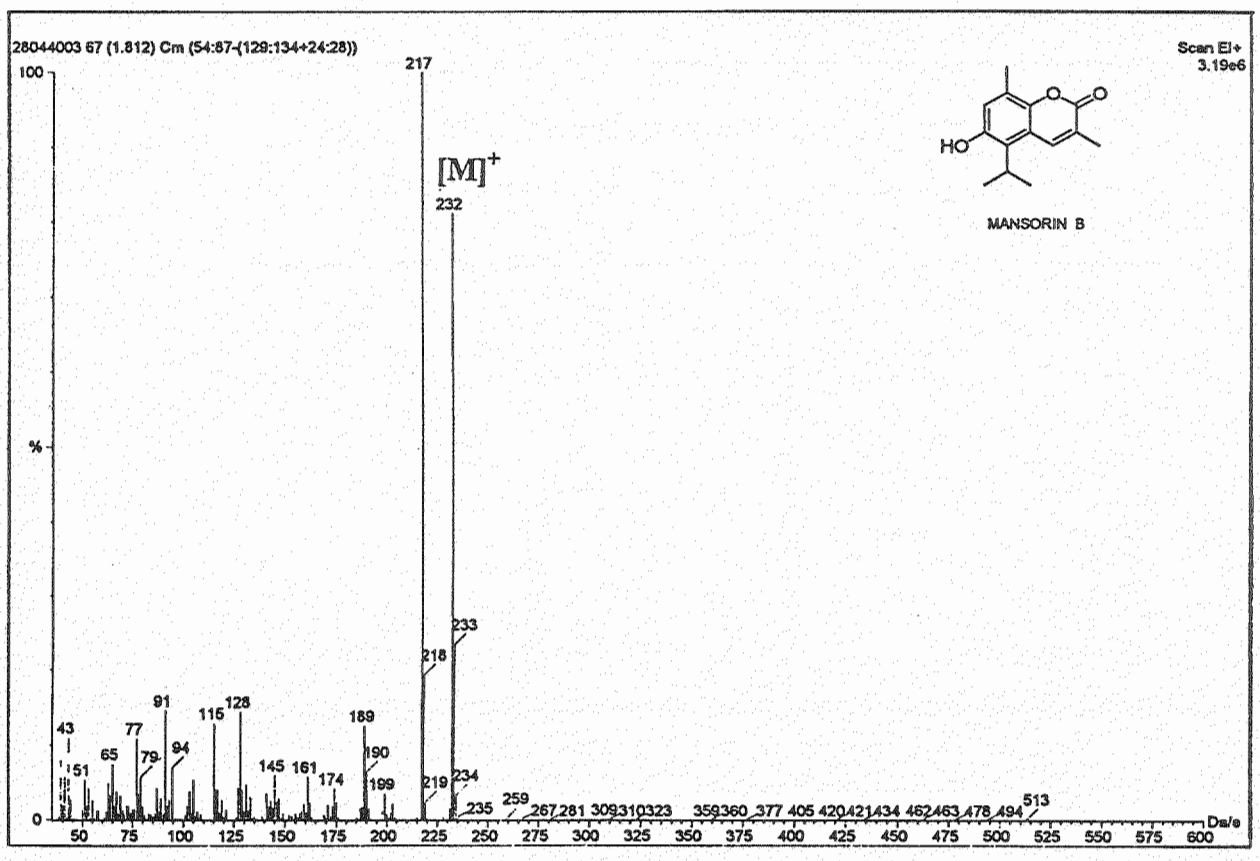


Fig 3.14 The mass spectrum of Compound 2

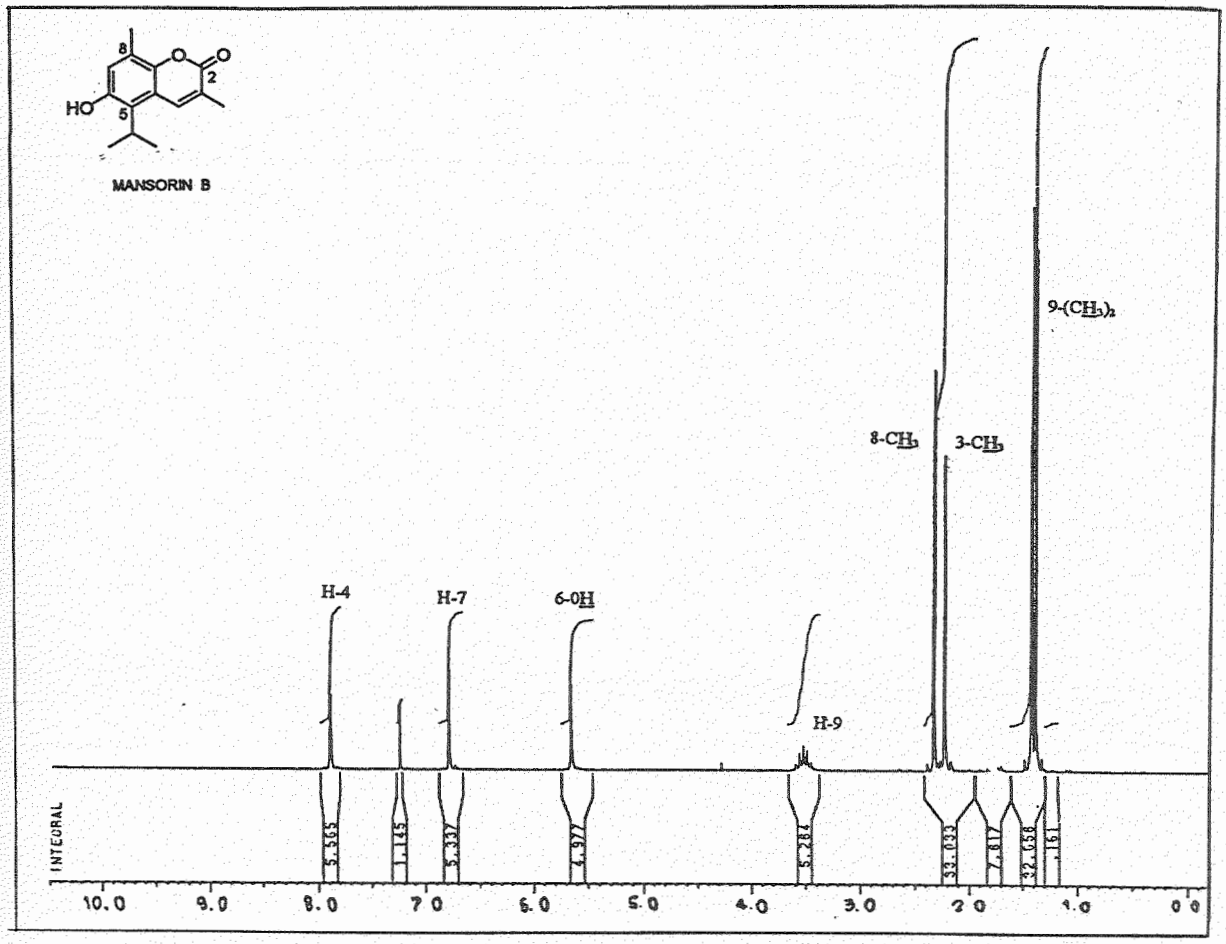


Fig 3.15 The ^{1}H-NMR spectrum of Compound 2

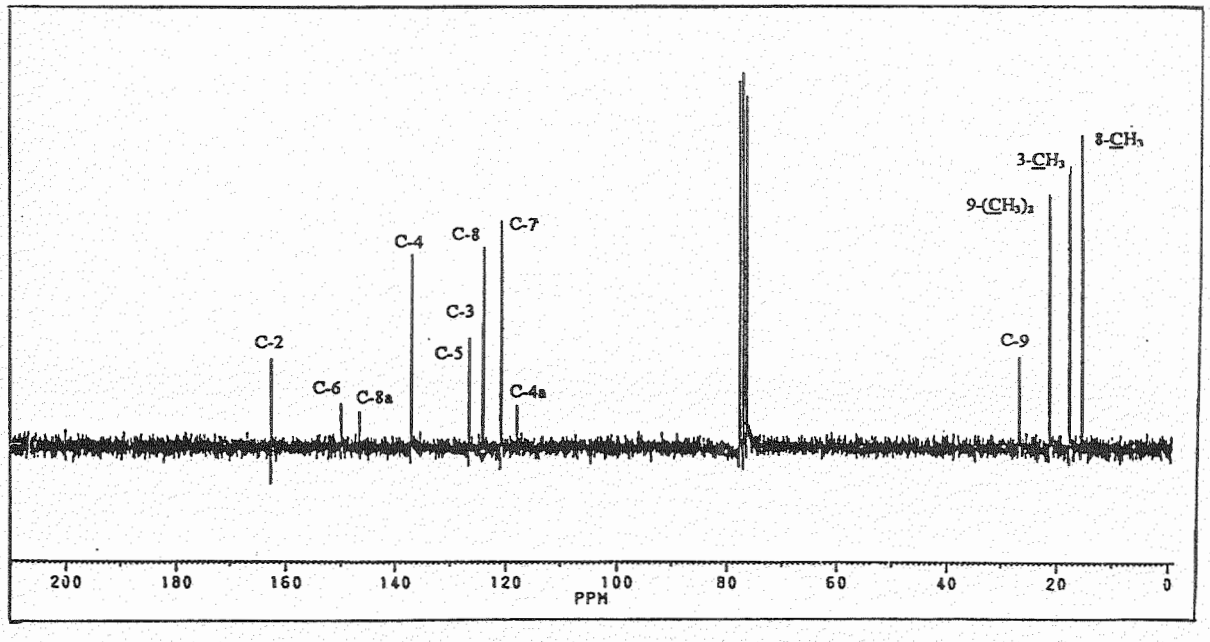


Fig 3.16 The ^{13}C-NMR spectrum of Compound 2

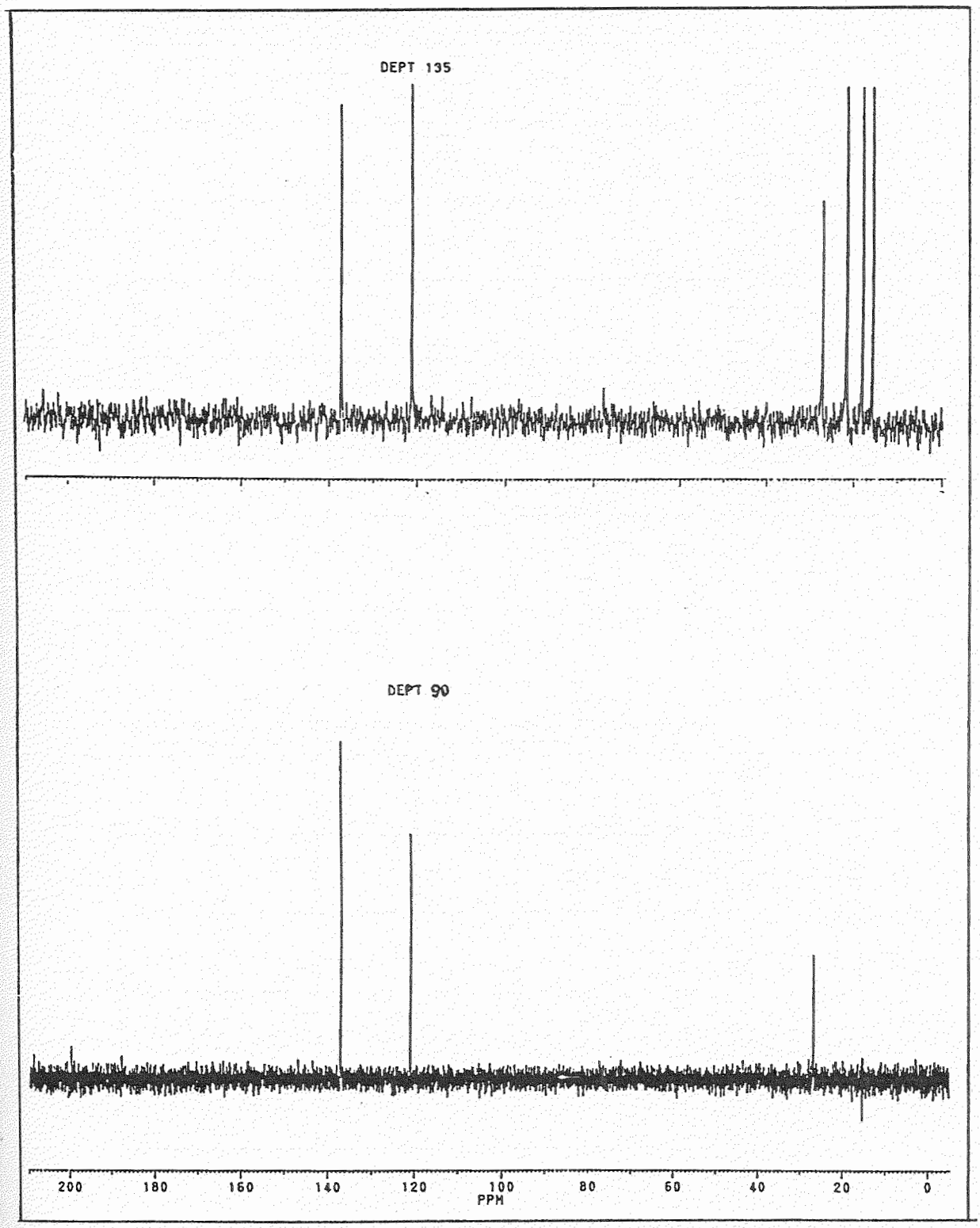


Fig 3.17 The DEPT 90 and 135 spectra of Compound 2

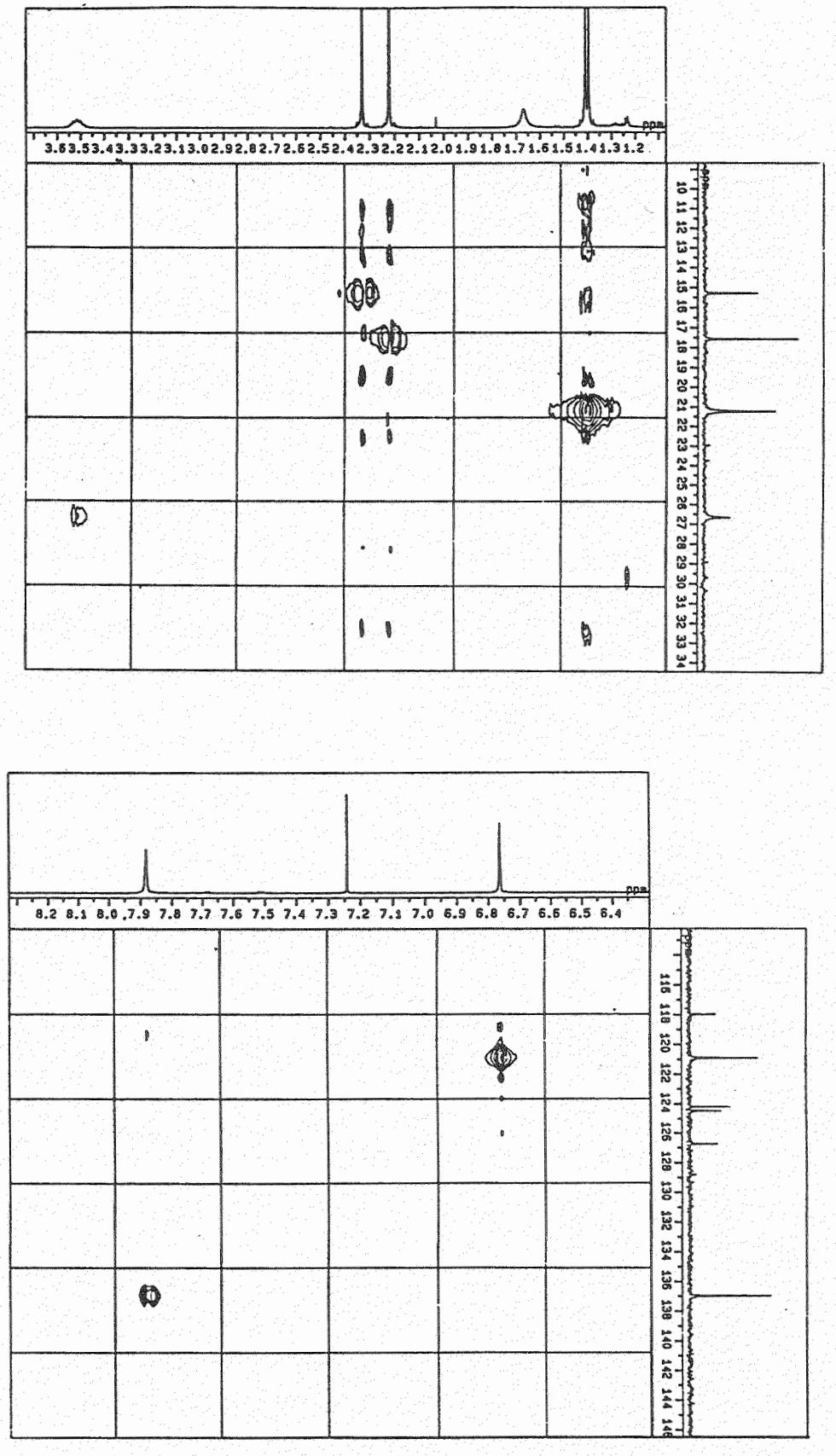


Fig 3.18 The HMQC spectra of Compound 2

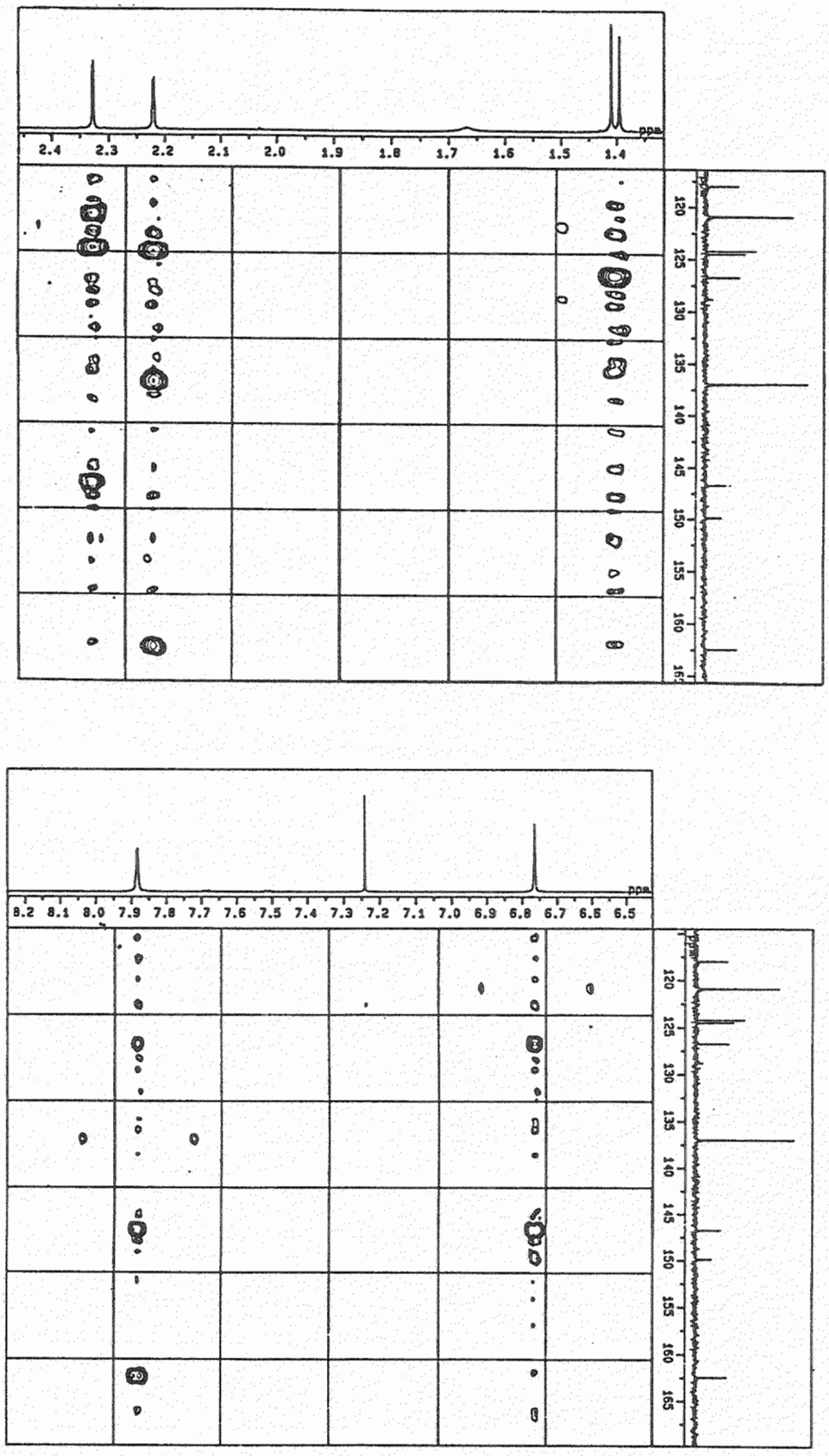
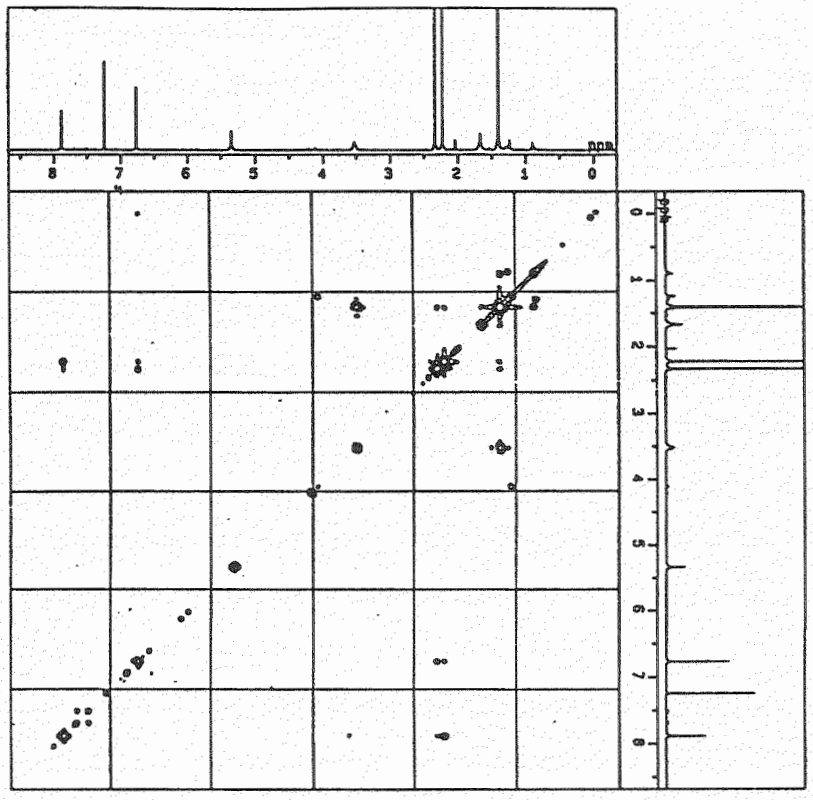


Fig 3.19 The HMBC spectra of Compound 2



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PD    :     1.5000 sec
RGAIN :         17

CLFRQ :  4512.64 Hz
CLPNT :        512
TOSCN :        256
CINWT :    10.00 usec
CINTV :   221.60 usec
CINT2 :   110.80 usec

PW1   :     6.80 usec
PW2   :    23.40 usec
PW3   :    11.70 usec
PI1   :250.0000 msec
PI2   :   1.0000 msec
PI3   :  48.9824 msec
JCNST :   145.00 Hz

OBNUC : 1H
OBFRQ :   500.00 MHz
OBSET : 162016.02 Hz
IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :        120
IRPW  :     55.0 usec
IRBP1 :          30
IRBP2 :           6
IRRNS :           0

ADBIT :          16
CTEMP :        27.3 c
CSPED :          11 Hz
SLVNT : CDCL3
RESOL :        8.81 Hz
CLRSO :        8.81 Hz
TLINE :           4
THTOP :     30.0000
THBTM :      0.5000

OPERATOR :
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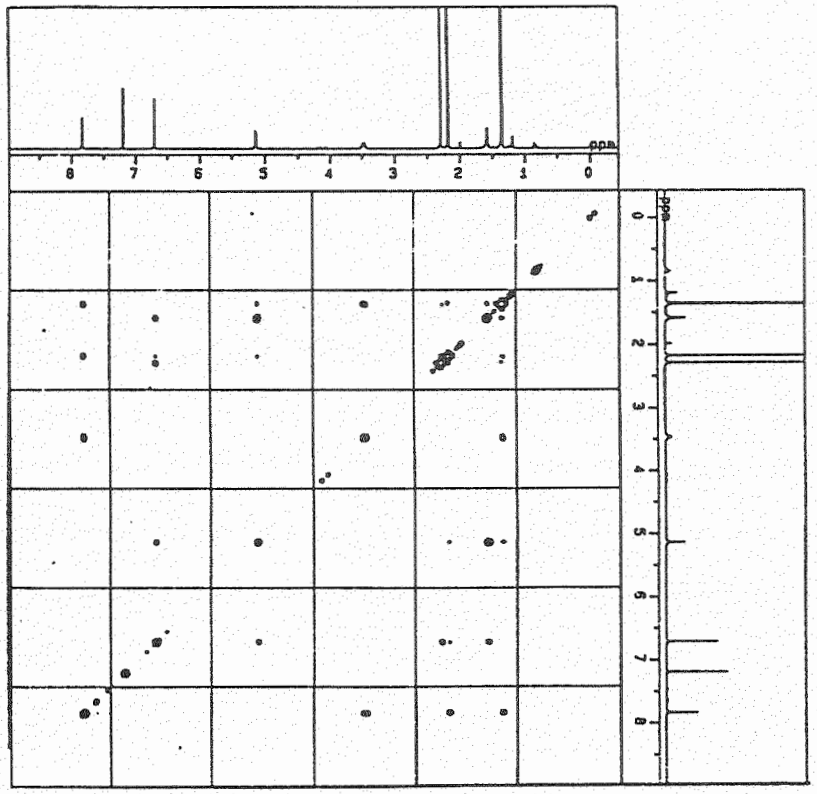
Fig 3.20 The COSY spectrum of Compound 2



```
##############################
# CHULALONGKORN UNIVERSITY   #
#         JNM-A500           #
##############################

SFILE : [.MNA]CPDS-NOESY
COMNT : CPDS-NOESY

EXMOD : NOESY
IRMOD : NON
POINT :        512
FREQU :  4710.32 Hz
SCANS :          16
DUMMY :           4
ACQTM :      0.1087 sec
PD    :      2.0000 sec
RGAIN :          17

CLFRQ :  4710.32 Hz
CLPNT :        512
TOSCN :        256
CINWT :    10.00 usec
CINTV :   212.30 usec
CINT2 :   106.15 usec

PW1   :     6.80 usec
PW2   :    23.40 usec
PW3   :    11.70 usec
PI1   :2108.6957 msec
PI2   :   1.0000 msec
PI3   :  48.9824 msec
JCNST :   145.00 Hz

OBNUC : 1H
OBFRQ :   500.00 MHz
OBSET : 162090.18 Hz
IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :        120
IRPW  :     55.0 usec
IRBP1 :          30
IRBP2 :           6
IRRNS :           0

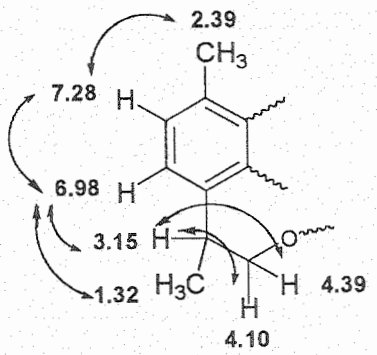
ADBIT :          16
CTEMP :        27.8 c
CSPED :           0 Hz
SLVNT : CDCL3
RESOL :        9.20 Hz
CLRSO :        9.20 Hz
TLINE :           6
THTOP :     30.0000
THBTM :      6.3500

OPERATOR :
```

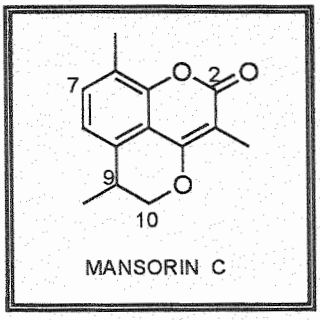
Fig 3.21 The ^{1}H-^{1}H NOESY spectrum of Compound 2

3.3.3 *Compound 3:* Mansorin C

Compound **3**, $[\alpha]_D^{18}$ +18.9 (c = 1.00, $CHCl_3$), m.p. 151-153 °C, was obtained as a white crystal. This compound showed a single spot on TLC with R_f value 0.25 (silica gel; hexane : dichloromethane = 3:7). The presence of an α,β-unsaturated ester (lactone) was implied by the IR band at 1705 cm^{-1} (Fig 3.22) and confirmed by the signal in the ^{13}C NMR spectrum at δ_C 164.2 ppm. The ^1H NMR spectrum (Fig 3.24) showed the *para*-substituted aromatic pattern signals; two doublet signals at δ_H 6.98 and 7.28 with J = 7.6 Hz. In addition, two doublet of doublet signals centered at δ_H 4.10 (1H, J = 6.7 and 11.0 Hz) and 4.39 (1H, J = 4.0 and 10.7 Hz) were assigned to a –CH-CH_2-O- group. The NMR data of Compound **3** were compared with those of isolated compound from *M. gagei*[2] and revealed that Compound **3** was 2,3-dihydro-3,6,9-trimethylnaphtho [1,8-bc]-pyran-7-oxa-8-one, herein, called as mansorin C. However, the proton assignments of H-6 and H-7 from the previous report[2] were not compatible with the present data, which was reinforced with the aids of NOESY experiment.



From the NOESY spectrum of Compound **3**, the aromatic proton at δ_H 6.98 coupled with the other aromatic proton (δ_H 7.28), methine proton (δ_H 3.15) and methyl group (δ_H 1.32) as well as the signal at δ_H 7.28 showed the correlation with another methyl group (δ_H 2.39). Moreover, the correlations between the methine proton (δ_H 3.15) and methylene protons (δ_H 4.10 and 4.39, 1H each) were observed. Therefore, the chemical shift values of H-6 and H-7 must be switched to δ_H 6.98 and 7.28, respectively (Table 3.7).



MANSORIN C

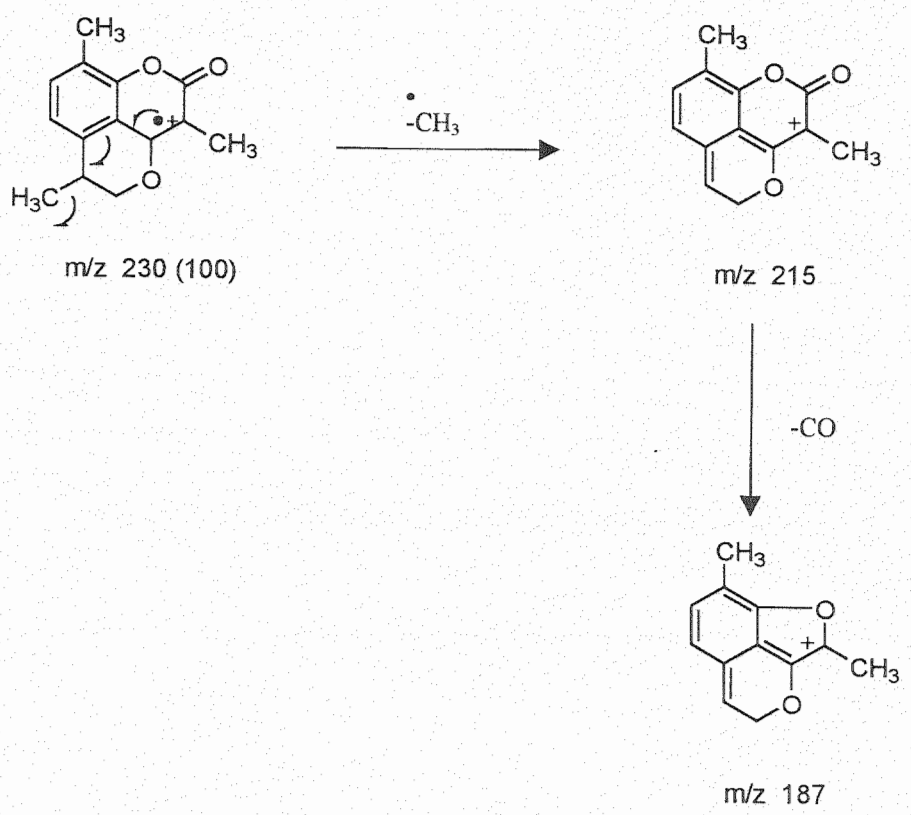
Table 3.7 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **3**

Position	Chemical Shift (ppm)[a]	
	δ_H (J in Hz)	δ_C
2	-	164.2
3	-	102.5
4	-	159.4
4a	-	120.0
5	-	110.8
6*	6.98, d, 1H (7.6)	132.4
7*	7.28, d, 1H (7.6)	124.0
8	-	134.4
8a	-	150.1
9	3.15, m, 1H	31.0
10	4.10, dd, 1H (6.7,11.0) 4.39, dd, 1H (4.0, 10.7)	72.5
3-CH_3	2.04, s, 3H	8.9
8-CH_3	2.39, s, 3H	17.0
9-CH_3	1.32, d, 3H (7.4)	15.4

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively

* Assignments differed from those in the literature[2]

The mass spectrum (Fig 3.23) displayed the molecular ion peak at m/z 230 ([M]$^+$), and other fragmentations at m/z 215 (M^+-CH_3) and 187 (M^+-CH_3-CO). The proposed fragmentation pattern of Compound **3** is shown in Scheme 3.4.



Scheme 3.4 The proposed fragmentation pattern of Compound **3**

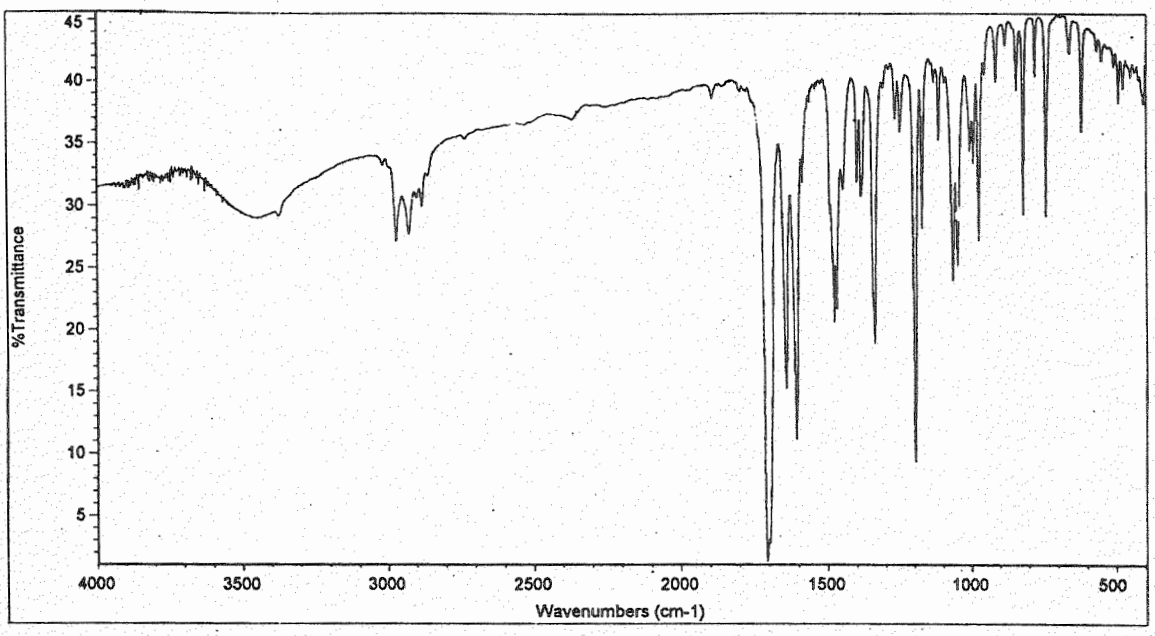


Fig 3.22 The FT-IR spectrum of Compound 3

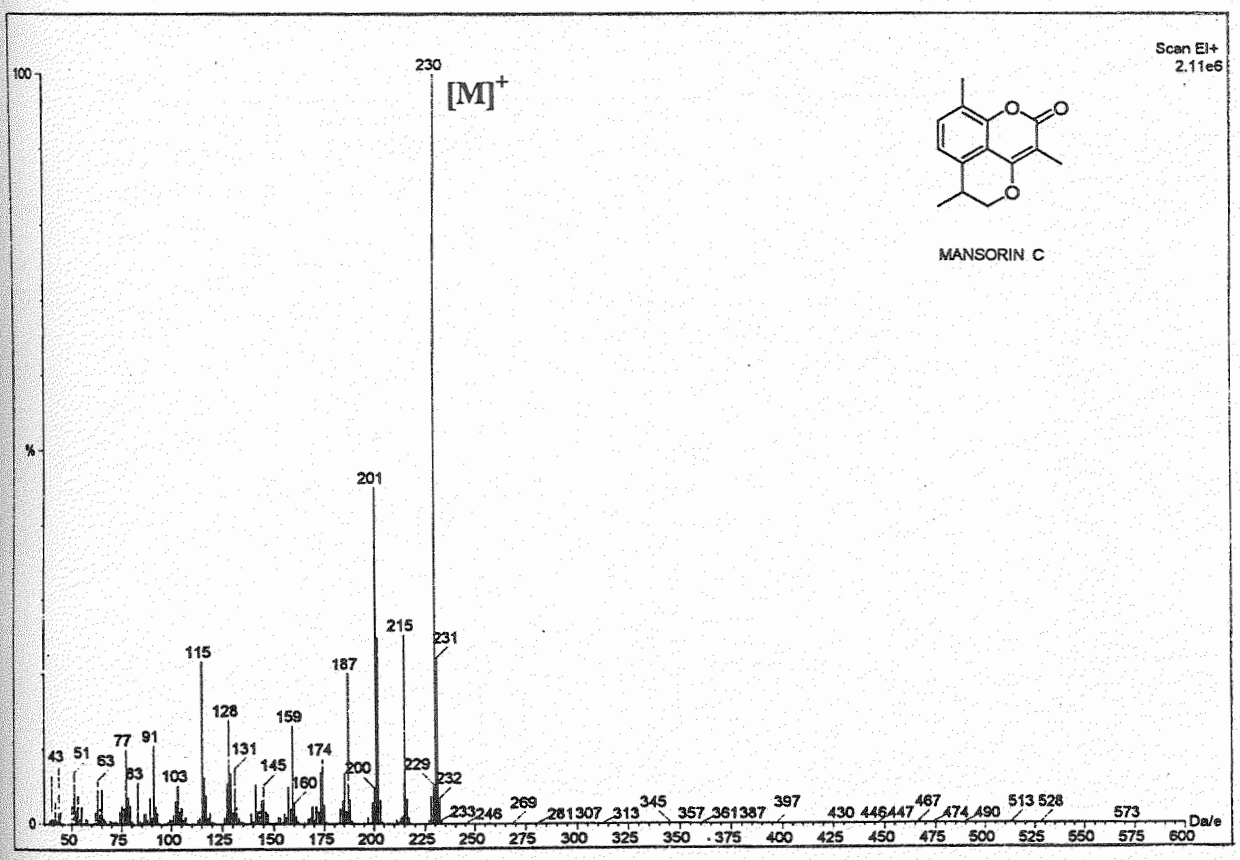


Fig 3.23 The mass spectrum of Compound 3

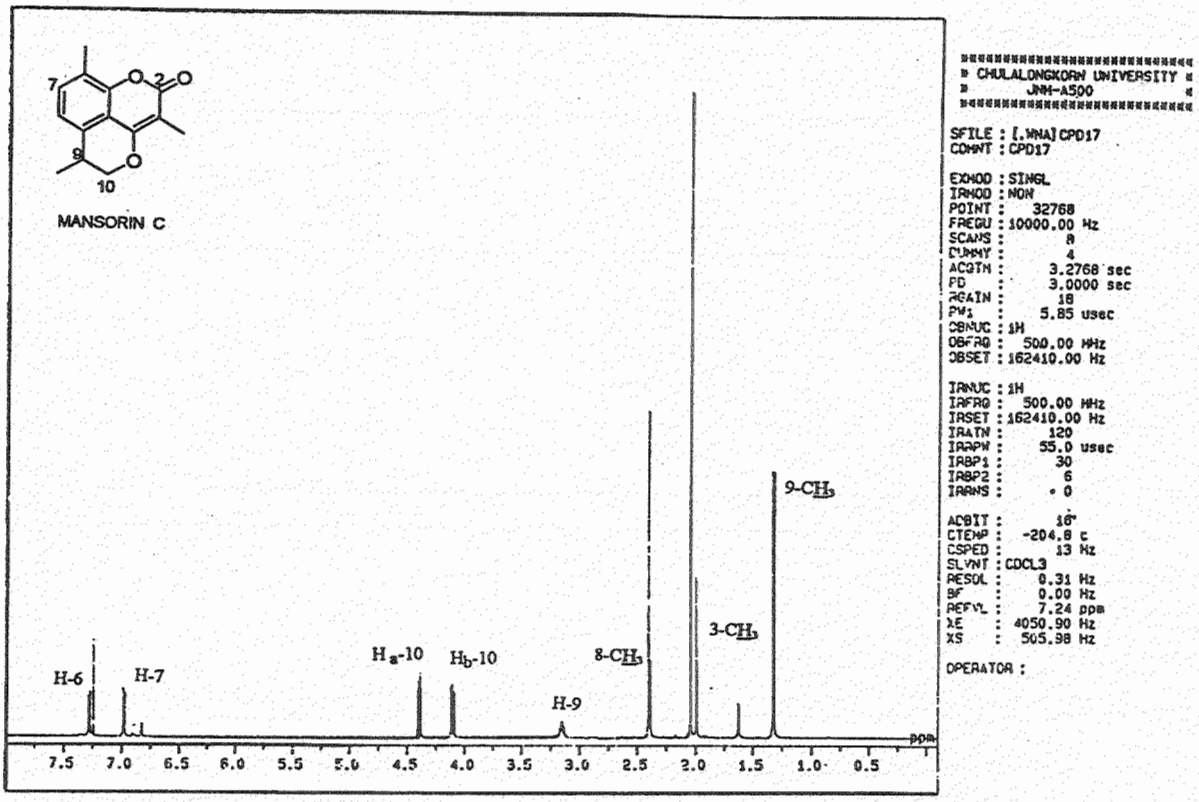


Fig 3.24 The ^{1}H-NMR spectrum of Compound 3

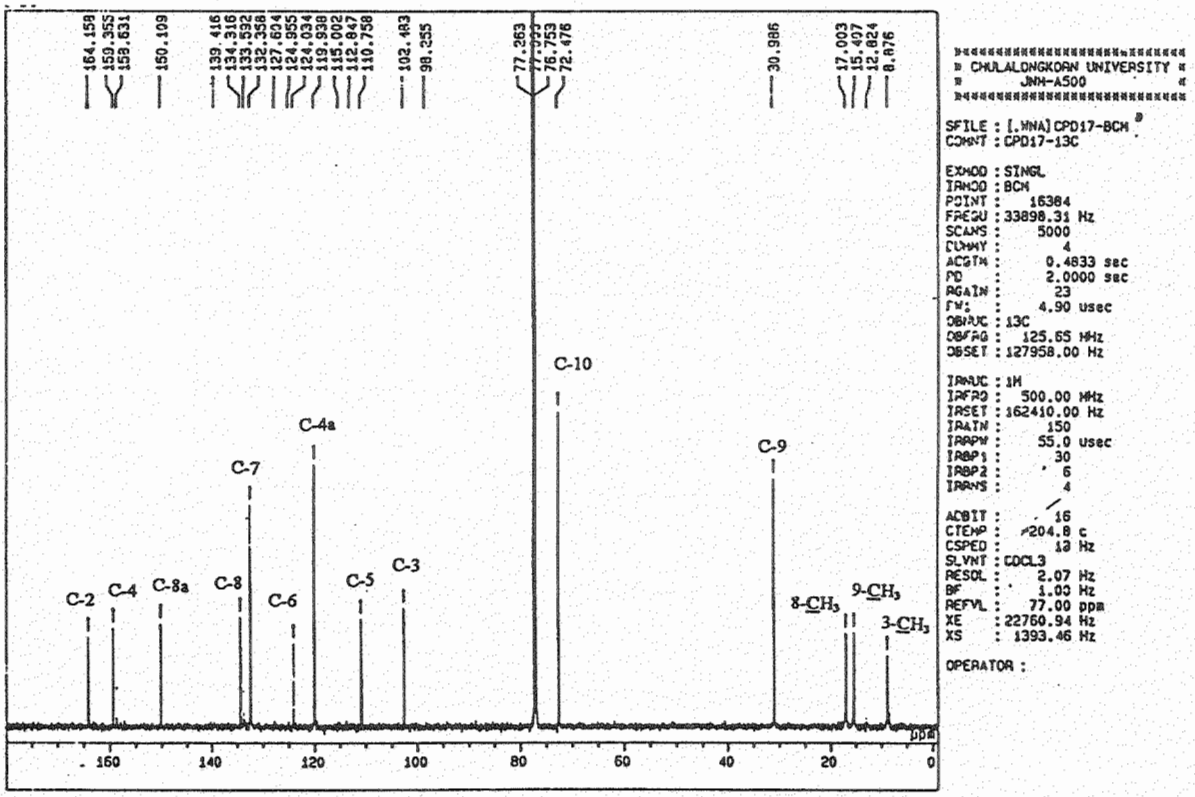


Fig 3.25 The ^{13}C-NMR spectrum of Compound 3

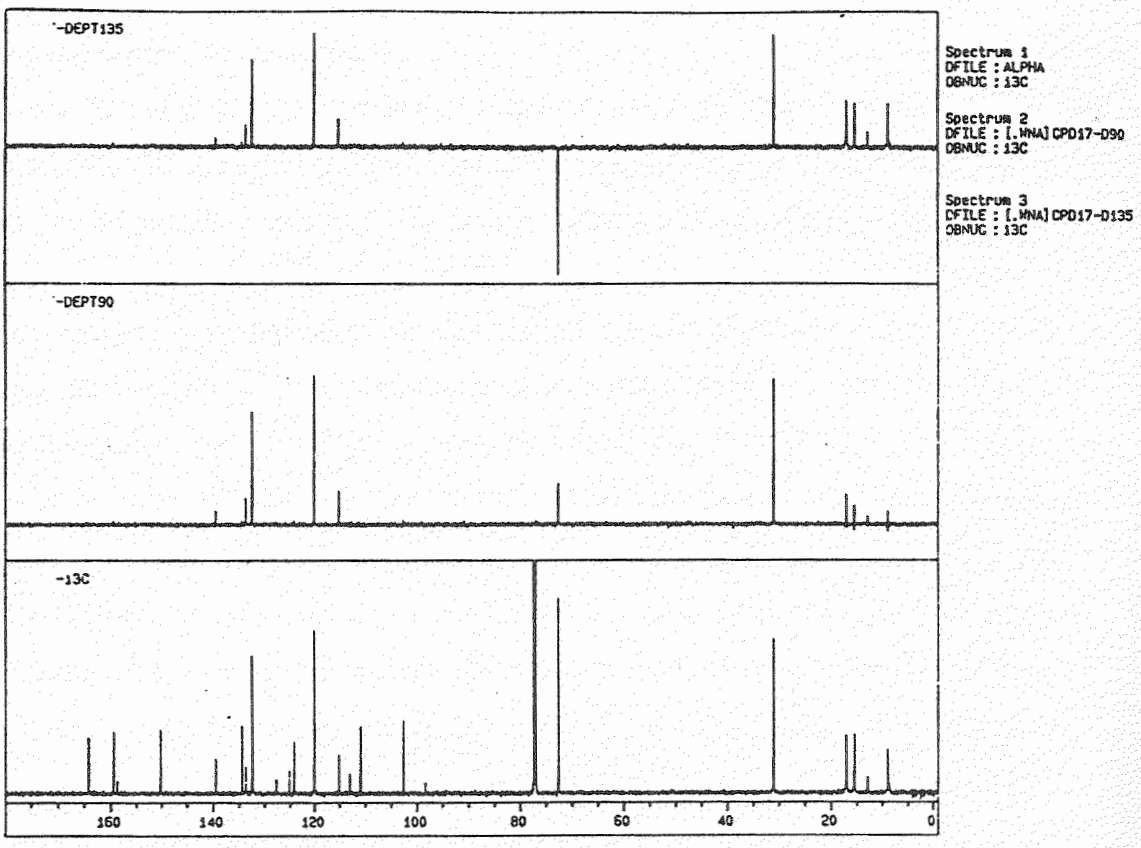


Fig 3.26 The DEPT 90 and 135 spectra of Compound 3

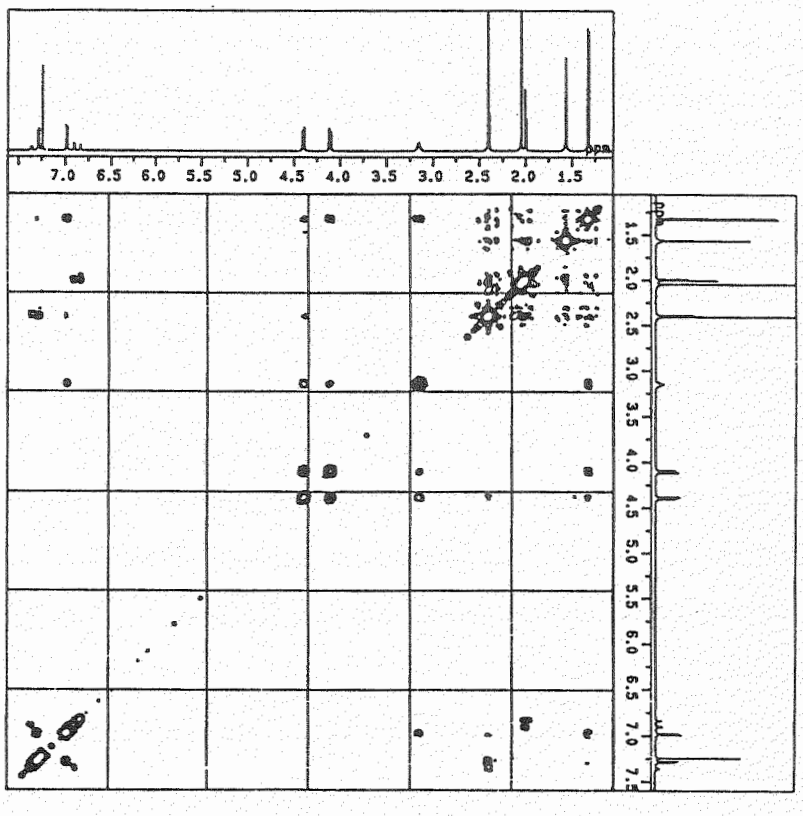


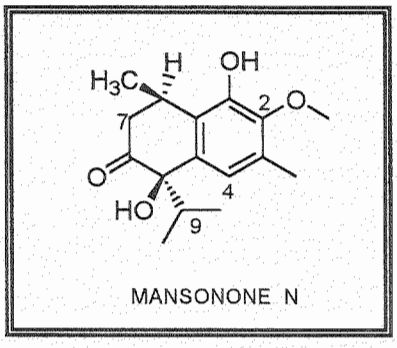
Fig 3.27 The ^{1}H-^{1}H NOESY spectrum of Compound 3

3.3.4 *Compound 4:* Mansonone N

Compound **4** was isolated as a colorless crystal; $[\alpha]_D^{20}$ +30.0 (c = 0.20, $CHCl_3$), m.p. 144-145 °C. A molecular ion at m/z 278 in the EIMS, ^1H NMR and ^{13}C NMR spectra of Compound **4** suggested a molecular formula of $C_{16}H_{22}O_4$. Two different kinds of chemical shifts were noticed in the ^{13}C NMR spectrum (Fig 3.30): six resonances between 120-145 ppm, typical of an aromatic moiety and high field signals from 15 to 80 ppm, characteristic of an aliphatic unit. A ketonic carbonyl was also identified in the low-field region of the ^{13}C NMR spectrum by a resonance at δ_C 212.1 (C-6). This signal was positioned on the aliphatic unit based on the informative data from the HMBC correlations with ^1H NMR signals (Fig 3.33) at δ_H 3.79 (H-8), 2.49 (H-7), 3.21 (H-7), and 2.15 (H-9). An isopropyl group could be noticeable from the ^1H NMR spectrum (Fig 3.29) by the presence of two methyl groups at δ_H 0.79 (d, J = 6.9 Hz, 9-CH_3) and δ_H 0.99 (d, J = 6.7 Hz, 9-CH_3) which correlated in the COSY spectrum with a methine resonance centered at δ_H 2.15 (sept, J = 6.9 Hz, H-9). This isopropyl unit also showed long-range heteronuclear correlations to the aliphatic group between the protons of the methyl units located at δ_H 0.79 (9-CH_3) and δ_H 0.99 (9-CH_3) in the ^1H NMR spectrum with the ^{13}C NMR resonance at δ_C 80.6 (C-5). The chemical shift of C-5 was typical of a quaternary oxygenated carbon. Other two methyl signals were detected in the ^1H NMR spectrum. The doublet at δ_H 1.16 (d, J = 6.9 Hz, 8-CH_3) was correlated in the COSY spectrum (Fig 3.34) with the aliphatic proton at δ_H 3.79 (m, H-8). Further analysis of the aliphatic area of the same spectrum gave evidence for the presence of a CH_3-CH-CH_2 unit. The second methyl group was a singlet located at δ_H 2.30 (s, 3-CH_3) suggesting an attachment to the aromatic system of the molecule. This hypothesis was supported by HMBC correlations between the δ_H 2.30 and the ^{13}C NMR resonances at δ_C 120.1 (C-4), 128.8 (C-3), and 144.4 (C-2).

The only proton in the aromatic region of the ^1H NMR spectrum was positioned on C-4 as a result of the long-range heteronuclear correlations observed between the ^1H NMR signal at δ_H 6.86 (H-4) and the ^{13}C NMR resonances located at δ_C 80.6 (C-5), 16.0 (3-CH_3), 124.6 (C-8a), and 144.4 (C-2). This assignment was supported by the ^1H-^1H NOESY spectrum (Fig 3.41) observed between proton at δ_H 6.86 (H-4) and the ^1H NMR signals at δ_H 2.15 (H-9), 2.30 (3-CH_3) and 0.99 (9-CH_3). Substitution of a methoxy group (δ_H 3.79) at the C-2 position of the aromatic moiety was also confirmed

through NOE correlations with the methyl group at δ_H 2.30 (3-CH_3). Spectrometric data permitted to establish the structure of Compound **4** as a new sesquiterpenoid derivative named mansonone N and their NMR data is shown in Table 3.8. Definitive evidence for this structure was obtained from a single-crystal X-ray analysis (see Appendix). The molecular structure and crystallographic numbering scheme are illustrated in Figure 3.36. Unfortunately, the measured crystal was an inversion twin and, therefore, the absolute configuration could not be determined.



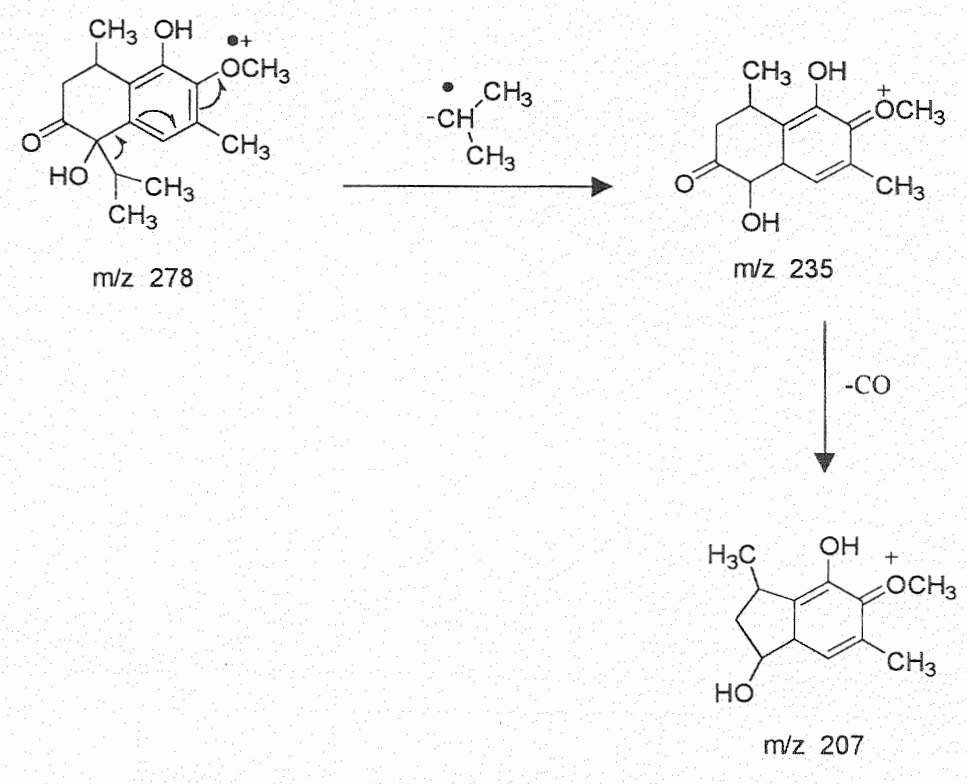
MANSONONE N

Table 3.8 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **4**

Position	Chemical Shift (ppm)[a]	
	δ_H (J in Hz)	δ_C
1	-	145.1
2	-	144.4
3	-	128.8
4	6.86, s, 1H	120.1
4a	-	136.5
5	-	80.6
6	-	212.1
7	2.49, dd, 1H (1.7, 12.5) 3.21, dd, 1H (7.8, 12.6)	43.8
8	3.79, m, 1H	32.4
8a	-	124.6
9	2.15, sept, 1H (6.9)	39.6
1-OH	5.84, s, 1H	-
2-OCH_3	3.79, s, 3H	60.6
3-CH_3	2.30, s, 3H	16.0
5-OH	4.05, s, 1H	-
8-CH_3	1.16, d, 3H (6.9)	22.0
9-$(CH_3)_2$	0.79, d, 3H (6.9) 0.99, d, 3H (6.9)	18.2 16.3

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively

The mass spectrum (Fig 3.28) displayed the molecular ion peak at m/z 278 ($[M]^+$), other fragmentations were detected at m/z 235 (M^+-C_3H_7) and 207 (M^+-C_3H_7-CO). The proposed fragmentation pattern of Compound **4** is shown in Scheme 3.5.



Scheme 3.5 The proposed fragmentation pattern of Compound **4**

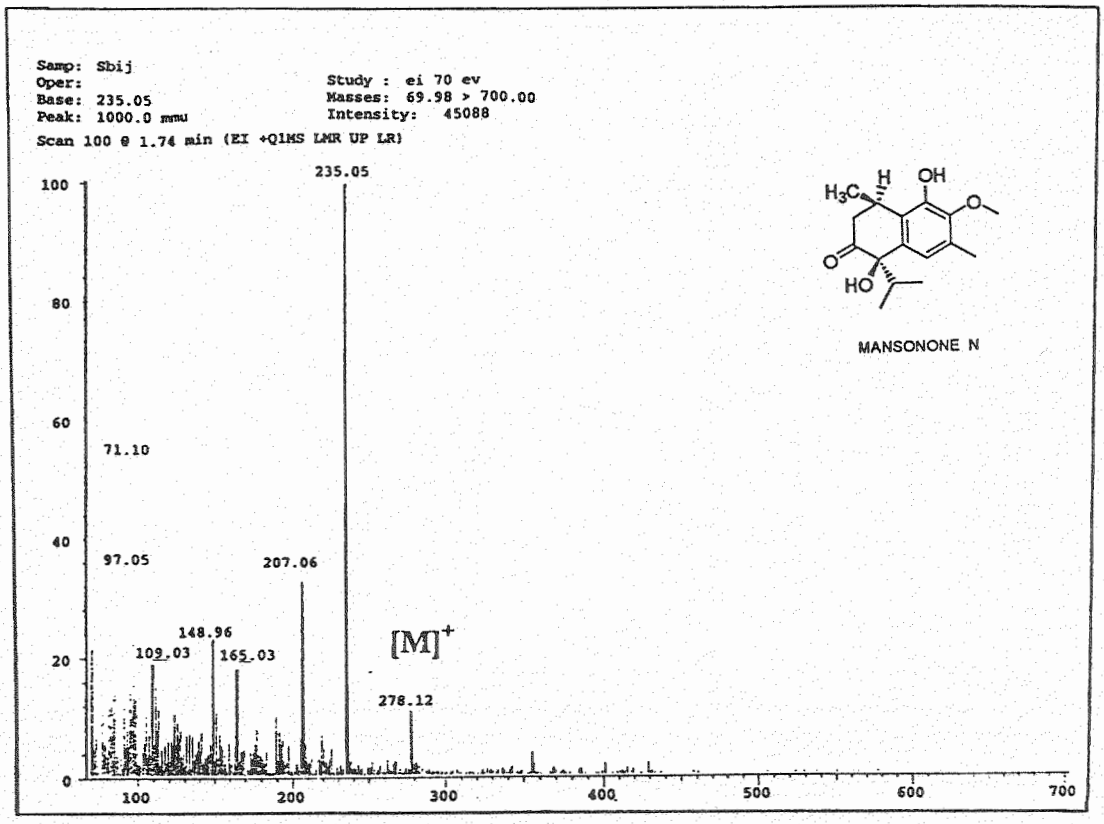


Fig 3.28 The mass spectrum of Compound 4

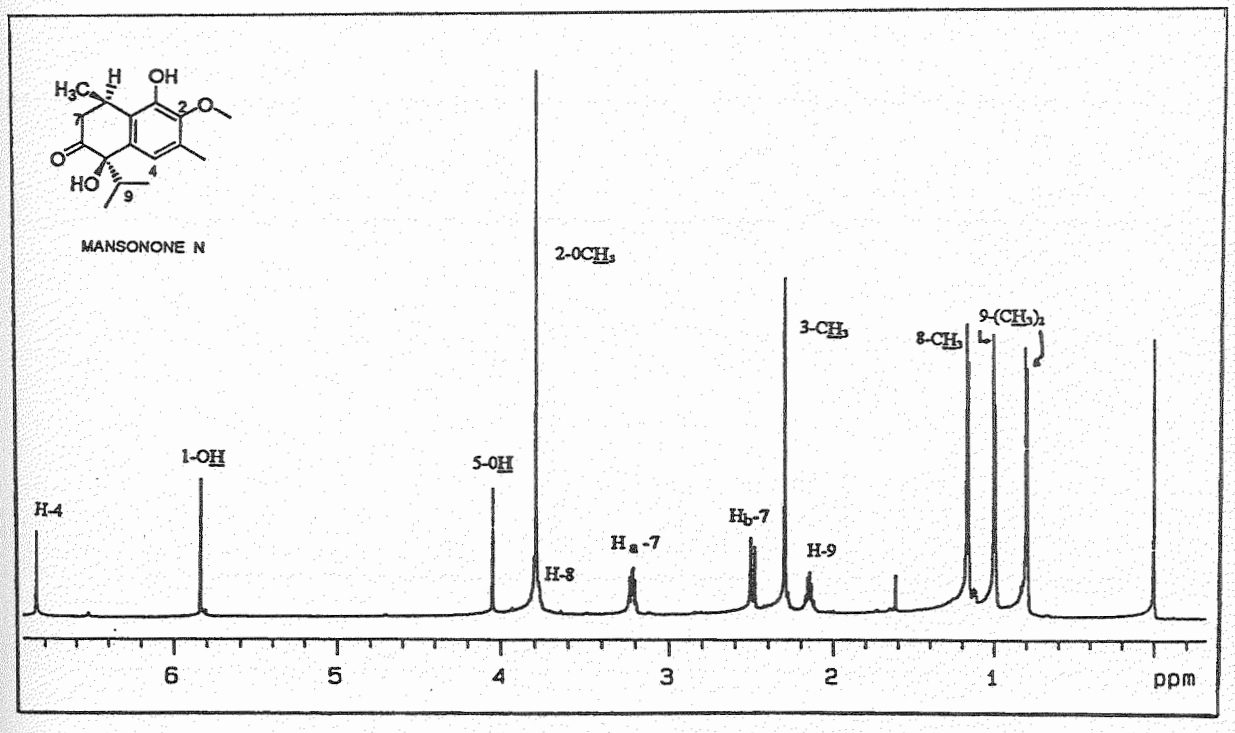


Fig 3.29 The ^{1}H-NMR spectrum of Compound 4

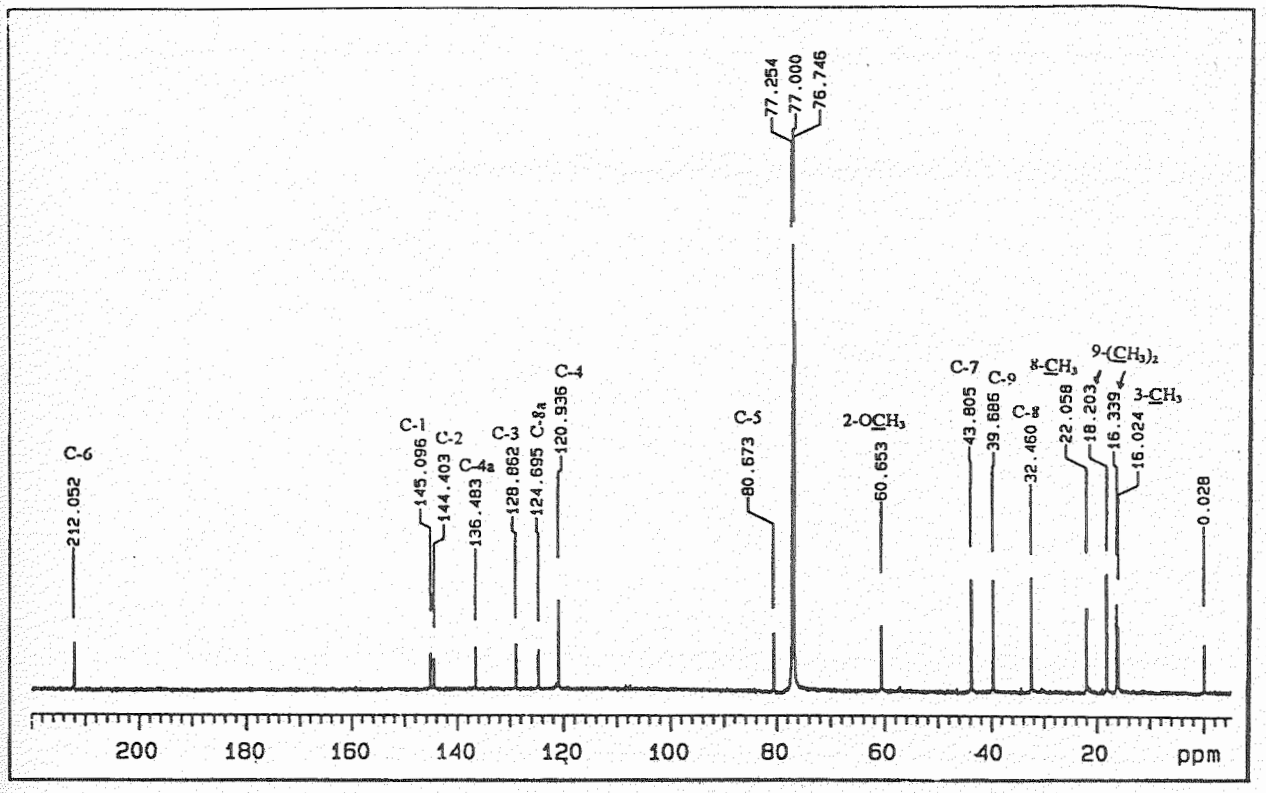


Fig 3.30 The ^{13}C-NMR spectrum of Compound 4

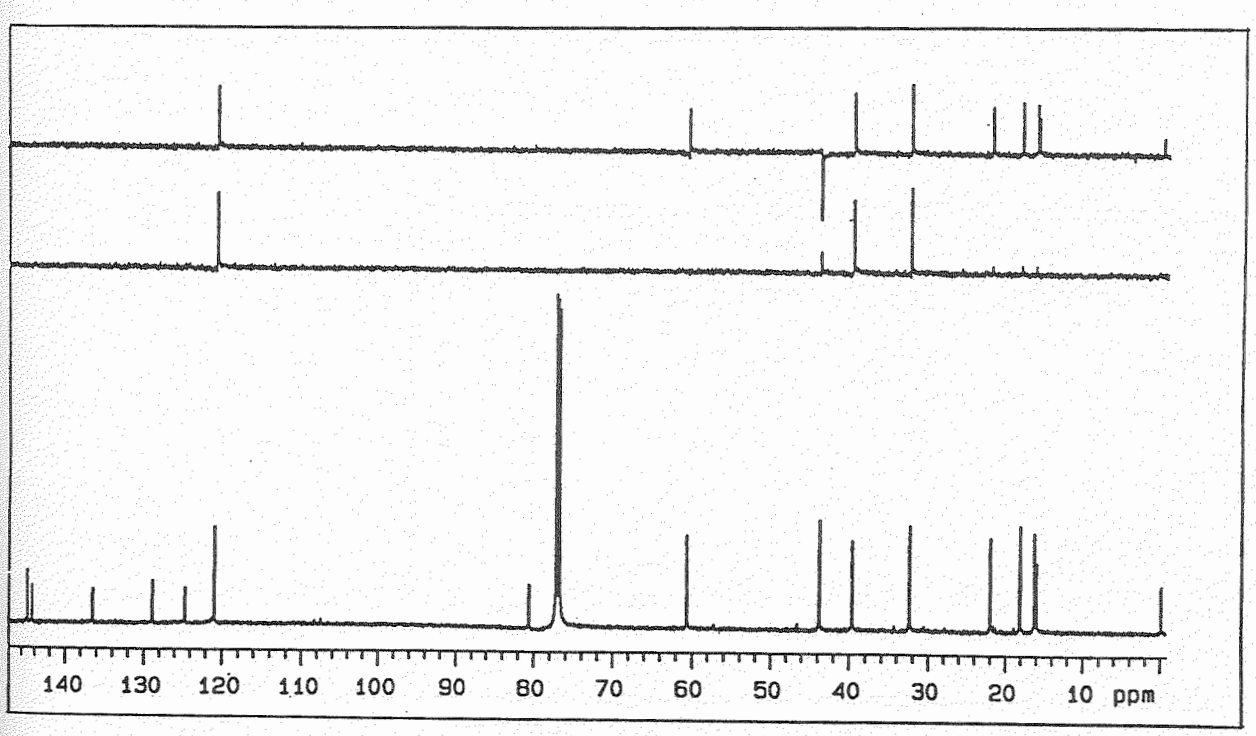


Fig 3.31 The DEPT 90 and 135 spectra of Compound 4

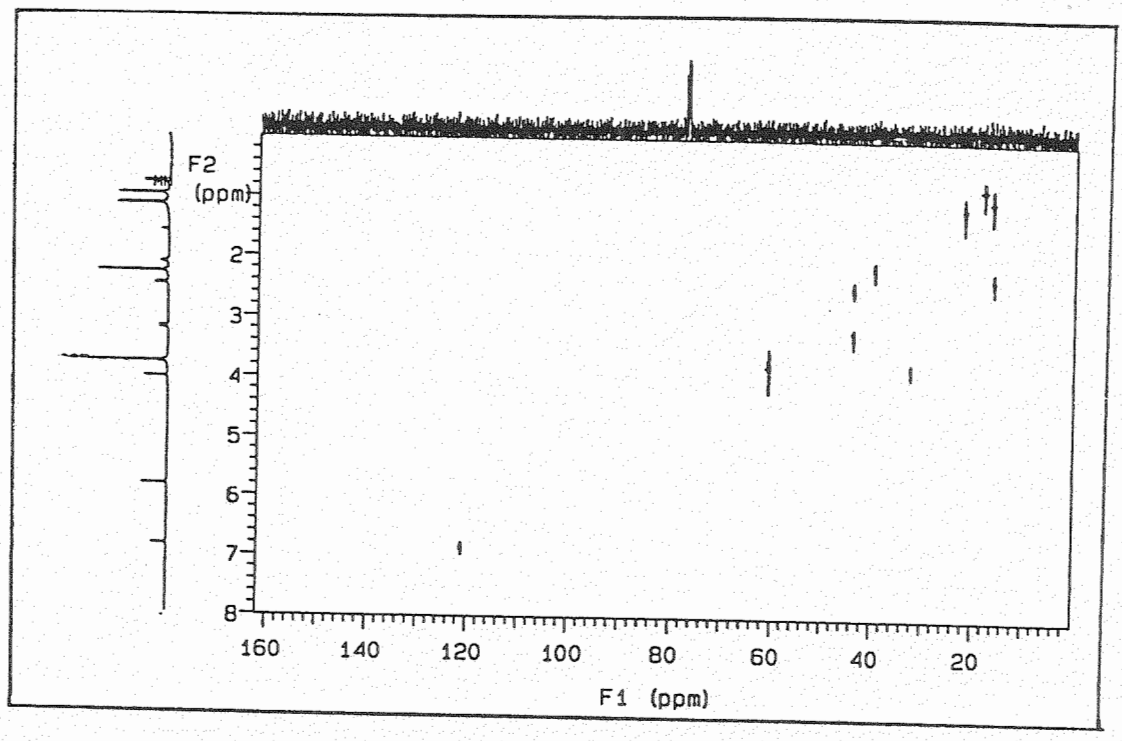


Fig 3.32 The HMQC spectrum of Compound 4

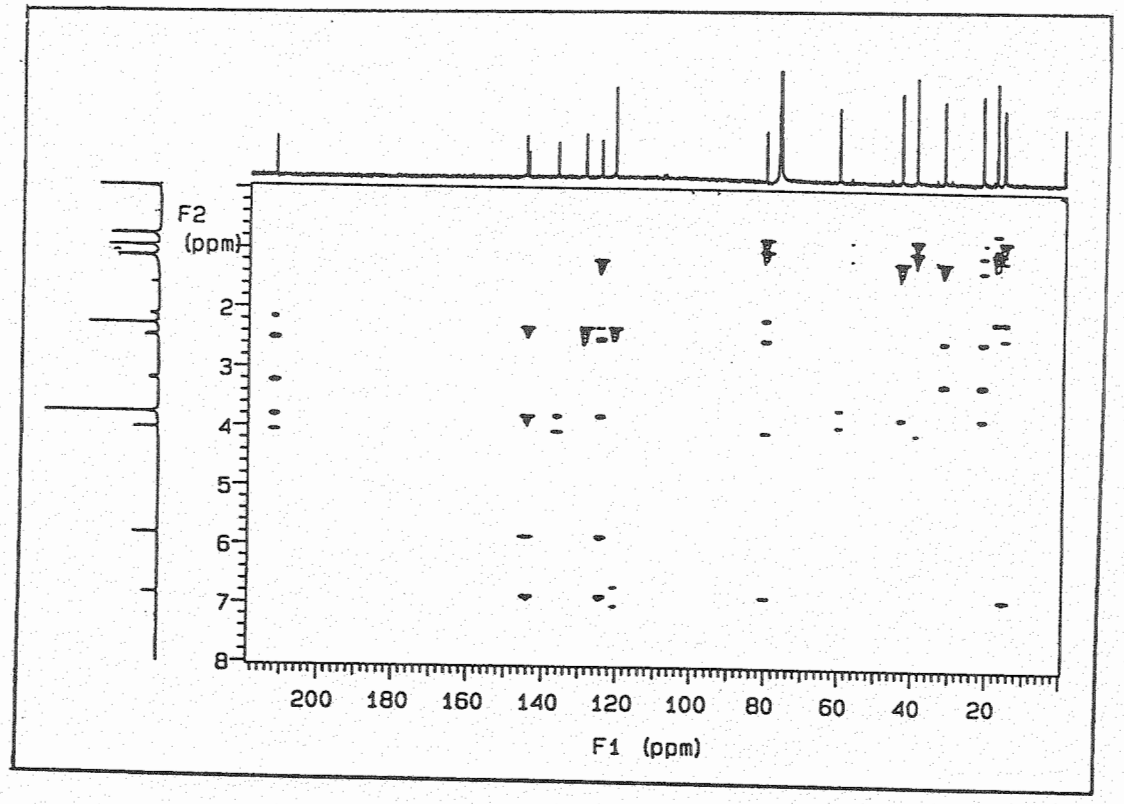


Fig 3.33 The HMBC spectrum of Compound 4

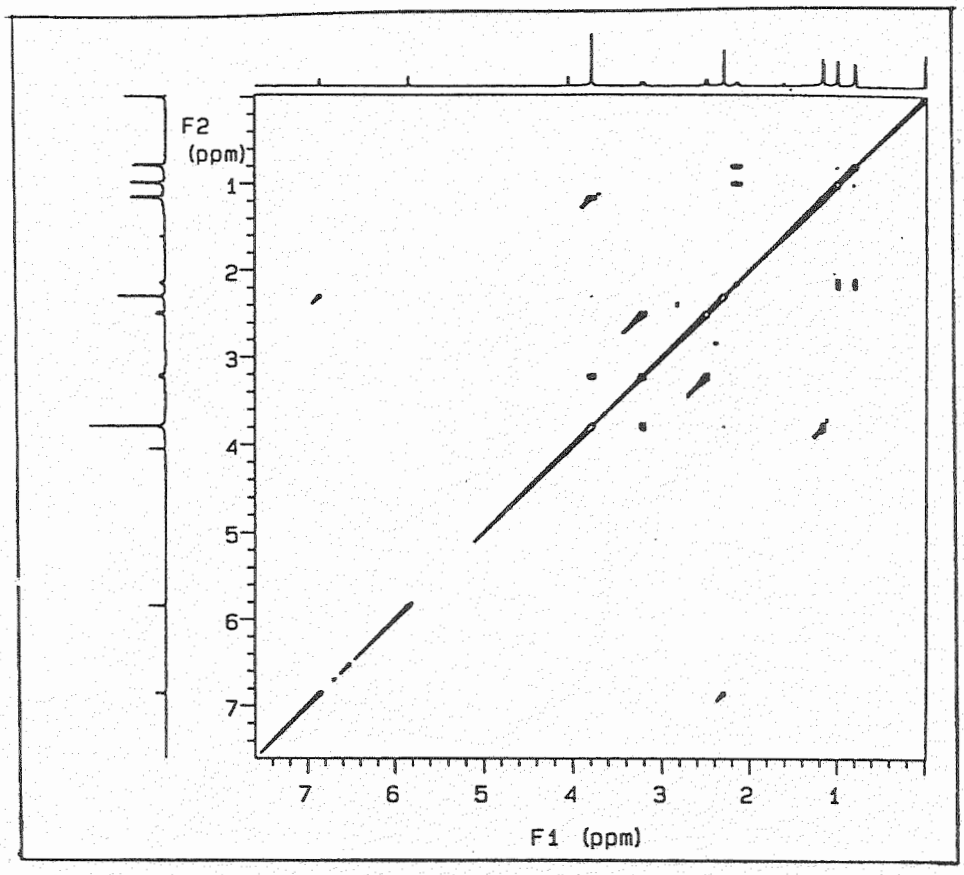


Fig 3.34 The COSY spectrum of Compound 4

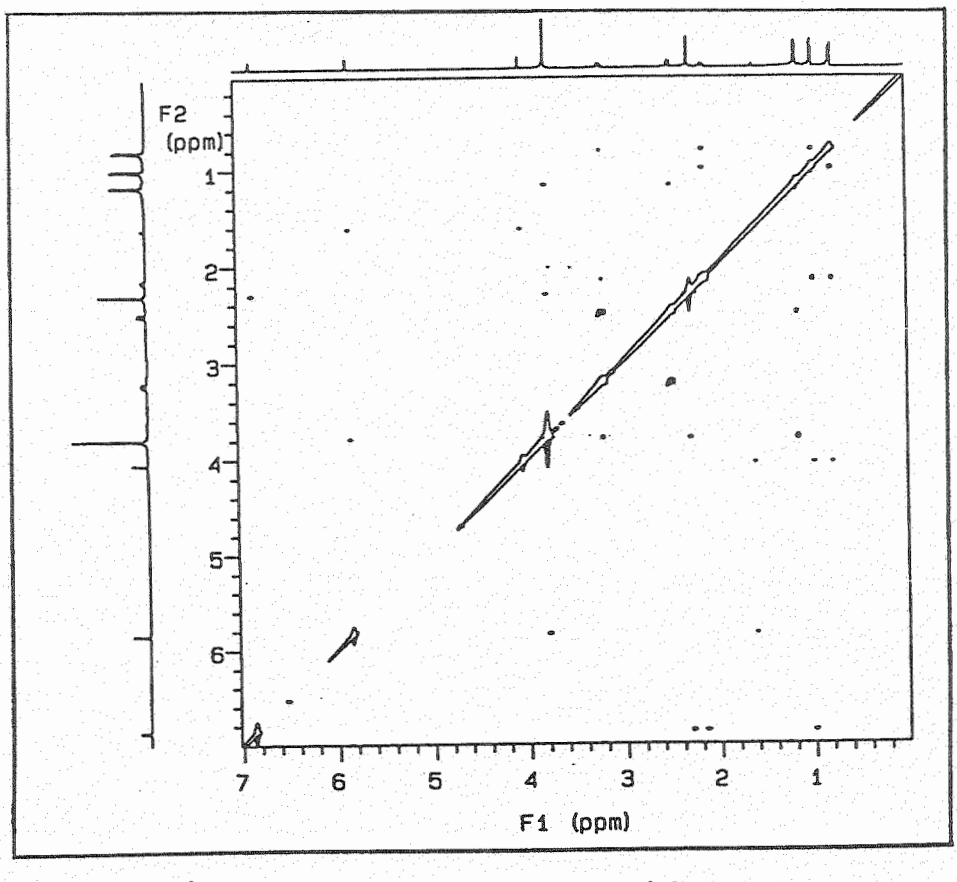


Fig 3.35 The ^{1}H-^{1}H NOESY spectrum of Compound 4

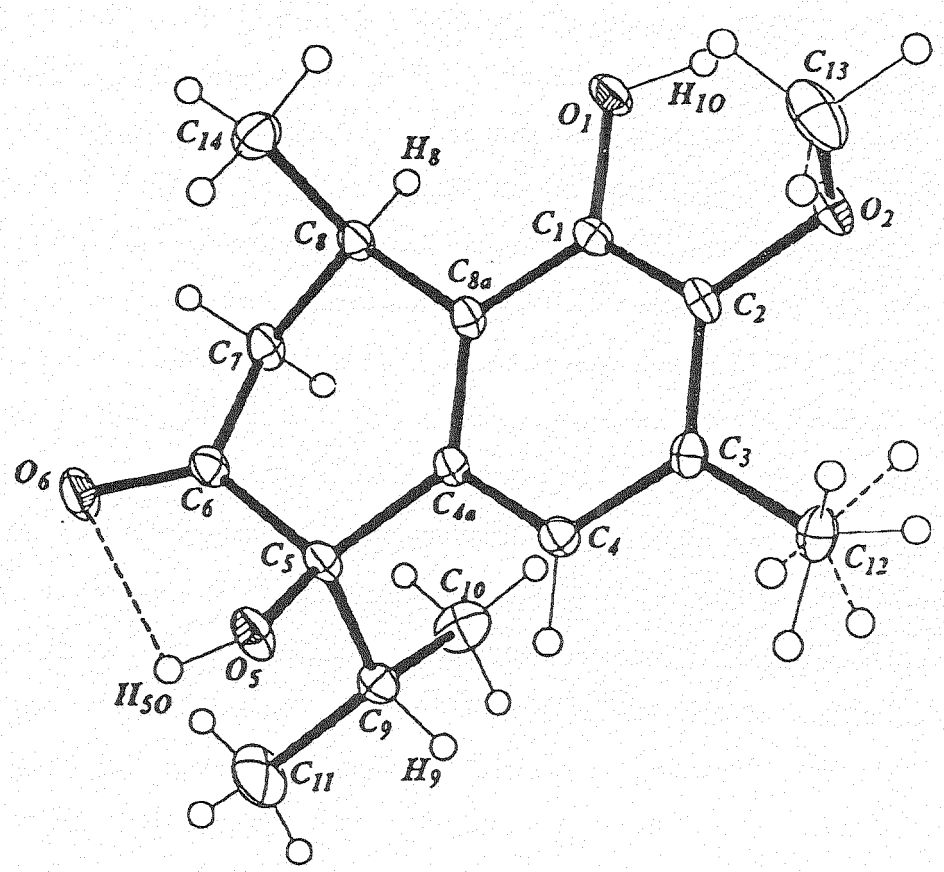
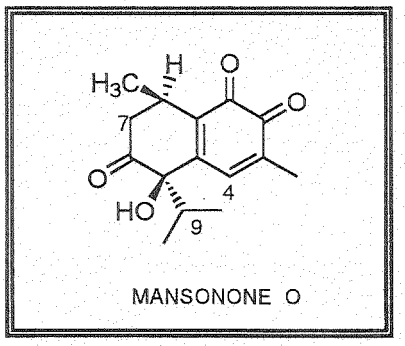


Fig 3.36 ORTEP of Compound 4

3.3.5 *Compound 5:* Mansonone O

Compound **5** was isolated as a violet orthorhombic; $[\alpha]_D^{20}$ –76.0 (c = 0.10, $CHCl_3$), m.p. 125-128°C. A molecular ion at m/z 262 in the EI-MS (Fig 3.37), ^1H NMR (Fig 3.38) and ^{13}C NMR spectra (Fig 3.39) of Compound **5** suggested a molecular formula of $C_{15}H_{18}O_4$. Comparison of the ^1H NMR and ^{13}C NMR spectra of this compound with those of Compound **4** (Table 3.9), it was manifestly observed that the non-aromatic moiety of these two molecules were identical. The ^1H NMR singlet signal at δ_H 7.06 was positioned at C-4 after the observation of a long-range heteronuclear correlation between this signal and the ^{13}C NMR resonance at δ_C 79.3 (C-5). Measurement of a NOE effect between the same ^1H NMR singlet at δ_H 7.06 and the signal at δ_H 2.00 (3H) permitted to locate of this methyl group at C-3. This result was confirmed by an HMBC correlation (Fig 3.42) observed between the ^{13}C NMR resonance at δ_C 15.5 (3-CH_3) and the ^1H NMR singlet at δ_H 7.06 (H-4). Finally, two ketonic carbonyl carbon signals seen in the ^{13}C NMR spectrum at δ_C 179.3 and 180.1 were located at C-1 and C-2, respectively, in agreement with the HMBC correlation observed between the ^{13}C NMR signal at δ_C 180.1 (C-2) and the ^1H NMR singlet at δ_H 7.06 (H-4). Compound **5** was identified as a new natural product and named mansonone O. Definitive evidence of this structure was obtained from a single-crystal X-ray analysis (see Appendix). The molecular structure and crystallographic numbering scheme are illustrated in Figure 3.44.



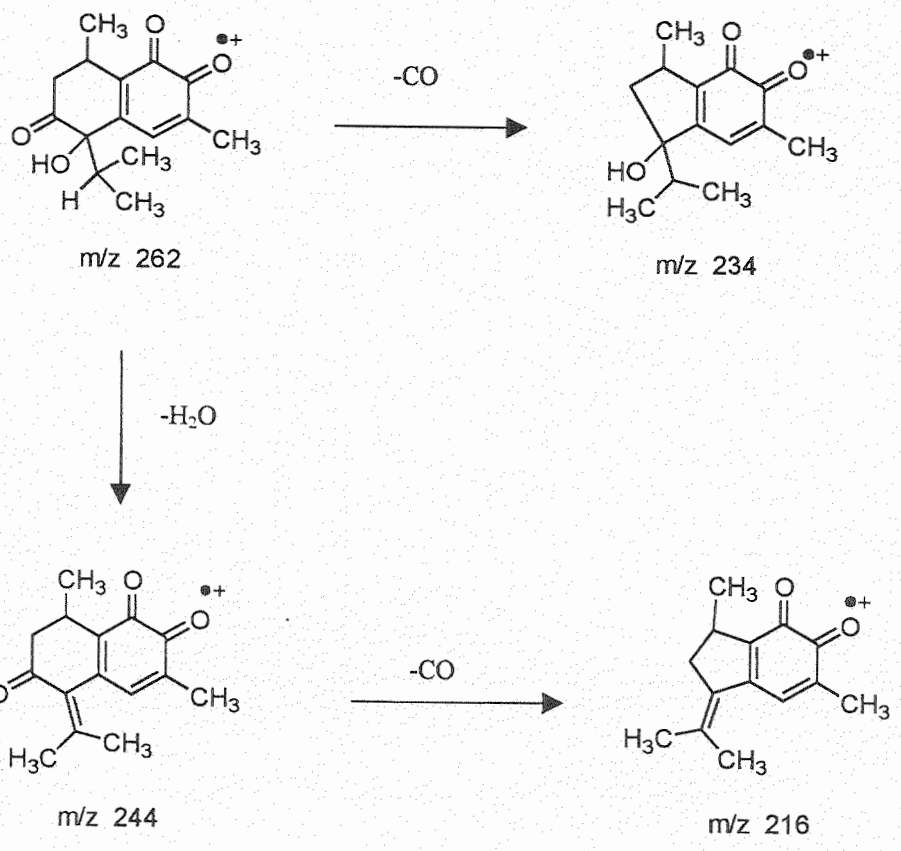
MANSONONE O

Table 3.9 ^{1}H-NMR and ^{13}C-NMR spectral data of Compounds **4** and **5**

Position	Chemical Shift (ppm)			
	4[a]		**5**[a]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	-	145.1	-	179.3
2	-	144.4	-	180.1
3	-	128.8	-	137.2
4	6.86, s, 1H	120.1	7.06, dd, 1H (1.5, 3.4)	136.0
4a	-	136.5	-	148.8
5	-	80.6	-	79.3
6	-	212.1	-	208.8
7	2.49, dd, 1H (1.7, 12.5) 3.21, dd, 1H (7.8, 12.6)	43.8	2.47, dd, 1H (1.2, 12.8) 3.10, dd, 1H (7.9, 13.1)	42.6
8	3.79, m, 1H	32.4	3.54, m, 1H	32.0
8a	-	124.6	-	137.0
9	2.15, sept, 1H (6.9)	39.6	2.21, sept, 1H (6.7)	38.4
1-OH	5.84, s, 1H	-	-	-
2-OCH_3	3.79, s, 3H	60.6	-	-
3-CH_3	2.30, s, 3H	16.0	2.00, d, 3H (1.5)	15.5
5-OH	4.05, s, 1H	-	3.95, s, 1H	-
8-CH_3	1.16, d, 3H (6.9)	22.0	1.06, d, 3H (6.7)	21.7
9-$(CH_3)_2$	0.79, d, 3H (6.9) 0.99, d, 3H (6.9)	18.2 16.3	0.89, d, 3H (7.0) 1.08, d, 3H (7.0)	17.7 16.9

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively

The mass spectrum (Fig 3.37) displayed the molecular ion peak at m/z 262 ([M^+]), and other fragmentations were detected at m/z 244 (M^+-H_2O), 234 (M^+-CO) and 216 (M^+-H_2O-CO). The proposed fragmentation pattern of Compound **5** is shown in Scheme 3.6.



Scheme 3.6 The proposed fragmentation pattern of Compound **5**

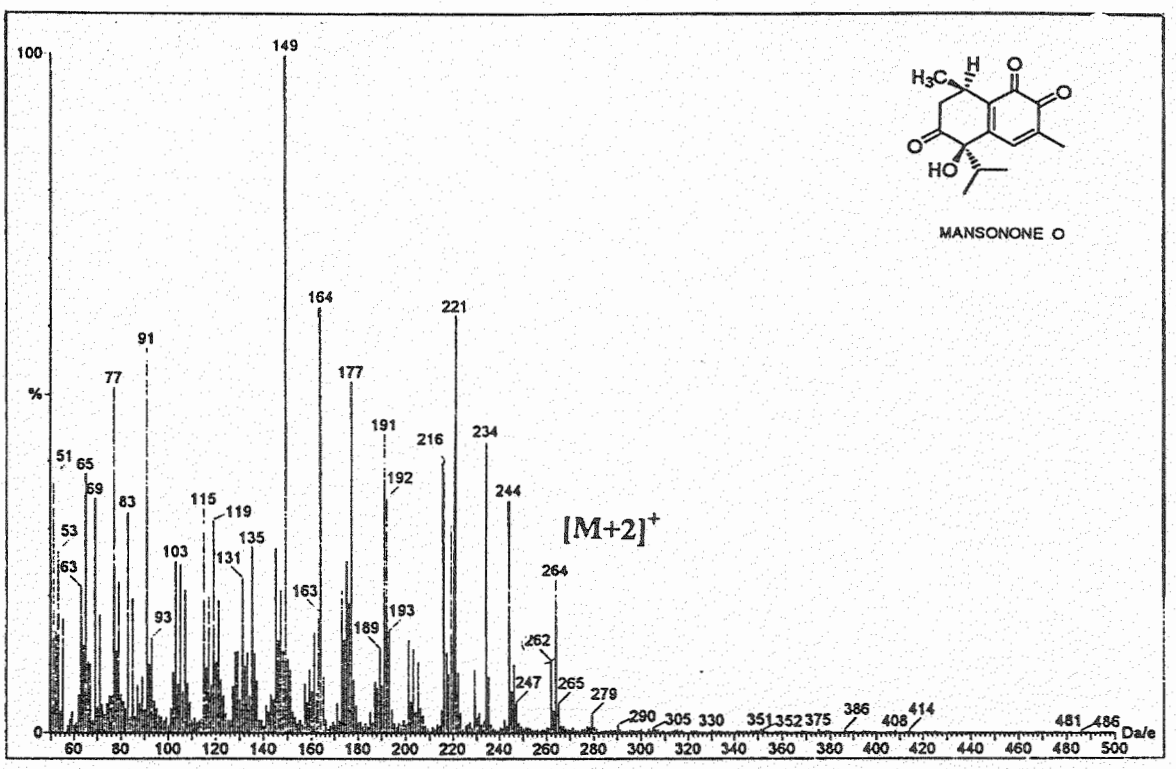


Fig 3.37 The mass spectrum of Compound 5

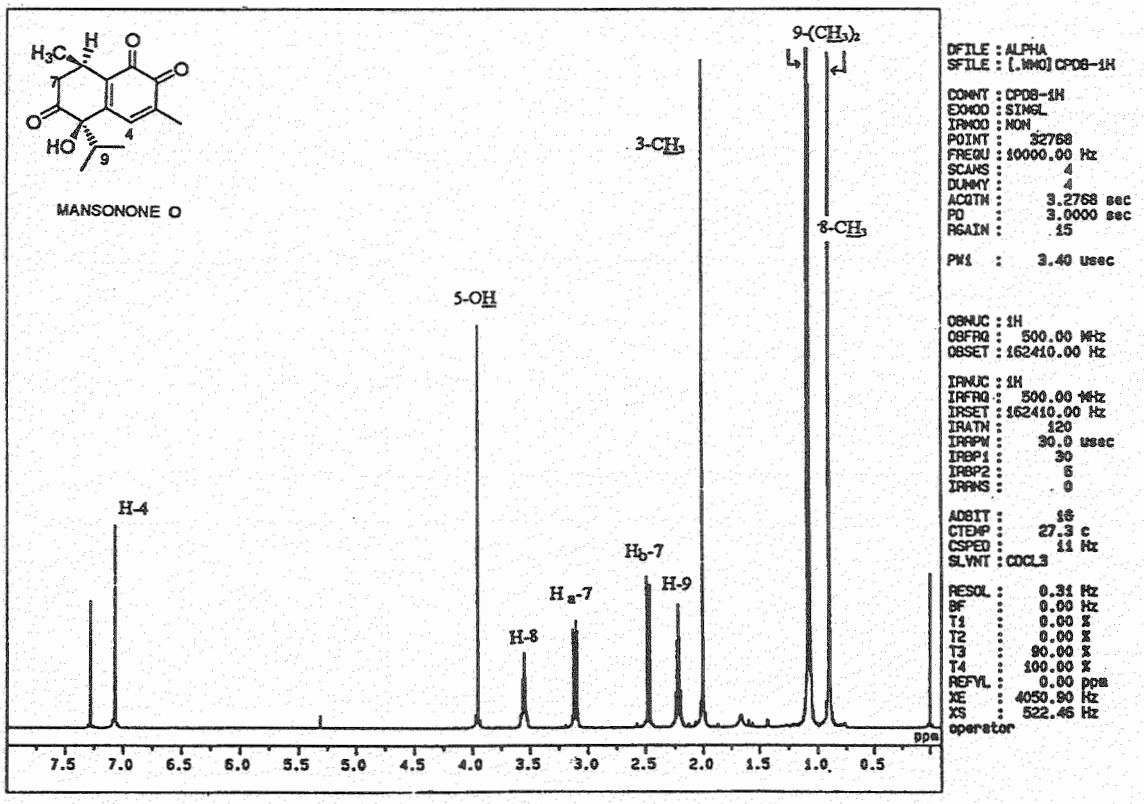


Fig 3.38 The ^{1}H-NMR spectrum of Compound 5

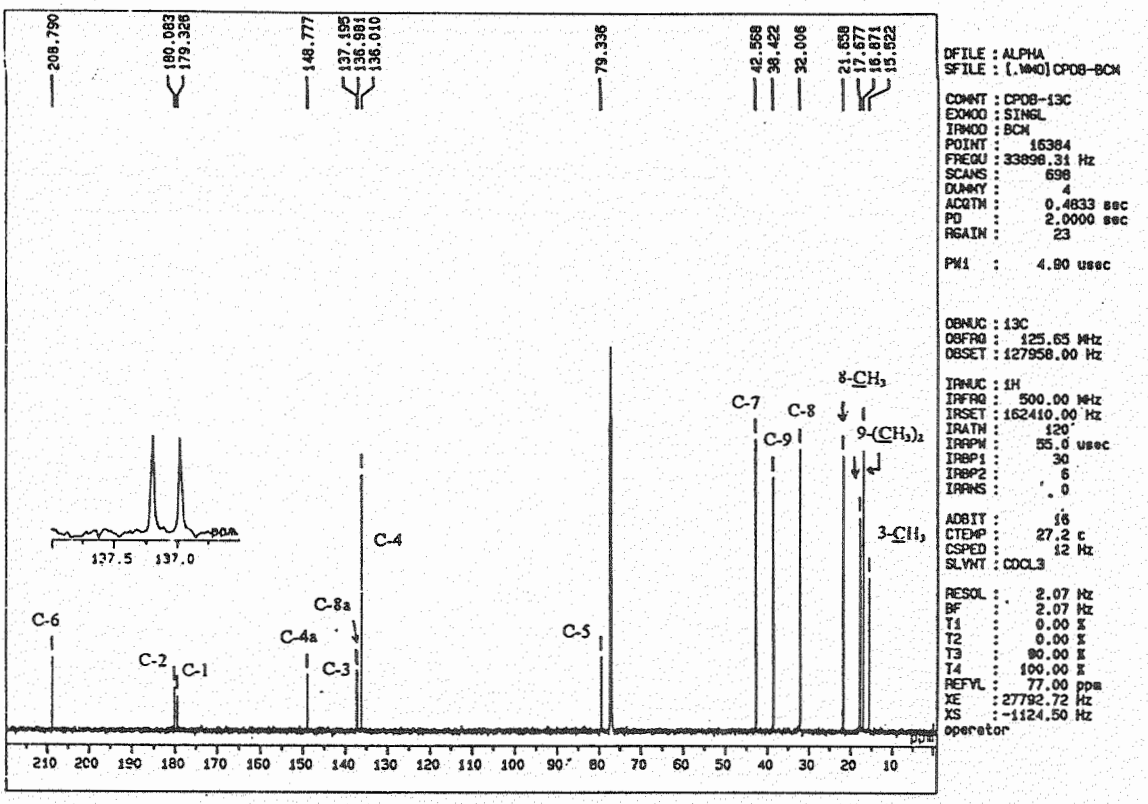


Fig 3.39 The ^{13}C-NMR spectrum of Compound 5

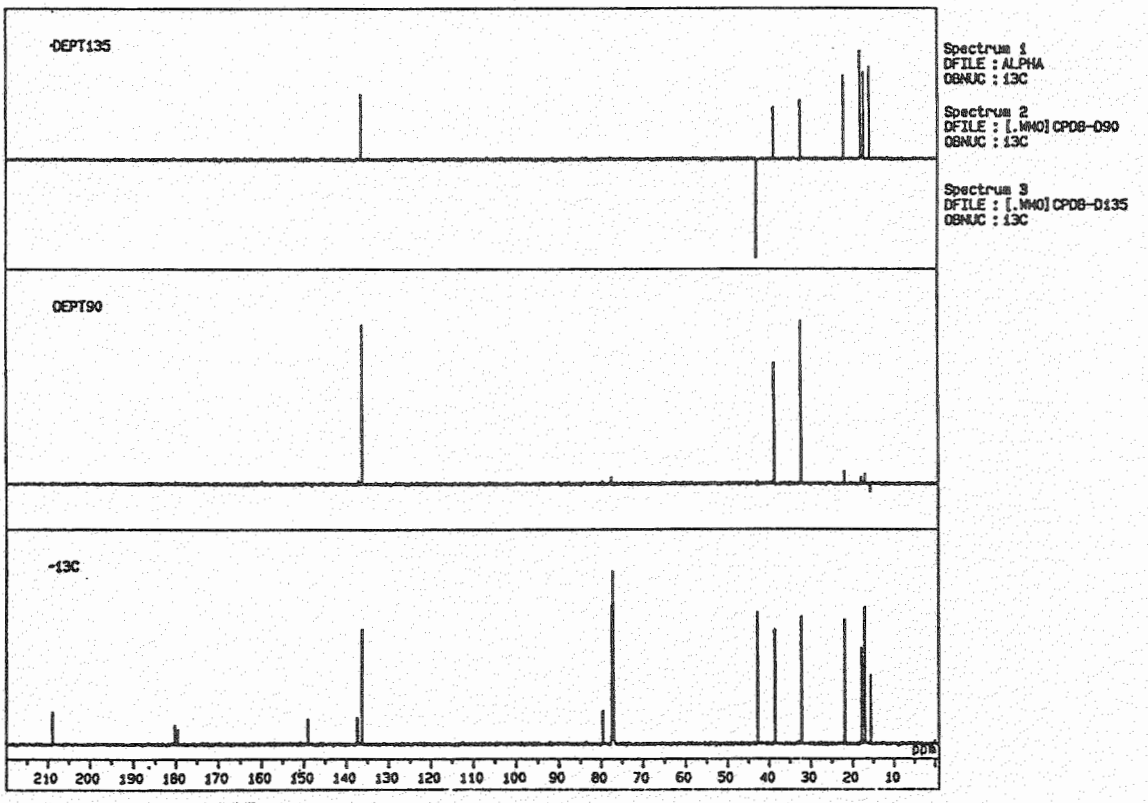


Fig 3.40 The DEPT 90 and 135 spectra of Compound 5

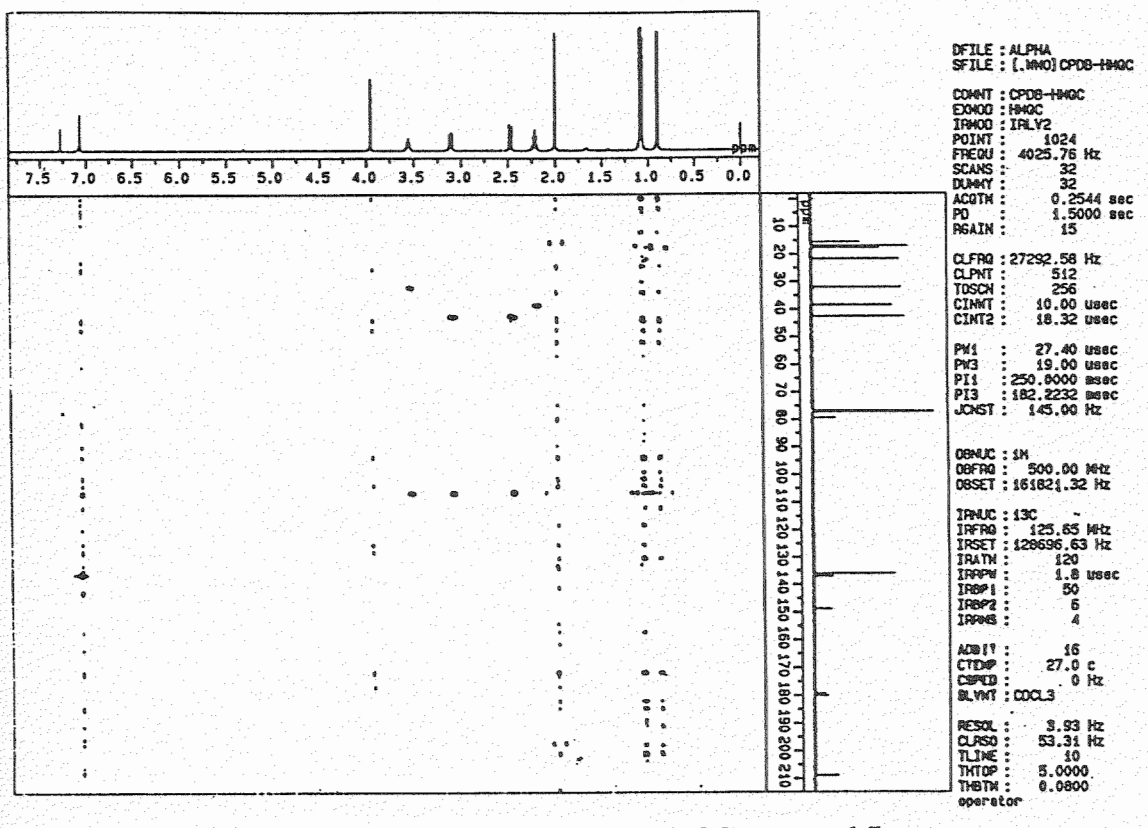


Fig 3.41 The HMQC spectrum of Compound 5

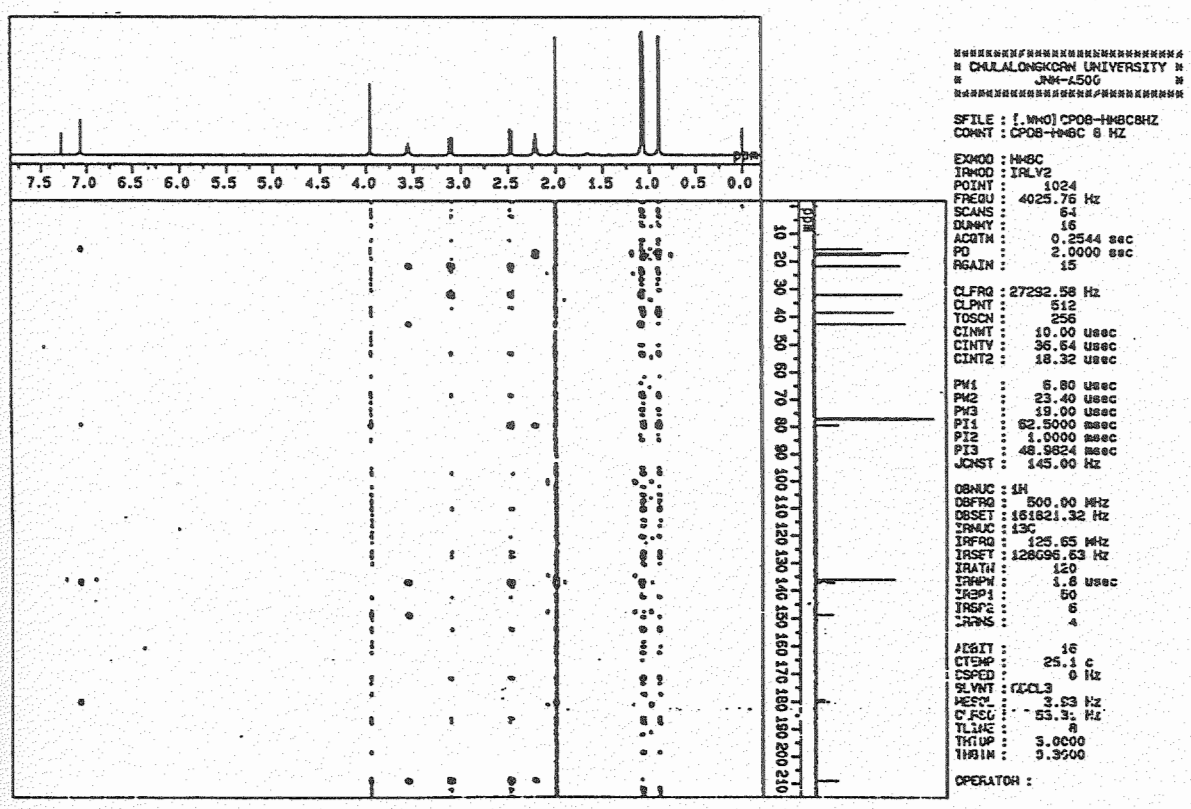
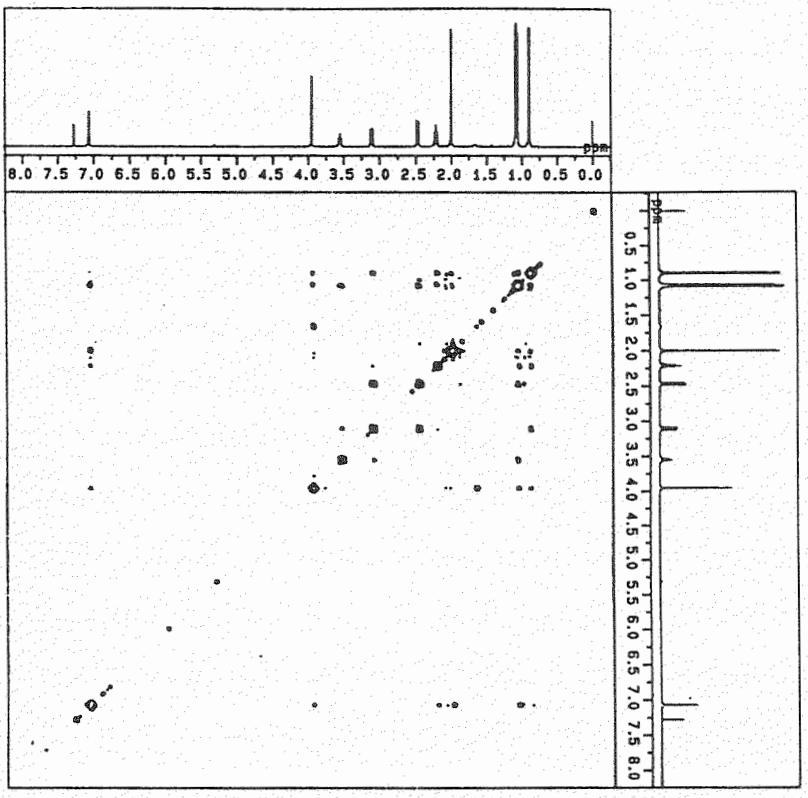


Fig 3.42 The HMBC spectrum of Compound 5

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2-FEB-1999 11:19:02.01

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* CHULALONGKORN UNIVERSITY *
*         JNM-A500         *
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RGAIN :       15

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ADBIT :        16
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CSPED :        12 Hz
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OPERATOR :
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Fig 3.43 The ^{1}H-^{1}H NOESY spectrum of Compound 5

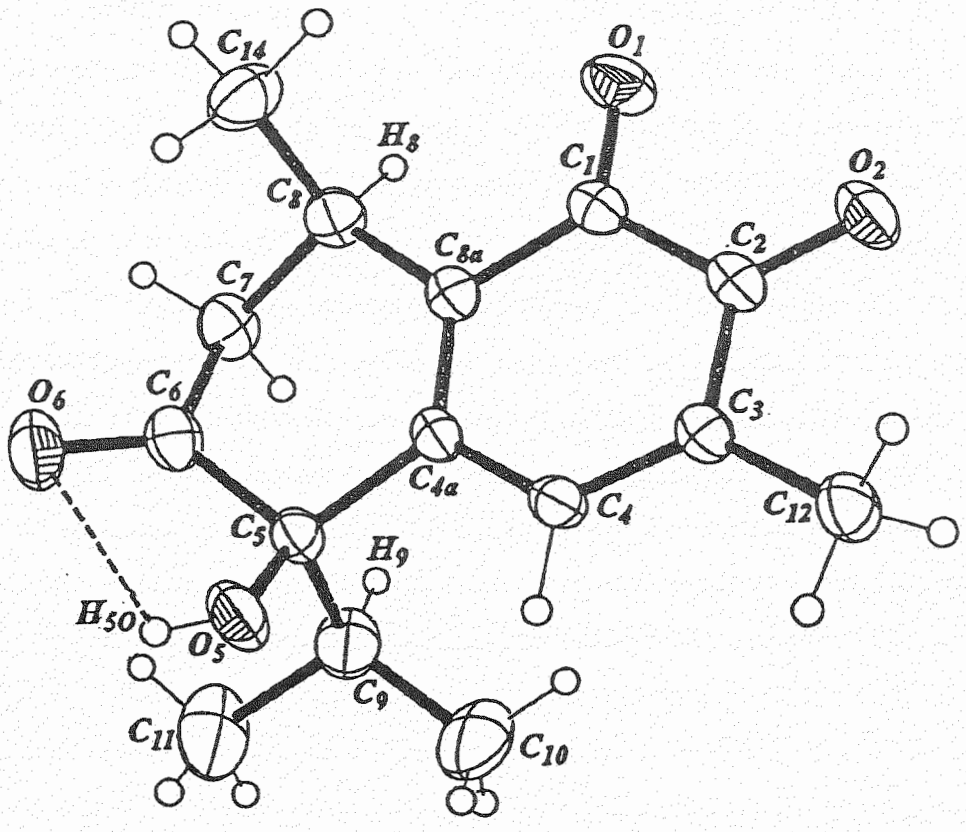
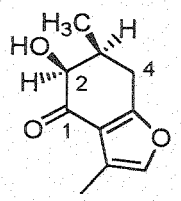


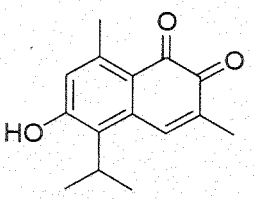
Fig 3.44 ORTEP of Compound 5

3.3.6 *Compound 6:* Mansonone P

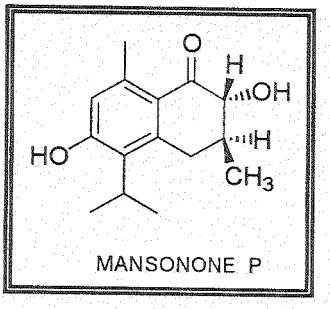
Commpound **6** was accomplishly isolated as pale yellow powder, $[\alpha]_D^{20}$ −13.5 (c = 0.20, $CHCl_3$), m.p. 175-176 °C. A molecular formula of $C_{15}H_{20}O_3$ was deduced for Compound **6** from the EIMS (m/z 248 $[M]^+$) (Fig 3.45), ^1H NMR (Fig 3.16) and ^{13}C NMR (Fig 3.47) spectra. As Compound **4**, the ^{13}C NMR spectrum of Compound **6** suggested the presence of an aromatic moiety with six signals located between 120 and 160 ppm as well as an aliphatic portion of the molecule represented by resonances found from 15 to 40 ppm. An isopropyl group was identified from the ^1H NMR spectral data by the presence of two methyl groups at δ_H 1.36 (d, J = 6.7 Hz, 9-$(CH_3)_2$) and a methine resonance centered at δ_H 3.32 (sept, J = 6.7 Hz, H-9). This unit was assigned to C-5 from HMBC correlations (Fig 3.50) observed between the septet at δ_H 3.32 and ^{13}C signals observed at δ_C 143.8 (C-5), 158.5 (C-4a) and 129.2 (C-6). Other long-range heteronuclear correlations between the ^1H NMR signals of the methyl unit at δ_H 1.31 (3-CH_3) and the ^{13}C NMR resonances found at δ_C 77.9 (C-2), 36.9 (C-3), and 35.1 (C-4) allowed the placement of the latter methyl group at C-3. The fourth methyl group was positioned on the aromatic ring by HMBC correlations between its ^1H NMR signals at δ_H 2.57 (8-CH_3) and the ^{13}C NMR resonances at δ_C 118.8 (C-7), 122.4, (C-8a) and 141.8 (C-8). A ketonic carbonyl was also identified in the low-field region of the ^{13}C NMR spectrum at δ_C 199.5 (C-1). This signal was shown to be linked to the aliphatic part of the molecule based upon the information derived from HMBC correlations with ^1H NMR signals at δ_H 3.89 (H-2), 4.29 (2-OH), and 2.00 (H-3). The structure was finally confirmed by comparison of ^1H and ^{13}C NMR data with desacetylcalaminthone[34] and mansonone G[14] (Table 3.10). Desacetylcalaminthone is a monoterpene derivative isolated from *Calamintha ashei* (Lamiaceae), with an aliphatic moiety closely related to that of Compound **6** while mansonone G, isolated for the first time from the heartwood of *Mansonia altissima*,[6] shares a common aromatic pattern with Compound **6**. The relative chiralities of carbons C-2 and C-3 were determined different from those of Compound **4** and **5** on the basis of the *trans* pseudo-diaxial coupling constants calculated between the ^1H NMR signals H-2 and H-3 (J = 12.5 Hz) as well as between H-3 and H-4 (J =11.8 Hz). The relative stereochemistry of Compound **6** was confirmed by a NOESY (Fig 3.51) correlation observed between ^1H NMR resonances at δ_H 3.89 (H-2) and 2.61 (H-4). Compound **6** was characterized as a novel natural product, named mansonone P.



DESACETYCALAMINTHONE

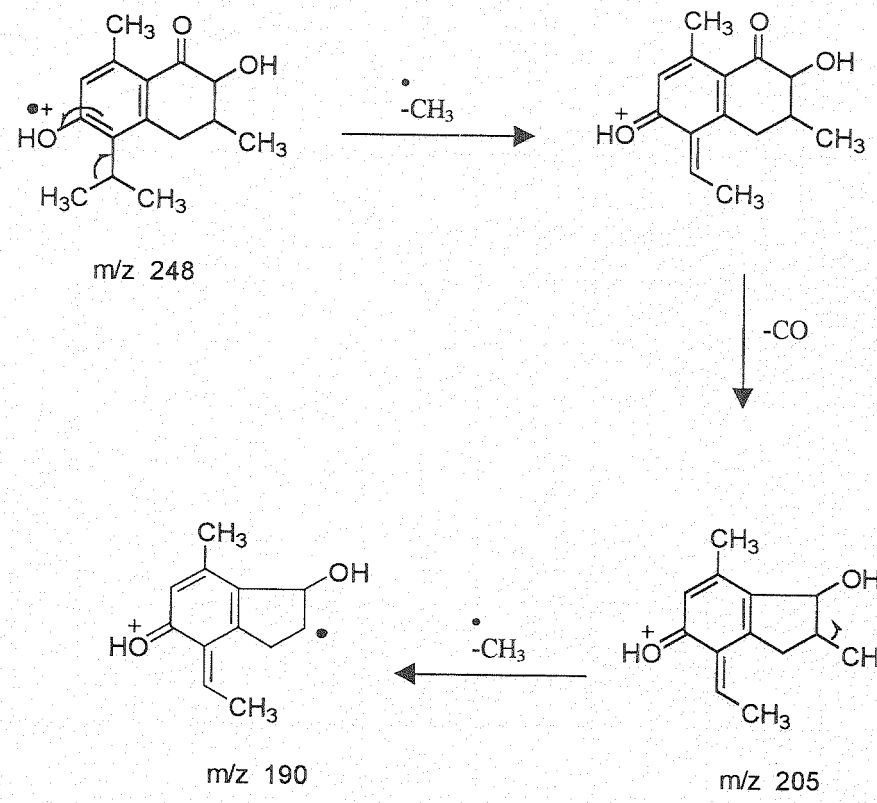


MANSONONE G



MANSONONE P

The mass spectrum (Fig 3.45) displayed the molecular ion peak at m/z 248 ($[M]^+$), and other fragmentations at m/z 205 (M^+-C_3H_7), 190 (M^+-C_3H_7-CH_3) and 175 (M^+-C_3H_7-CH_3-CH_3). The proposed fragmentation pattern of Compound **6** is shown in Scheme 3.7.



Scheme 3.7 The proposed fragmentation pattern of Compound **6**

Table 3.10 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **6**, desacetylcalaminthone and mansonone G

Position	6[a]		Descetylcalaminthone[b]		Mansonone G[c]
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	-	199.5	-	194.7	
2	3.89, dd, 1H (2.4, 12.5)	77.9	4.37, dd, 1H (2.0, 4.6)	75.9	
3	2.00, m, 1H (12.5, 11.7)	36.9	2.81, dddd, 1H (1.9, 4.6, 5.5, 7.1)	35.4	
4	3.16, dd, 1H (4.9, 17.1) 2.61, dd, 1H (11.9, 17.4)	35.1	3.16, dd, 1H (5.5, 17.6) 2.77, dd, 1H (1.9, 17.6)	30.0	
4a	-	129.2			
5	-	143.8			
6	-	158.5			
7	6.49, s, 1H	118.8			6.49, s, 1H
8	-	141.8			
8a	-	122.4			
9	3.32, sept, 1H (6.7)	27.2			3.48, sept, 1H (7.0)
2-OH	4.29, d, 1H (2.2)	-			
3-CH_3	1.31, d, 3H (6.4)	18.8			
6-OH	5.67, s, 1H	-			
8-CH_3	2.57, s, 3H	22.8			2.47, s, 3H
9-$(CH_3)_2$	1.36, d, 6H (6.7)	20.1 (2C)			1.38, d, 6H (7.0)

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively
[b] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 400 and 100 MHz, respectively
[c] ^{1}H NMR spectra was measured in $CDCl_3$-CD_3OD (1:1) at 60 MHz

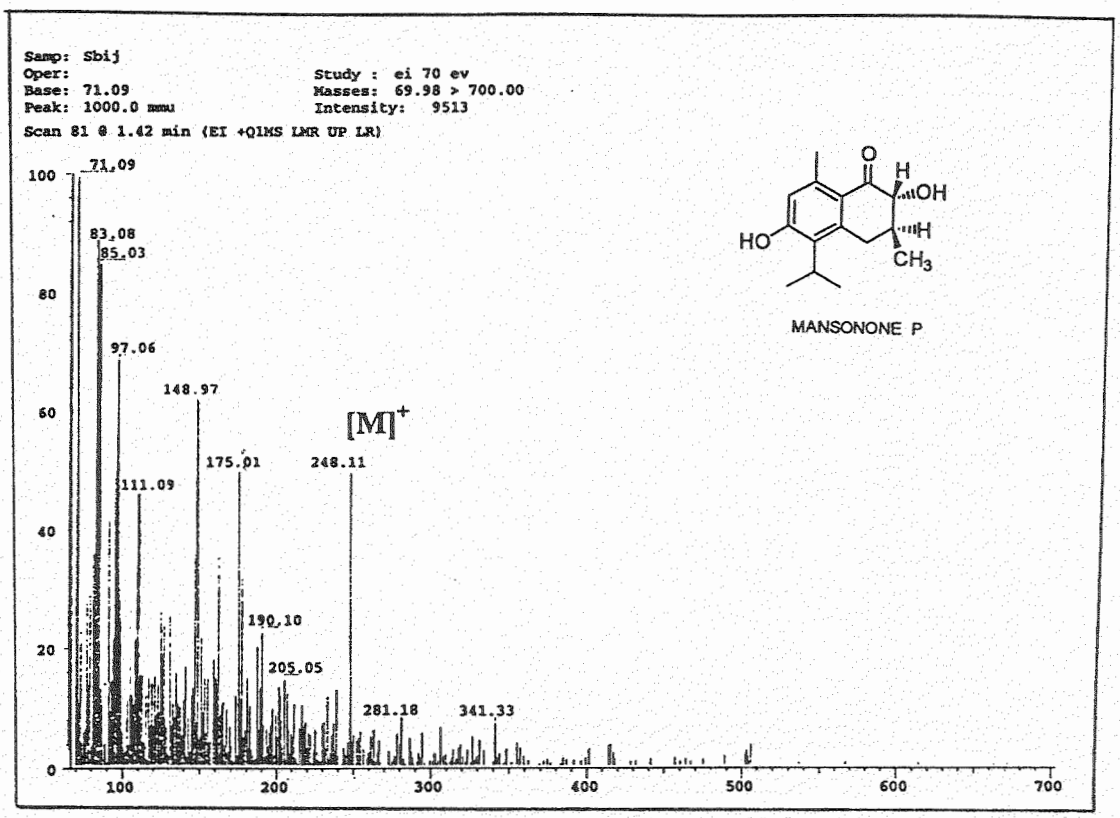


Fig 3.45 The mass spectrum of Compound 6

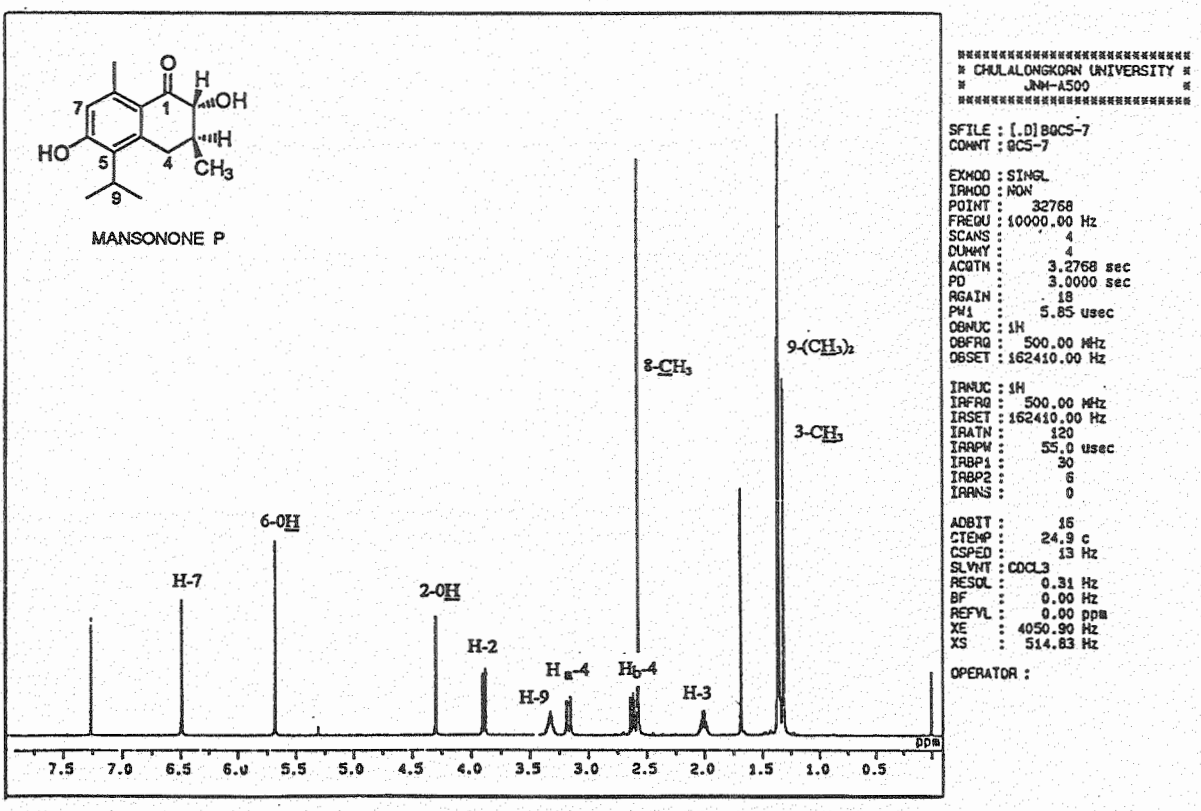
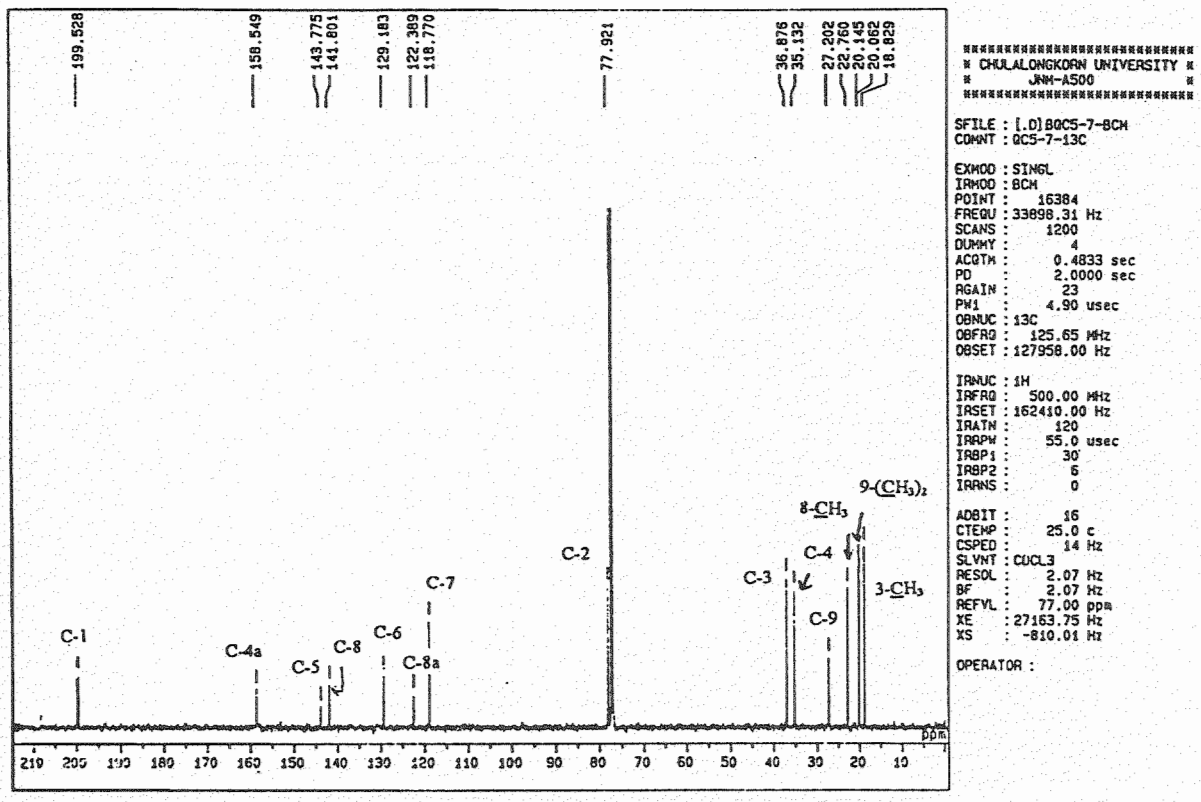


Fig 3.46 The ^{1}H-NMR spectrum of Compound 6

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OBFRQ :   125.65 MHz
OBSET : 127958.00 Hz

IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :      120
IRAPW :     55.0 usec
IRBP1 :       30
IRBP2 :        6
IRANS :        0

ADBIT :       16
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CSPED :       14 Hz
SLVNT : CDCL3
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BF    :     2.07 Hz
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OPERATOR :
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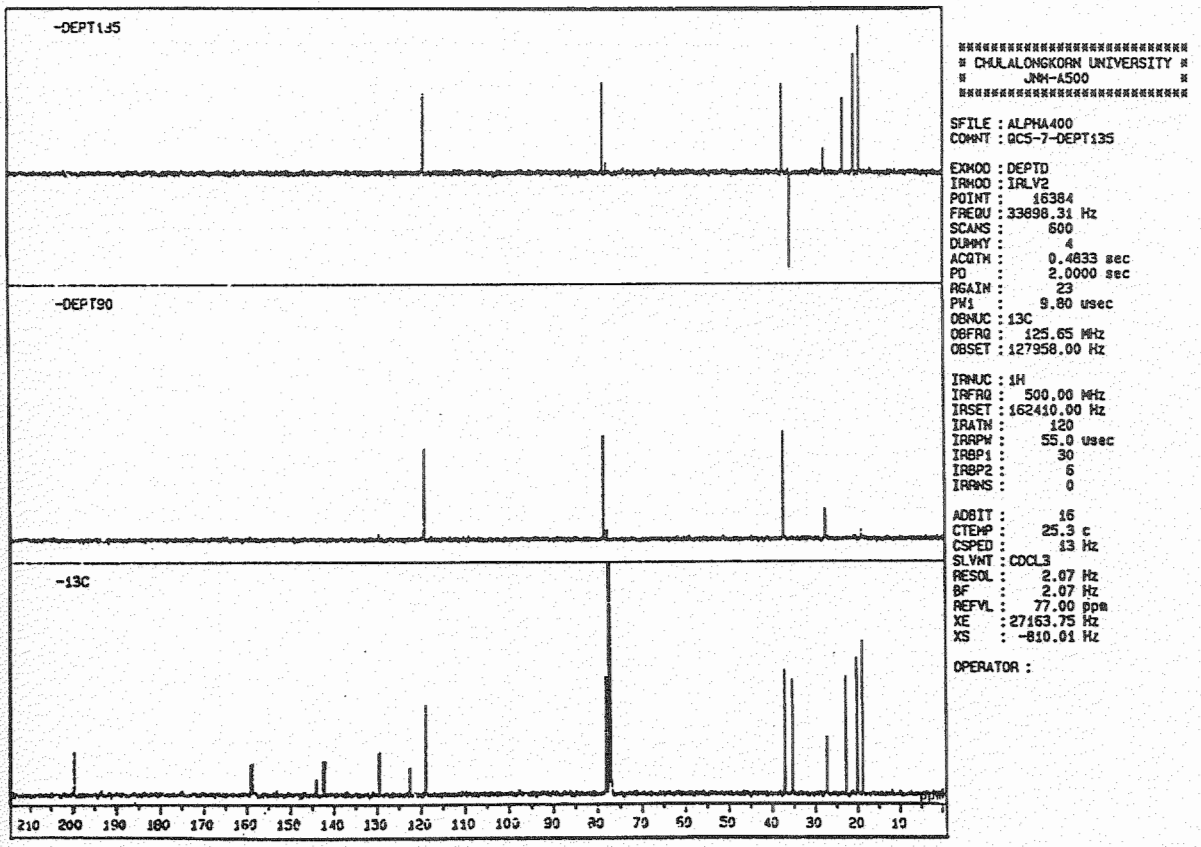
Fig 3.47 The ^{13}C-NMR spectrum of Compound 6

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OBNUC : 13C
OBFRQ :   125.65 MHz
OBSET : 127958.00 Hz

IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :      120
IRAPW :     55.0 usec
IRBP1 :       30
IRBP2 :        6
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BF    :     2.07 Hz
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OPERATOR :
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Fig 3.48 The DEPT 90 and 135 spectra of Compound 6

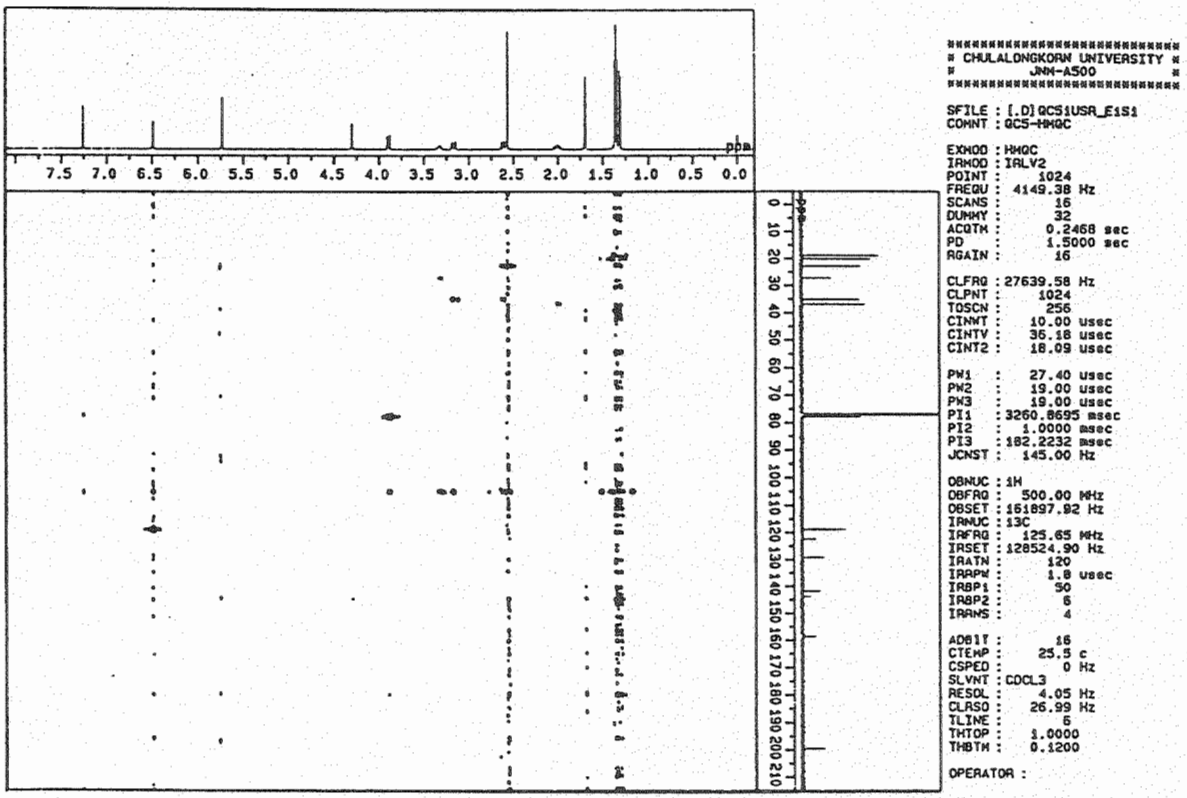


Fig 3.49 The HMQC spectrum of Compound 6

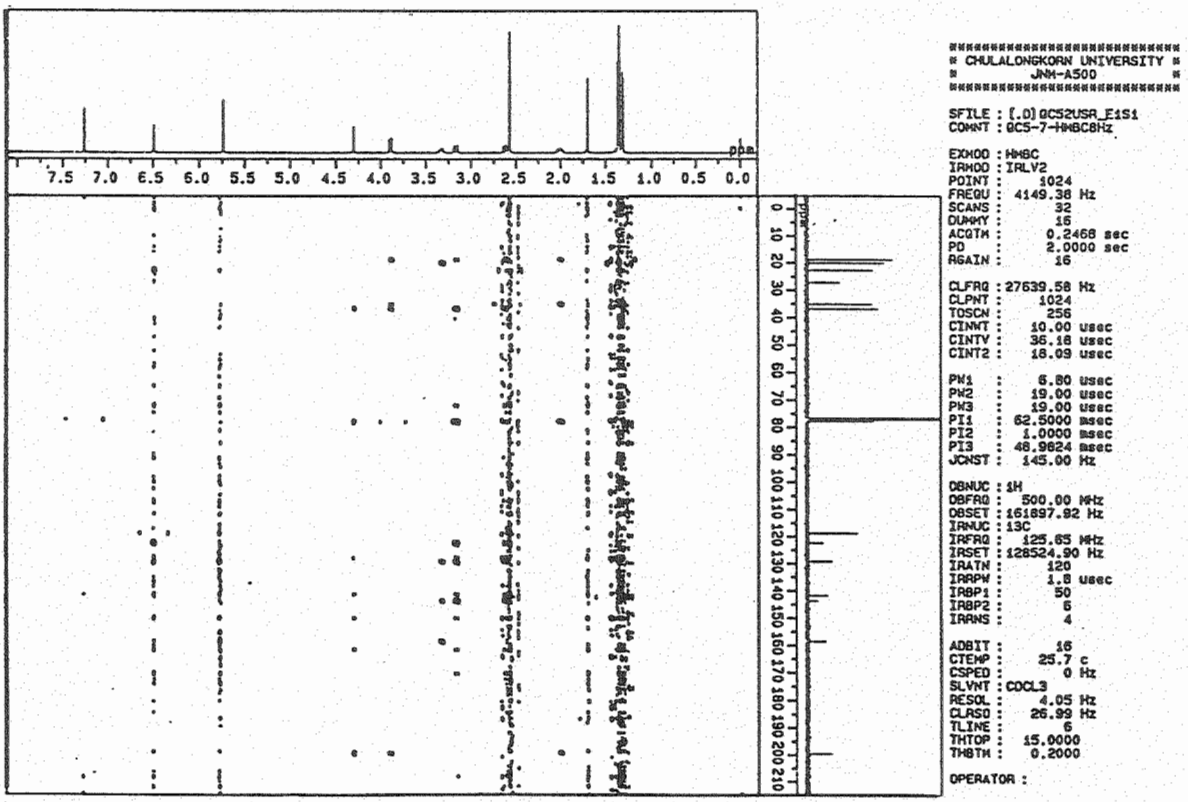
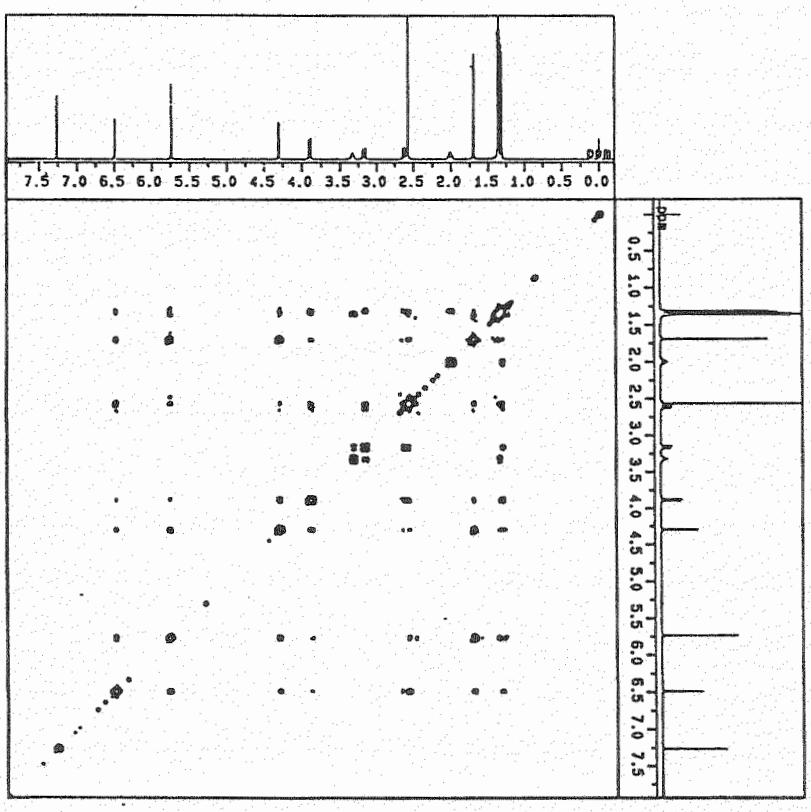


Fig 3.50 The HMBC spectrum of Compound 6

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* CHULALONGKORN UNIVERSITY  *
*         JNM-A500          *
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PI1   : 1847.8261 msec
PI2   :  1.0000 msec
PI3   : 48.9824 msec
JCNST :  145.00 Hz

OBNUC : 1H
OBFRQ :  500.00 MHz
OBSET : 161851.53 Hz
IRNUC : 1H
IRFRQ :  500.00 MHz
IRSET : 162410.00 Hz
IRATN :      120
IRPW  :    55.0 usec
IRBP1 :       30
IRBP2 :        6
IRRNS :        0

ADBIT :       16
CTEMP :     25.8 c
CSPED :        0 Hz
SLVNT : CDCL3
RESOL :     7.96 Hz
CLRSO :     7.96 Hz
TLINE :        6
THTOP :  12.0000
THBTM :   0.1500

OPERATOR :
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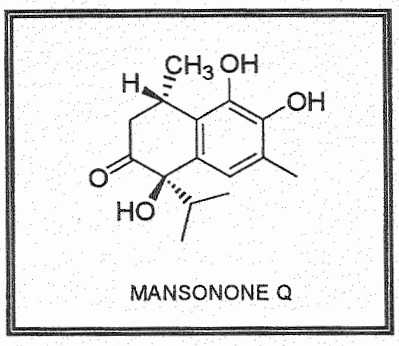
Fig 3.51 The ^{1}H-^{1}H NOESY spectrum of Compound 6

3.3.7 *Compound 7:* Mansonone Q

Commpound 7 was obtained as pale yellow powder, $[\alpha]_D^{20}$ +9.5 (c = 0.10, $CHCl_3$), m.p. 105-106 °C. The ^1H and ^{13}C NMR spectra of this compound were very similar to those of Compound **4** (Table 3.11). The two compounds differed by the absence of signals for the methoxy group of δ_H 3.79 in the ^1H NMR spectrum (Fig 3.53), and δ_C 60.6 in the ^{13}C NMR spectrum (Fig 3.54). This methoxy group was replaced by a hydroxyl group firmly characterized by the appearance of a ^1H NMR singlet signal at δ_H 4.65 ppm. The position of the hydroxyl unit was confirmed by long-range heteronuclear correlations, with the ^{13}C NMR resonances at δ_C 141.2 (C-1), 140.1 (C-2), and 122.1 (C-3). The EIMS (m/z 264 $[M]^+$) (Fig 3.52) of Compound 7 as well confirmed the substitution. Compound 7 was identified as a new natural product named mansonone Q. The relative chiralities of carbons C-5 and C-8 were determined as *R** and *S**, respectively. They were attributed on the basis of differences observed in the coupling constants of H-7 (2H) and H-8 (J = 10.1 and 4.6 Hz) of mansonone Q compared with those of the structurally-related mansonones N (J = 1.7 and 7.8 Hz) and O (J = 1.2 and 7.9 Hz) as well as after observation of a lower-field chemical shift of the 8-CH_3 group at δ_H 1.52 for mansonone Q, which was at δ_H 1.16 and 1.06 for mansonones N and O, respectively. This attribution was in good agreement with the NOE effects noticed between the 8-CH_3 group at δ_H 1.52 and the signal at δ_H 0.93 belonging to one of the methyl groups of the isopropyl moiety (9-CH_3) and the NOE between the proton signal at δ_H 2.50 and the 8-CH_3 and 9-CH_3 groups positioned at δ_H 1.52 and 0.86, respectively. The relatively large coupling constant obtained between ^1H NMR signals at δ_H 3.04 and 3.60 (J = 10.1 Hz) was attributed to a *cis* arrangement of these two protons, due to the ring tension resulting from the steric hindrance between the isopropyl moiety and the 8-CH_3 group. If this compound was isolated more, further studies are planned for the determination the absolute configuration of the isolated compounds by LC/NMR analysis of derivatives obtained by the Mosher esterification.



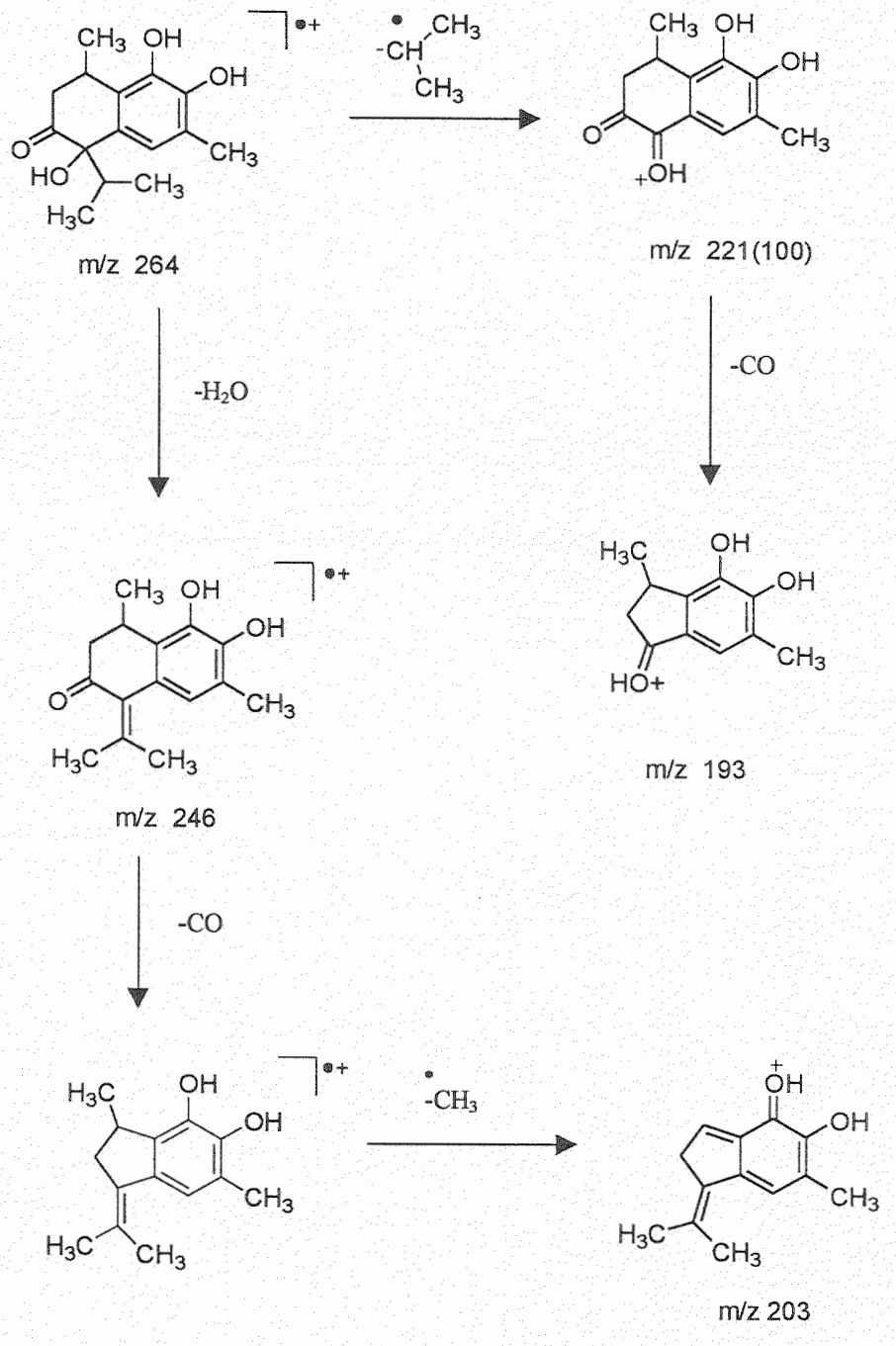
MANSONONE Q

Table 3.11 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound 7

Position	Chemical Shift[a]	
	δ_H (J in Hz)	δ_C
1	-	141.2
2	-	140.1
3	-	122.1
4	6.88, s, 1H	119.9
4a	-	133.0
5	-	80.4
6	-	212.5
7	2.50, dd, 1H (4.6, 16.8) 3.04, dd, 1H (10.1, 16.8)	40.5
8	3.60, m, 1H	29.3
8a	-	123.7
9	2.10, sept, 1H (6.9)	38.0
1-OH	5.46, s, 1H	-
2-OH	4.65, s, 1H	-
3- CH_3	2.25, s, 3H	15.6
5-OH	3.88, s, 1H	-
8- CH_3	1.52, d, 3H (7.3)	23.4
9-$(CH_3)_2$	0.86, d, 3H (6.7) 0.86, d, 3H (6.7)	16.7 15.6

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively

The mass spectrum (Fig 3.52) displayed the molecular ion peak at m/z 264 ($[M]^+$), other fragmentations were detected at m/z 221 (M^+-C_3H_7), 246 (M^+-H_2O), 203 (M^+-H_2O-C_3H_7) and 193 (M^+-C_3H_7-CO). The proposed fragmentation pattern of Compound 7 is shown in Scheme 3.8.



Scheme 3.8 The proposed fragmentation pattern of Compound 7

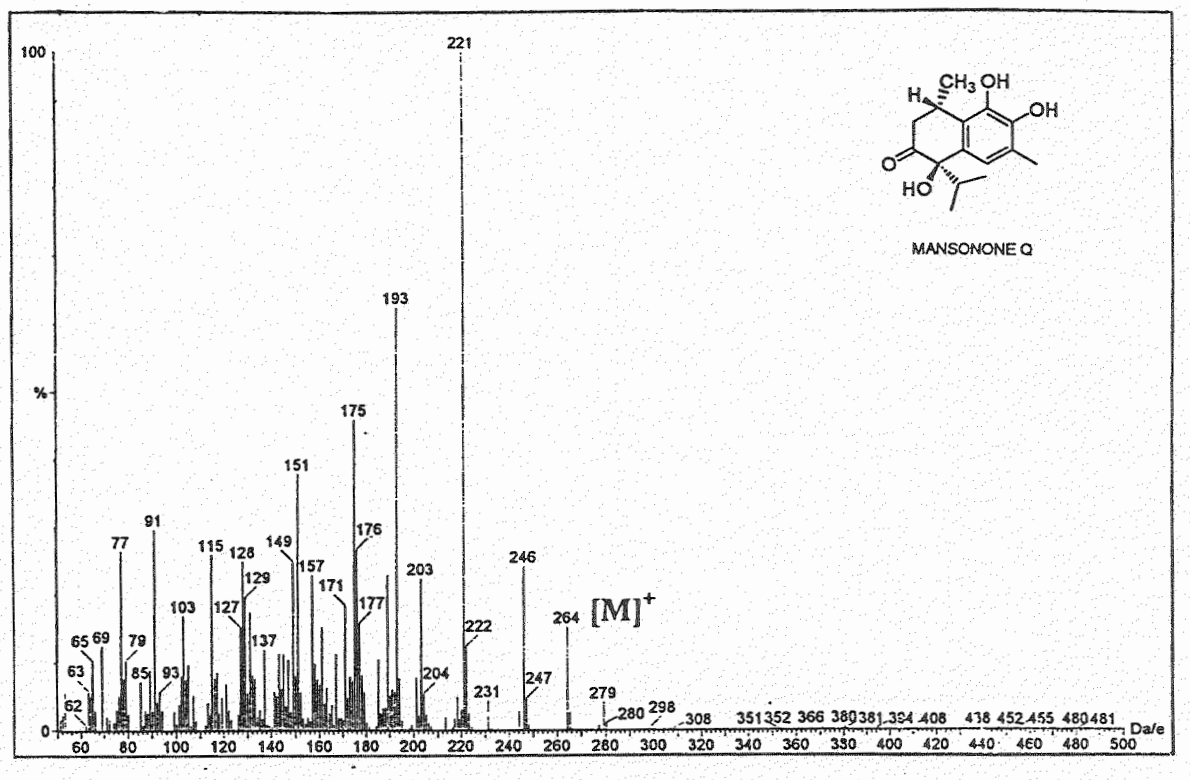


Fig 3.52 The mass spectrum of Compound 7

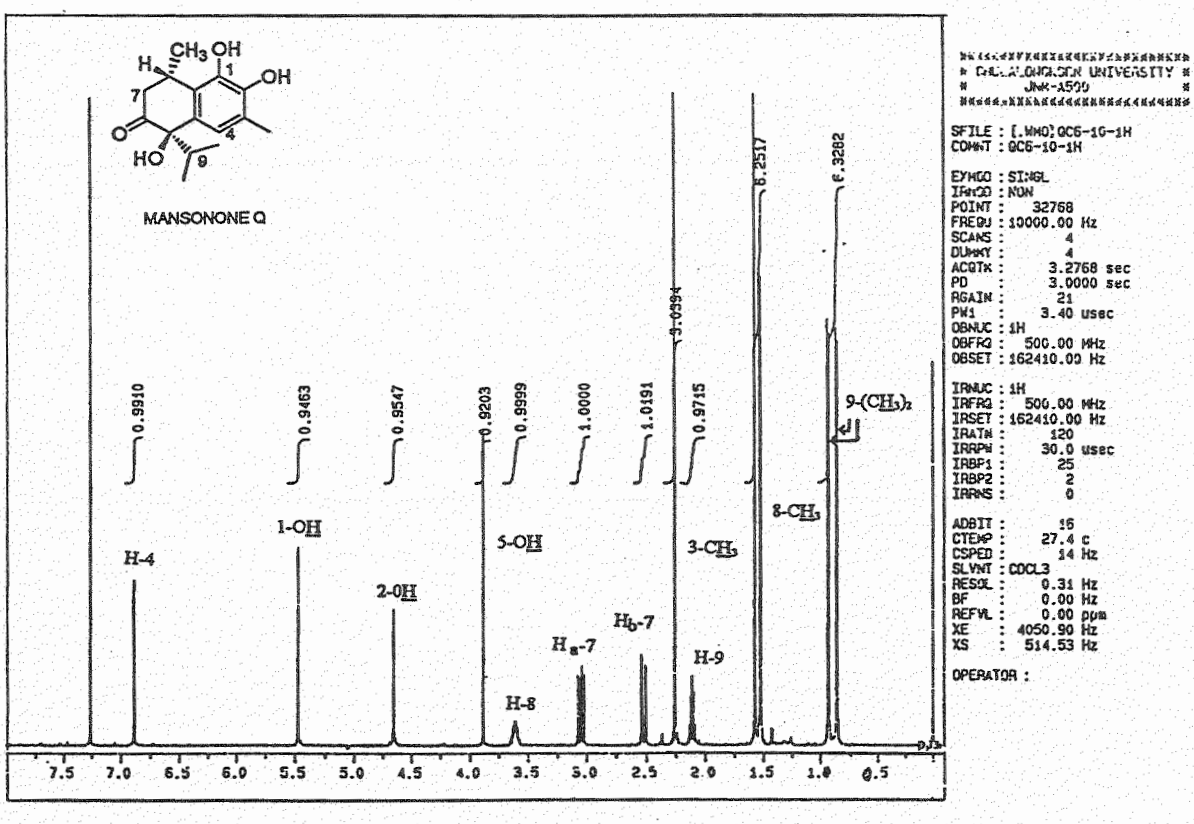


Fig 3.53 The ^{1}H-NMR spectrum of Compound 7

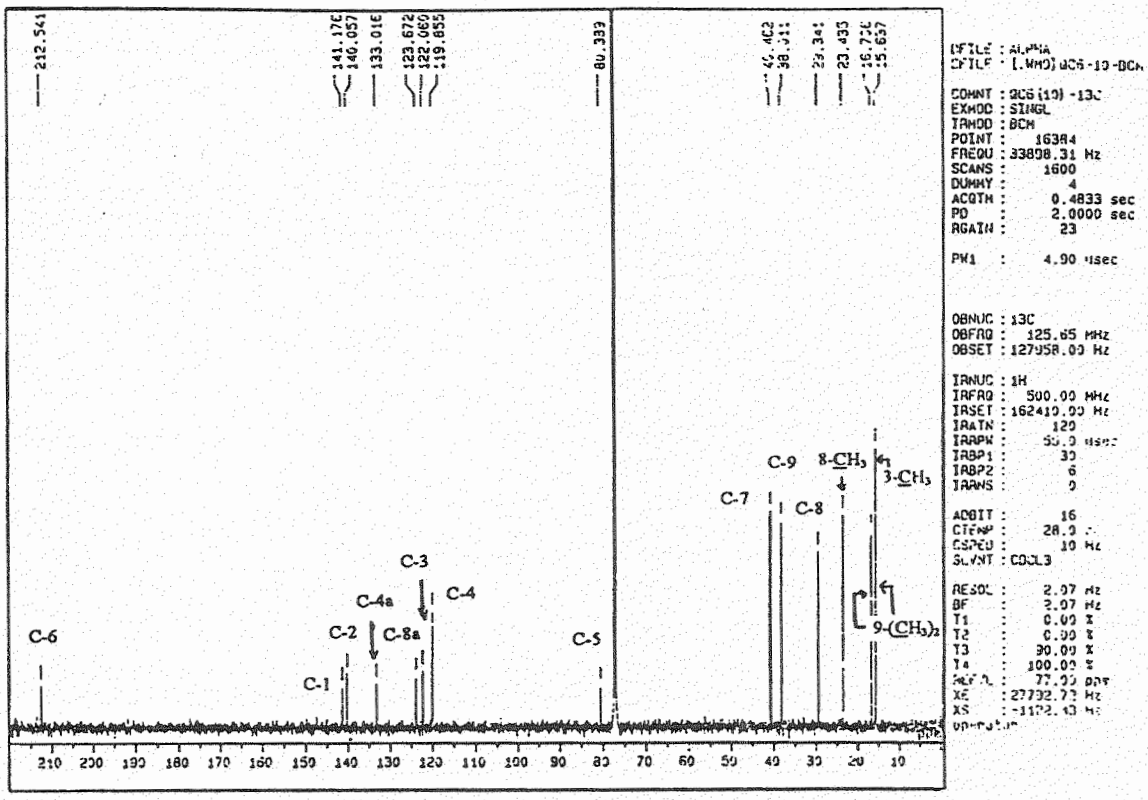


Fig 3.54 The ^{13}C-NMR spectrum of Compound 7

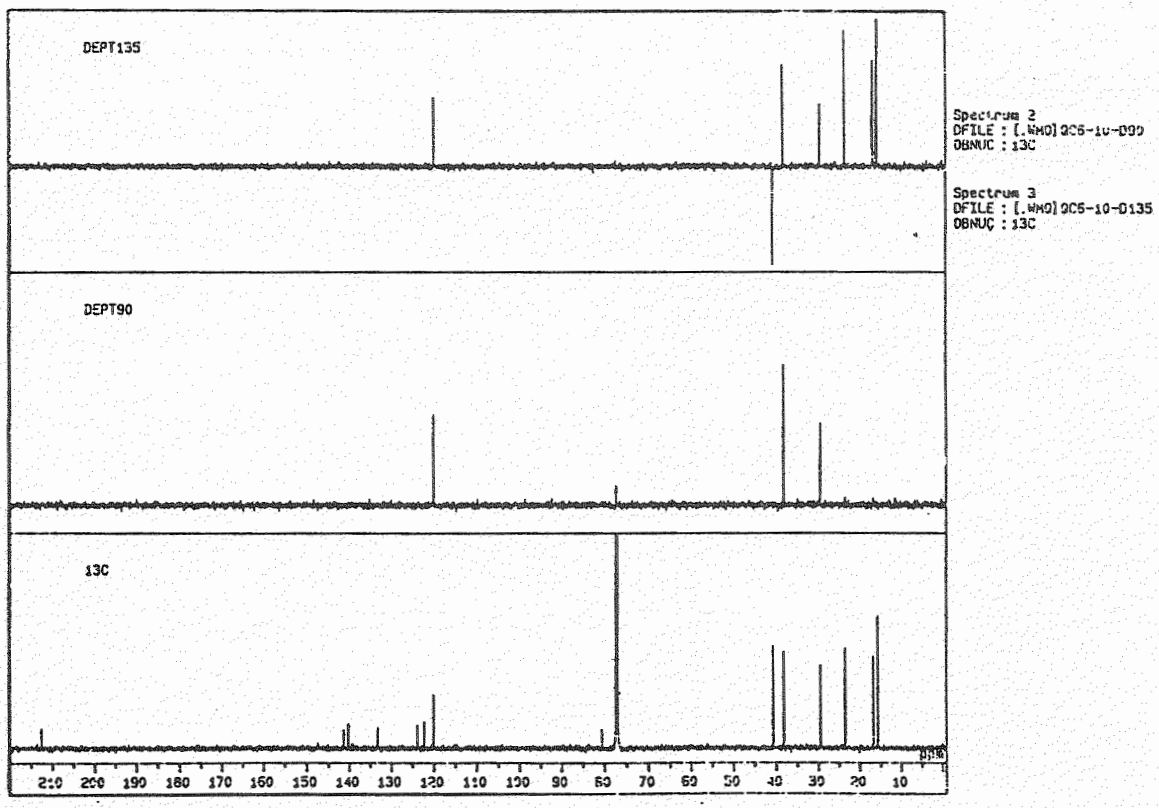
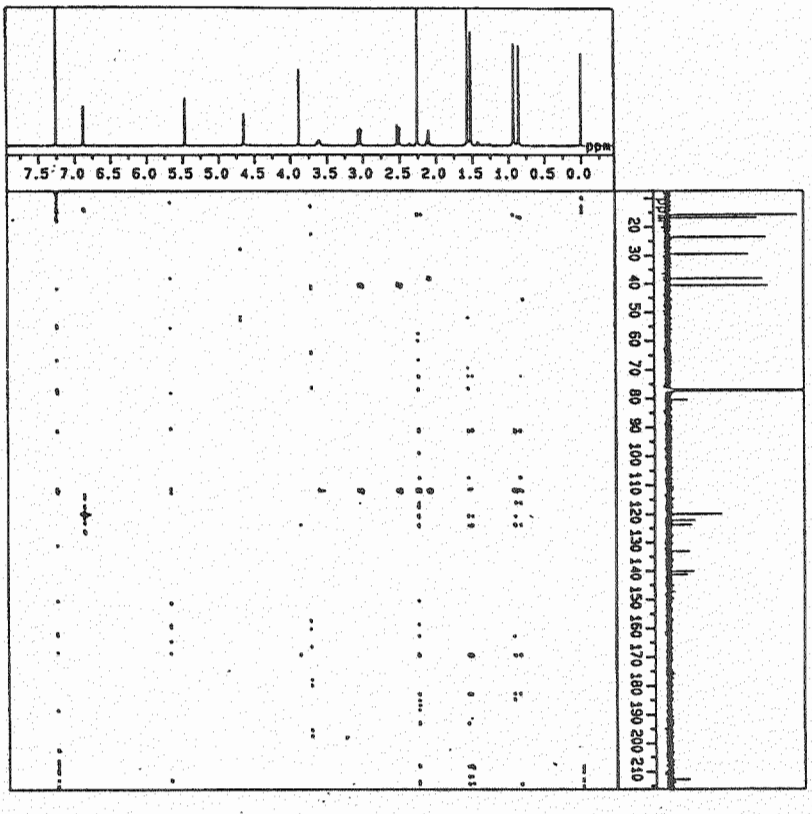


Fig 3.55 The DEPT 90 and 135 spectra of Compound 7

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CHULALONGKORN UNIVERSITY
          JNM-A500

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SLVNT : CDCL3
RESOL :      4.10 Hz
CLRSO :     51.29 Hz
TLINE :        10
THTOP :   10.0000
THSTM :    0.2000

OPERATOR :
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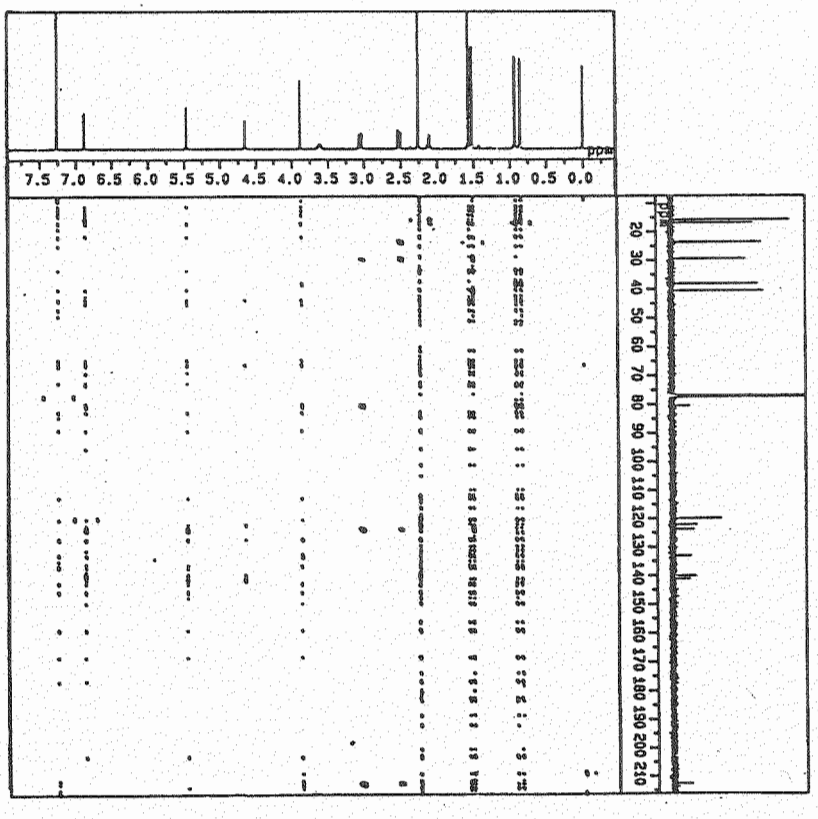
Fig 3.56 The HMQC spectrum of Compound 7




```
CHULALONGKORN UNIVERSITY
          JNM-A500

SFILE : [.T]QC62USR_E1S1
COMNT : QC6-10-1H

EXMOD : HMBC
IRMOD : IRLV2
POINT :      1024
FREQU :   4199.92 Hz
SCANS :        32
DUMMY :        16
ACQTM :    0.2438 sec
PD    :    2.0000 sec
RGAIN :        21

CLFRQ : 26260.50 Hz
CLPNT :       512
TOSCN :       256
CINNT :     10.00 usec
CINTV :     38.08 usec
CINT2 :     19.94 usec

PW1   :      6.80 usec
PW2   :     13.60 usec
PW3   :     19.00 usec
PI1   :  62.5000 msec
PI2   :    1.0000 msec
PI3   :  48.9824 msec
JCNST :    145.00 Hz

OBNUC : 1H
OBFRQ :    500.00 MHz
OBSET : 161792.32 Hz
IRNUC : 13C
IRFRQ :    125.65 MHz
IRSET : 129360.77 Hz
IRATN :       120
IRRPW :       1.8 usec
IRBP1 :       100
IRBP2 :         2
IRANS :         4

ADBIT :        16
CTEMP :      28.1 c
CSPED :         0 Hz
SLVNT : CDCL3
RESOL :      4.10 Hz
CLRSO :     51.29 Hz
TLINE :        10
THTOP :    5.0000
THSTM :    0.5000

OPERATOR :
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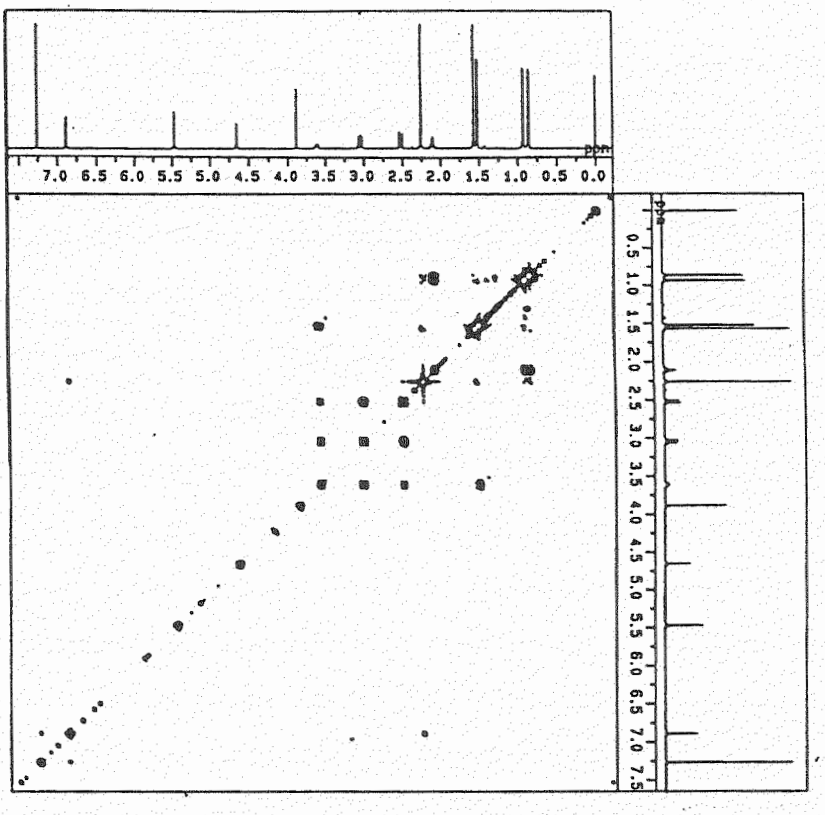
Fig 3.57 The HMBC spectrum of Compound 7




```
CHULALONGKORN UNIVERSITY
JNM-A500

SFILE : [.WMO]QC6-10-COSY
COMNT : QC6-10-H-H COSY

EXMOD : COSY
IRMOD : NON
POINT :      512
FREQU :  3938.56 Hz
SCANS :        8
DUMMY :        4
ACQTM :   0.1300 sec
PD    :   2.0000 sec
RGAIN :       21

CLFRQ :  3938.56 Hz
CLPNT :      512
TOSCN :      256
CINNT :    10.00 usec
CINTV :   253.90 usec
CINT2 :   126.95 usec

PW1   :     6.80 usec
PW2   :    13.60 usec
PW3   :     6.80 usec
PI1   : 3260.8695 msec
PI2   :   1.0000 msec
PI3   :  48.9824 msec
JCNST :   145.00 Hz

OBNUC : 1H
OBFRQ :   500.00 MHz
OBSET : 161777.68 Hz
IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :       120
IRAPW :     30.0 usec
IRAP1 :       25
IRAP2 :        2
IRANS :        0

ADBIT :       16
CTEMP :     26.9 c
CSPED :       14 Hz
SLVNT : CDCL3
RESOL :     7.69 Hz
CLASO :     7.69 Hz
TLINE :       10
THTOP :  30.0000
THBTM :   0.6000

OPERATOR :
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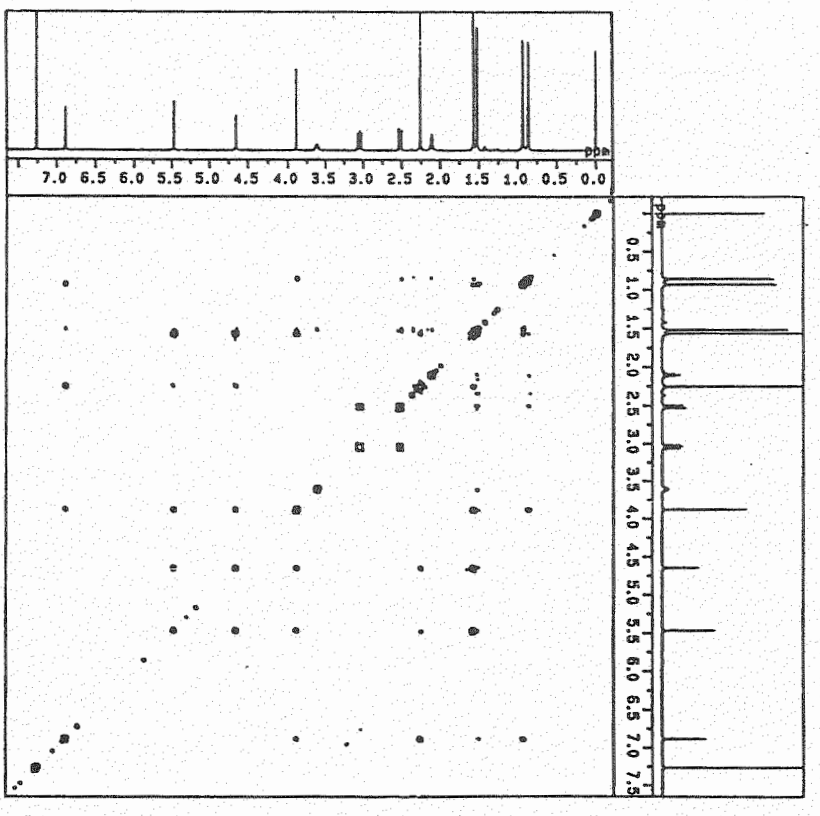
Fig 3.58 The COSY spectrum of Compound 7




```
CHULALONGKORN UNIVERSITY
JNM-A500

SFILE : [.WMO]QC6-10-NOESY
COMNT : QC6-10-NOESY

EXMOD : NOESY
IRMOD : NON
POINT :      512
FREQU :  3938.56 Hz
SCANS :        8
DUMMY :        4
ACQTM :   0.1300 sec
PD    :   2.0000 sec
RGAIN :       21

CLFRQ :  3938.56 Hz
CLPNT :      512
TOSCN :      256
CINNT :    10.00 usec
CINTV :   253.50 usec
CINT2 :   126.95 usec

PW1   :     6.80 usec
PW2   :    13.60 usec
PW3   :     6.80 usec
PI1   : 3260.8695 msec
PI2   :   1.0000 msec
PI3   :  48.9824 msec
JCNST :   145.00 Hz

OBNUC : 1H
OBFRQ :   500.00 MHz
OBSET : 161777.68 Hz
IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162410.00 Hz
IRATN :       120
IRAPW :     30.0 usec
IRAP1 :       25
IRAP2 :        2
IRANS :        0

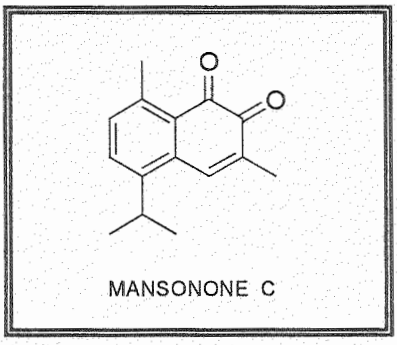
ADBIT :       16
CTEMP :     27.4 c
CSPED :        0 Hz
SLVNT : CDCL3
RESOL :     7.69 Hz
CLASO :     7.69 Hz
TLINE :       10
THTOP :  15.0000
THBTM :   0.3000

OPERATOR :
```

Fig 3.59 The ^{1}H-^{1}H NOESY spectrum of Compound 7

3.3.8 *Compound 8:* Mansonone C

Compound **8** was fruitfully isolated as orange needles, m.p 113-114°c. This compound showed a single spot on TLC with R_f value 0.5 (silica gel; hexane : ethyl acetate = 7:3). The ^{1}H NMR spectrum of this compound showed the presence of a methyl singlet signal at δ_H 2.60, a methyl doublet at δ_H 2.05 (J = 2.0 Hz), an isopropyl group at δ_H 3.36 (m, 1H) and 1.27 (d, 6H, J = 7.0 Hz), two doublet of the *ortho*-coupled aromatic protons at δ_H 7.16 (1H, J = 8.2 Hz) and 7.40 (1H, J = 8.2 Hz) and an olefinic doublet at δ_H 7.63 (J = 1.5 Hz). The presence of a naphthoquinone ring was manifestly supported by eight carbon signals between δ_C 129.3 and 145.3, as well as, two carbonyl carbon signals at δ_C 181.9 and 182.3 which were supported by IR spectrum (Fig 3.60) at ν_{max} 1550 (aromatic moiety) and 1664 cm^{-1} (α,β-unsaturated ketone; quinone).[35] According to a number of 1,2-naphthoquinones were isolated from *Mansonia* plant,[5-10] the ^{1}H and ^{13}C NMR data of Compound **8** were compared and found to be identical with those of mansonone C.[5] In addition, the mass spectrum (Fig 3.61) of this compound did not show $[M]^+$ peak but displayed the $[M+2]^+$ peak at m/z 230, which was the characteristic of 1,2-naphthoquinones.[36]



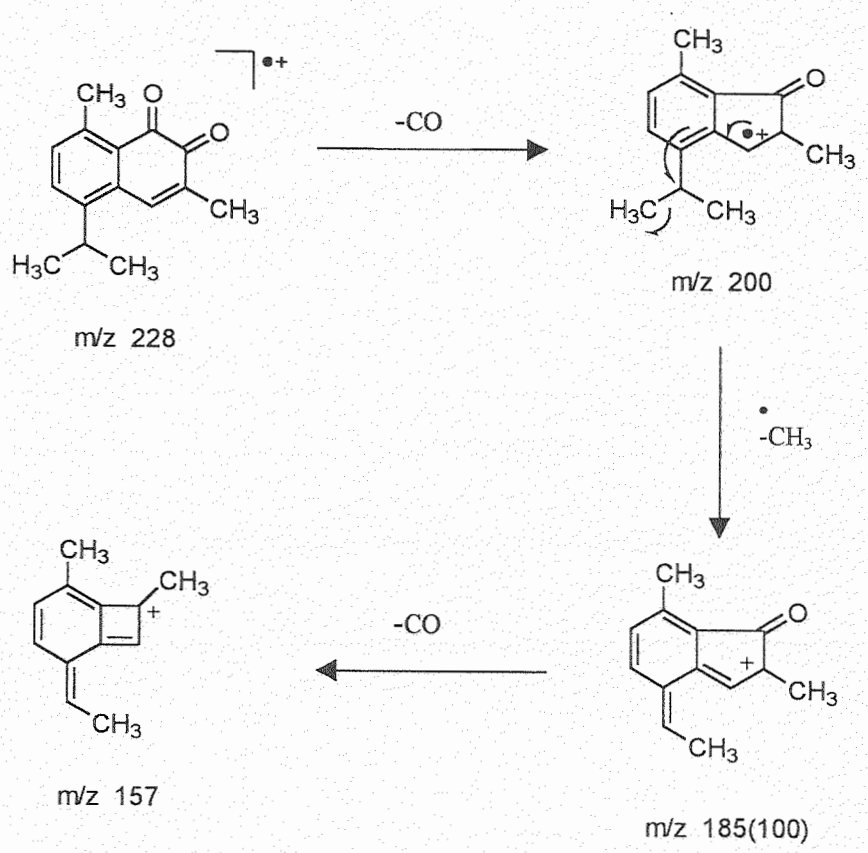
MANSONONE C

Table 3.12 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **8**

	Chemical Shift[a]	
Position	δ_H (J in Hz)	δ_C
1	-	182.3
2	-	181.9
3	-	129.3
4	7.63, d, 1H (1.5)	138.0
4a	-	135.0
5	-	132.4
6*	7.16, d, 1H (8.2)	131.9
7*	7.40, d, 1H (8.2)	134.1
8	-	145.3
8a	-	143.0
9	3.36, m, 1H	28.3
3-CH_3	2.02, d, 3H (2.0)	16.0
8-CH_3	2.60, s, 3H	22.9
9-$(CH_3)_2$	1.27, d, 1H (7.0)	23.7 (2C)

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 200 and 50 MHz, respectively
* The chemical shift values were inter-changeable

The mass spectrum (Fig 3.61) showed the molecular ion peak at m/z 228 ($[M]^+$), and other fragmentations at m/z 200 (M^+-CO), 185 (M^+-CO-CH_3) and 157 (M^+-CO-CH_3-CO). The proposed fragmentation pattern of Compound **8** is shown in Scheme 3.9.



Scheme 3.9 The proposed fragmentation pattern of Compound **8**

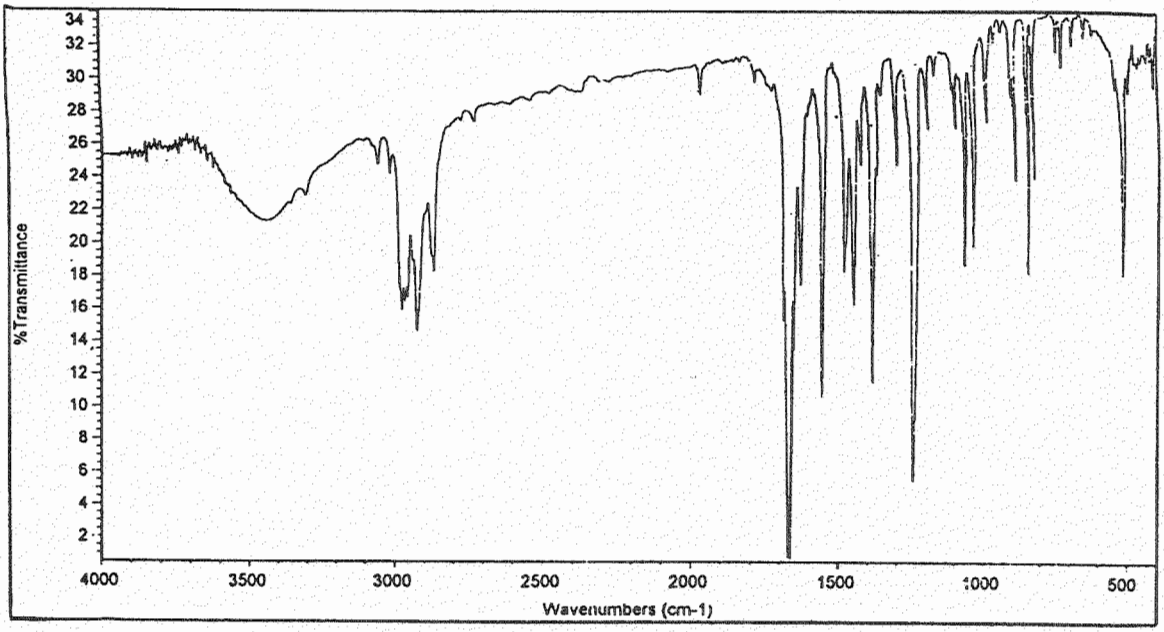


Fig 3.60 The FT-IR spectrum of Compound 8

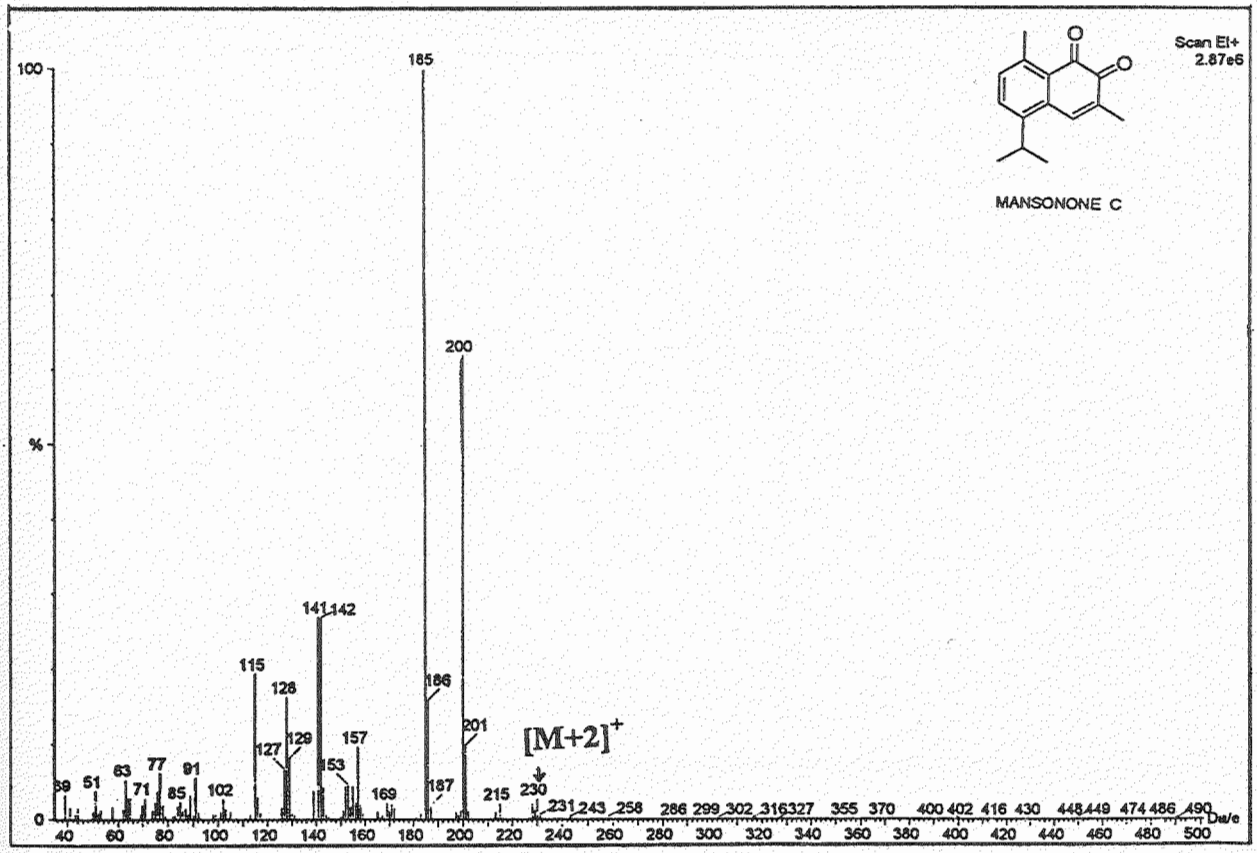


Fig 3.61 The mass spectrum of Compound 8

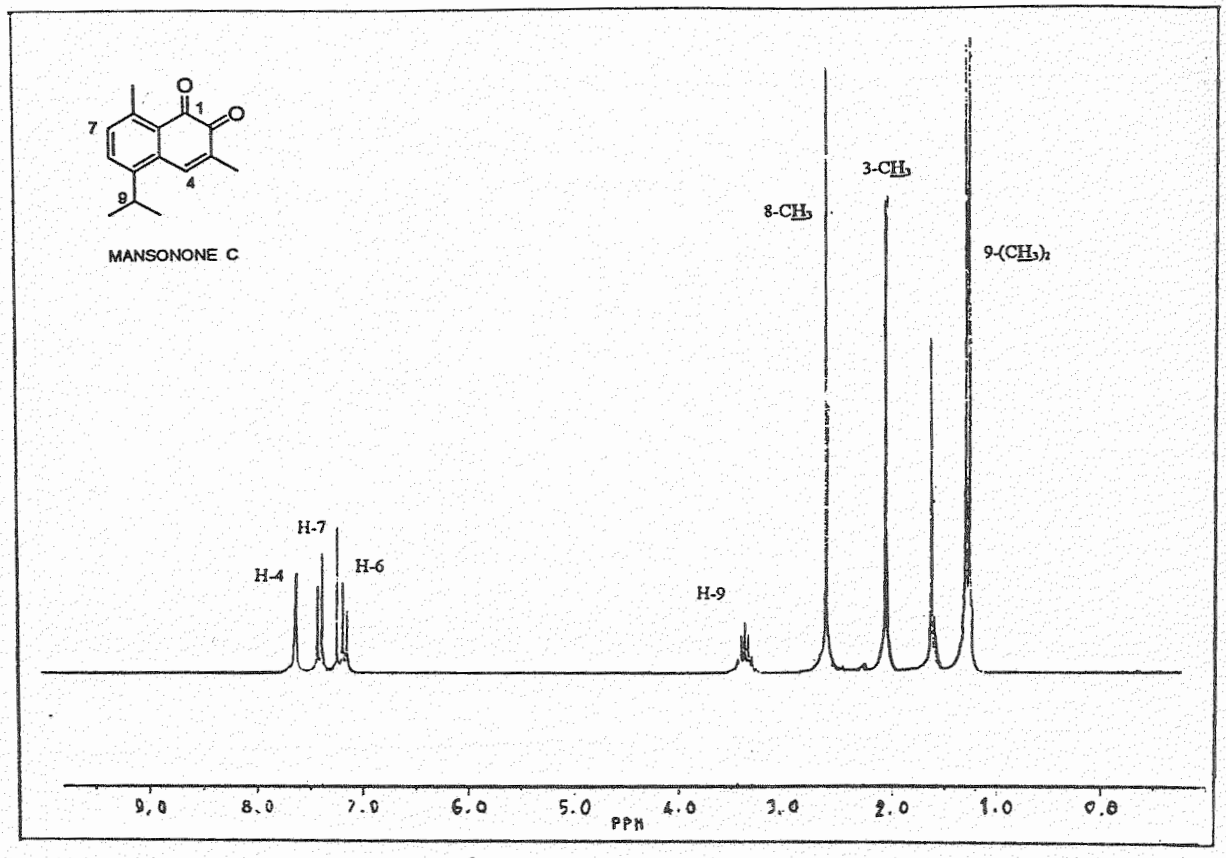


Fig 3.62 The ^{1}H-NMR spectrum of Compound 8

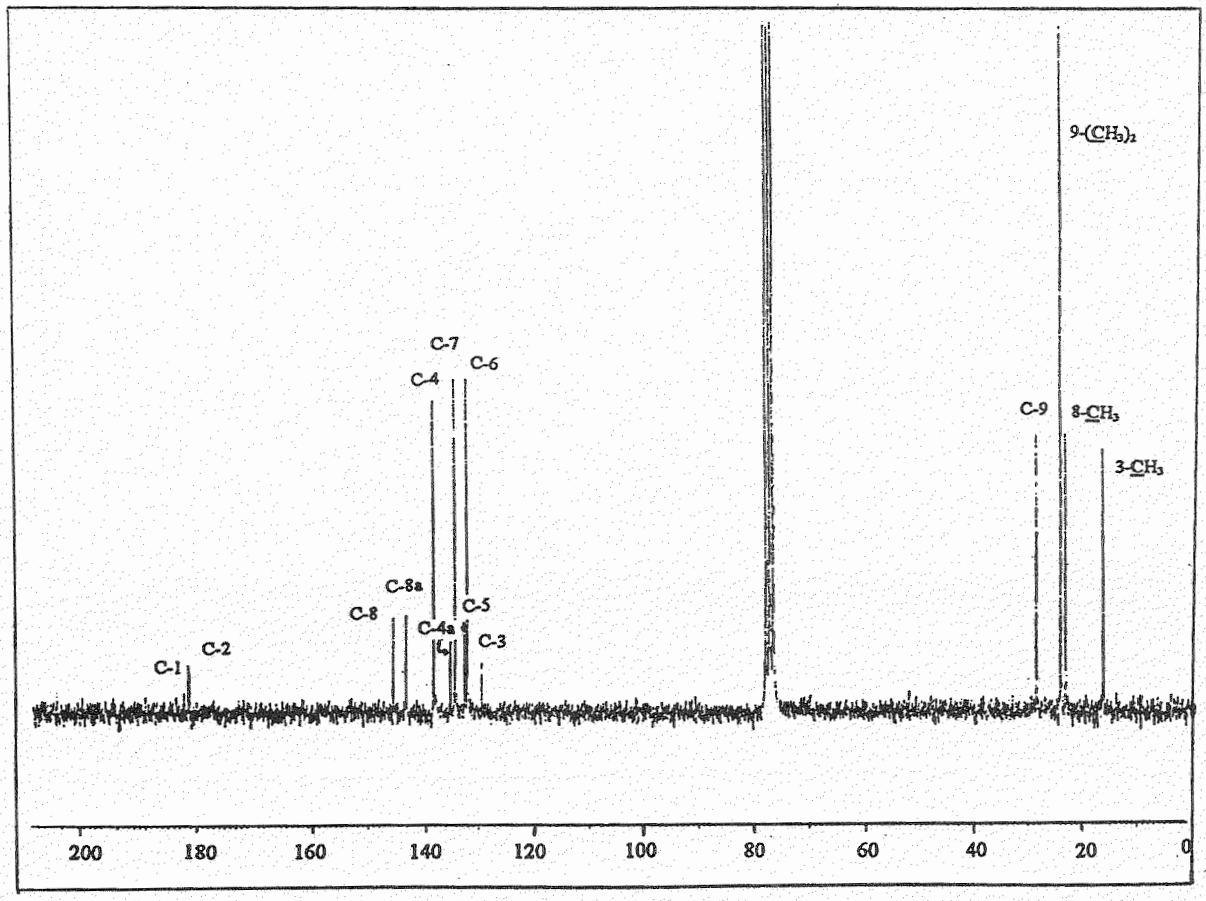


Fig 3.63 The ^{13}C-NMR spectrum of Compound 8

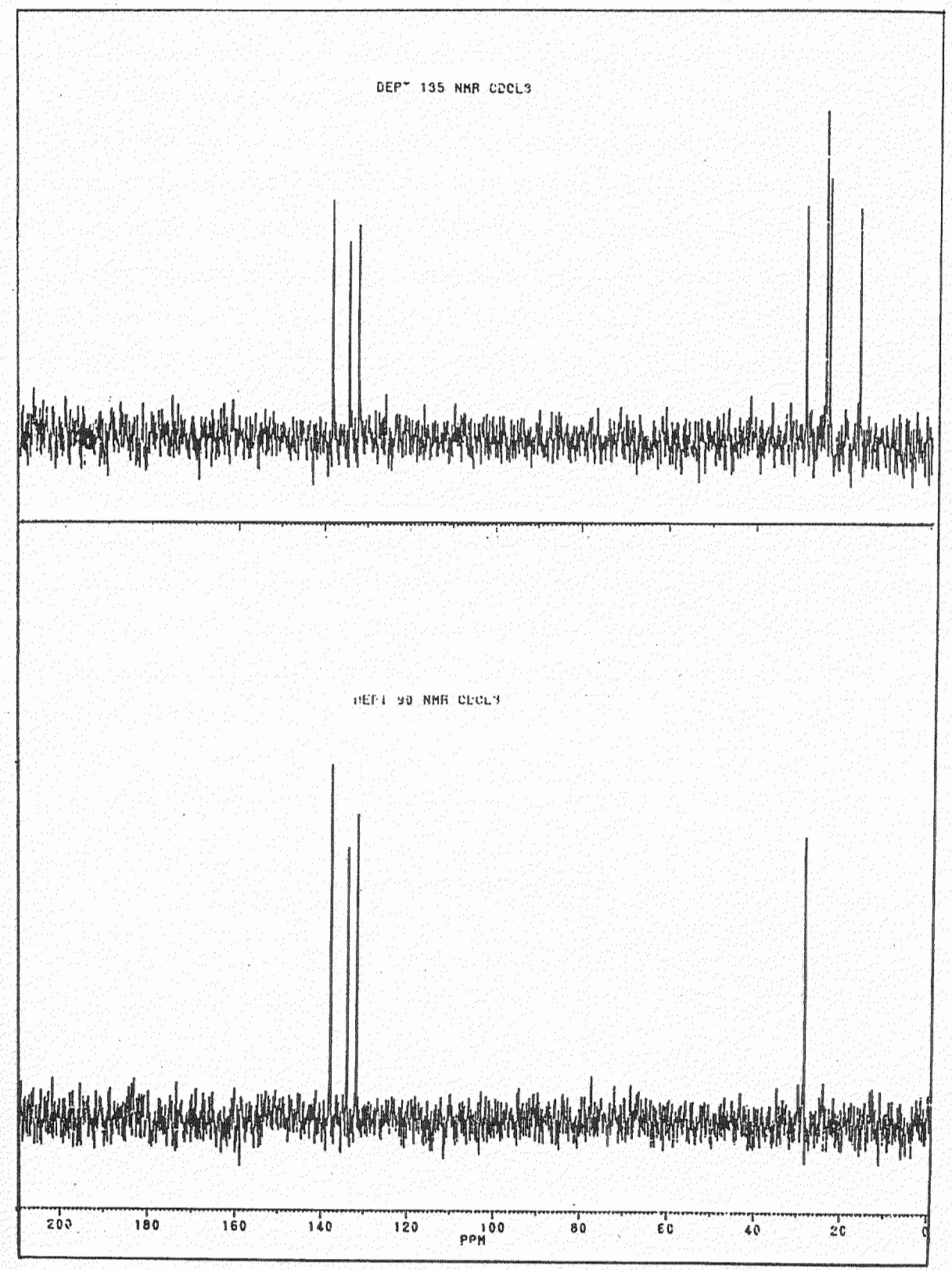
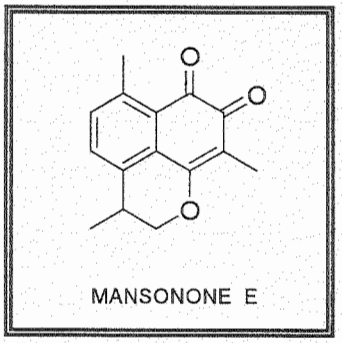


Fig 3.64 The DEPT 90 and 135 spectra of Compound 8

3.3.9 *Compound 9:* Mansonone E

Compound **9** was isolated as orange powder, m.p 118-120°C. This compound showed a single spot on TLC with R_f value 0.4 (silica gel; hexane : ethyl acetate = 7:3). Its molecular formula was established as $C_{15}H_{14}O_3$ by EIMS and ^{13}C NMR data. The ^1H NMR spectrum of Compound **9** (Fig 3.66) showed the presence of two methyl singlets at δ_H 1.95 and 2.65, a methyl doublet at δ_H 1.37 (J = 7.0 Hz), two aromatic protons at δ_H 7.26 and 7.35 (J = 7.9 Hz each) which were in *ortho* to each other, a methine multiplet at δ_H 3.10 and a oxygenated methylene doublet of doublet at δ_H 4.23 (1H, J = 5.2 and 10.7 Hz), 4.41 (1H, J = 4.0 and 10.7 Hz). The ^{13}C NMR spectrum of Compound **9** (Fig 3.67) displayed a total of 15 carbon signals; two ketonic carbons at δ_C 180.2 and 182.2, eight aromatic and/or olefinic carbons between δ_C 126.9 and 162.4, and five aliphatic carbons between δ_C 7.8 and 71.4.

According to a number of 1,2-naphthoquinones have been reported in the literature, the ^1H and ^{13}C NMR spectral data of Compound **9** were compared to those of 1,2-naphthoquinones and disclosed that NMR data of Compound **9** were identical to those of mansonone E[13] (Table 3.13). Thus, the structure of Compound **9** is 2,3-dihydro-3,6,9-trimethylnaphtho[1,8-bc]pyran-7,8-dione (mansonone E), previously isolated from *Mansonia altissima*.[5]



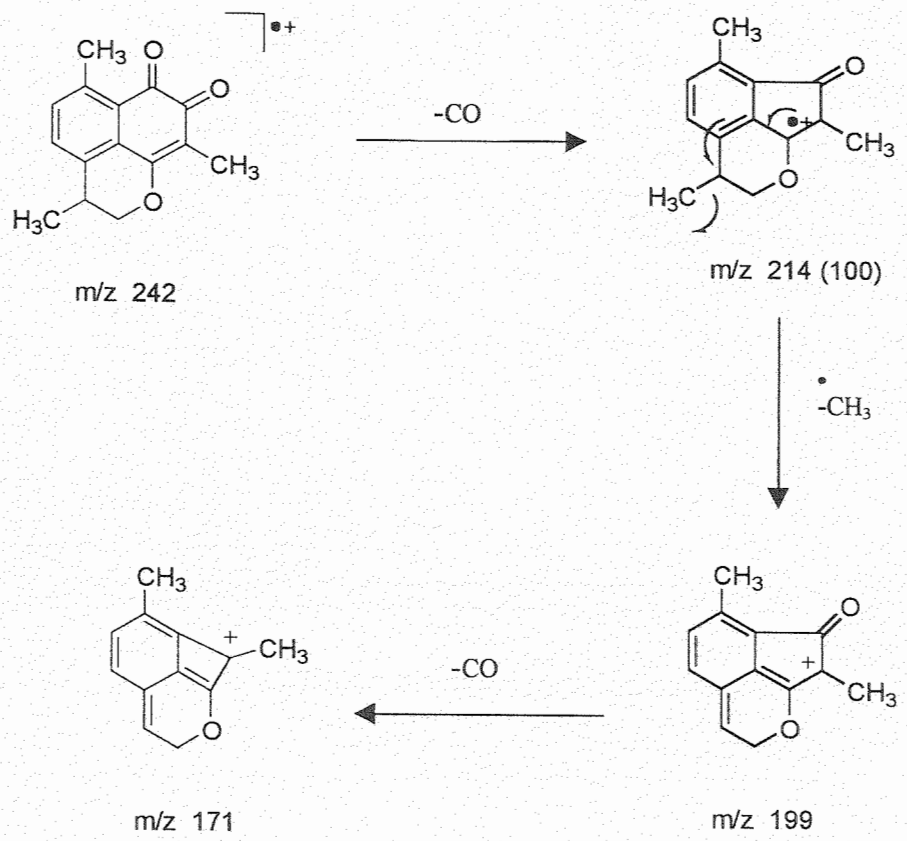
MANSONONE E

Table 3.13 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **9** and mansonone E[13]

Position	Chemical Shift (ppm)			
	9[a]		Mansonone E[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	-	182.2	-	182.2
2	-	180.2	-	180.2
3	-	116.3	-	116.8
4	-	162.4	-	162.4
4a	-	126.9	-	126.8
5	-	136.9	-	136.9
6	7.35, d, 1H (7.9)	132.6	7.35, d, 1H (7.8)	132.6
7	7.26, d, 1H (7.9)	134.8	7.25, d, 1H (7.8)	134.9
8	-	142.9	-	142.8
8a	-	127.3	-	127.3
9	3.10, m, 1H	31.3	3.10, m, 1H	31.3
10	4.41, dd, 1H (4.0, 10.7) 4.23, dd, 1H (5.2, 11.0)	71.4	4.41, br d, 1H (3.9, 10.7) 4.23, dd, 1H (5.1, 10.3)	71.4
3-CH_3	1.95, s, 3H	7.8	1.94, s, 3H	7.8
8-CH_3	2.65, s, 3H	22.5	2.63, s, 3H	22.5
9-CH_3	1.37, d, 3H (7.0)	17.6	1.37, d, 3H (6.8)	17.5

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively
[b] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 600 and 150.8 MHz, respectively

The mass spectrum (Fig 3.65) displayed the molecular ion peak at m/z 242 ($[M]^+$), and other fragmentations were detected at m/z 214 (M^+-CO), 199 (M^+-CO-CH_3) and 171 (M^+-CO-CH_3-CO). The proposed fragmentation pattern of Compound **9** is shown in Scheme 3.10.



Scheme 3.10 The proposed fragmentation pattern of Compound **9**

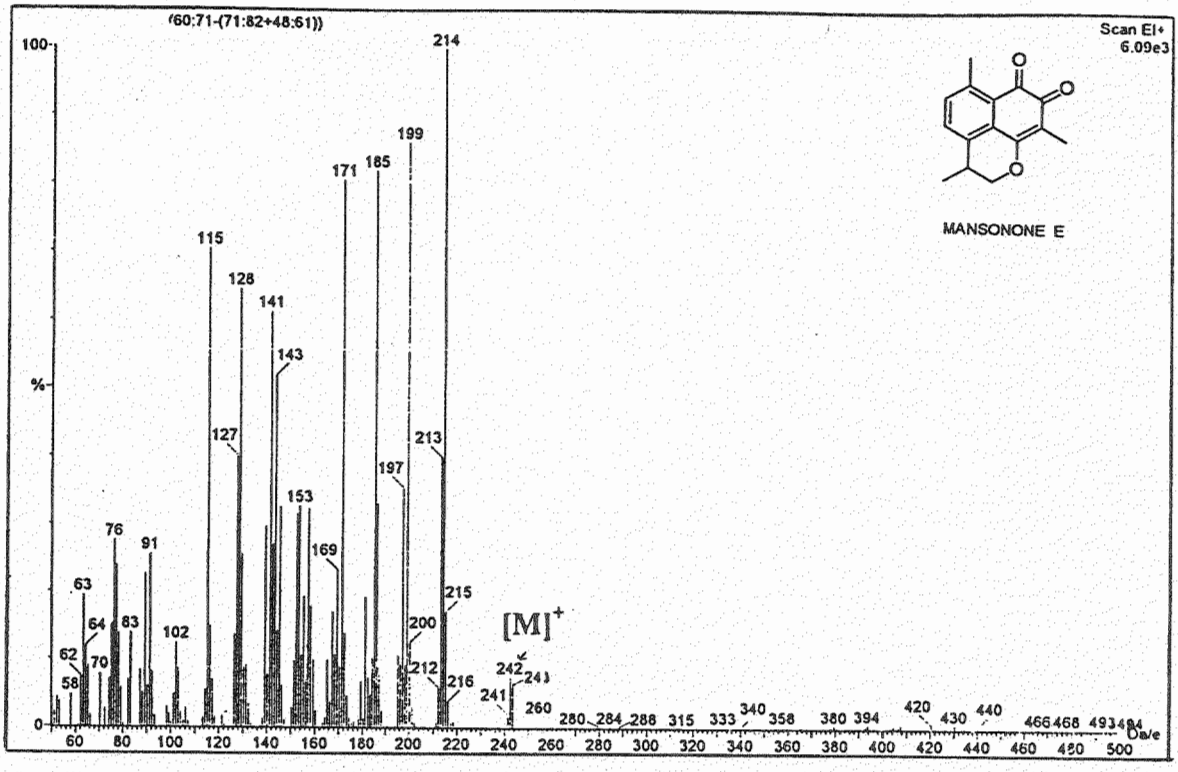


Fig 3.65 The mass spectrum of Compound 9

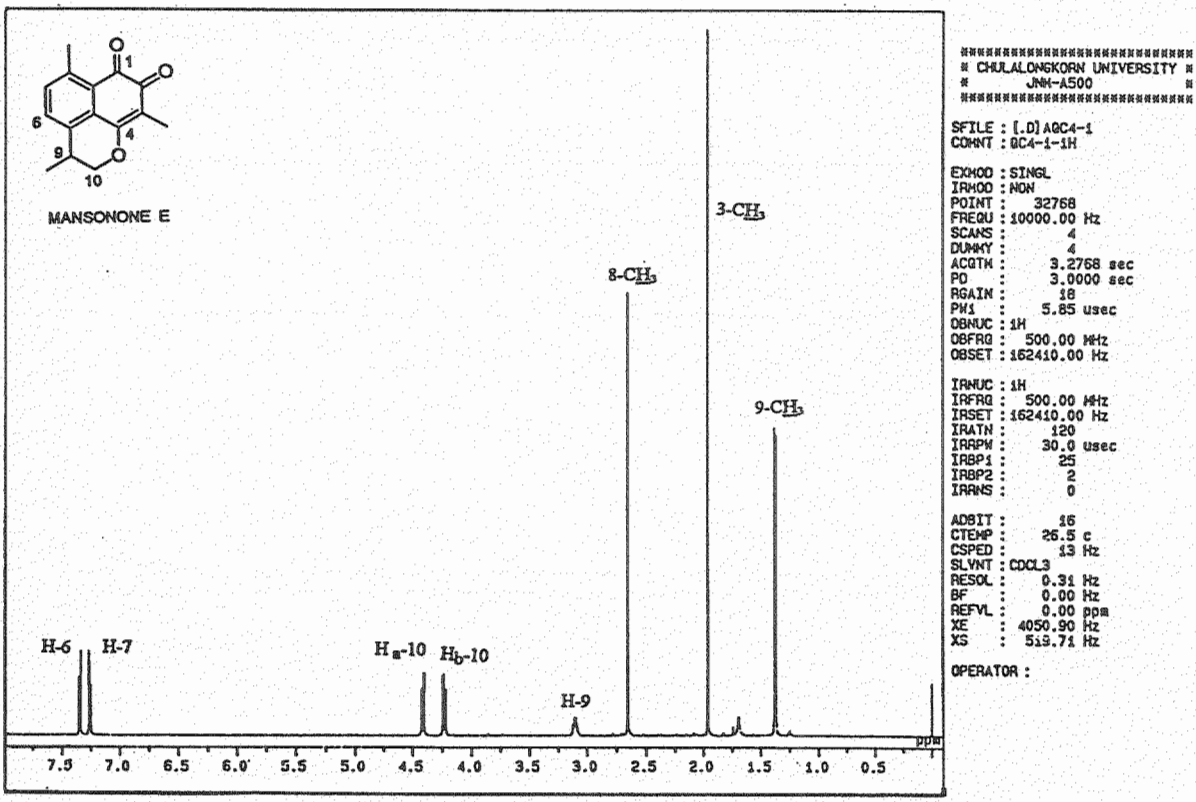


Fig 3.66 The ^{1}H-NMR spectrum of Compound 9

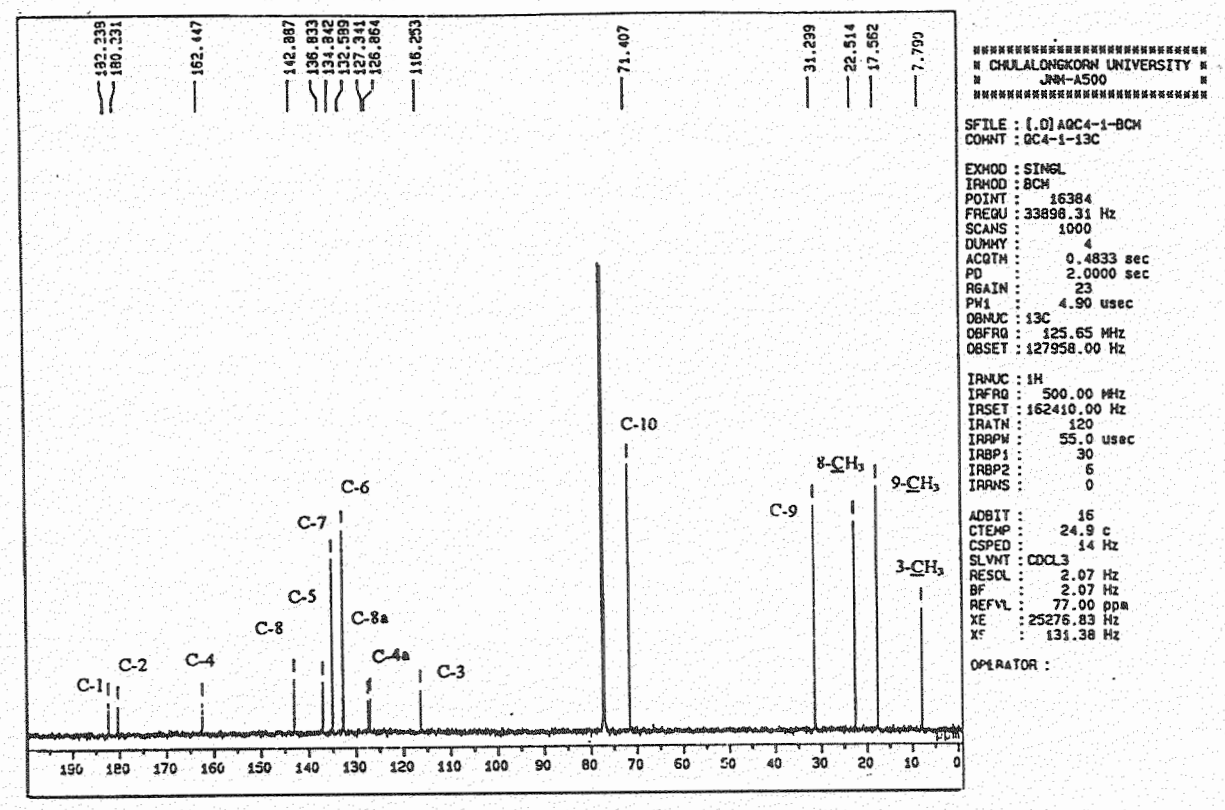


Fig 3.67 The ^{13}C-NMR spectrum of Compound 9

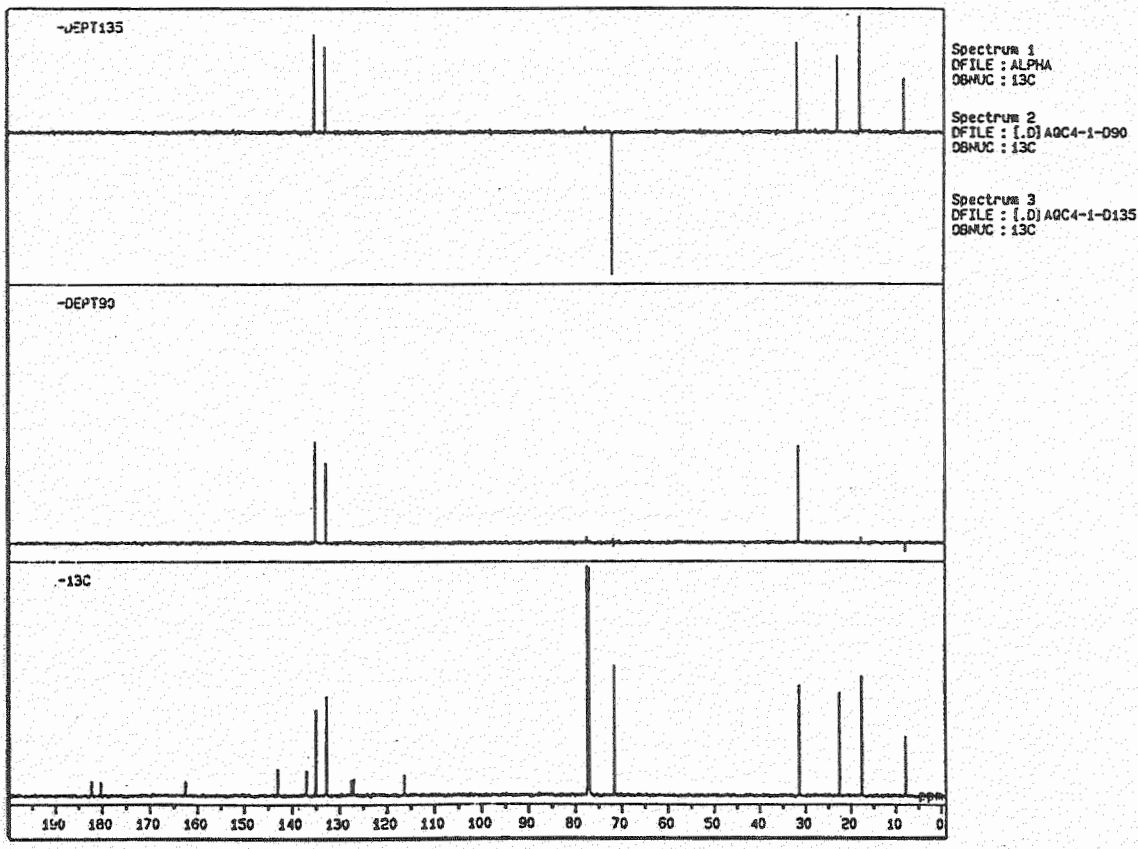
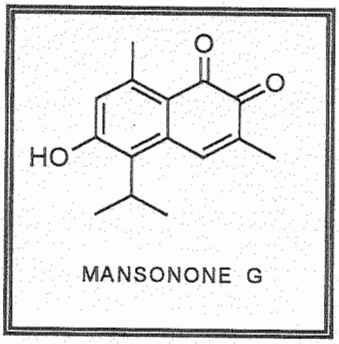


Fig 3.68 The DEPT 90and 135 spectra of Compound 9

3.3.10 *Compound 10:* Mansonone G

Compound **10** was isolated as orange powder, m.p 204-206°C. This compound showed a single spot on TLC with R_f value 0.15 (silica gel; hexane : ethyl acetate = 7:3). Its IR spectrum showed absorptions ascribable to a hydroxy group at ν_{max} 3300 cm^{-1}, an aromatic at 1585 and 1551 cm^{-1}, and an α,β-unsaturated ketone (quinone) at 1644 cm^{-1}; the latter was evident from a signal at δ_C 181.2 and 183.8 in the ^{13}C NMR spectrum (Fig 3.72). Moreover, in the HMBC spectrum (Fig 3.76), the carbonyl carbon signal at δ_C 183.8 showed correlations with the olefinic proton at δ_H 7.70 and methyl group at δ_H 2.06. Thus, the chemical shift values of C-1 and C-2 were δ_C 181.2 and 183.8, respectively. The ^1H NMR data of Compound **10** (Table 3.14) were similar to those obtained for Compound **8**, except for the absence of the set of *ortho*-coupled proton signals and the presence of aromatic proton singlet at δ_H 7.70 (1H). This indicated the substituent (a hydroxy group) at 6- or 7-position in aromatic ring. The location of the hydroxy group assigned for C-6 was confirmed by the NOESY correlations that an aromatic proton at δ_H 6.50 showed correlations with 8-CH_3 (δ_H 2.58). The complete assignment of all protons and carbons was elucidated by analysis of HMQC and HMBC experiments. Thus, the structure of Compound **10** was assigned as 6-hydroxy mansonone C. Base on the literature reported, 6-hydroxy mansonone C was designated as mansonone G which previously isolated from *Mansonia* plant.[6]



MANSONONE G

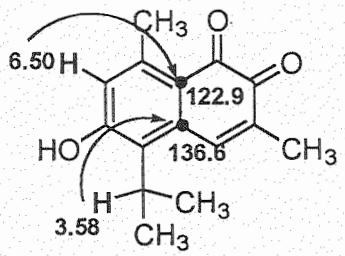
The NMR data of mansonone G[2] were identical to those of Compound **10** (Table 3.14), except for the carbon assignments of C-4a, C-8 and C-8a.

Table 3.14 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **10** and mansonone G[2]

Position	Chemical Shift (ppm)[a]		
	10 and **Mansonone G**	**Mansonone G**	**10**
	δ_H (J in Hz)	δ_C	δ_C
1	-	181.2	181.2
2	-	183.8	183.8
3	-	135.7	135.7
4	7.70, s, 1H	140.4	140.4
4a*	-	122.9	136.6
5	-	134.7	134.7
6	-	164.4	164.4
7	6.50, s, 1H	120.6	120.6
8*	-	136.6	147.8
8a*	-	147.8	122.9
9	3.58, m, 1H	28.1	28.1
3-CH_3	2.06, s, 3H	15.7	15.7
8-CH_3	2.58, s, 3H	23.6	23.6
(9-CH_3)$_2$	1.42, d, 6H (7.0)	21.4	21.4

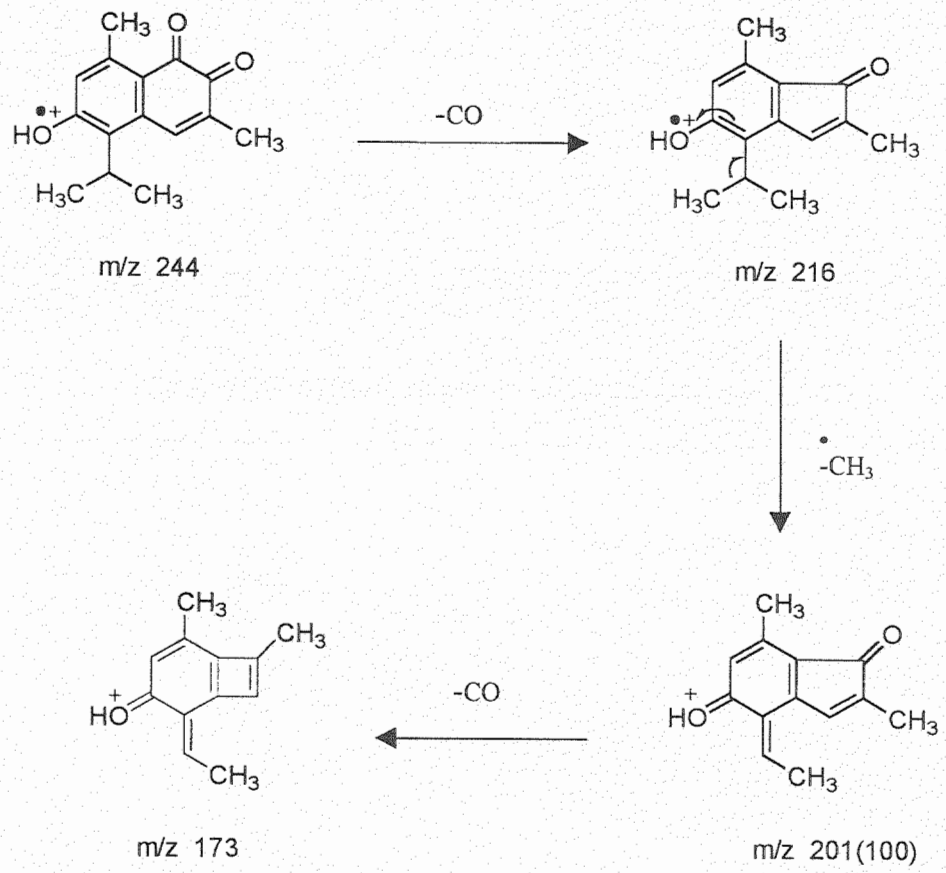
[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125 MHz, respectively
*Assignment differed from those in leterature[2]

In the HMBC correlation of Compound **10**, the aromatic proton signal at δ_H 6.50 (H-7) showed a long-range correlation with the carbon signal at δ_C 122.9 as well as the methine proton signal at δ_H 3.58 (H-9) showed a long-range correlation with the carbon signal at δ_C 136.6 as shown below.



Therefore, the appropriate carbon assignments of C-4a, C-8 and C-8a of Compound **10** or mansonone G were must be 136.6, 147.8 and 122.9, respectively.

The mass spectrum (Fig 3.70) displayed the molecular ion peak at m/z 244 ($[M]^+$), and other fragmetation at m/z 216 (M^+-CO), 201 (M^+-CO-CH_3) and 173 (M^+-CO-CH_3-CO). The proposed fragmentation pattern of Compound **10** is presented in Scheme 3.11.



Scheme 3.11 The proposed fragmentation pattern of Compound **10**

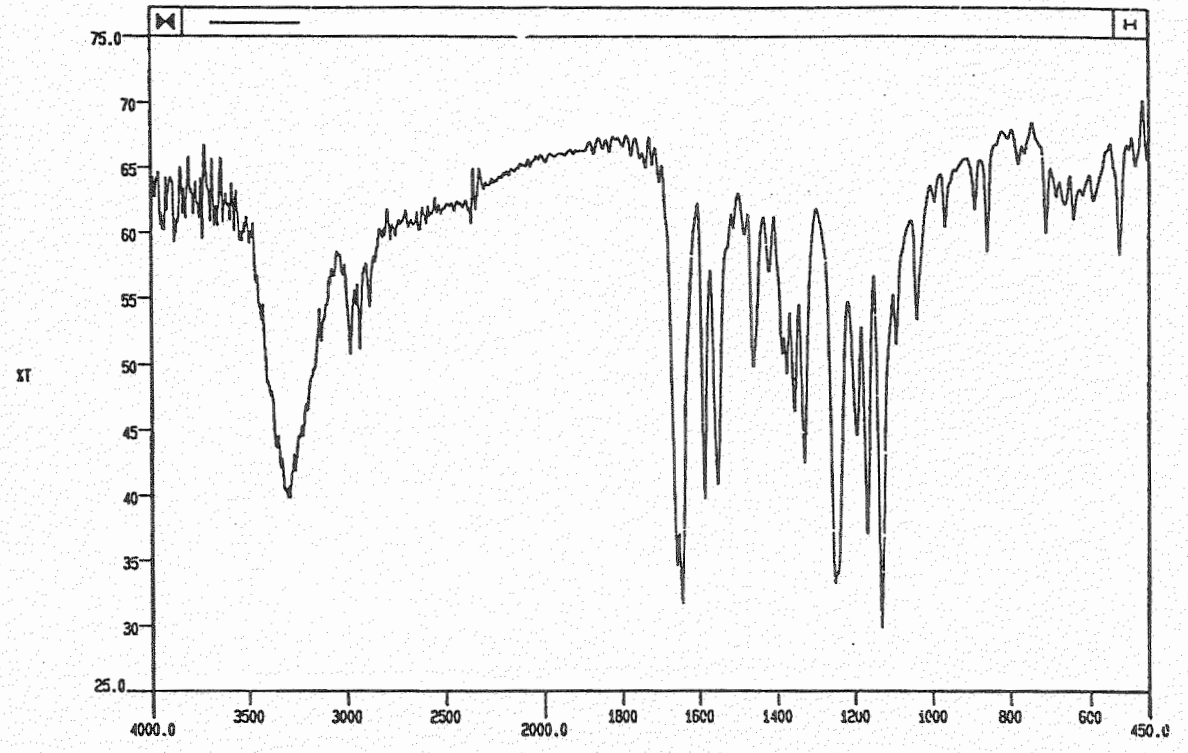


Fig 3.69 The FT-IR spectrum of Compound 10

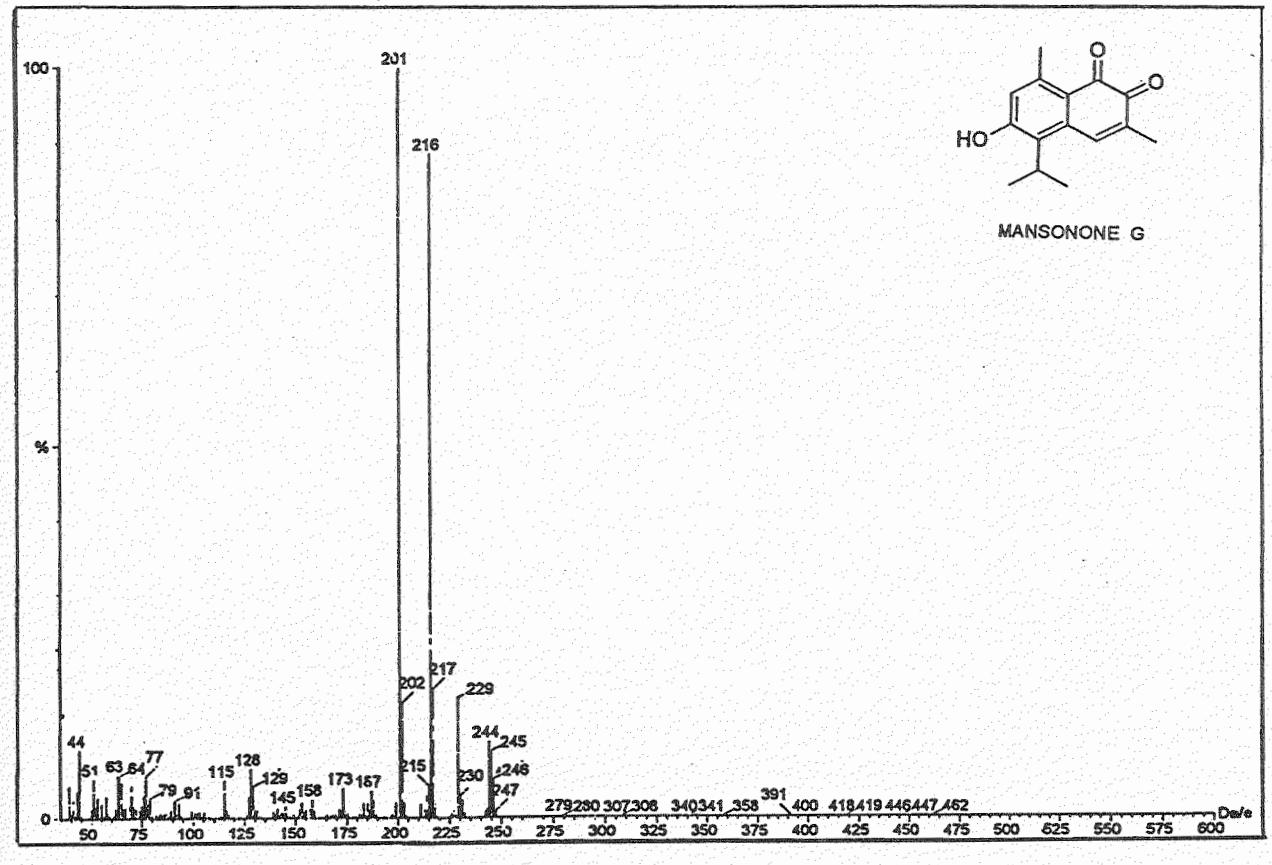


Fig 3.70 The mass spectrum of Compound 10

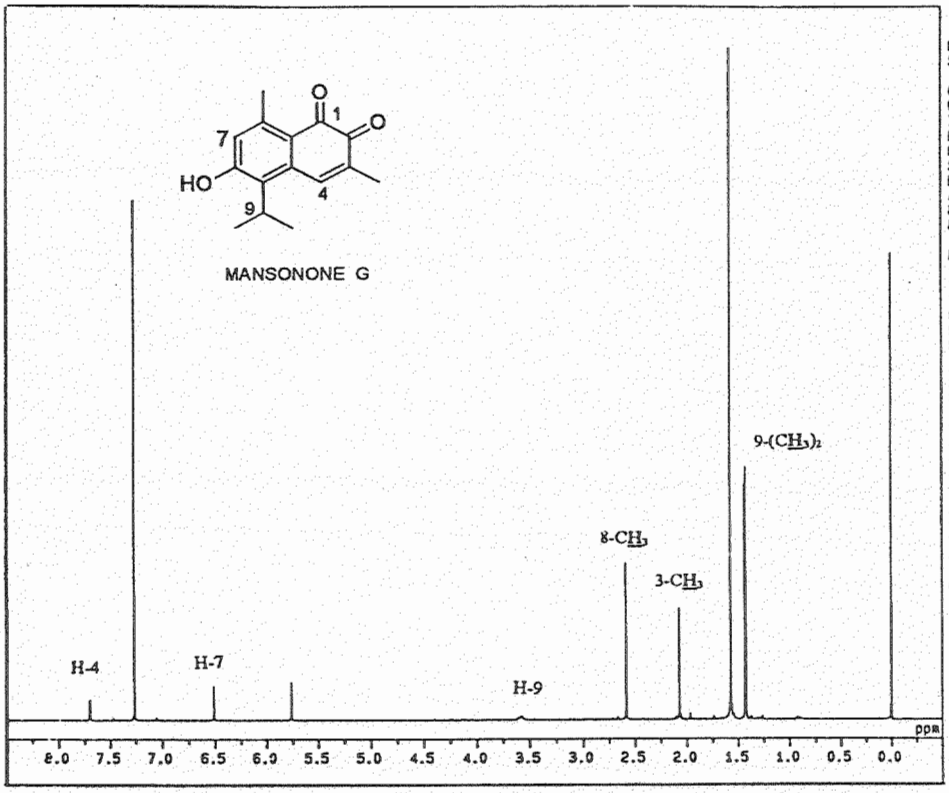


Fig 3.71 The ^{1}H-NMR spectrum of Compound 10

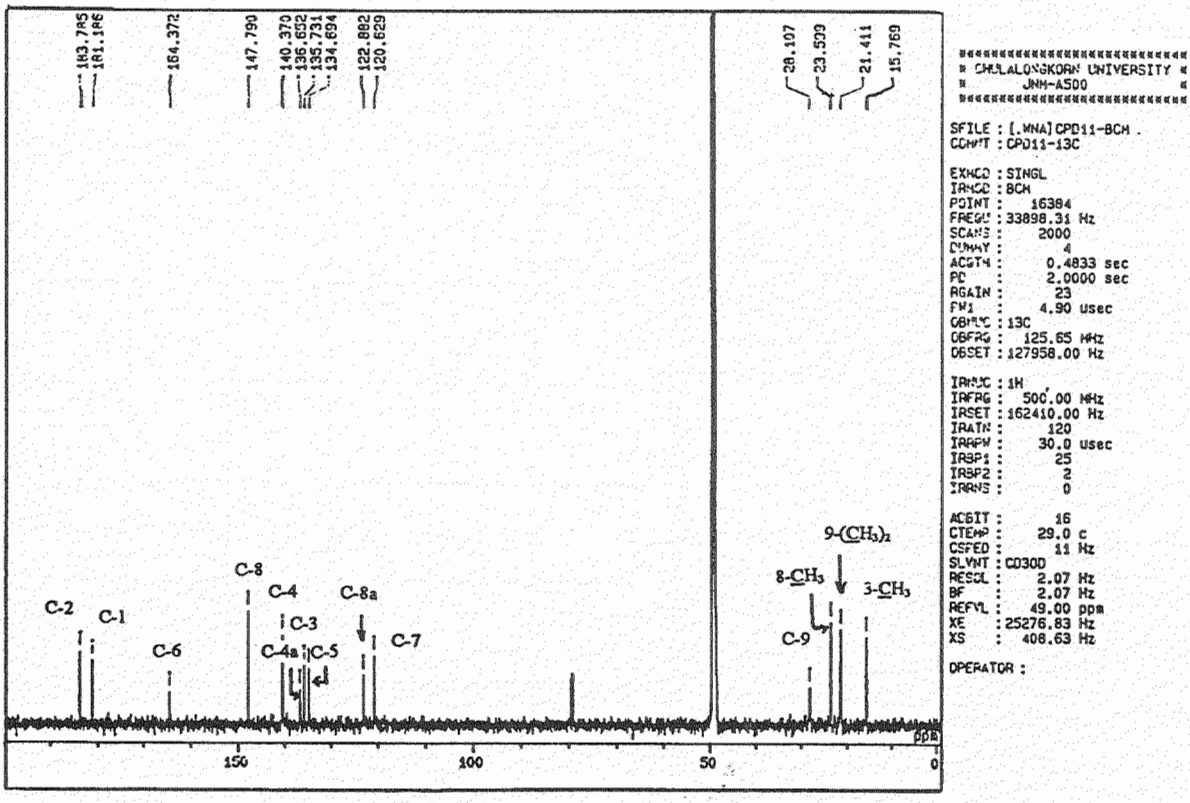


Fig 3.72 The ^{13}C-NMR spectrum of Compound 10

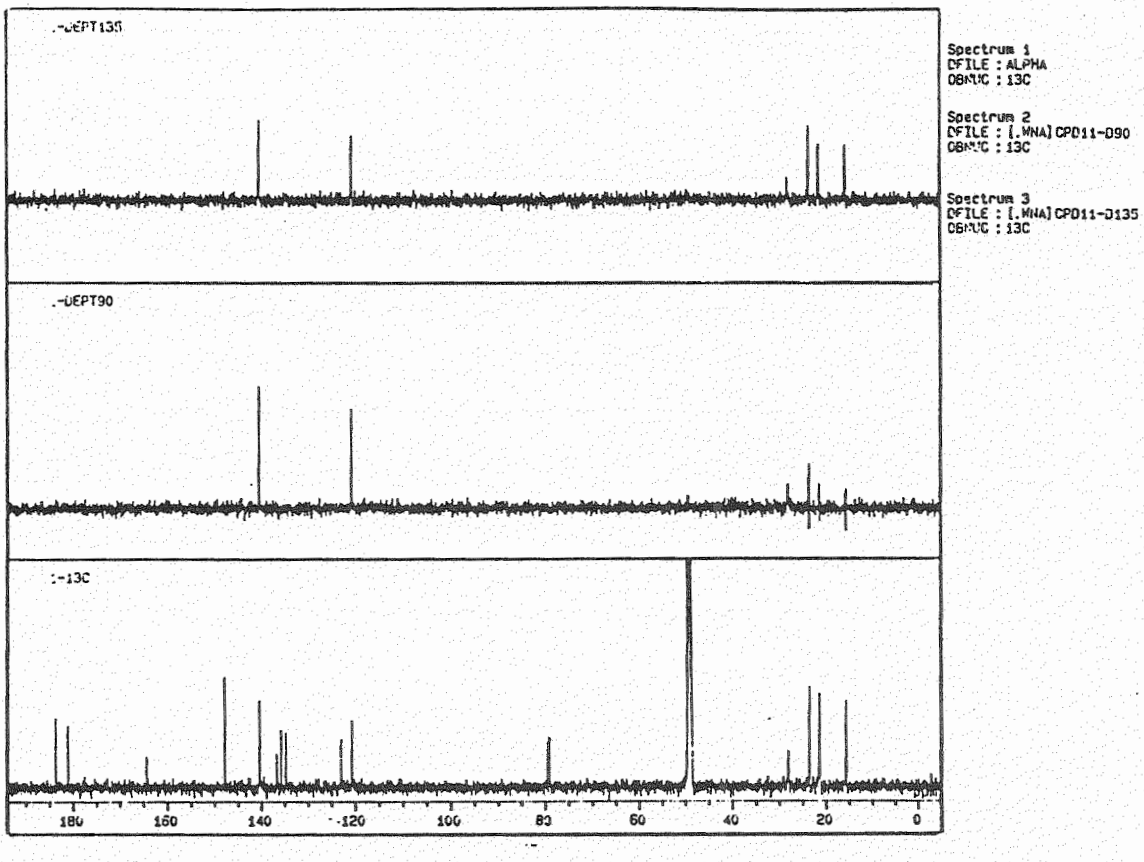


Fig 3.73 The DEPT 90 and 135 spectra of Compound 10

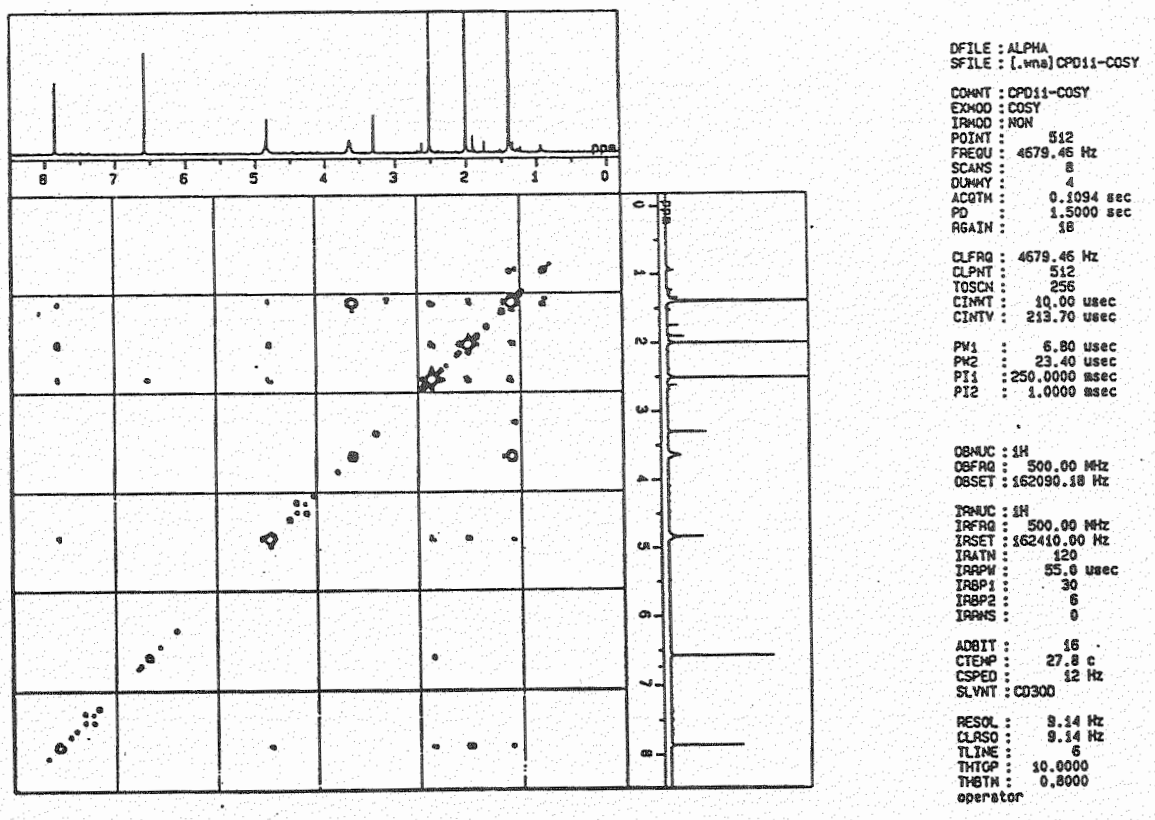


Fig 3.74 The COSY spectrum of Compound 10

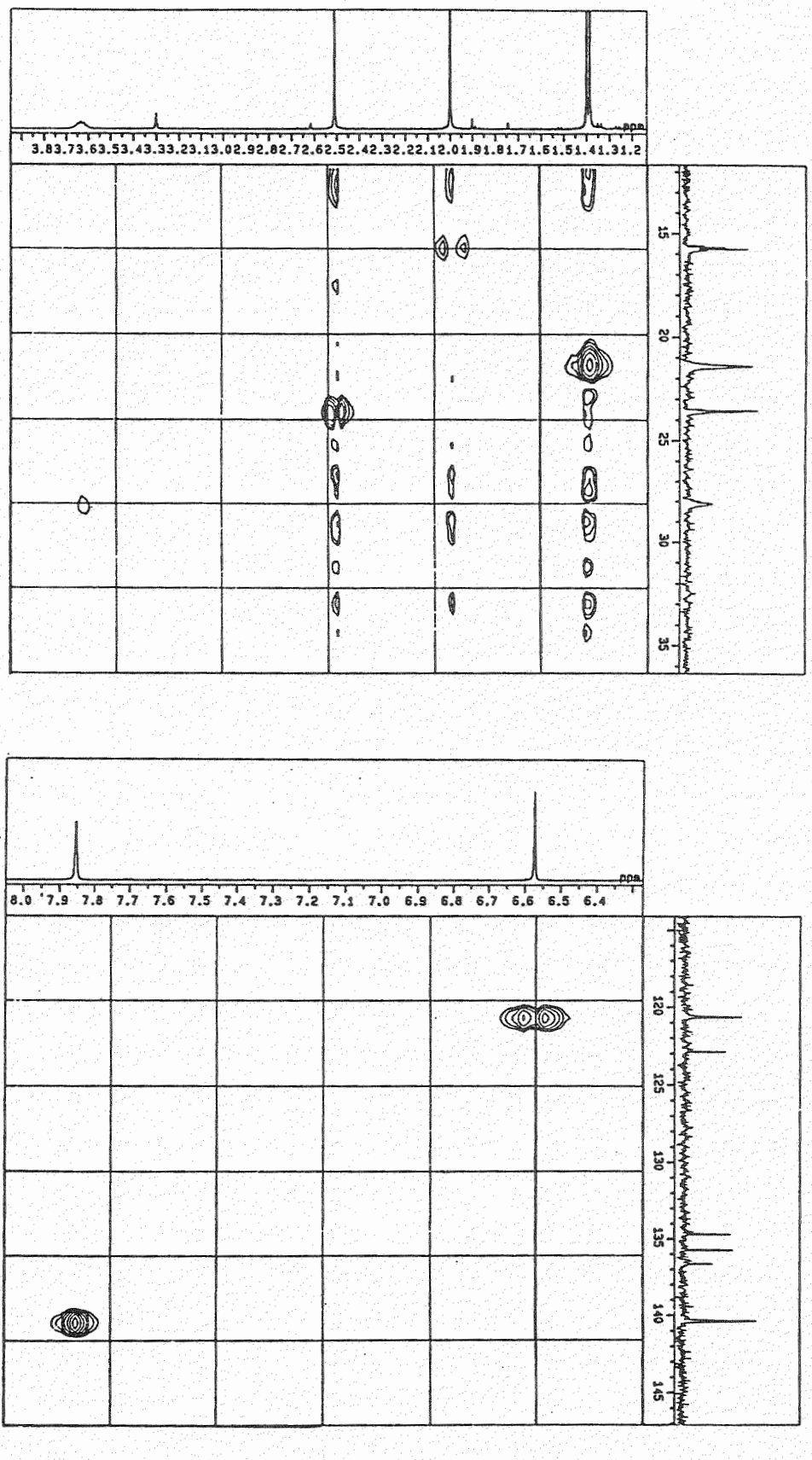


Fig 3.75 The HMQC spectra of Compound **10**

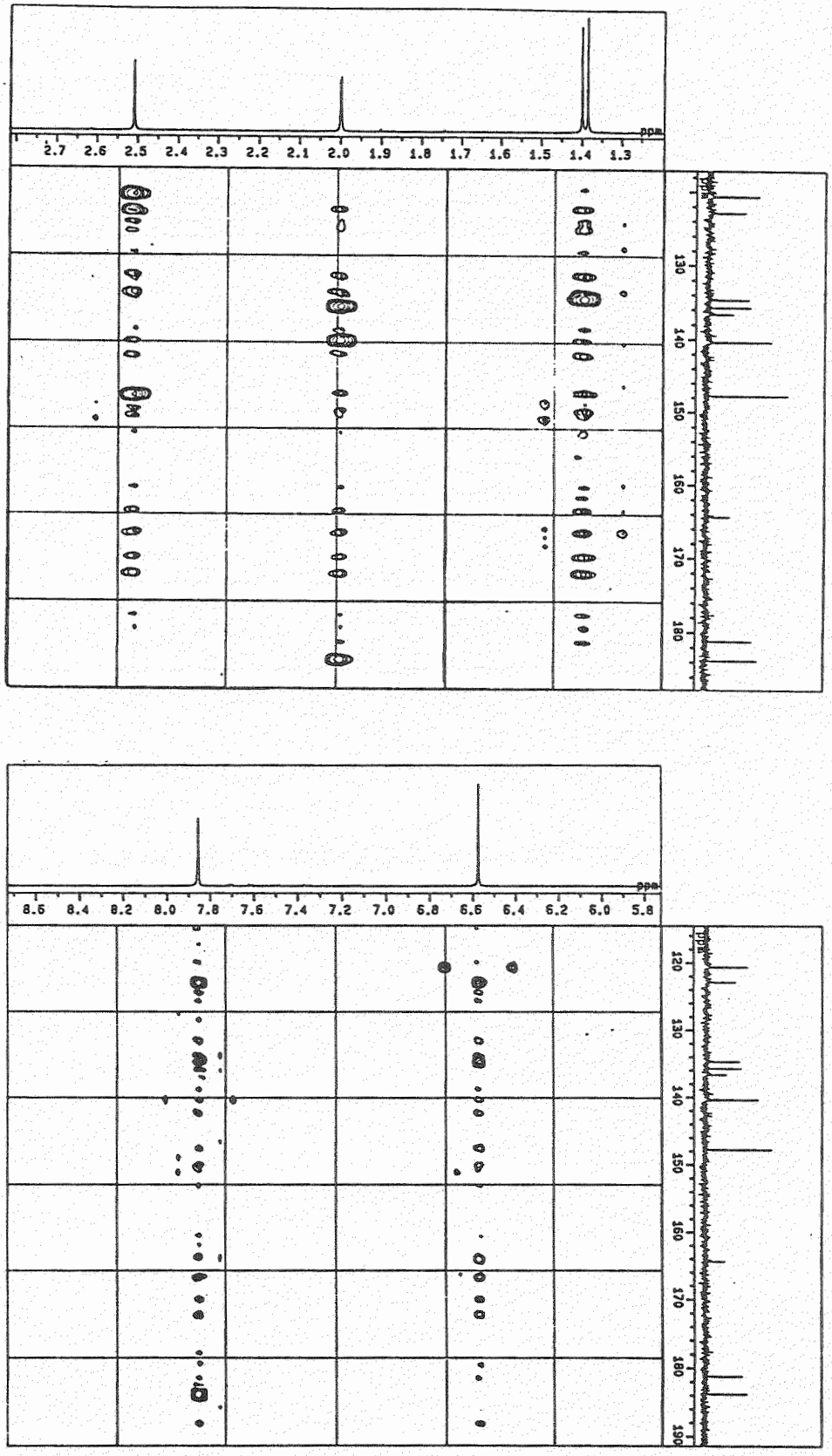


Fig 3.76 The HMBC spectra of Compound 10

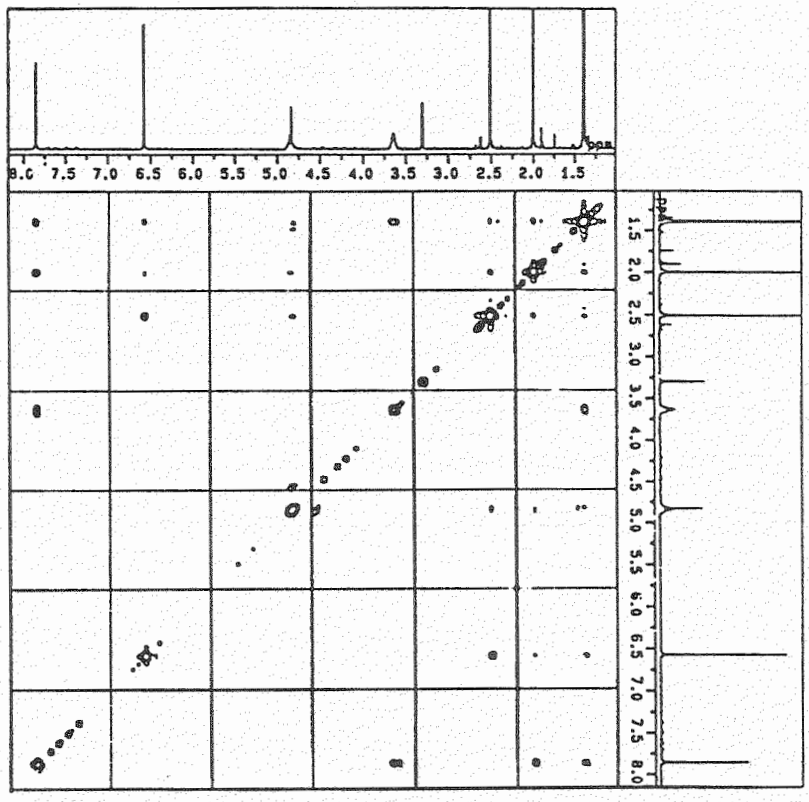
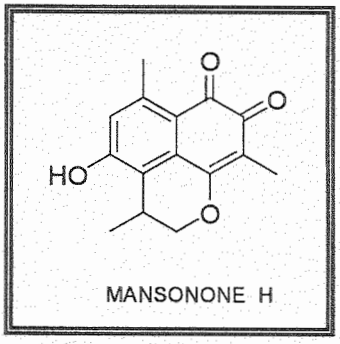


Fig 3.77 The ^{1}H-^{1}H NOESY spectrum of Compound 10

3.3.11 *Compound 11:* Mansonone H

Compound **11**, decomposed at 270°C, was isolated as red platelet. This compound showed a single spot on TLC with R_f value 0.38 (silica gel; hexane : ethyl acetate = 3:7). The molecular formula was proposed to be $C_{15}H_{14}O_4$ according to the elemental analysis result: Found %C 69.41 and %H 5.67; calcd. for $C_{15}H_{14}O_4$ MW. 258.27: %C 69.76 and %H 5.42. Its IR spectrum (Fig 3.78) showed the presence of a hydroxy group (3100-3300 cm^{-1}), an α,β-unsaturated ketone (1670 cm^{-1}) and an aromatic moiety (1567 cm^{-1}). Its ^1H NMR data was similar to that of mansonone E[13] (Table 3.15), except for the absence of a set *ortho*-coupled portons and the presence of a singlet of aromatic proton at δ_H 6.71 (1H, s). Therefore, there is a substituent, a hydroxy group, at C-6 or C-7. The location of this hydroxy at the C-6 position was confirmed by the strong NOESY correlation (Fig 3.83) between δ_H 6.71 and 2.55 (8-CH_3) instead of between δ_H 6.71 and 1.31 (9-CH_3). Thus, the structure of Compound **11** was proposed as 6-hydroxy mansonone E.

Comparison the NMR data of Compound **11** with those of 1,2-naphthoquinones reported in the literature revealed that Compound **11** was identical with mansonone H or 6-hydroxy mansonone E (Table 3.16) previously isolated from *Mansonia altissima*[6] and *Mansonia gagei*.[2]



MANSONONE H

Table 3.15 ^{1}H-NMR spectral data of Compound **11** and mansonone E[13]

Position	Chemical Shift (ppm)	
	11[a]	**Mansonone E**[b]
6	-	7.35, d, 1H (7.8)
7	6.71, s, 1H	7.25, d, 1H (7.8)
9	3.27, m, 1H	3.10, m, 1H
10	4.46, dd, 1H (0.9, 10.7) 4.33, dd, 1H (3.9, 11.0)	4.41, dd, 1H (3.9, 10.7) 4.23, dd, 1H (5.1, 10.7)
3-CH_3	1.89, s, 3H	1.94, s, 3H
8-CH_3	2.55, s, 3H	2.63, s, 3H
9-CH_3	1.31, d, 3H (7.0)	1.37, d, 3H (6.8)

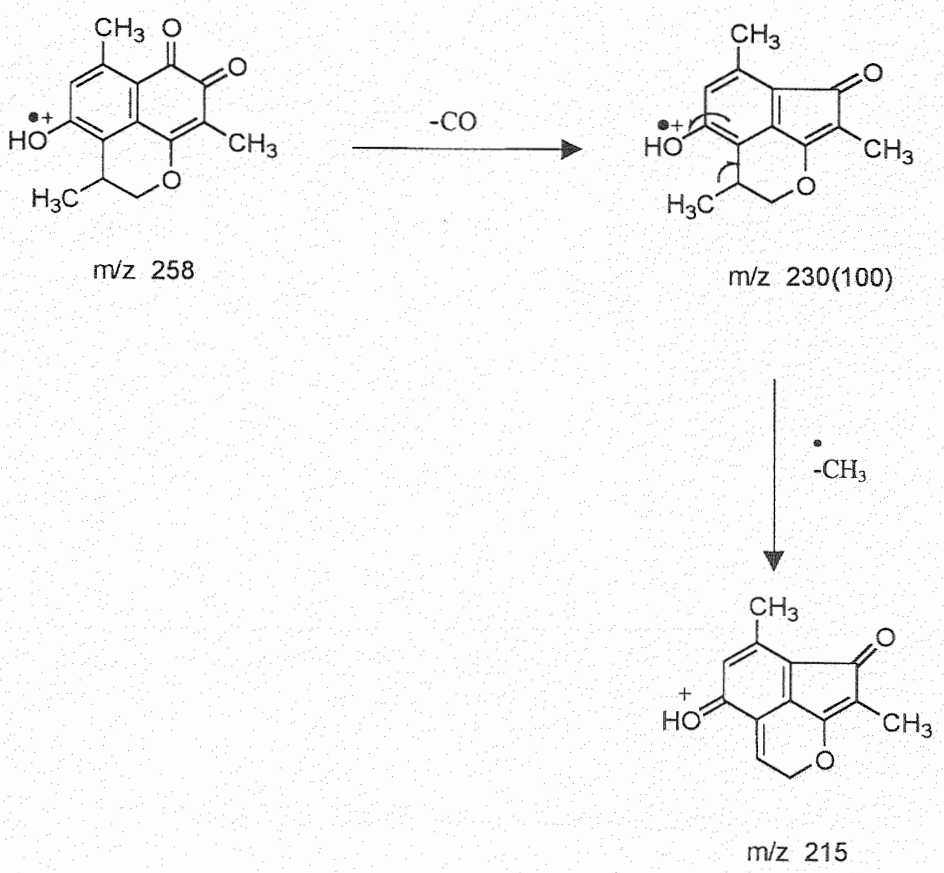
[a] ^{1}H NMR spectra were measured in CD_3OD at 500 MHz, respectively
[b] ^{1}H NMR spectra were measured in CD_3Cl_3 at 600 MHz, respectively

Table 3.16 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **11** and mansonone H[13]

Position	Chemical Shift (ppm)			
	11[a]		**Mansonone H**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	-	182.2	-	183.2
2	-	181.2	-	180.2
3	-	116.5	-	114.8
4	-	164.8	-	165.6
4a	-	127.4	-	129.4
5	-	120.3	-	118.9
6	-	161.7	-	156.0
7	6.71, s, 1H	120.3	6.33, s, 1H	121.8
8	-	147.4	-	148.3
8a	-	129.6	-	129.2
9	3.27, m, 1H	27.4	3.21, m, 1H	27.5
10	4.46, dd, 1H (0.9, 10.7) 4.33, dd, 1H (3.9, 11.0)	73.4	4.40, br d, 1H (10.3) 4.28, dd, 1H (3.5, 10.3)	73.8
3-CH_3	1.89, s, 3H	7.8	1.85, s, 3H	7.9
6-OH	-	-	-	-
8-CH_3	2.55, s, 3H	23.4	2.48, s, 3H	23.8
9-CH_3	1.31, d, 3H (7.0)	17.3	1.24, d, 3H (7.3)	17.4

[a] ^{1}H and ^{13}C NMR spectra were measured in CD_3OD at 500 and 125 MHz, respectively
[b] ^{1}H and ^{13}C NMR spectra were measured in CD_3OD at 600 and 150.8 MHz, respectively

The mass spectrum (Fig 3.79) displayed the molecular ion peak at m/z 258 ($[M]^+$), and other fragmentations were detected at m/z 230 (M^+-CO) and 215 (M^+-CO-CH_3). The proposed fragmentation pattern of Compound **11** is shown in Scheme 3.12.



Scheme 3.12 The proposed fragmentation pattern of Compound **11**

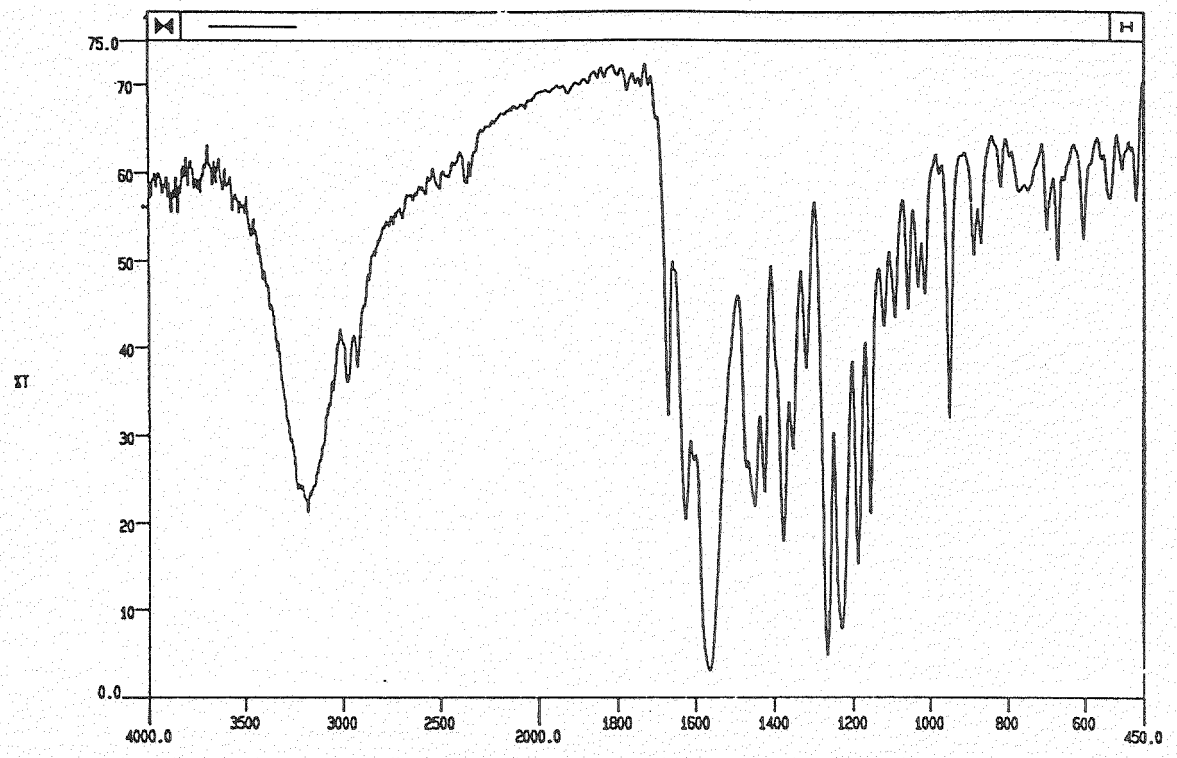


Fig 3.78 The FT-IR spectrum of Compound 11

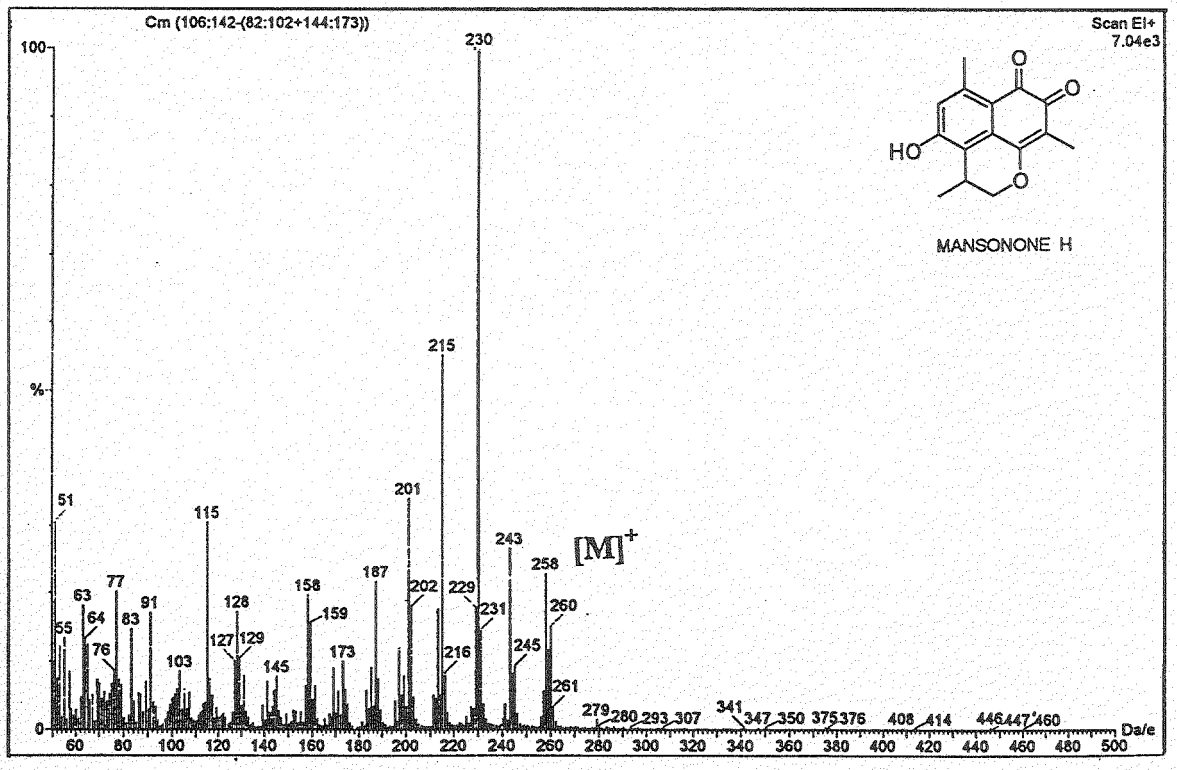


Fig 3.79 The mass spectrum of Compound 11

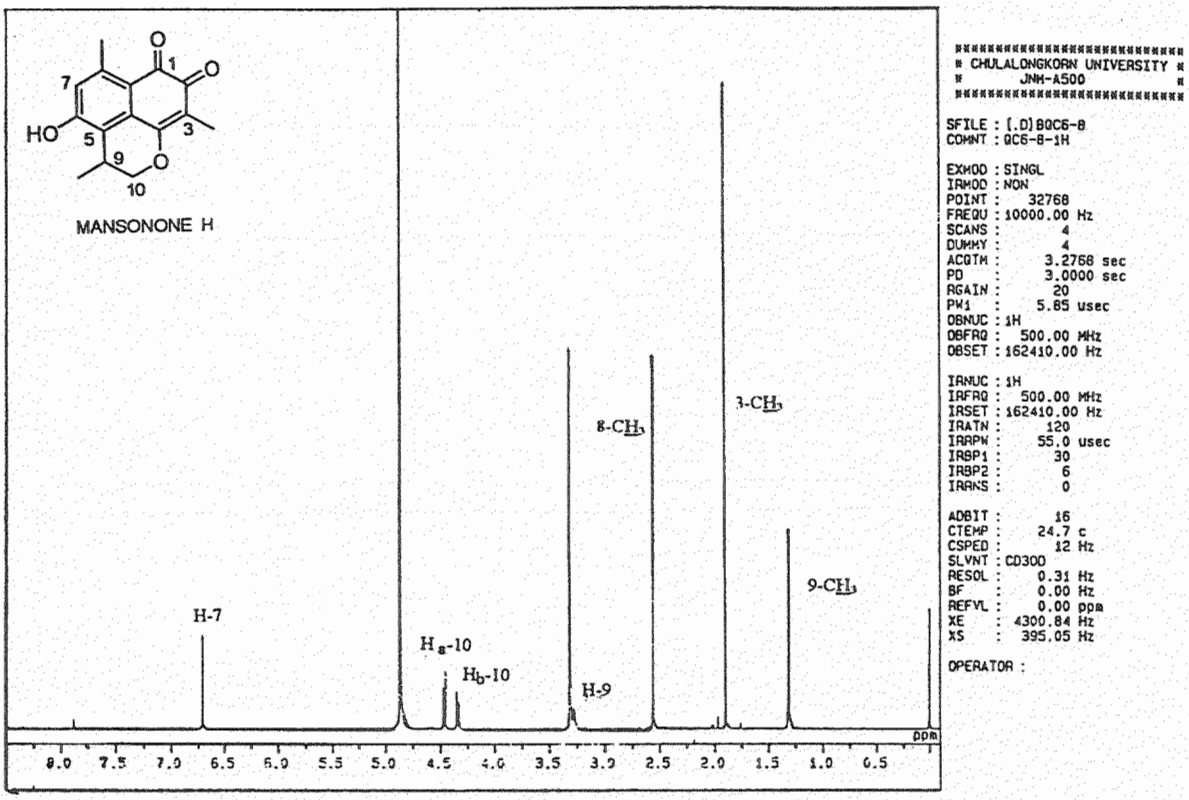


Fig 3.80 The ^{1}H-NMR spectrum of Compound 11

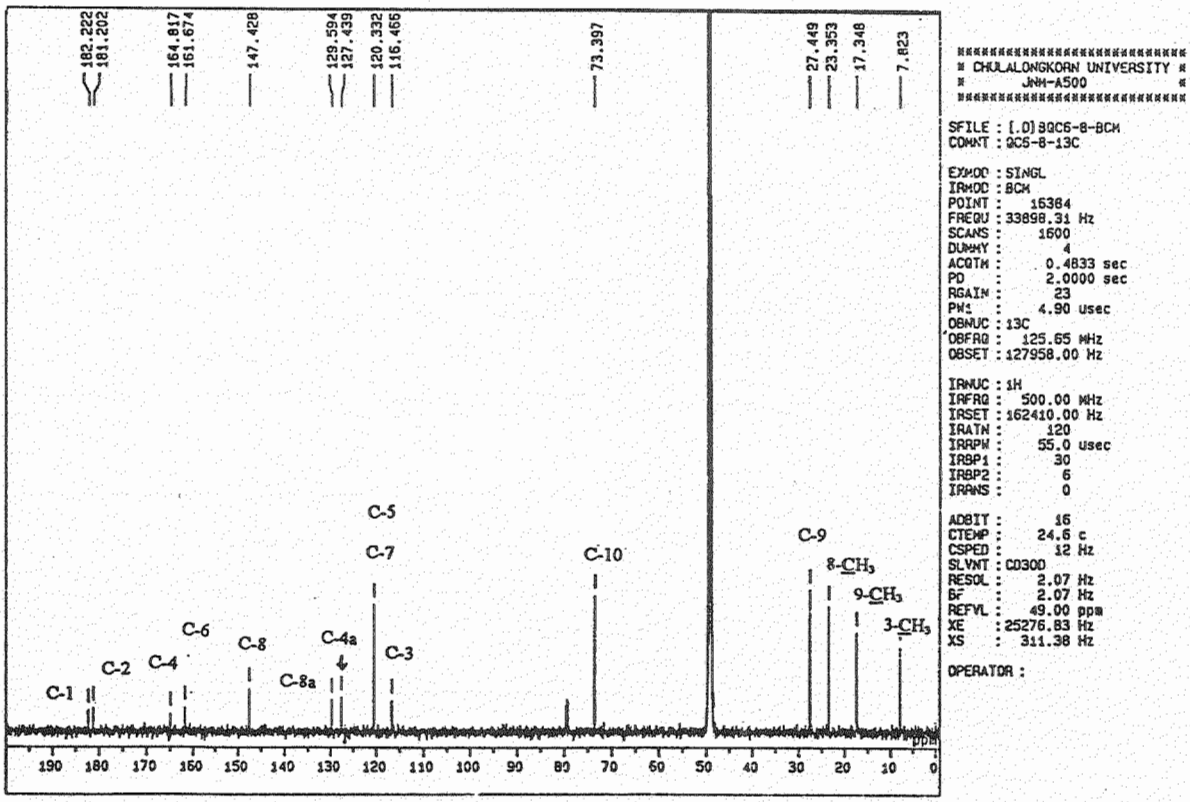


Fig 3.81 The ^{13}C-NMR spectrum of Compound 11

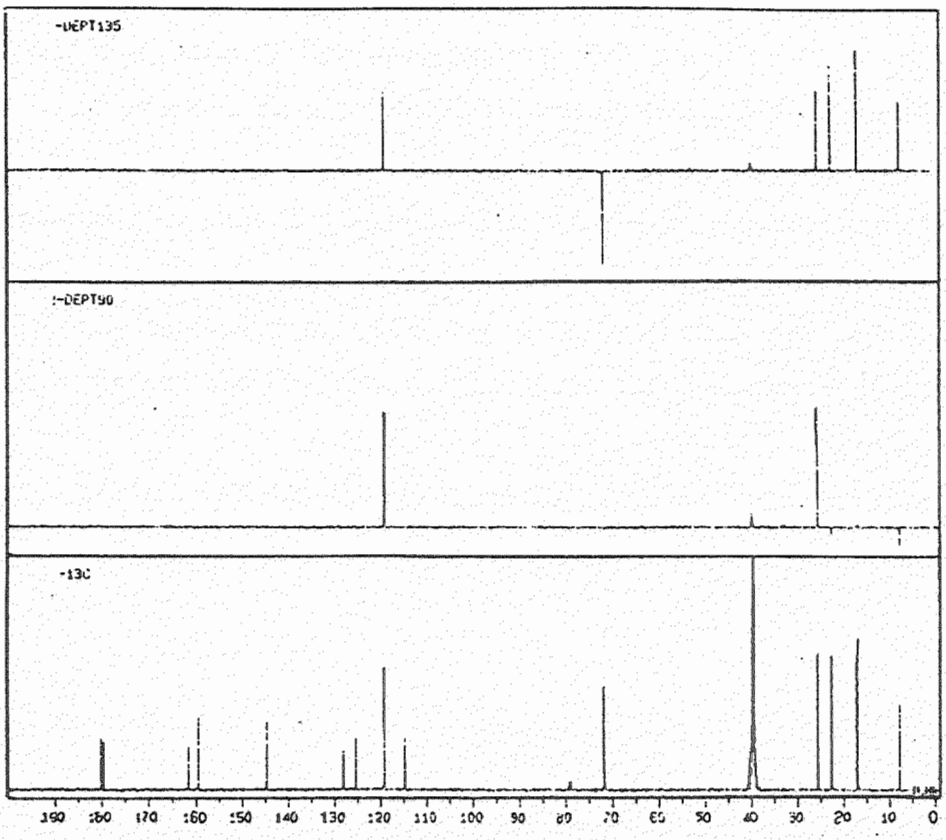
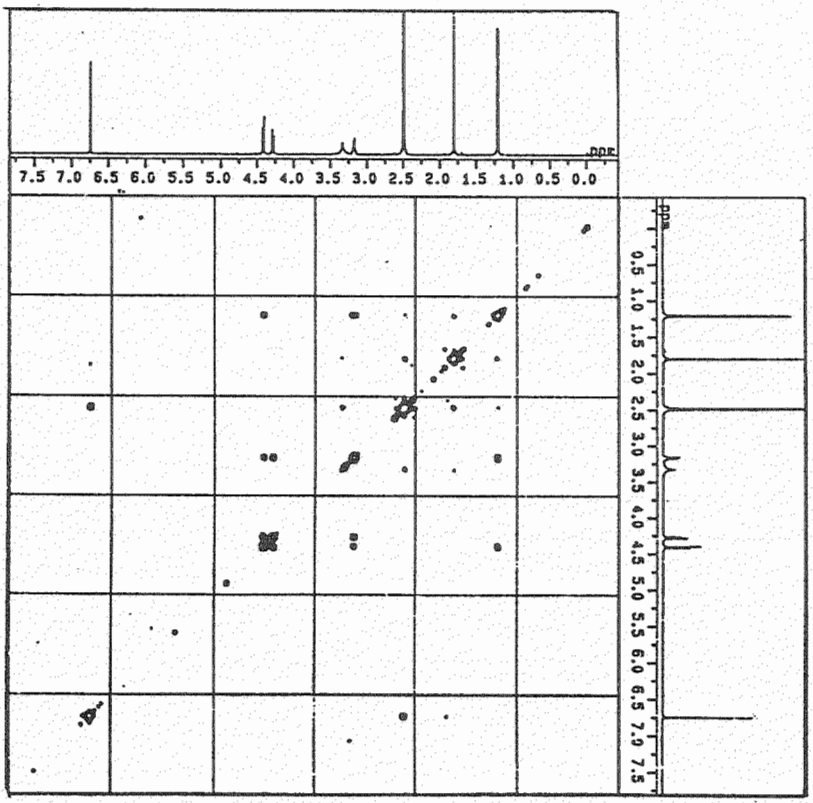


Fig 3.82 The DEPT 90 and 135 spectra of Compound 11

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IRMOD : HMG
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SCANS :       24
DUMMY :        4
ACQTM :   0.1240 sec
PD    :   2.0000 sec
RGAIN :       19

CLFRQ :  4128.62 Hz
CLPNT :      512
TOSCN :      256
CINNT :    10.00 usec
CINT1 :   242.20 usec
CINT2 :   121.10 usec

PW1   :    11.70 usec
PW2   :    23.40 usec
PW3   :    11.70 usec
PI1   : 1195.6522 msec
PI2   :   1.0000 msec
PI3   :  80.6756 msec
JCNST :   145.00 Hz

OBSMC : 1H
OBFRQ :   500.00 MHz
OBSET : 161770.35 Hz
IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 161384.19 Hz
IRATN :      250
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IRBP1 :       30
IRBP2 :        6
IRANS :        0

A/BIT :       1E
CTEMP :     23.7 C
CSHED :        0 Hz
SLVNT : DMSO
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CLRES :     8.06 Hz
TLINE :        8
THTOF :   10.0000
THOTH :    0.1900

OPERATOR :
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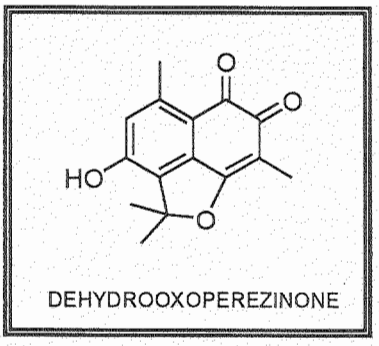
Fig 3.83 The ^{1}H-^{1}H NOESY spectrum of Compound 11

3.3.12 *Compound 12:* Dehydrooxoperezinone

Compound **12,** m.p 230-232 °C, was isolated as yellow powder. This compound showed a single spot on TLC with R_f value 0.4 (silica gel; hexane : ethyl acetate = 3:7). The molecular formula was proposed to be $C_{15}H_{14}O_4$ according to the elemental analysis result: Found %C 68.73 and %H 4.27; calcd. for $C_{15}H_{14}O_4$ MW. 258.27: %C 69.76 and %H 5.42, identical to that of Compound **11**. Its ^{1}H NMR data were further compared to those of Compound **11** (Table 3.17) and found that the signals of -CH(CH$_3$)-CH$_2$-O- unit (δ_H 3.27 (1H), 4.33 (1H), 4.46 (1H) and 1.31 (3H)) in Compound **11** were replaced by the signals of -C(CH$_3$)$_2$-O- unit (δ_H 1.76 (6H)) in Compound **12**; this observation was confirmed by the equivalent methyl carbon signal at δ_H 25.9 in ^{13}C NMR spectrum (Fig 3.86). Therefore, the structure of Compound **12** could be deduced to be 3-hydroxy-2,2,5,8-tetramethyl-2H-naphtho[1,8-bc]furan-6,7-dione or dehydrooxoperezinone which was a known naturally occurring compound, previously isolated from the roots of *Aristolochia liukiuensis* Hatsusima by Kazuhito and coworkers in 1992.[17] The NMR data of Compound **12** and dehydrooxoperezinone were in good agreement which showed in Table 3.18.

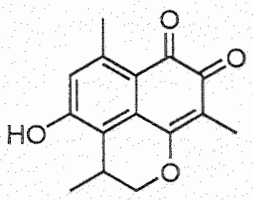


DEHYDROOXOPEREZINONE

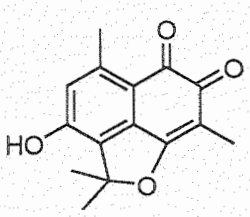
Table 3.17 ^{1}H-NMR spectral data of Compounds **11** and **12**

Position	Chemical Shift (ppm)	
	11[a]	**12**[a]
7	6.71, s, 1H	6.68, s, 1H
9	3.27, m, 1H	-
10	4.46, dd, 1H (0.9, 10.7) 4.33, dd, 1H (3.9, 11.0)	-
3-CH_3	1.89, s, 3H	1.87, s, 3H
8-CH_3	2.55, s, 3H	2.59, s, 3H
9-CH_3	1.31, d, 3H (7.0)	-
9-$(CH_3)_2$	-	1.76, s, 6H

[a] ^{1}H NMR spectra were measured in CD_3OD at 500 MHz, respectively



MANSONONE H (**11**)



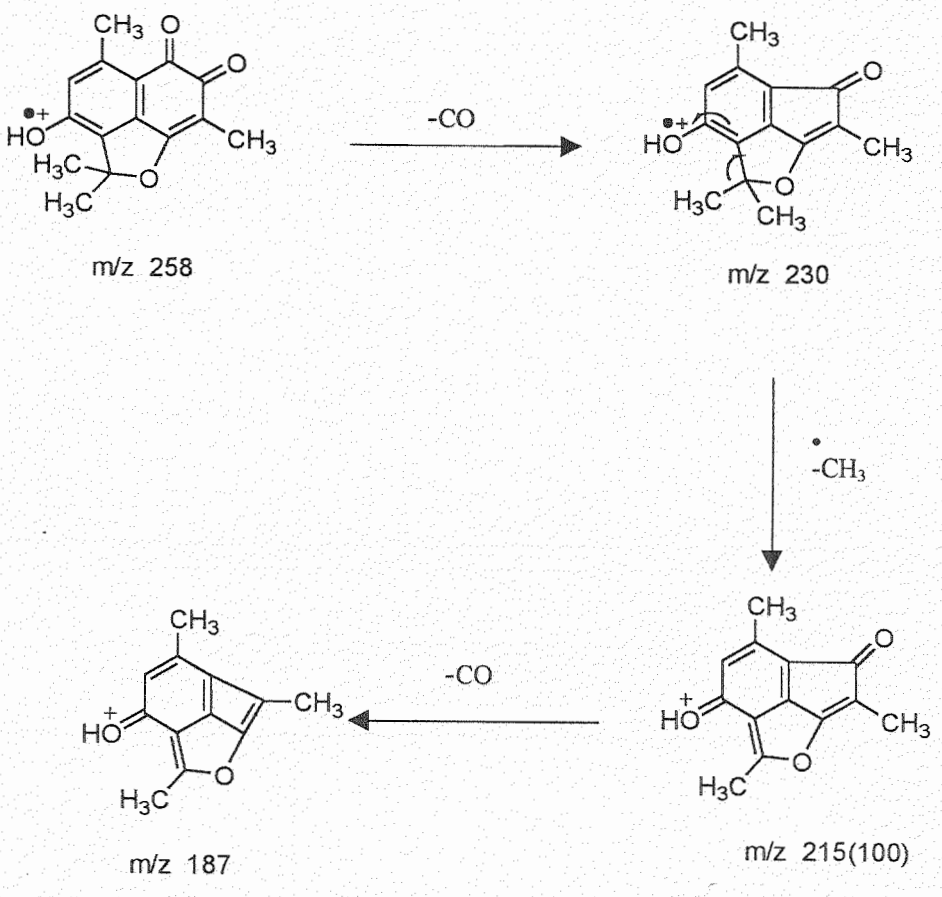
DEHYDROOXOPEREZINONE (**12**)

Table 3.18 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **12** and Dehydrooxoperezinone[17]

Position	Chemical Shift (ppm)			
	12[a]		**Dehydrooxoperezinone**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	-	182.6	-	179.9
2	-	178.8	-	179.0
3	-	109.0	-	107.3
4	-	170.8	-	167.3
4a	-	148.6	-	145.9
5	-	121.4	-	120.0
6	-	159.7	-	156.8
7	6.68, s, 1H	120.3	6.75, s, 1H	116.4
8	-	137.5	-	135.7
8a	-	132.8	-	130.3
9	-	97.9	-	95.7
3-CH_3	1.87, s, 3H	7.8	1.79, s, 3H	7.8
6-OH	-	-	11.55, br, 1H	-
8-CH_3	2.59, s, 3H	20.6	2.54, s, 3H	19.9
9-$(CH_3)_2$	1.76, s, 3H	25.9 (2C)	1.71, s, 3H	25.5 (2C)

[a] ^{1}H and ^{13}C NMR spectra were measured in CD_3OD at 500 and 125 MHz, respectively
[b] ^{1}H and ^{13}C NMR spectra were measured in CD_3SOCD_3 at 500 and 125 MHz, respectively

The mass spectrum (Fig 3.84) displayed the molecular ion peak at m/z 258 ($[M]^+$), and other fragmentations were shown at m/z 230 (M^+-CO) 215 (M^+-CO -CH_3) and 187 (M^+-CO -CH_3-CO). The proposed fragmentation pattern of Compound **12** is presented in Scheme 3.13.



Scheme 3.13 The proposed fragmentation pattern of Compound **12**

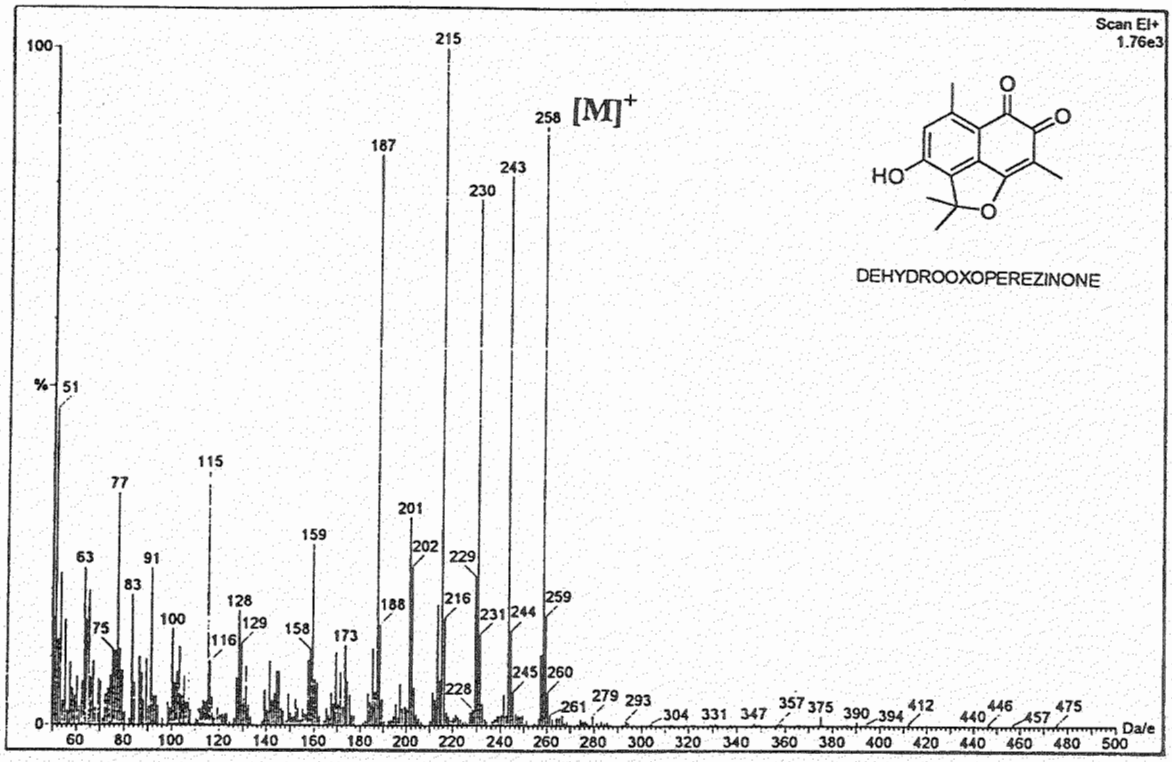


Fig 3.84 The mass spectrum of Compound 12

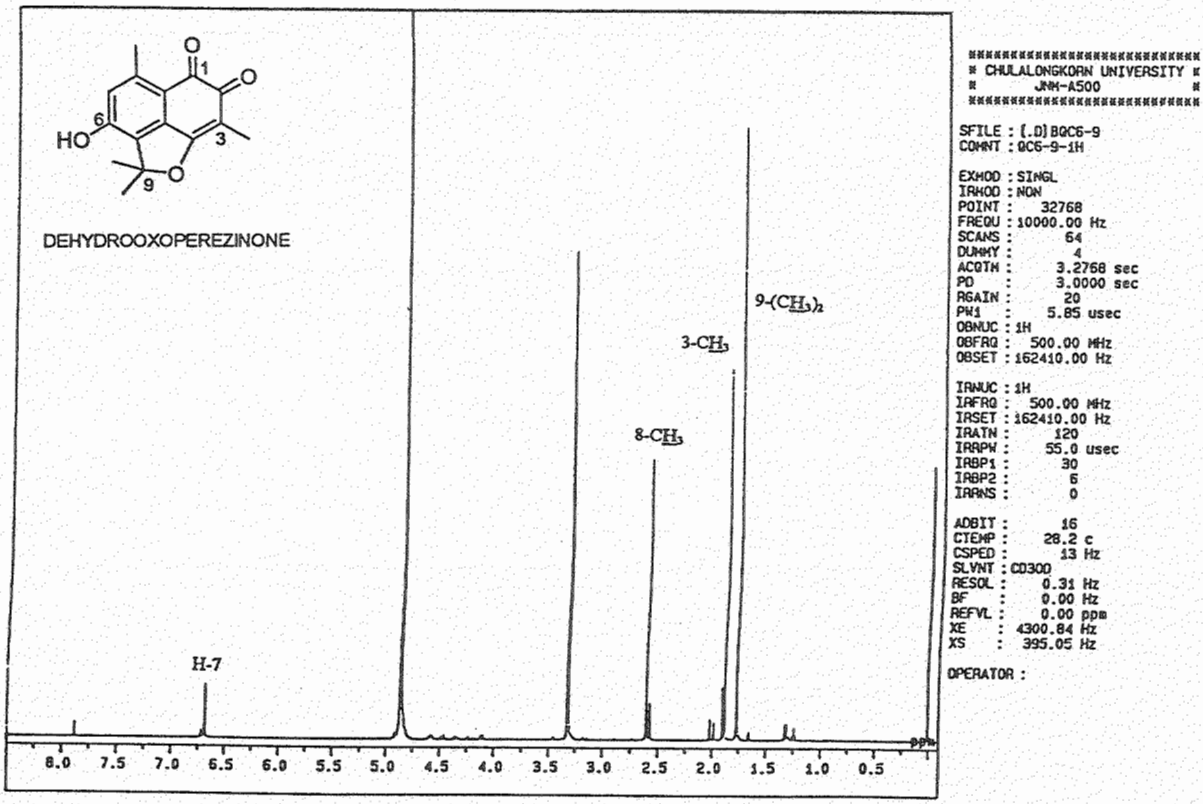


Fig 3.85 The ^{1}H-NMR spectrum of Compound 12

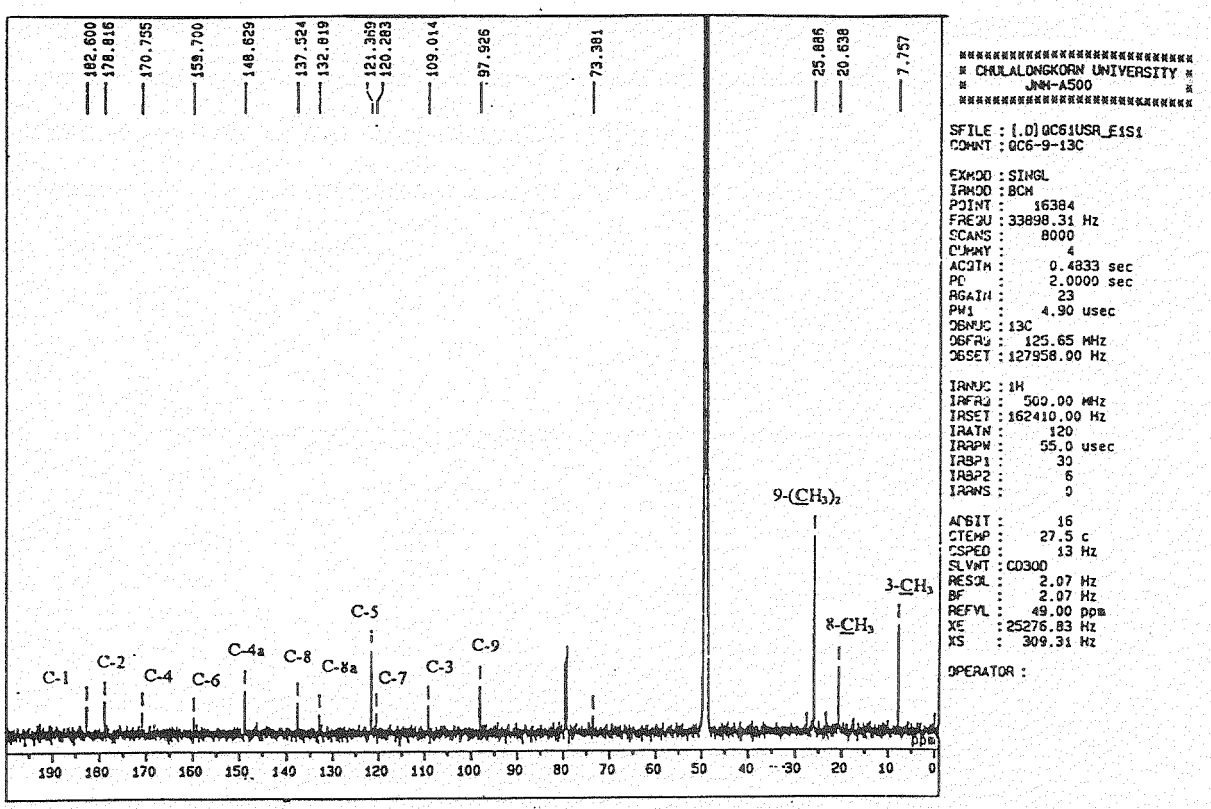


Fig 3.86 The ^{13}C-NMR spectrum of Compound 12

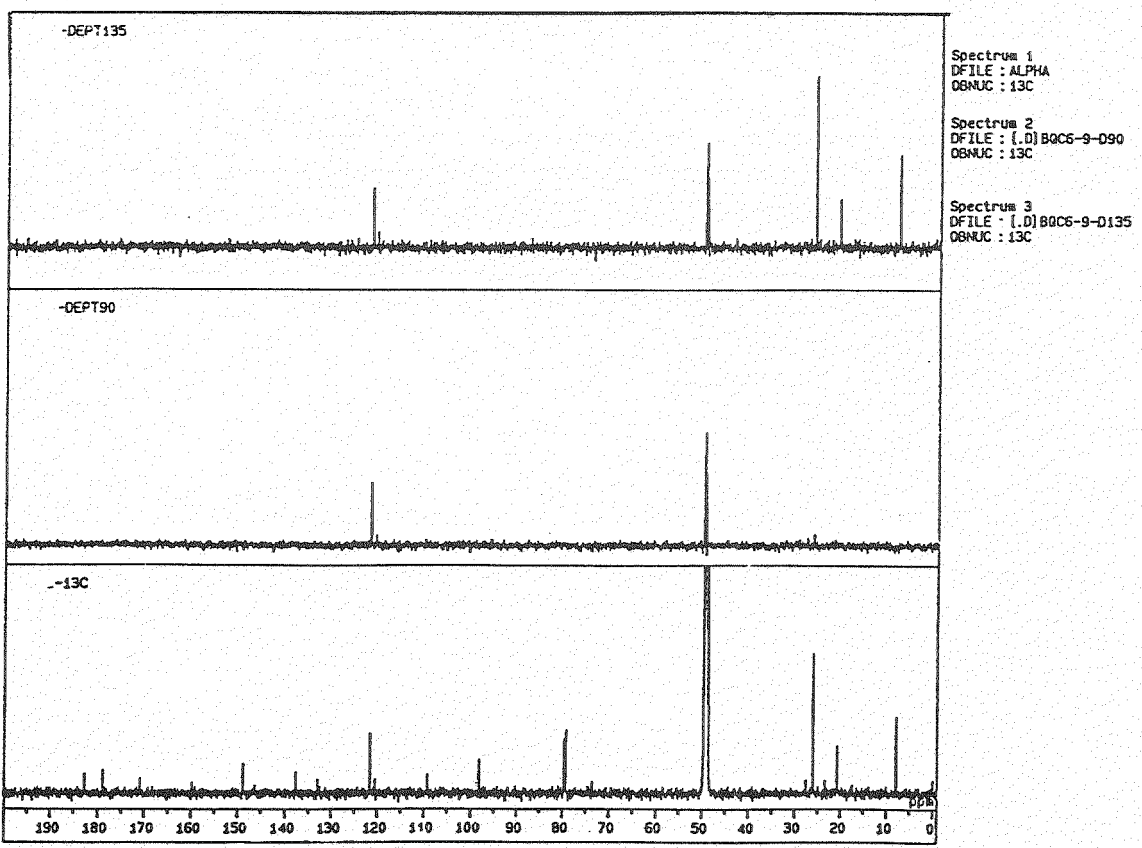


Fig 3.87 The DEPT 90 and 135 spectra of Compound 12

3.4 HPLC Analysis of the Dichloromethane Extract and Isolated Compounds

Dichloromethane extract and ten isolated compounds were detected by HPLC Column (Nova-Pak RP-18) using acetonitrile:H_2O = 20:80 → 100:0 in 30 minutes. The chromatograms of dichloromethane extract and those isolated compounds are shown in Fig 3.88 and 3.89, respectively.

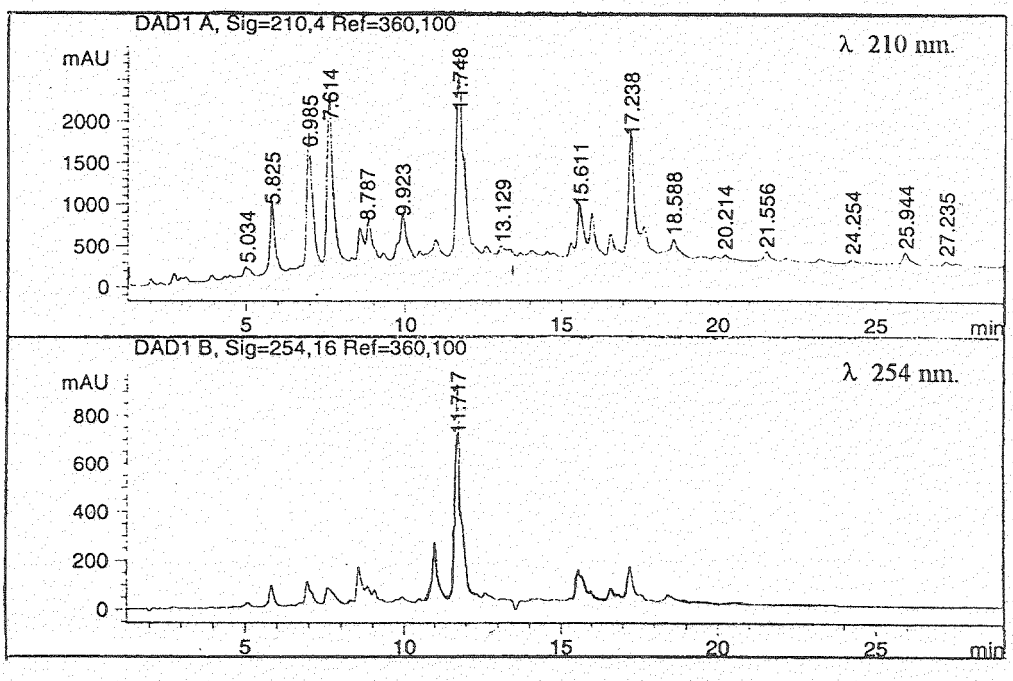


Fig 3.88 The HPLC chromatogram of the dichloromethane extract

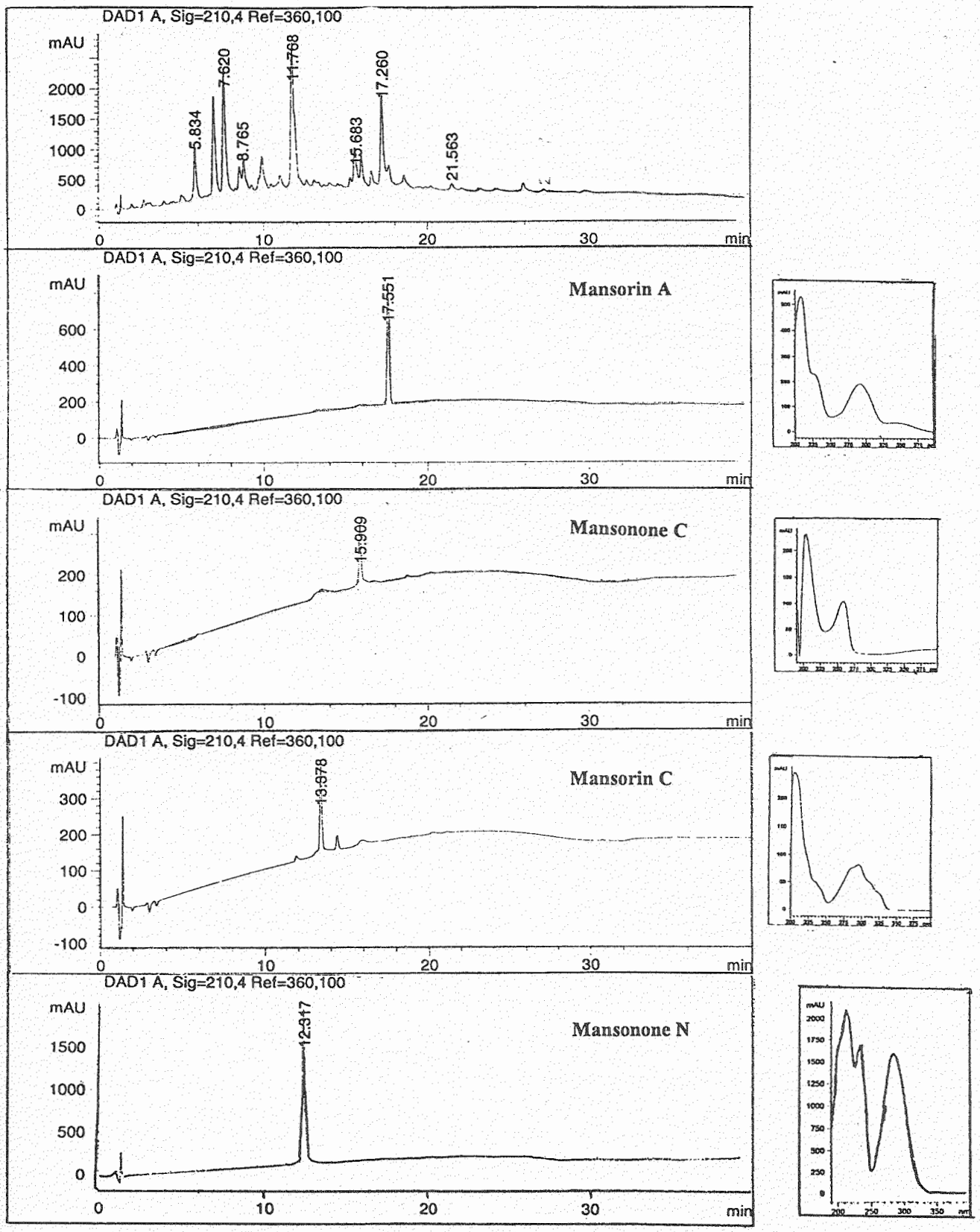
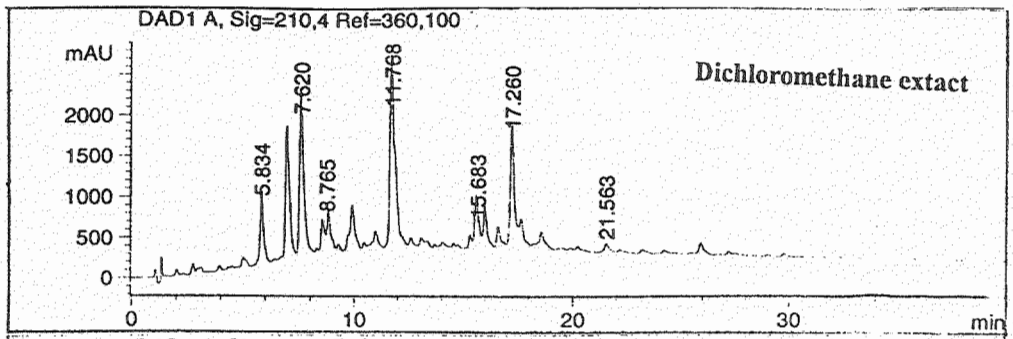


Fig 3.89 The HPLC chromatogram of ten compounds isolated from the dichloromethane extract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10
20
30
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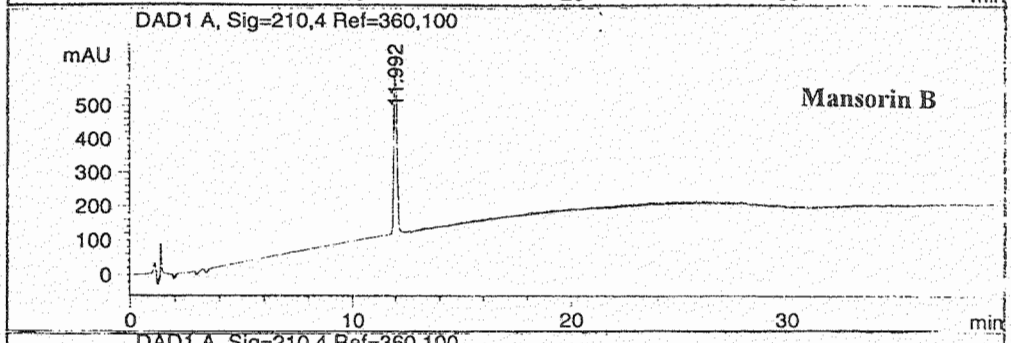
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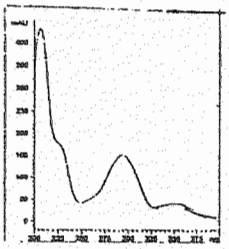

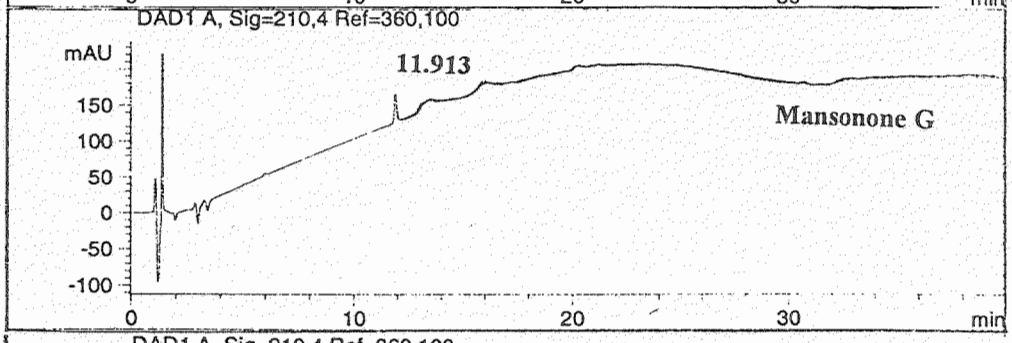
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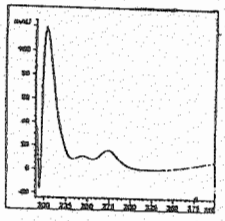
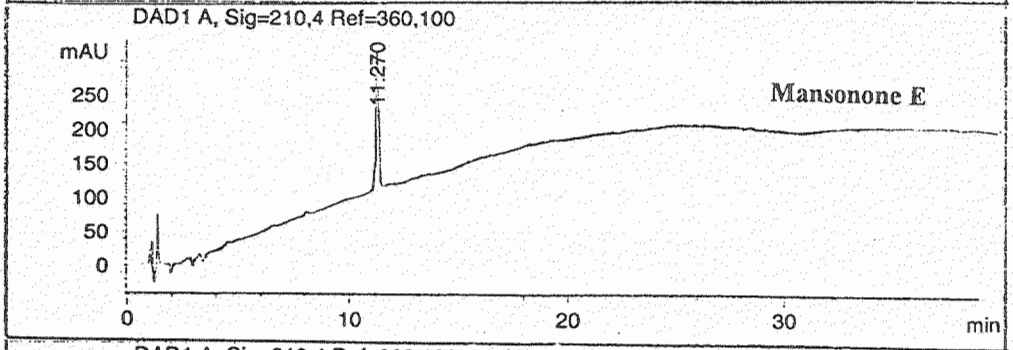
30
min

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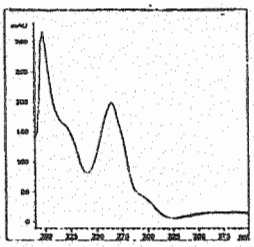

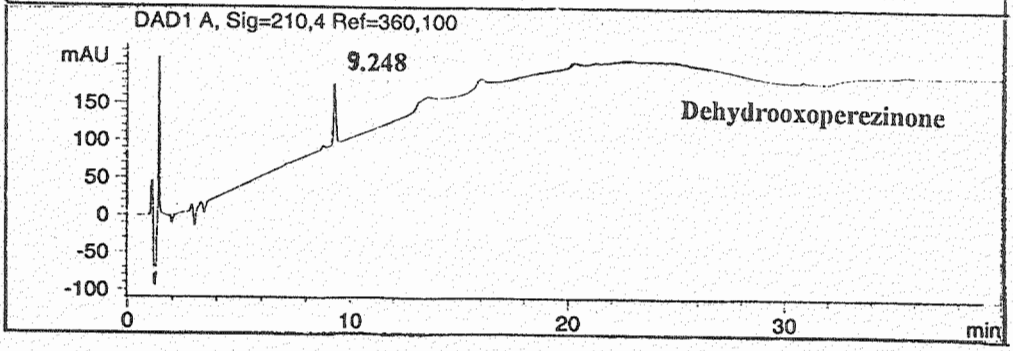
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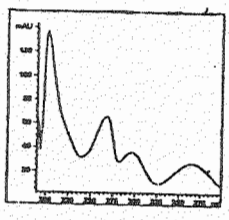


Fig 3.89 (Cont.)

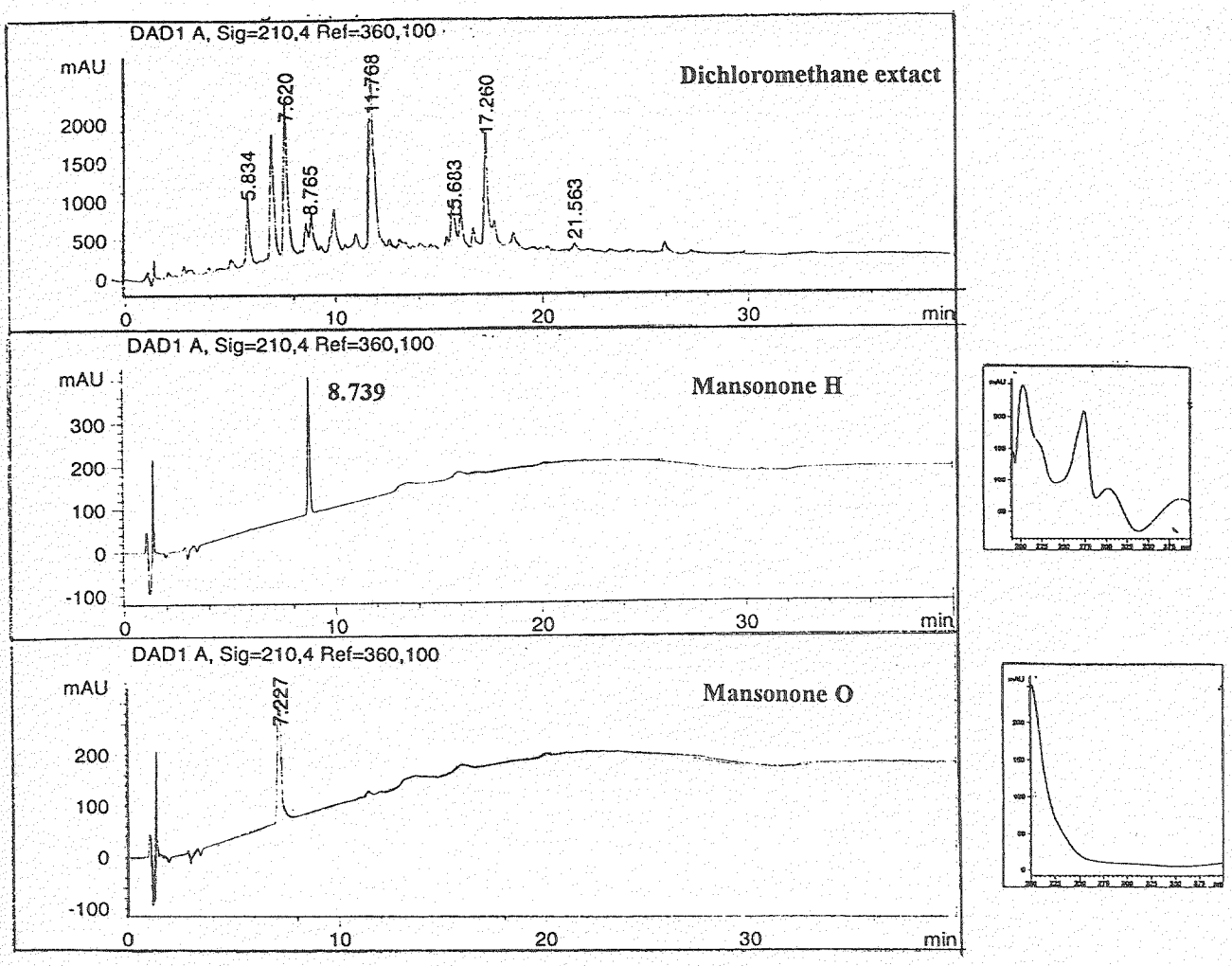


Fig 3.89 (Cont.)

From HPLC Chromatogram (Fig 3.88), it lucidly showed the remaining high intensity peaks at R_t. 5.8, 6.9 and 7.6 mins. This maybe due to the high polarity of compounds that can not isolated by silica gel column chromatography.

3.5 Biological Activities of Isolated Compounds of the Dichloromethane Extract

As it has been aforementioned, the dichloromethane extract displayed the most intriguing results for preliminary screening test (see Tables 3.1-3.3 and Figs 3.1-3.2). As a consequent the constituents have been thoroughly investigated. All isolated compounds from this extract were reassayed for brine shrimp cytotoxicity, antifungal, larvicidal, antioxidant, antithrombin and anticancer activities in order to confirm those biological activities. The biological activity studies are tabulated as shown in Tables 3.19-3.22.

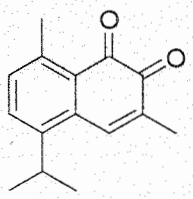
Table 3.19 Brine shrimp cytotoxicity test of the isolated compounds from the dichloromethane extract of the heartwoods of *M.gagei*

Compound	LC_{50}	Activity
Mansorin A	88.58	Medium
Mansorin C	201.79	Low
Mansonone C	2.47	High
Mansonone G	2.24	High
Mansonone H	58.52	Medium

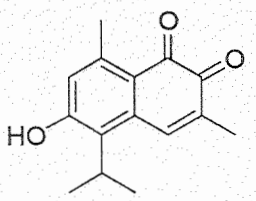
Note

High activity	($LC_{50} < 10$	μg/mL)
Medium activity	($10 \leq LC_{50} \leq 100$	μg/mL)
Low activity	($LC_{50} < 1000$	μg/mL)

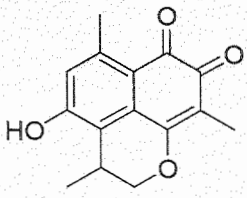
From Table 3.19, these compounds can be categorized into two classes; 1,2-naphthoquinones (mansonones C, G and H) and coumarins (mansorins A and C).



MANSONONE C



MANSONONE G



MANSONONE H

Base upon the biological activity results of a series of 1,2-naphthoquinones, mansonones C, G and H, the influence of substituent on the activity could be observed. To illustrate this, mansonones C and G had the same main structure except for the substituent at 6-position. Both compounds revealed the same trend of high toxicity activity against brine shrimp whatever the substituent at 6-position was either H or OH. Comparison of mansonones G and H, the results of brine shrimp cytotoxicity test manifestly revealed that a pyran ring in mansonone H decreased this activity.

As clearly demonstrated the results in Table 3.19, it seemed that the isolated 1,2-naphthoquinones exhibited higher toxicity than the isolated coumarins. These observations, however, could not draw any final conclusion. More compounds in these two types were needed for further studying the structure-activity relationship (SAR).

Table 3.20 Antifungal, larvicidal and radical scavenging activities of the isolated compounds from the dichloromethae extract of the heartwoods of *M. gagei*

Compounds	*C. cucumerinum*[a]	*C. albicans*[a]	*A. aegypti*[b]	DPPH[c]
Mansorin A (**1**)	2.5	> 10	> 50	> 10
Mansorin B (**2**)	0.6	> 10	> 50	> 10
Mansorin C (**3**)	> 10	> 10	> 50	> 10
Mansonone N (**4**)	> 10	> 10	> 50	2.5
Mansonone O (**5**)	> 10	> 10	n.t.	> 10
Mansonone P (**6**)	> 10	> 10	> 50	> 10
Mansonone C (**8**)	0.6	0.15	6.25	> 10
Mansonone E (**9**)	0.6	2.5	> 50	> 10
Mansonone G (**10**)	> 10	> 10	> 50	> 10
Mansonone H (**11**)	> 10	> 10	> 50	> 10
Dehydrooxoperezinone (**12**)	> 10	> 10	> 50	> 10
Nystatin	0.2	1	-	-
Rotenone	-	-	3	-
Quercetin	-	-	-	0.5

[a] Minimal amount (μg) of compound to inhibit growth on a silica gel TLC plate
[b] Minimal concentration (ppm) of compound required to kill all the larvae after 24 hours
[c] Minimal amount (μg) of compound required to show a radical scavenging activity on a silica gel TLC plate
n.t. not tested

From the previous study on the bioactivities of mansonones, many reports[18-21] indicated the antifungal properties of mansonones as phytoalexins, especially against Dutch elm disease (DED) by the fungi *Ophiostoma ulmi* (Buism.) Nannf. and *Ophiostoma novo-ulmi* Brasier. In the plant genus *Ulmus*, mansonones E, F and G were found to be the most potent antifungal compounds.

From this examination (Table 3.20), the isolated compounds from *M. gagei* were tested for antifungal activity against *Cladosporium cucumerinum* and *Candida albicans*. Mansonones C and E were found to be the only active products against *C. albicans* with a minimal amount of 0.15 μg, and 2.5 μg, respectively to ensure inhibition of the yeast

growth. Mansonones C and E as well as mansorins A and B were found to be active against *C. cucumerinum* with minimal inhibitory amount of 0.6, 0.6, 2.5 and 0.6 µg, respectively. The antifungal potency of mansorin B and mansonones C and E was close to that of nystatin employed as a control.

Mansonone C was the only isolated compound to possess toxic property against the larvae of the yellow fever-transmitting mosquito *Aedes aegypti* at 50 ppm. Dilution tests were performed and the minimal amount of compound to kill all the larvae after 24 hours was calculated as 6.25 ppm. This activity could not be detected in the raw extract probably due to the low concentration of the active compound in the dichloromethane extract (0.0046 %). In comparison, rotenone used as the reference compound was twice more active.

Mansonone N was finally the only isolated compound to exhibit radical scavenging properties in the DPPH, the other compounds being inactive on the TLC assay at a tested amount of 10 µg. A limit of activity at 2.5 µg was determined for mansonone N in the same assay. Quercetin used as a reference substance was 5 times more active.

Table 3.21 The antithrombin activity of the isolated compounds from the dichloromethane extract of the heartwoods of *M. gagei*

Compounds	% Inhibition
Mansorin A (**1**)	24.31
Mansorin B (**2**)	19.84
Mansorin C (**3**)	45.61
Mansonone N (**4**)	25.18
Mansonone O (**5**)	44.94
Mansonone P (**6**)	39.09
Mansonone Q (**7**)	n.t.
Mansonone C (**8**)	45.9
Mansonone E (**9**)	52.84
Mansonone G (**10**)	96.37
Mansonone H (**11**)	97.57
Dehydrooxoperezinone (**12**)	99.65

n.t. : not tested

On comparing the activities of various 1,2-naphthoquinones obtained from dichloromethane extract (Table 3.21), antithrombin property was significant enhanced by hydroxy group at the C-6 position as in mansonones G, H and dehydrooxoperezinone.

Mansorins A-C and mansonones N-Q exhibited with %inhibition values ranging between 19.84 and 45.61 %inhibition which were lower than those for mansonones C-H and dehydrooxoperezinone and had no activity influence by substituents. The fact maybe that the activity mainly depends on the 1,2-naphthoquinone basic skeleton.

Table 3.22 The anticancer activity of the isolated compounds from the dichloromethane extract of the heartwood of *M.gagei*.

Compounds	% Activities				
	Mouse Leukemia	Human Myeloma	Human Colon Adenocarcinoma	Human Leukemia	Human Breast Carcinoma
Mansorin A (**1**)	70.8	87.5	18.2	29.3	66.9
Mansorin B (**2**)	67.0	64.5	0.0	87.0	95.3
Mansorin C (**3**)	76.8	71.5	9.3	17.2	79.4
Mansonone N (**4**)	4.0	48.0	0.0	17.1	0.0
Mansonone O (**5**)	n.t.	n.t.	n.t.	n.t.	n.t.
Mansonone P (**6**)	82.3	66.2	34.9	28.8	96.0
Mansonone Q (**7**)	n.t.	n.t.	n.t.	n.t.	n.t.
Mansonone C (**8**)	98.8	100.0	98.7	100.0	100.0
Mansonone E (**9**)	100.0	100.0	96.5	99.3	98.3
mansonone G (**10**)	96.0	100.0	96.8	100.0	80.7
Mansonone H (**11**)	n.t.	n.t.	n.t.	n.t.	n.t.
Dehydrooxopere-zinone (**12**)	n.t.	n.t.	n.t.	n.t.	n.t.
Methotrexate	97.1	65.1	69.8	69.2	24.3

n.t. : not tested

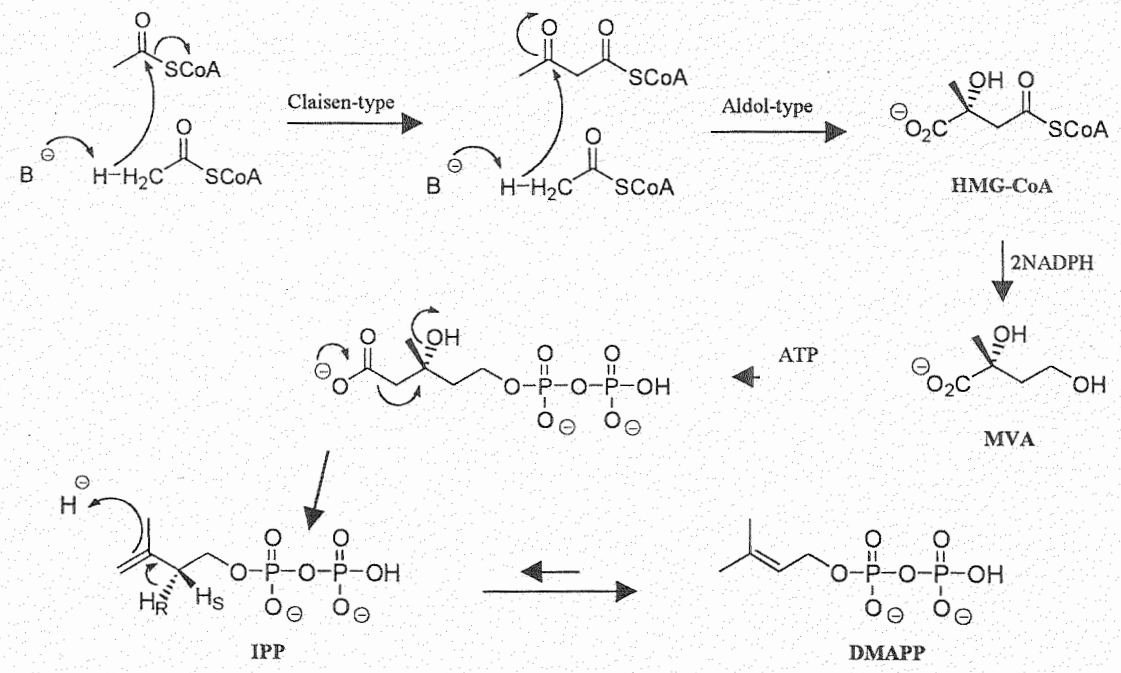
According to the results in Table 3.22, mansonones C, G and H demonstrated very potent inhibitors against a number of cancer cell lines. Mansonone P displayed selective inhibition toward Mouse Leukemia and Human Breast Carcinoma with 82.3 and 96.0 %inhibition, respectively. Mansorin A exhibited the selective activity against Human

Myeloma with 87.5 %inhibition as well as mansorin B displayed the certain inhibitory activity against Human Leukemia and Human Breast Carcinoma with 87.0 and 95.3 %inhibition.

3.6 Proposed Biogenesis of Mansonones

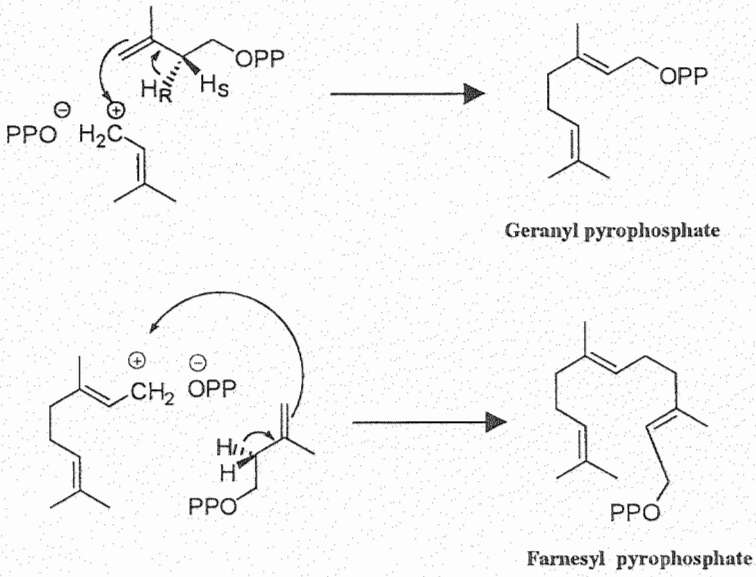
According to no complete assumption about biogenesis of mansonones, the sesquiterpenoid compounds containing cadinane skeleton, herein the biogenesis of mansonones were proposed based upon the literature[37-38] and related structures of isolated mansonones from *M. gagei*.

The focal point of sesquiterpene biogenesis is the naturally occurring compound, farnesol, whose formation from acetyl CoA, *via* mevalonic acid (Scheme 3.14). The initial step, catalysed by the enzyme acetoacetyl-SCoA thiolase, involves a Claisen ester condensation between two molecules of acetyl-SCoA. The second step, catalysed by the enzyme hydroxymethylglutaryl-SCoA (HMG-SCoA) synthase, is formally an aldol reaction, and the subsequent reduction of HMG-ScoA, to produce mevalonic acid (MVA) uses two moles of NADPH, and is catalysed by the enzyme HMG-CoA reductase.Mevalonic acid is subsequently pyrophosphorylated to produce MVA-5-pyrophosphate, and this suffers decarboxylation to yield isopentenyl pyrophoshate (IPP), the first of the biogenetic isoprene units. A stereospecific isomerization then ensures to provide the other five carbon unit, dimethylallyl pyrophosphate (DMAPP).



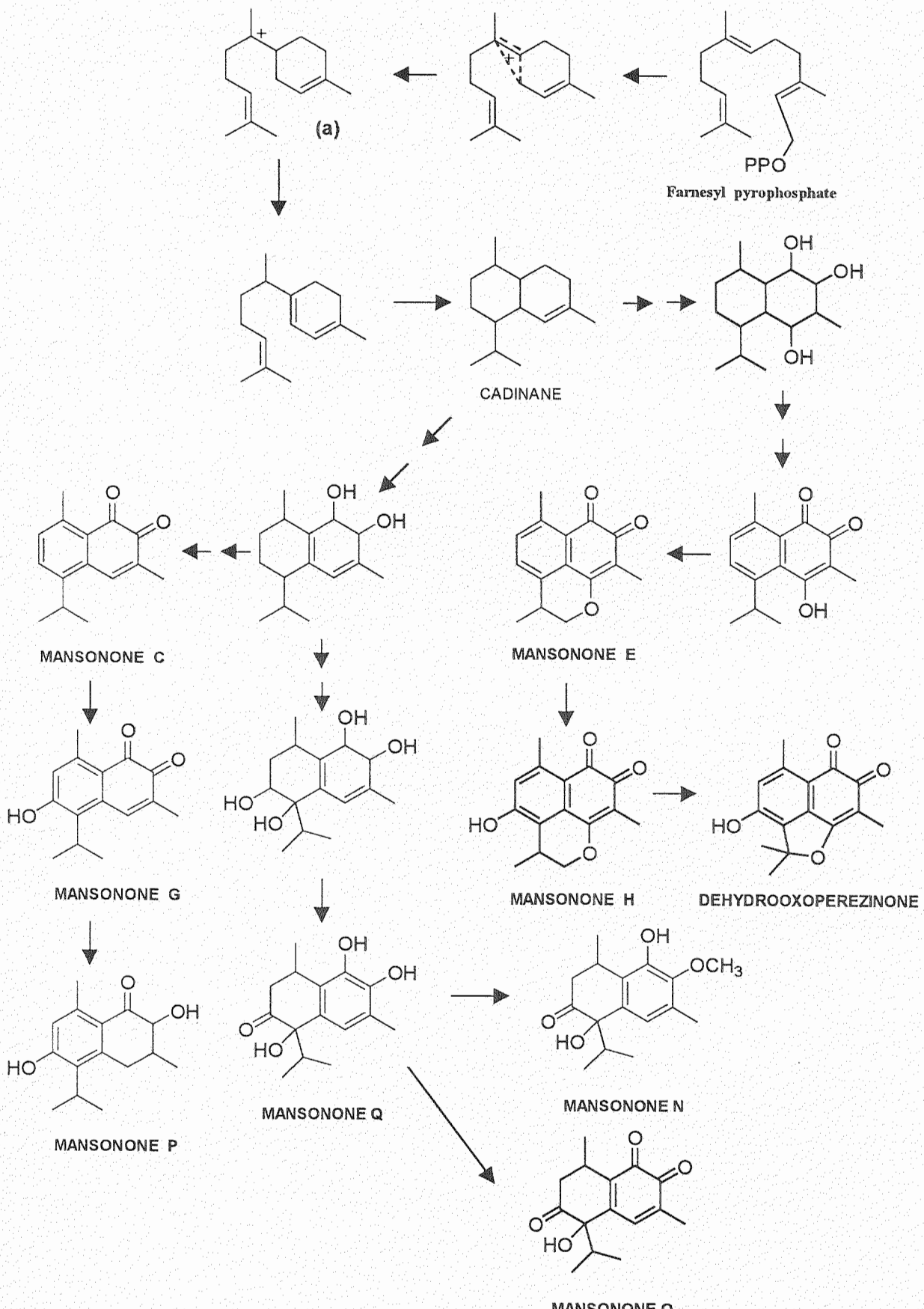
Scheme 3.14 The formation of IPP and DMAPP *via* mevalonic acid

Combination of these two five-carbon units produces the monoterpenes (Scheme 3.15), and it is now well established that prior ionization of DMAPP occurs to yield a tightly held ion-pair, which is highly electrophilic. This suffers nucleophilic attack by IPP to produce geranyl pyrophosphate. The geranyl pyrophosphate condenses with IPP to produce farnesyl pyrophosphate and this is the progenitor of all other sesquiterpenes (Scheme 3.15).

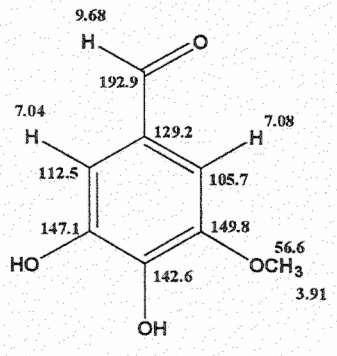


Scheme 3.15 The formation of Geranyl and Farnesyl pyrophosphates

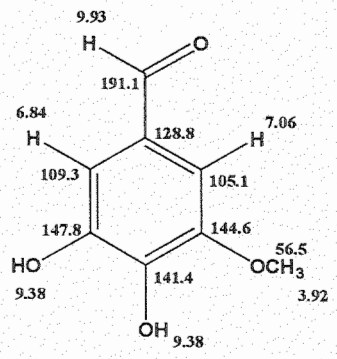
The cadinane series of sesquiterpenes were derived from cyclisation of cation (**a**) occurring by removal of the pyrophosephate anion to obtain cadinane compound. The subsequent modification of structure due to rearrangements and functional group changes by enzymes or atmosphere led to obtain a series of mansonones (Scheme 3.16).



Scheme 3.16 The proposed biogenesis of isolated mansonones



COMPOUND 13



SIMULATION ON ACD/LABS

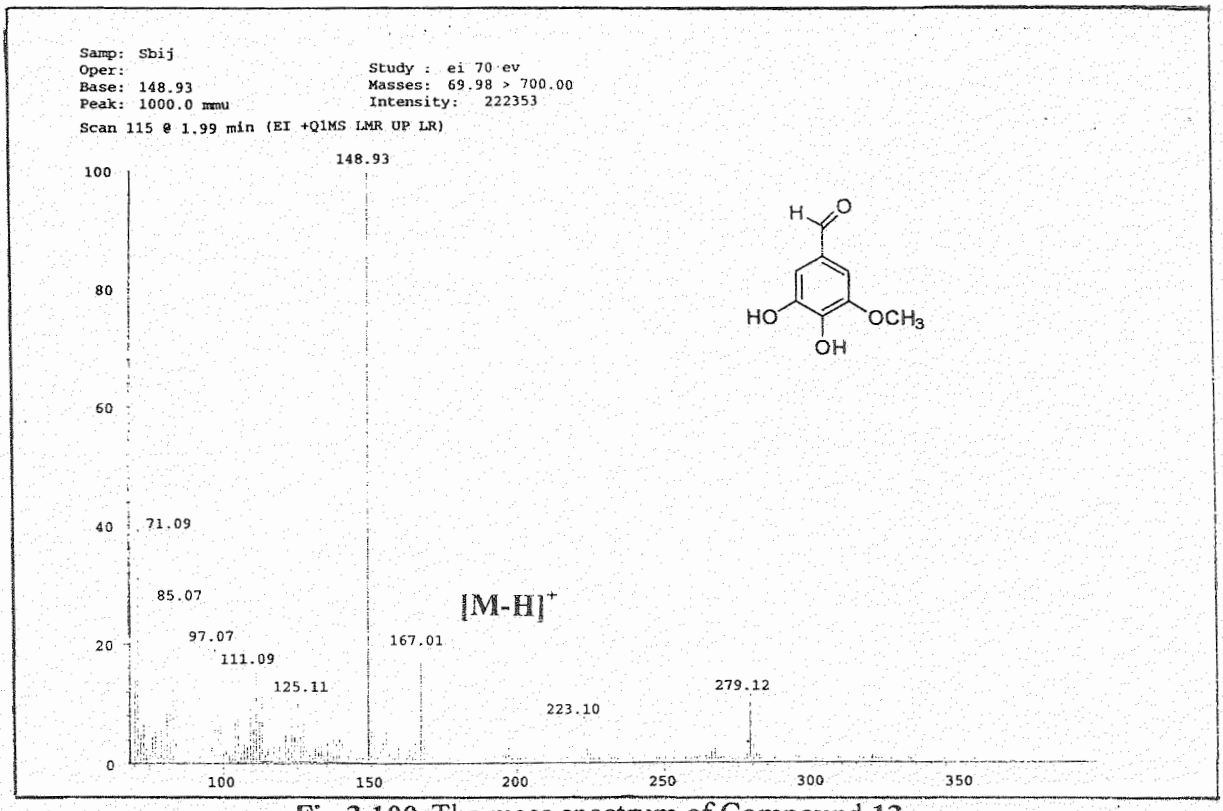


Fig 3.100 The mass spectrum of Compound **13**

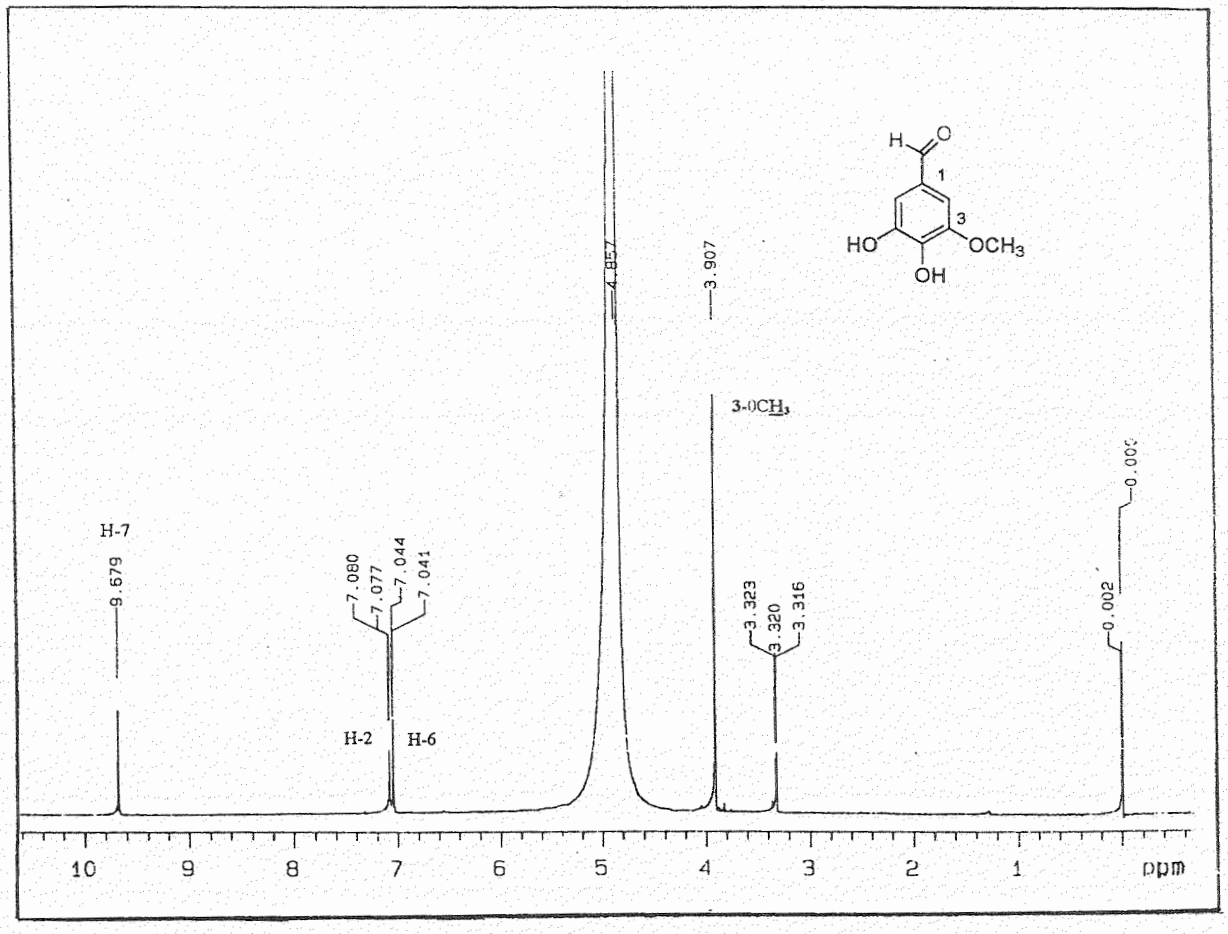


Fig 3.101 The ^{1}H-NMR spectrum of Compound **13**

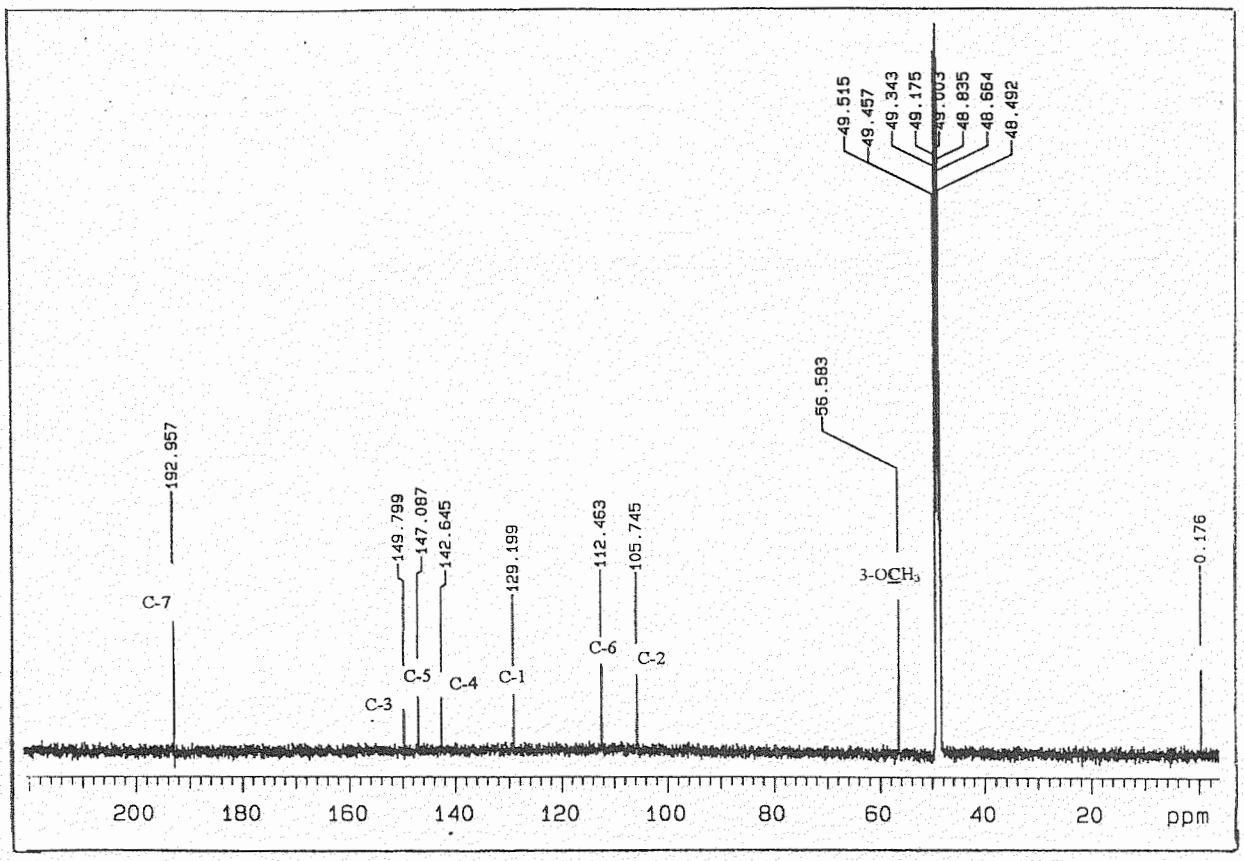


Fig 3.102 The ^{13}C-NMR spectrum of Compound **13**

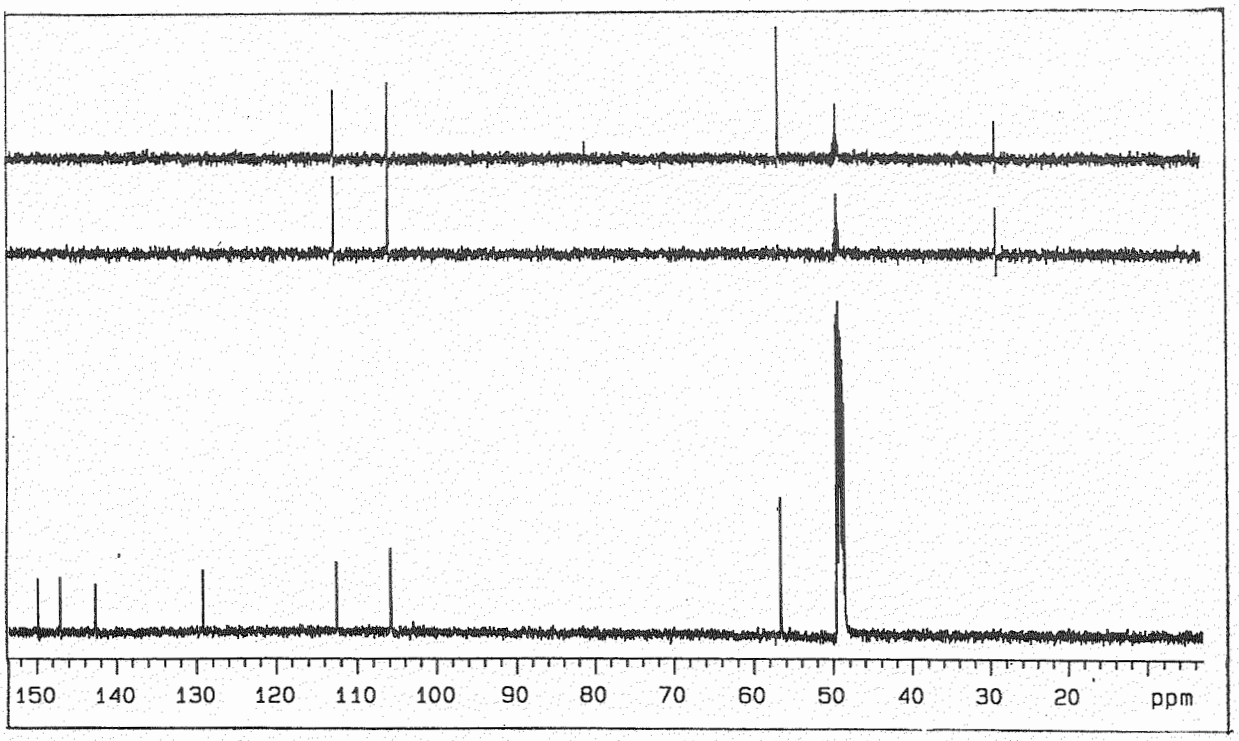


Fig 3.103 The DEPT 90 and 135 spectra of Compound **13**

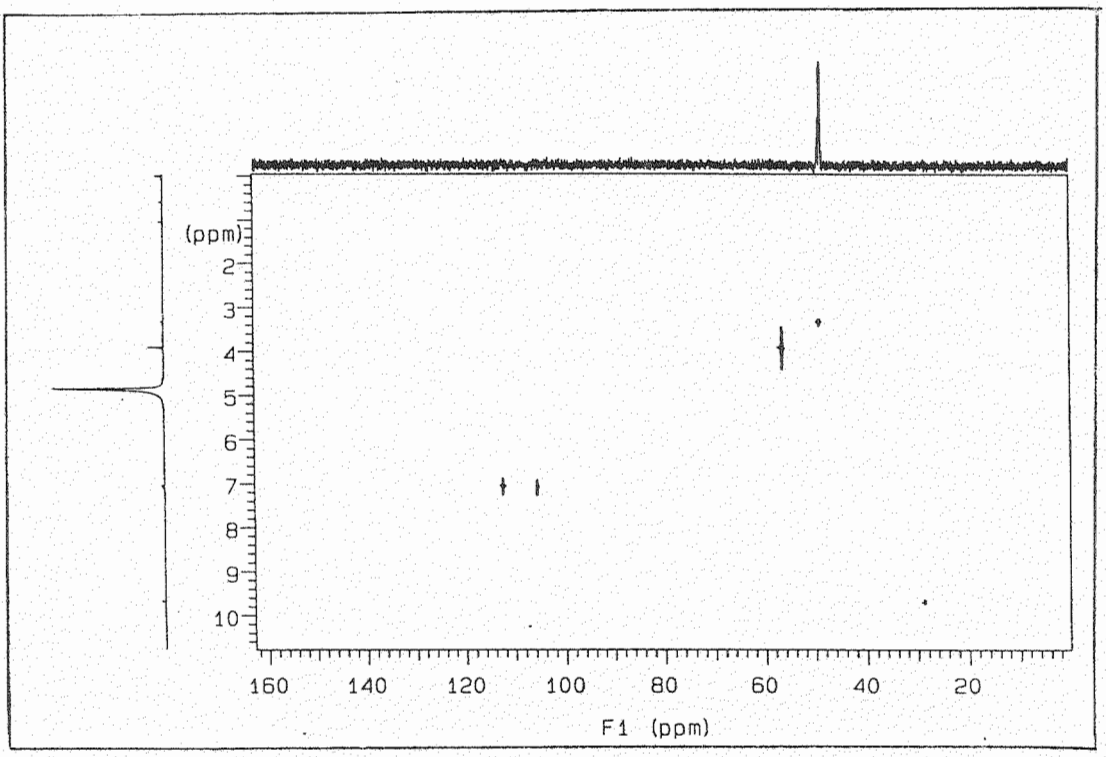


Fig 3.104 The HMQC spectrum of Compound **13**

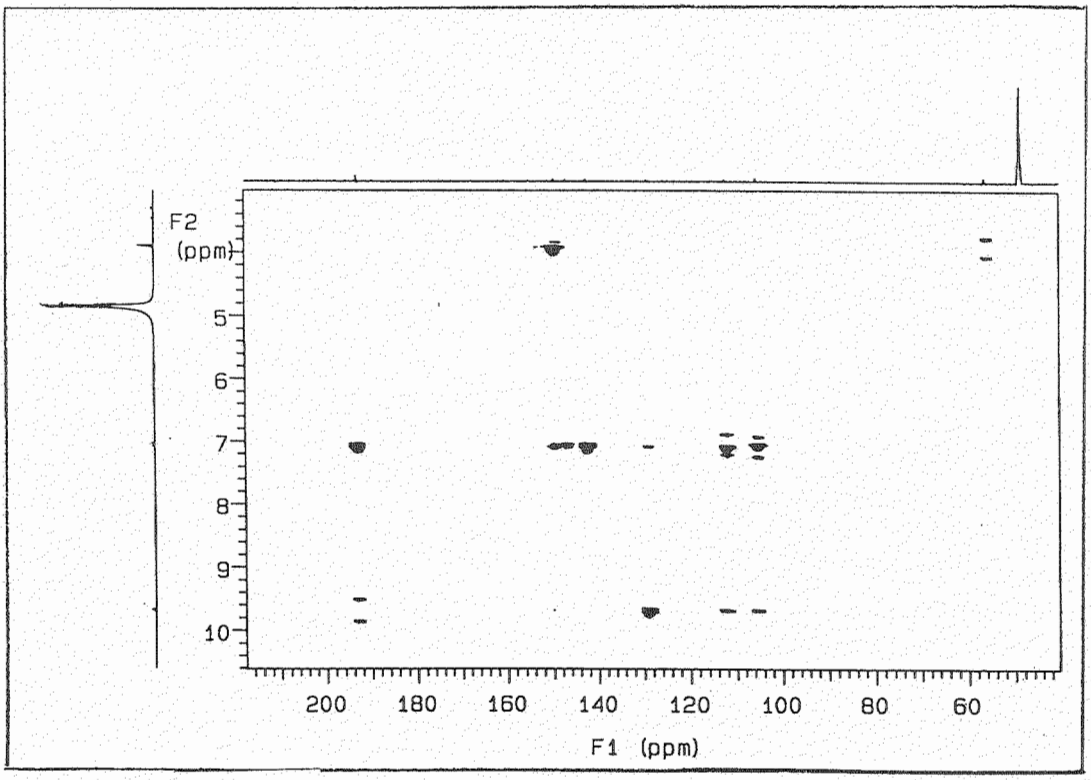


Fig 3.105 The HMBC spectrum of Compound **13**

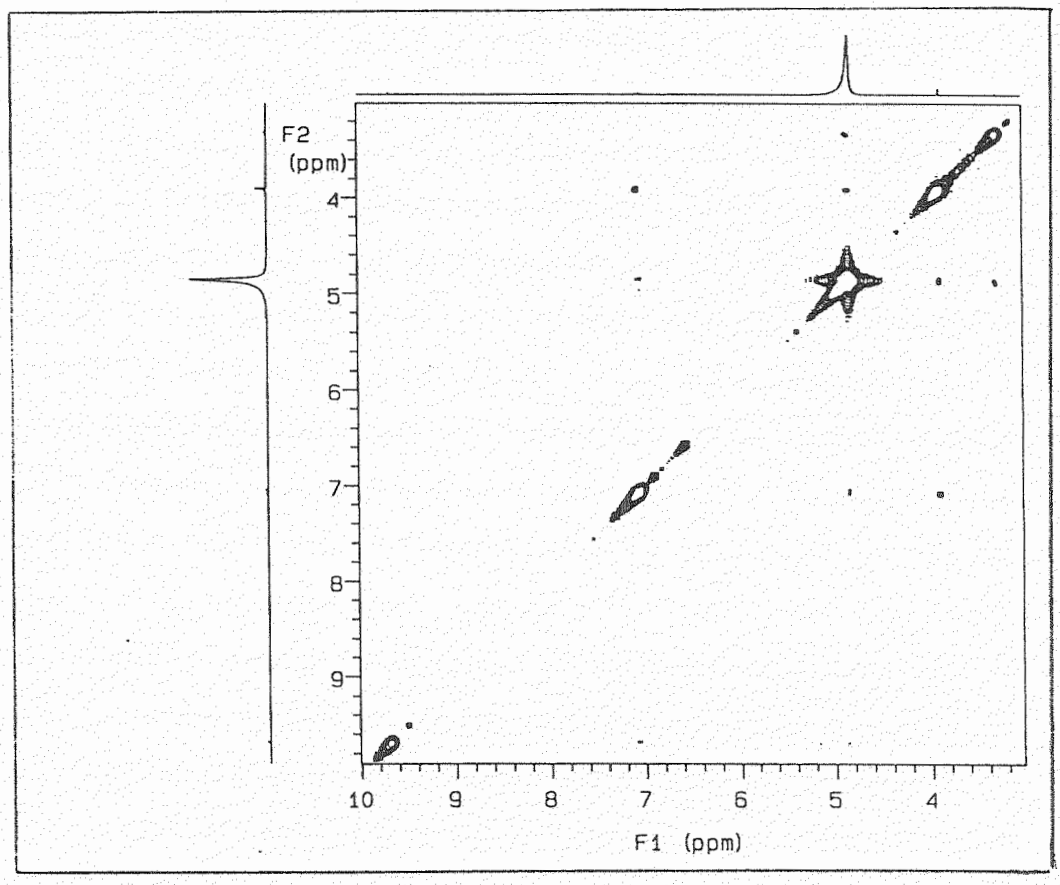
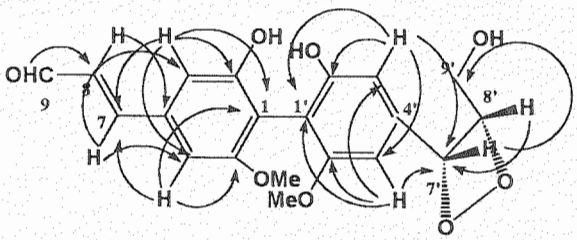


Fig 3.106 The COSY spectrum of Compound **13**

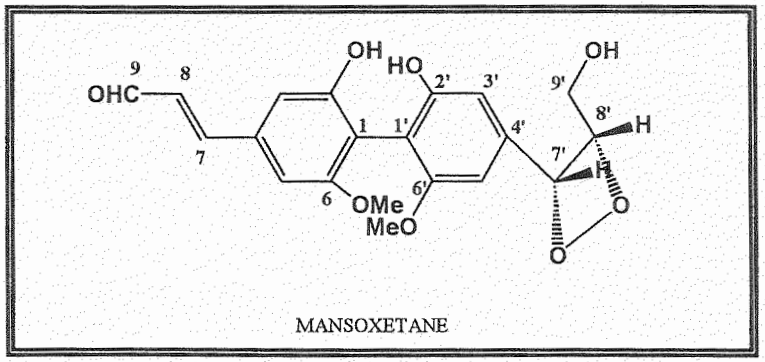
3.8.2 *Compound 14*: mansoxetane

Compound **14**, named mansoxetane, was obtained as a pale brown solid; $[\alpha]_D^{15}$ -12.8 (c = 0.27, $CHCl_3$). High-resolution FABMS analysis (Fig 3.107) gave m/z 389.1199 $(M+H)^+$ and established the molecular formula as $C_{20}H_{20}O_8$, which indicated Compound **14** to have the unsaturation number of 11. The presence of a *trans*-cinnamaldehyde residue in the molecule was suggested based on the observation of the characteristic signals at δ_H 9.66 and δ_C 193.5 ascribing to an aldehyde moiety and at δ_H 7.35 (d, J = 15.9 Hz) and δ_H 6.60 (dd, J = 15.9 and 7.6 Hz) arising from a *trans*-olefin, as well as on the HMBC correlations between these olefinic protons and the aromatic carbons on the benzene ring. A set of *meta*-coupled protons was observed at δ_H 6.90 and δ_H 6.76 in the ^{1}H-NMR spectrum (Fig 3.108), indicating that this benzene ring had four substituents, two of which were methoxy (δ_H 3.92 and δ_C 149.2) and hydroxy (δ_C 144.6). From both of the aromatic protons just mentioned above, HMBC correlations with the β-carbon (δ_C 152.8) of the acrylaldehyde function were observed, revealing that this part existed at the *ortho* position toward both of two *meta*-coupled protons. Furthermore, the HMBC correlations between both of these aromatic protons and the sp^2 quaternary carbon (δ_C 135.9) on the benzene ring were observed, indicating that this benzene ring had linkage with other unit at the *para* position of the acrylaldehyde group. All HMBC correlations are shown below.



From the ^{1}H (Fig 3.110) and ^{13}C NMR (Fig 3.109) spectral data, the presence of one more C6-C3 unit in Compound **14** was suggested. In analogy with the phenylpropanoid unit described above, the benzene ring in this residue had four substituents, two of which were methoxy (δ_H 3.90 and δ_C 147.2) and hydroxy (δ_C 144.2). From both of two *meta*-coupled aromatic protons (δ_H 6.70 and 6.57) on this benzene ring, HMBC correlations between the sp^3 carbon (δ_C 76.3) in one of the C3 unit were observed,

indicating that the propane unit was present at the *ortho* position towards both of two *meta*-coupled protons. The chemical shifts of three carbons in the propane unit were observed at δ_C 76.3, 78.8 and 61.3, revealing that they were the aliphatic carbons bearing oxygen functions, respectively. Further investigation of the structure of Compound **14** was performed by acetylation of this compound. Compound 14 (4.8 mg) was dissolved in dry pyridine (0.5 mL) was treated with dist. Acetic anhydride (0.25 mL) under reflux at room temperature for 10 hours. After the reaction was completed, dryness the products by flowing N_2 gas to take off the residue of pyridine and acetic anhydride. A triacetyl derivative, Compound **14a**, was obtained upon purification using silica gel column chromatography and confirmed by observation the molecular peak at m/z 514 in mass spectrum (Fig 3.114), three singlet signals of acetyl groups at δ_H 2.05, 2.29 and 2.31 (3H each) in ^{1}H NMR spectrum (Fig 3.115) and three carbonyl signals at δ_C 167.5, 167.9 and 170.3 as well as three methyl carbon signals at δ_C 20.3, 20.6 and 20.7 detected in ^{13}C NMR spectrum (Fig 3.116). Based on the result of acetylation of Compound **14**, the terminal carbon of the propane unit was proved to be a primary alcohol. Considering the molecular formula, the remaining two carbons constituted oxetane ring. A clear NOE observation between two protons (δ_H 4.90 and 4.05) on the oxetane ring revealed the stereochemical relation of the primary alcohol and the benzene ring to be *cis*. Furthermore, HMBC correlations between both of the aromatic protons and the sp^2 quaternary carbon (δ_C 133.2) on the benzene ring were observed, indicating that this benzene ring had linkage with another residue at the *para* position of the functionalized propane group. All the above spectroscopic analyses enabled to construct the dimeric structure Compound **14** that had a bridge between the *para* positions of each phenylpropanoid unit. To the best of our knowledge, this is the first example of biphenylneolignan[39] possessing an oxetane ring. The NMR data of Compound **14** are shown in Table 3.24.



MANSOXETANE

Table 3.24 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **14**

Position	Chemical Shift (ppm)[a]	
	δ_H (J in Hz)	δ_C
1	-	135.9
2	-	144.6
3	6.90, d, 1H (1.8)	111.3
4	-	126.7
5	6.76, d, 1H (1.8)	104.0
6	-	149.2
7	7.35, d, 1H (15.6)	152.8
8	6.60, dd, 1H (7.9, 15.6)	127.3
9	9.66, d, 1H (7.6)	193.5
1′	-	133.2
2′	-	144.2
3′	6.70, d, 1H (1.8)	108.2
4′	-	127.4
5′	6.57, d, 1H (1.8)	102.3
6′	-	147.2
7′	4.90, d, 1H (8.2)	76.3
8′	4.05, ddd, 1H (3.3, 3.3, 8.2)	78.8
9′	3.60, m, 1H 3.90, overlapped, 1H	61.3
2- and 2′-OH	5.56, br s, 2H	-
6-OMe	3.92, s, 3H	56.3
6′-OMe	3.90, s, 3H	56.2
9′-OH	2.30, s, 1H	-

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 400 and 100 MHz, respectively

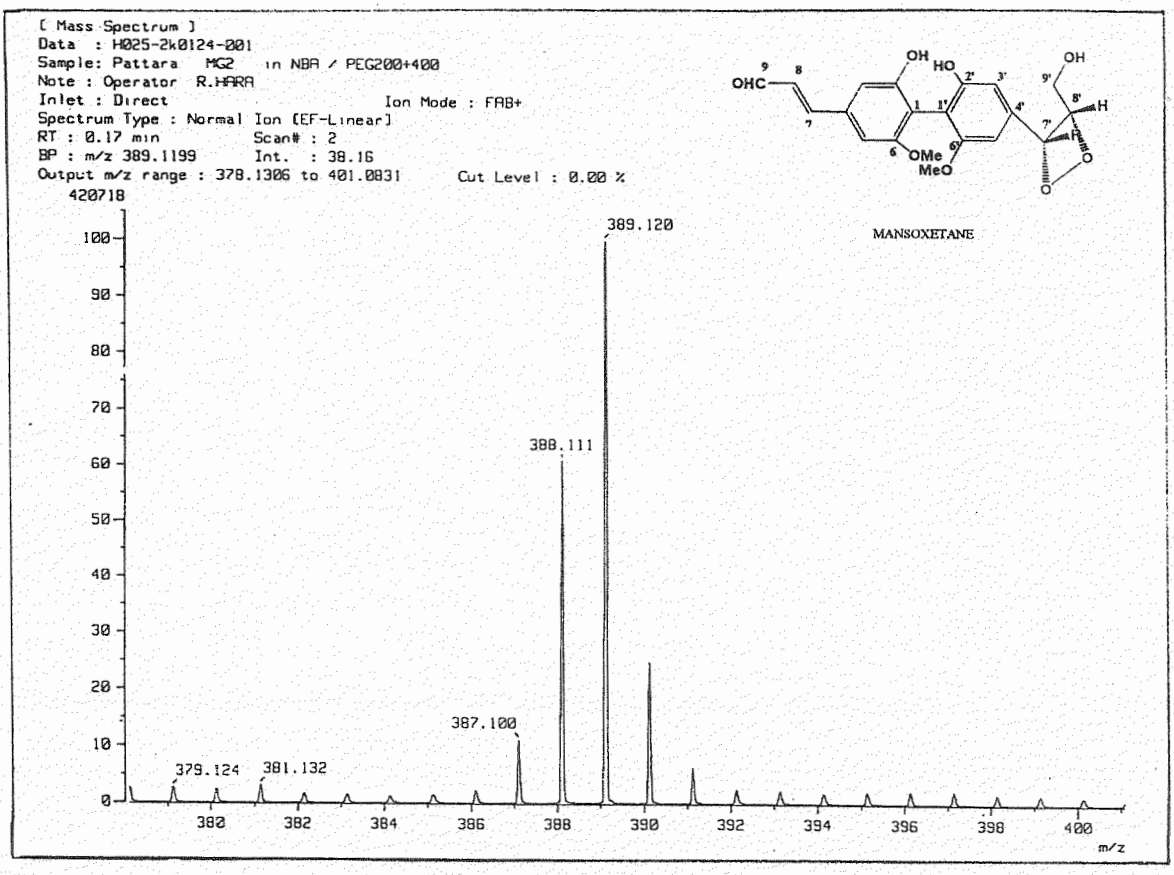


Fig 3.107 The high-resolution mass spectrum of Compound 14

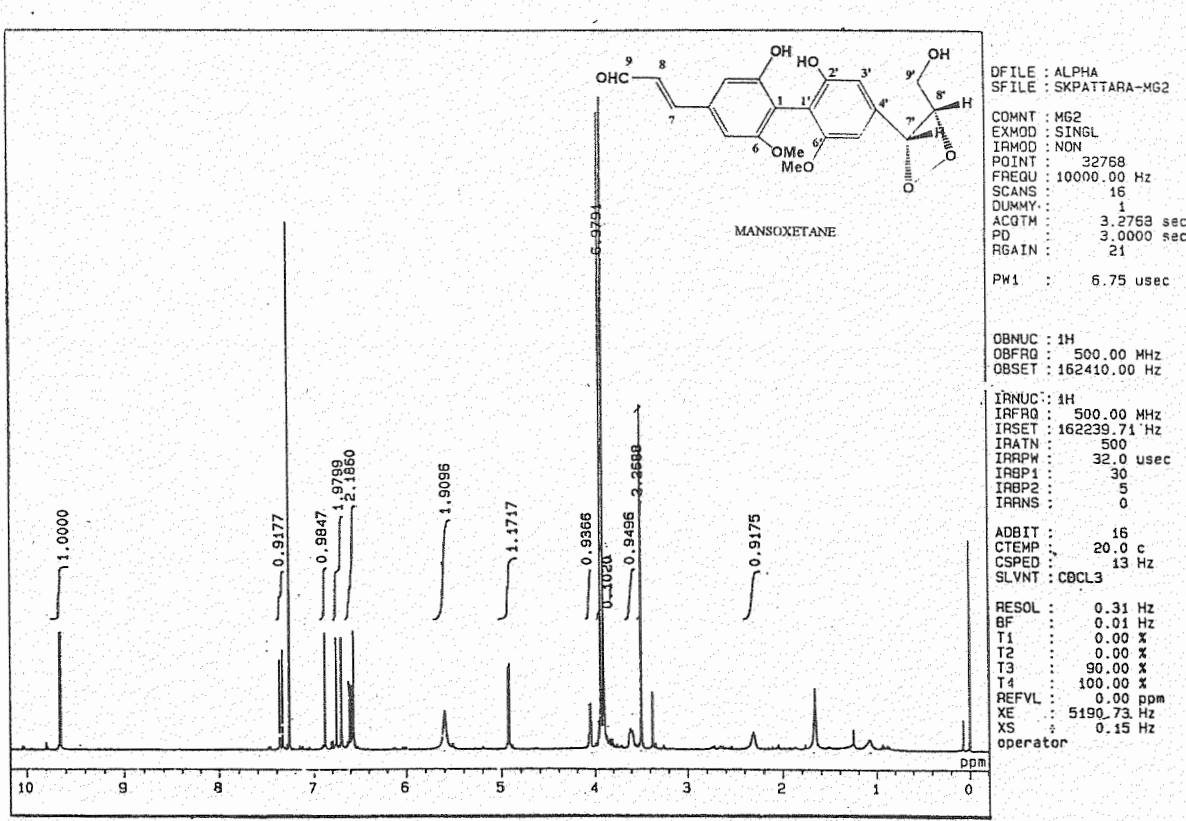


Fig 3.108 The ^{1}H-NMR spectrum of Compound 14

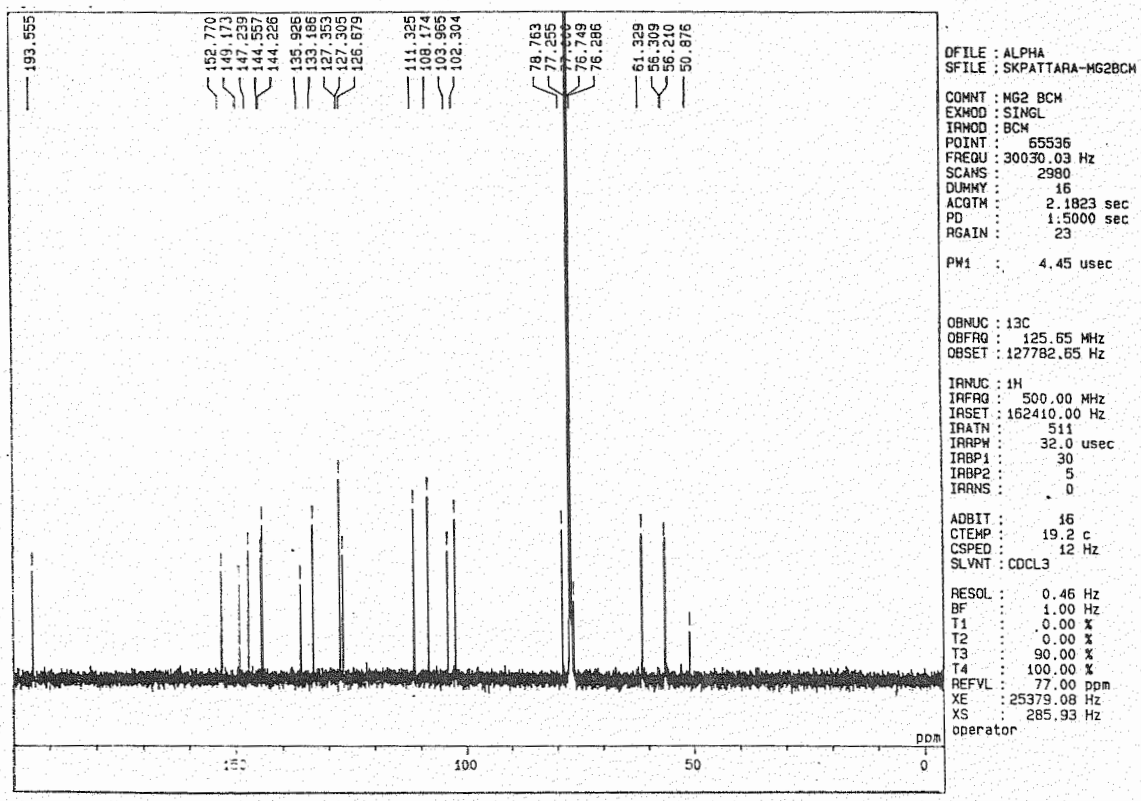


Fig 3.109 The ^{13}C-NMR spectrum of Compound **14**

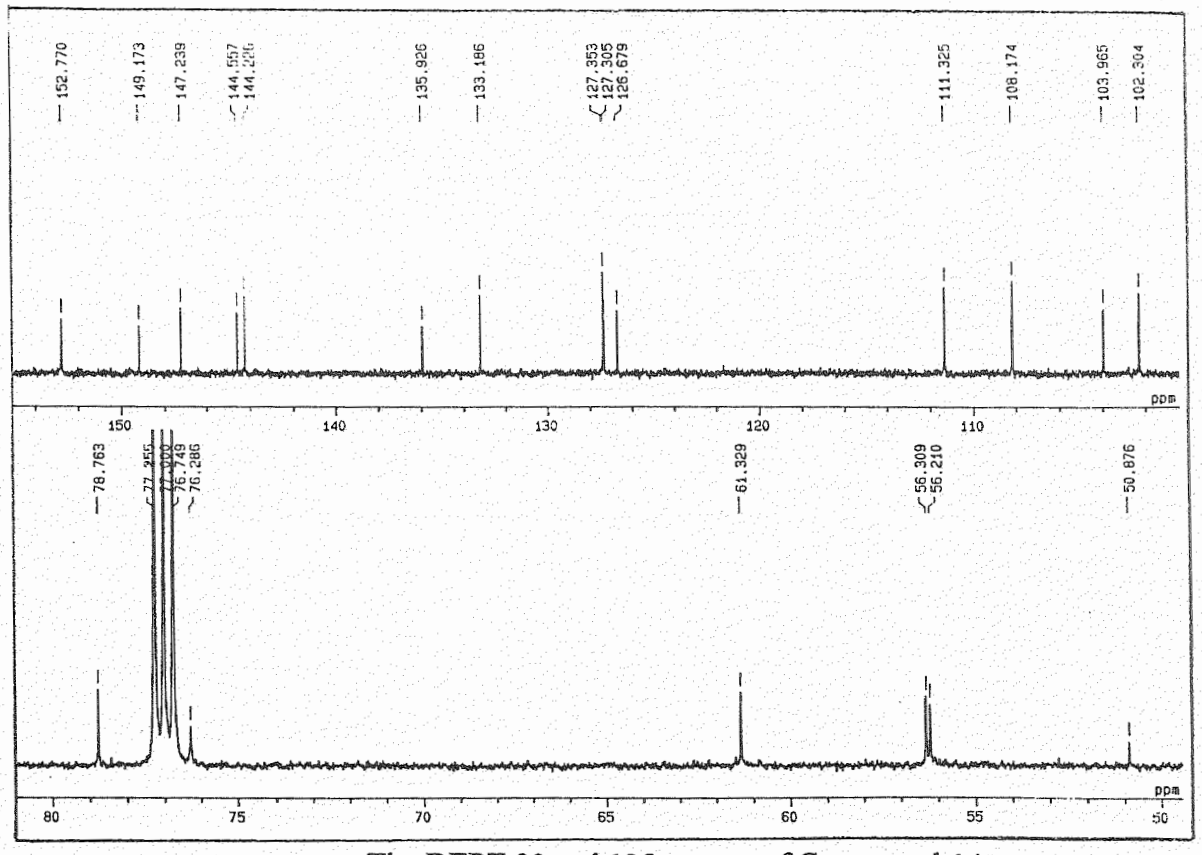


Fig 3.110 The DEPT 90 and 135 spectra of Compound **14**

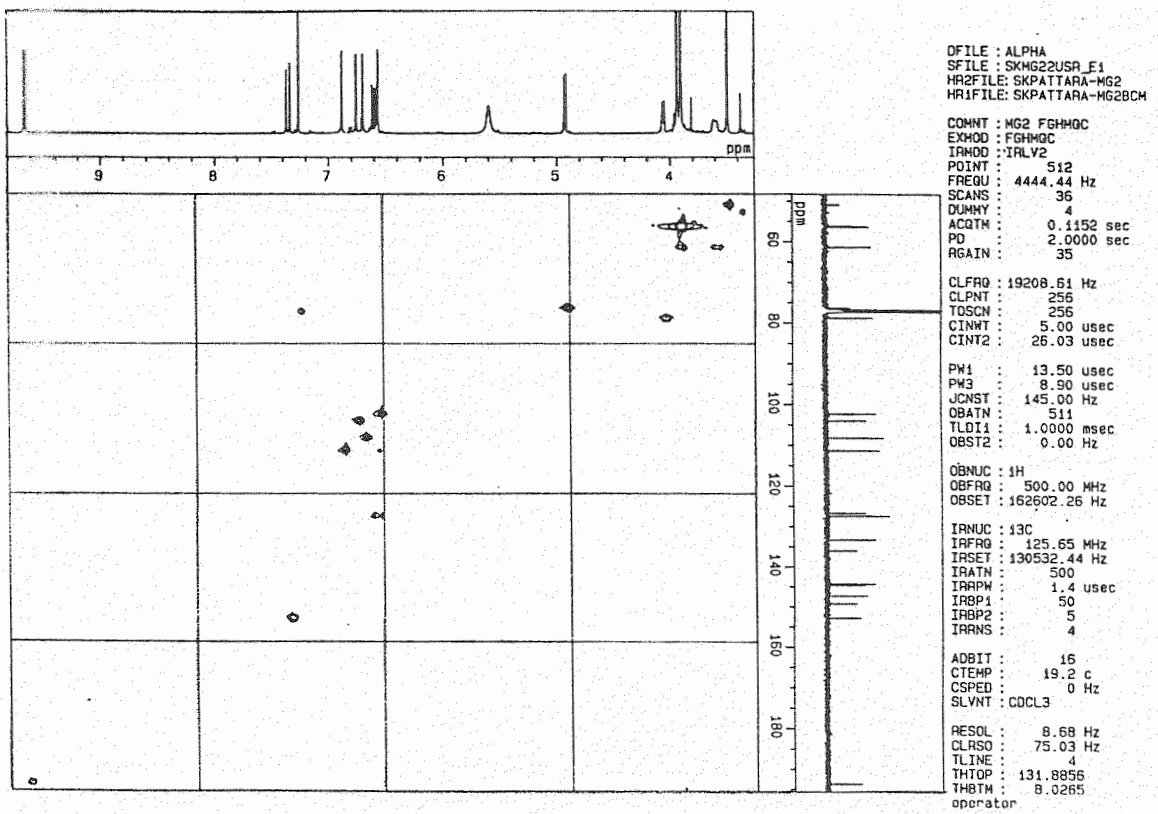


Fig 3.111 The HMQC spectrum of Compound 14

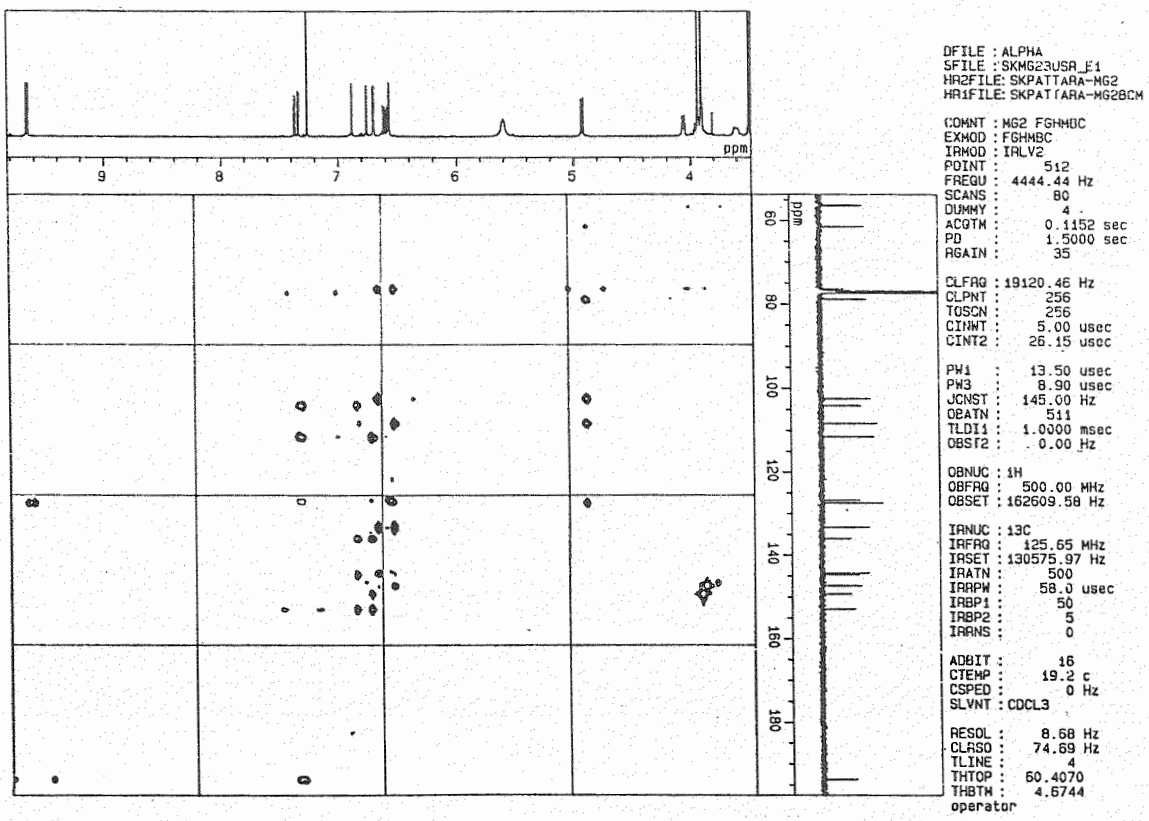
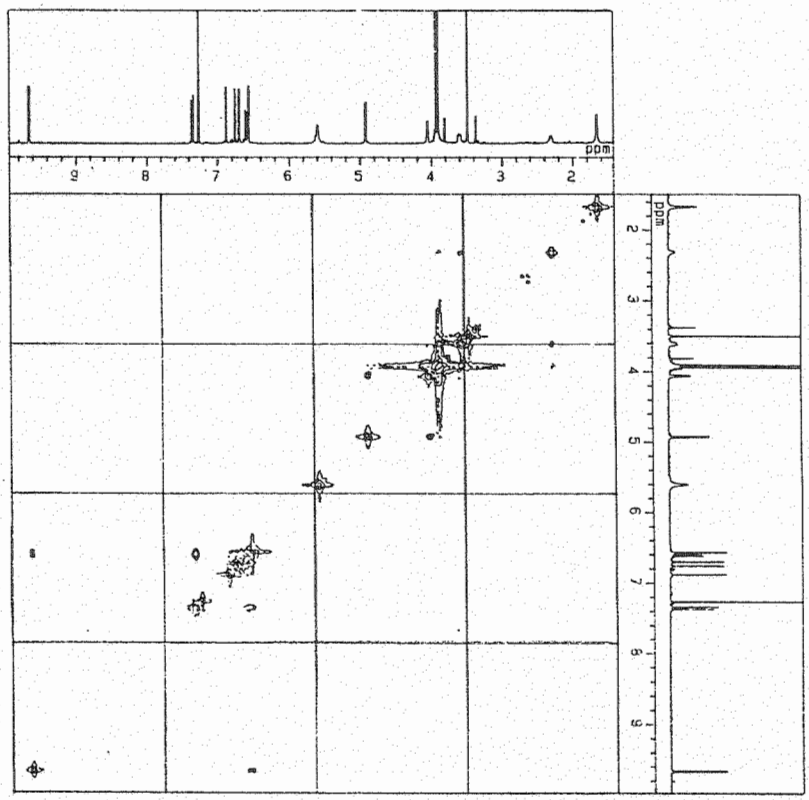


Fig 3.112 The HMBC spectrum of Compound 14

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PD    :   1.5000 sec
RGAIN :       22

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PW2   :    27.00 usec
PI1   : 120.0000 msec
PI2   :   1.0000 msec


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operator
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Fig 3.113 The COSY spectrum of Compound **14**

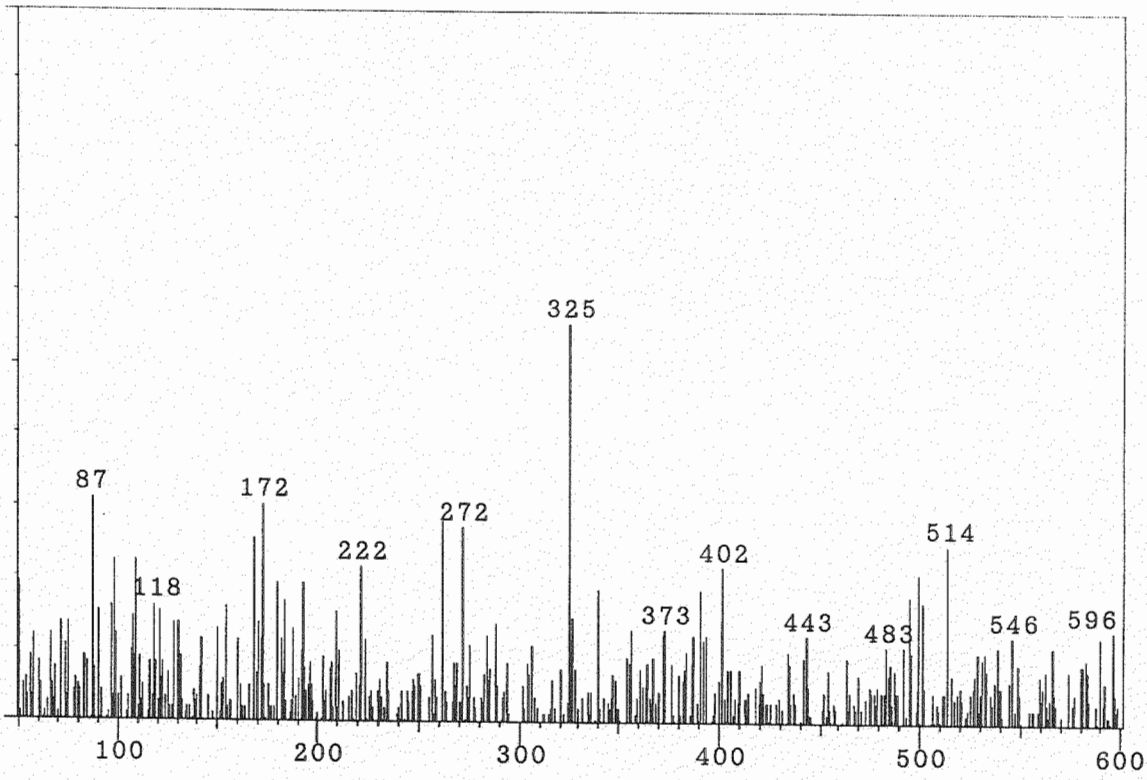

Fig 3.114 The mass spectrum of Compound **14a**

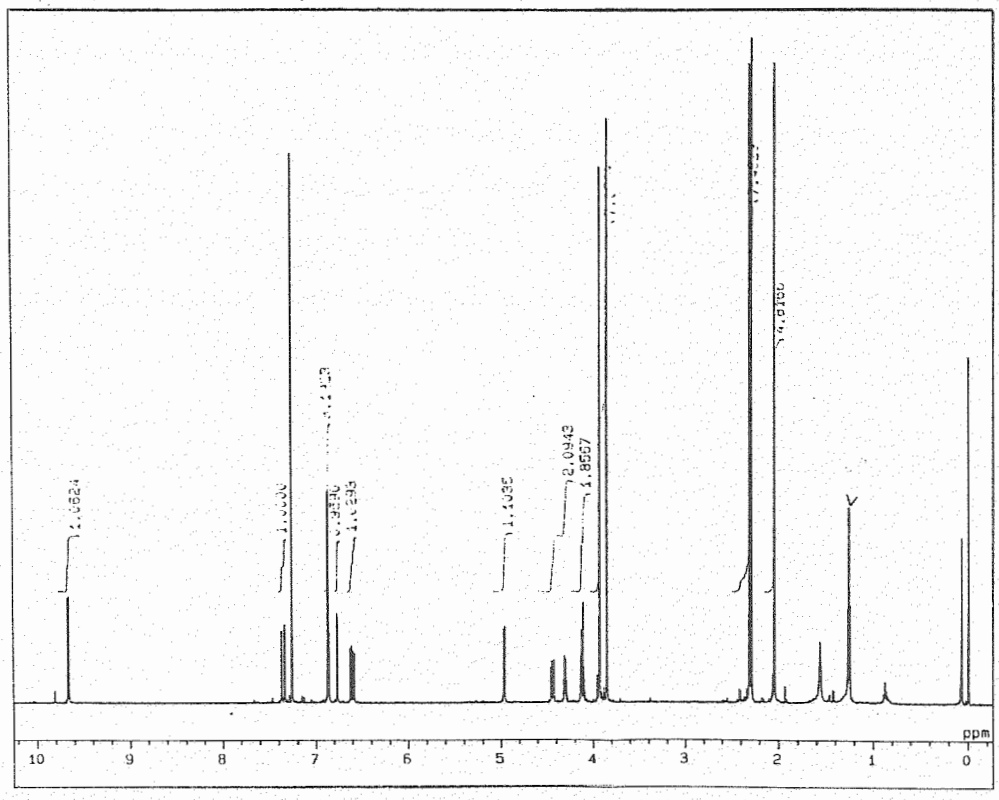

Fig 3.115 The ^{1}H NMR spectrum of Compound **14a**

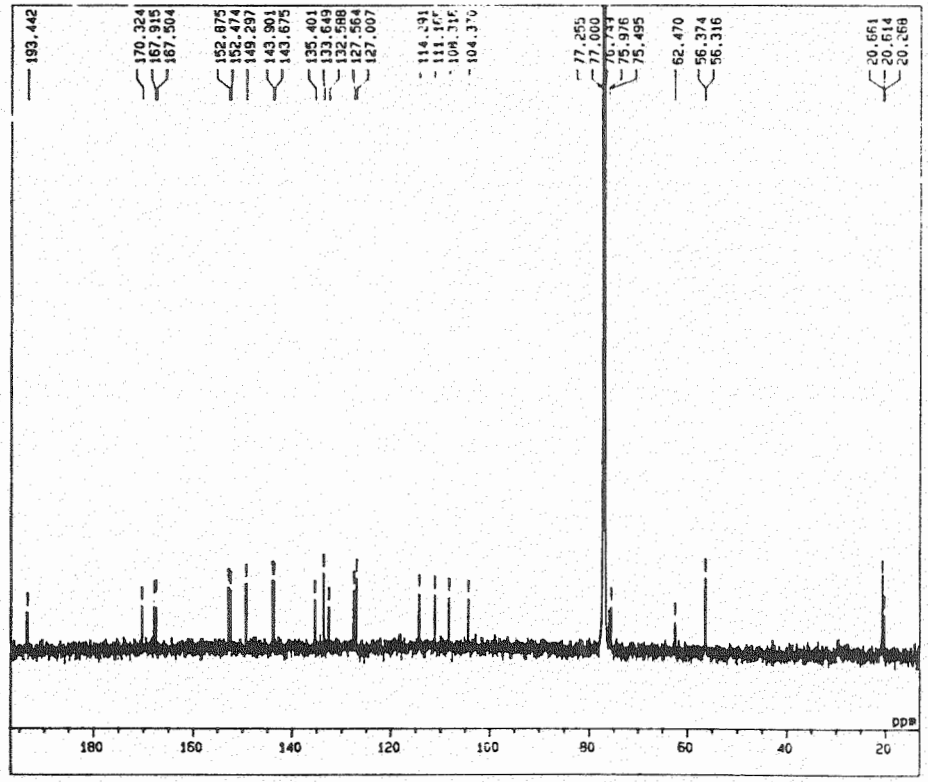

Fig 3.116 The ^{13}C NMR spectrum of Compound **14a**

3.9 The Biological Activity of Isolated Compounds from the Ethyl Acetate Extract

Two additional compounds were isolated from ethyl acetate extract: 3-methoxy-4,5-dihydroxybenzaldehyde (**13**) and mansoxetane (**14**). The former was examined for its bioactivities and the results are shown in Tables 3.25-3.26. The latter, nevertheless, was unfortunately isolated in an inadequate amount, thus the bioactivity is impossible to be evaluated.

Table 3.25 Antifungal, larvicidal and radical scavenging activities of 3-methoxy-4,5-dihydroxybenzaldehyde (**13**)

Compound	*Cladosporium Cucumerinum*[a]	*Candida Albicans*[a]	*Aedes aegypti*[b]	DPPH[a]
3-methoxy-4,5-dihydroxybenzaldehyde	>10	>10	>50	5.0

[a] : tested amount: 10 μg of pure compound
[b] : tested amount: 50 ppm of pure compound

Table 3.26 The anticancer activity of 3-methoxy-4,5-dihydroxybenzaldehyde (**13**)

Compounds	% Activities				
	Mouse Leukemia	Human Myeloma	Human Colon Adenocarcinoma	Human Leukemia	Human Breast Carcinoma
3-methoxy-4,5-dihydroxybenzaldehyde	70.8	87.5	18.2	29.3	66.9

From the result in above table, this compound possessed the cytotoxicity activity against various cancer cell-lines as well as the radical scavenging property towards DPPH.

PART III

3.10 Separation of the Methanolic Extract from the Heartwoods*

To extend the investigation of chemical composition of the heartwoods of *M.gagei*, the methanolic extract was throughly analyzed by HPLC compared with those of dichloromethane and ethyl acetate extracts (Fig 3.117). It was noticed that the major components of methanolic extract were in fact similar to those of dichloromethane and ethyl acetate extracts, except that the peaks of polar compounds (R_t 5-10 min) in methanolic extract were more intense than those of less polar compounds.

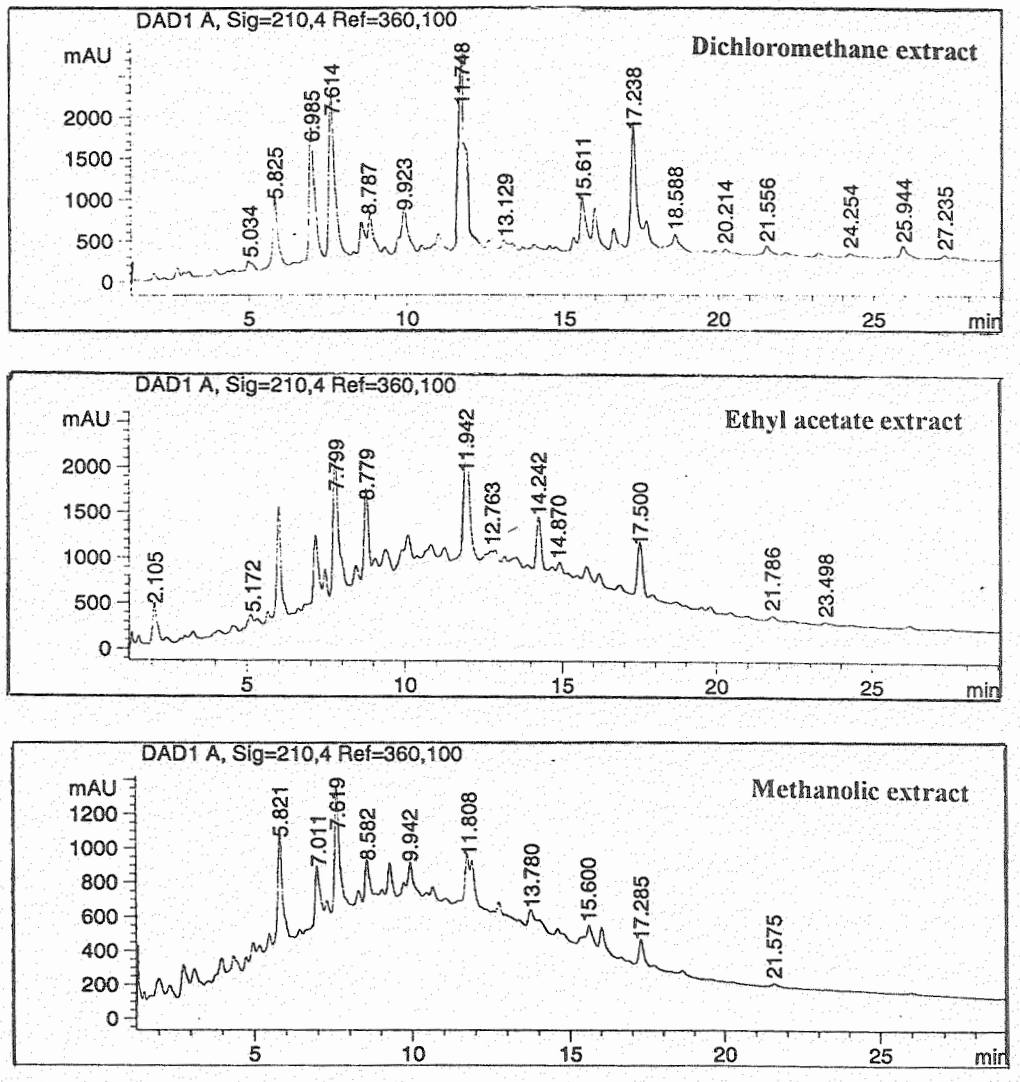

Fig 3.117 HPLC chromatogram of dichloromethane, ethyl acetate and methanolic Extracts (λ 210 nm)

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* This portion of research work was performed at Graduated School of Pharmaceutical Science, Chiba University, Chiba, Japan beyond the financial support under the United Nations Educational, Scientific and Cultural Organization (UNESCO)

3.10.1 Fractionation

The MeOH extract (20.10 g) was first subjected to fractionation using silica gel column chromatography eluting by the gradient of $CHCl_3$ and methanol. The fractions were collected and combined according to TLC results to furnish four fractions, MA-MD. The results of fractionation are shown in Table 3.27.

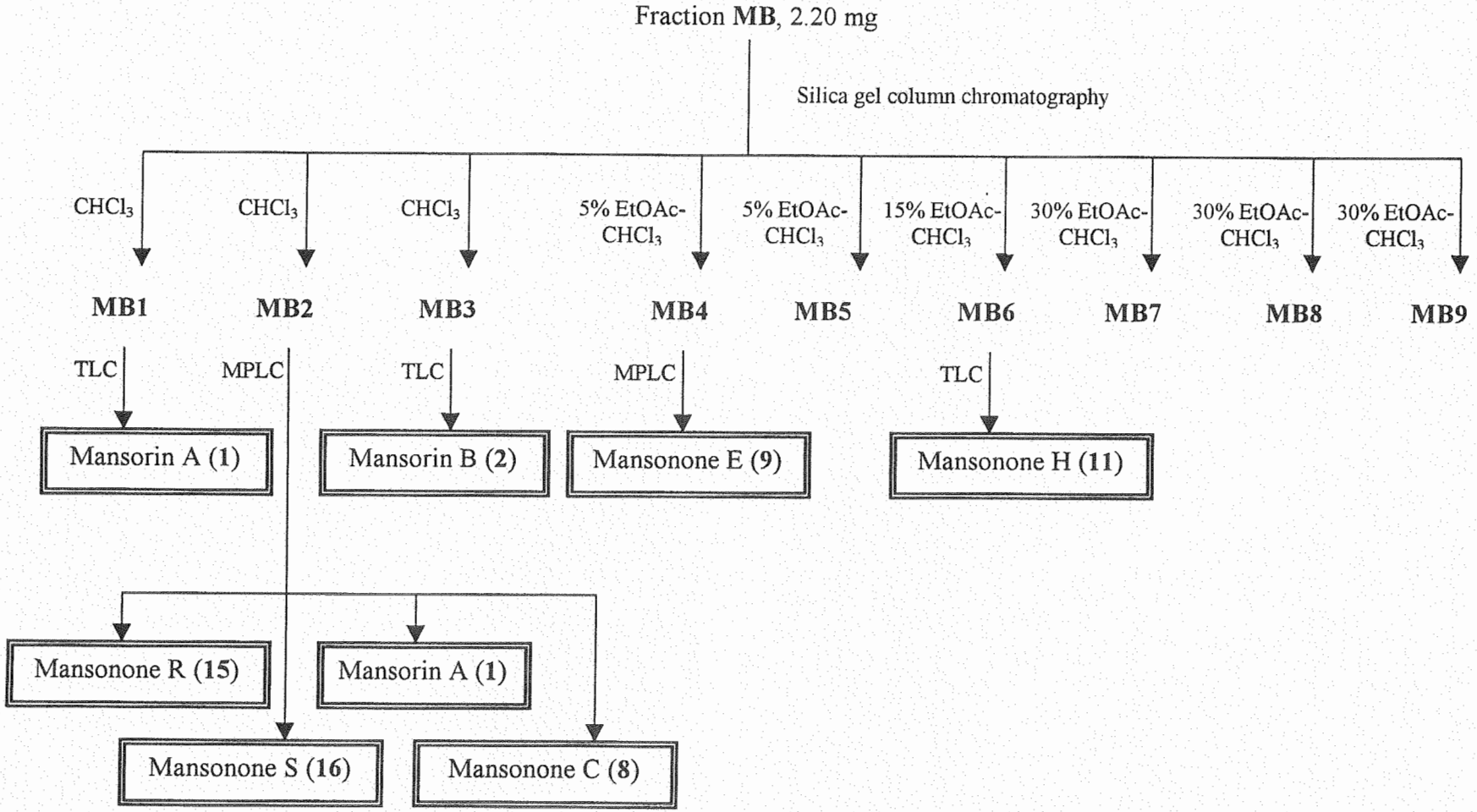
Table 3.27 The fractionation by quick column chromatography of the methanol extract from the heartwoods of *M. gagei*.

Fraction No./ Eluent (% vol. by vol.)	Remarks	Weight (g)
MA : 0-5% Methanol in $CHCl_3$	solid in yellow oil	0.04
MB : 10 % Methanol in $CHCl_3$	dark brown oil	2.20
MC : 20-40 % Methanol in $CHCl_3$	dark brown solid	6.29
MD : 50-100 % Methanol in $CHCl_3$	dark brown solid	5.03

3.10.2 Separation

3.10.2.1 Fraction MB

Fraction MB was further separated by subjection to silica gel column using the gradient of Hexane-EtOAc and EtOAc-MeOH giving nine fractions, MB1-MB9, monitoring by analytical TLC. Comparing R_f of major constituents of fractions MB1,MB3 and MB6 with the isolated compounds from the dichloromethane extract on analytical TLC plates revealed that fraction MB1,MB3 and MB6 contained mansorin A (**1**), mansorin B (**2**) and mansonone H (**11**), respectively. Fractions MB2 (56.5 mg) and MB4 (40.3 mg) were further subjected to MPLC with silica gel prepacked column using Hexane:EtOAc = 85:15 as eluent. Mansonones R (**15**) (2.7 mg) and S (**16**) (27.4 mg), mansorin A (**1**) (11.9 mg), mansonone C (**8**) (3.3 mg) were gained from fraction MB2 and Mansonone E (4.8 mg) was derived from fraction MB4. Mansonones R and S had not been isolated from dichloromethane and ethyl acetate extracts, and characterized as new naturally occurring compounds. The results of separation are summarized as shown in Scheme 3.20.



Scheme 3.20 Isolation diagram of fraction **MB** of the methanolic extract from the heartwoods of *M. gagei*

3.10.2.2 Fraction MC

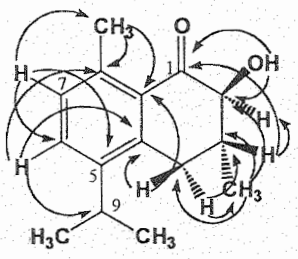
Fraction MC was first fractionated on a silica gel column using a gradient of $CHCl_3$-MeOH giving nine fractions, MC1-MC-9, monitoring by analytical TLC (silica gel) plates. A part of fraction MC3 (56.0 mg) was further separated by MPLC with silica gel prepacked column using the gradient of $CHCl_3$-EtOAc and EtOAc-MeOH as eluent, yielding 6.3 mg of mansoxetane (**14**), previously obtained from ethyl acetate extract.

3.11 Structural Elucidation

3.11.1 *Compound 15:* Mansonone R

Compound **15**, as pale yellow powder, was established as $C_{15}H_{20}O_2$ based on EIMS and NMR data. The ^1H NMR spectrum of Compound **15** (Fig 3.119) disclosed the presence of a set of *ortho*-coupled protons, an isopropyl group, two methyl groups and two methylene protons. The ^{13}C NMR spectrum of Compound **15** (Fig 3.120) also indicated the presence of these mentioned groups and an α-hydroxy ketone moiety.

The HMBC spectrum of Compound **15** (Fig 3.122) showed the correlations of the set of *ortho*-coupled proton signals in an aromatic ring at δ_H 7.14 and 7.38 with the methyl carbon at δ_C 22.6 (8-CH_3) and the methine carbon of the isopropyl group at δ_C 28.3, respectively. In addition, this methyl group (8-CH_3) was correlated to an aromatic carbon at δ_C 130.5. Furthermore, one of two methylene protons (δ_H 3.21) showed correlation with two aromatic carbons at δ_C 140.6 and 130.5, and the other (δ_H 2.63) was coupled with a methine carbon at δ_C 37.4 as well as another methyl group at δ_C 19.0 (3-CH_3). HMBC correlations were also observed between the methyl group (3-CH_3) and a carbon bearing a hydroxy group (δ_H 78.5). All HMBC correlations of Compound **15** are shown below:



The relative stereochemistry of Compound **15** was determined primarily on the basis of J value obtained from the ^1H NMR spectrum. The large coupling constant observed between H-2 and H-3 (J = 12.8 Hz) implied a *trans*-diaxial orientation for this

proton pair. Compound **15** was thus identified as a new natural product and named mansonone R. The NMR data of this compound are presented in Table 3.28.

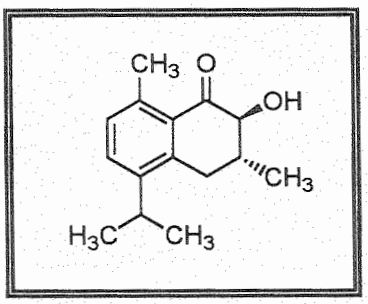


Table 3.28 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **15**

Position	Chemical Shift (ppm)[a]	
	δ_H (J in Hz)	δ_C
1	-	201.4
2	3.95, dd, 1H (2.7, 12.8)	78.5
3	2.10, m, 1H	37.4
4	2.63, dd, 1H (11.9, 17.1) 3.21, overlapped, 1H	33.7
4a	-	140.6
5	-	144.4
6	7.38, d, 1H (7.9)	128.8
7	7.14, d, 1H (7.9)	130.0
8	-	139.0
8a	-	130.5
9	3.19, m, 1H	28.3
2-OH	4.15, d, 1H (2.7)	-
3-CH_3	1.34, d, 3H (6.4)	19.0
8-CH_3	2.62, s, 3H	22.6
9-$(CH_3)_2$	1.24, d, 3H (6.4) 1.25, d, 3H (6.4)	22.9 23.5

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 400 and 100 MHz, respectively

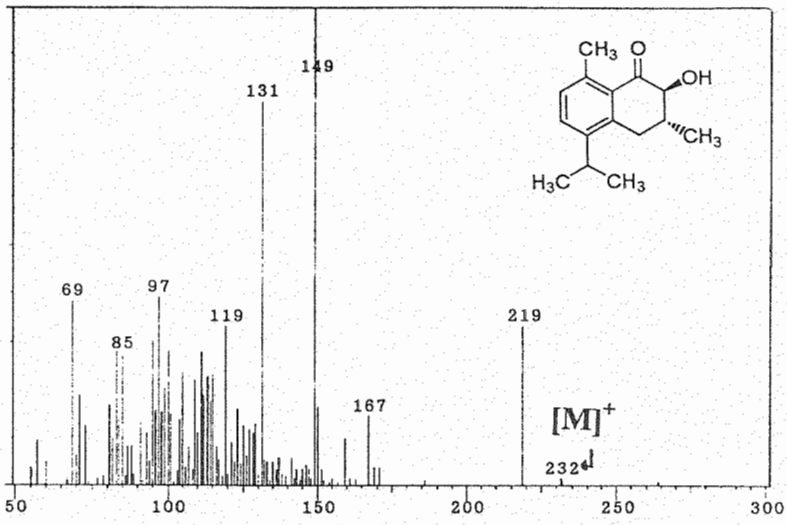


Fig 3.118 The mass spectrum of Compound **15**

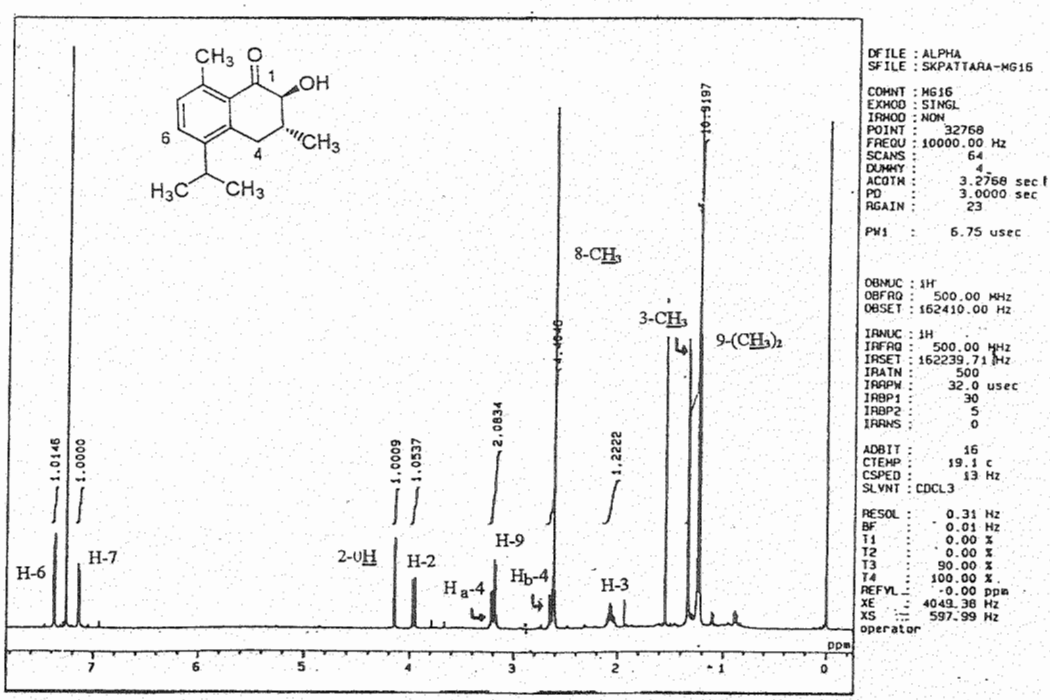


Fig 3.119 The ^{1}H-NMR spectrum of Compound **15**

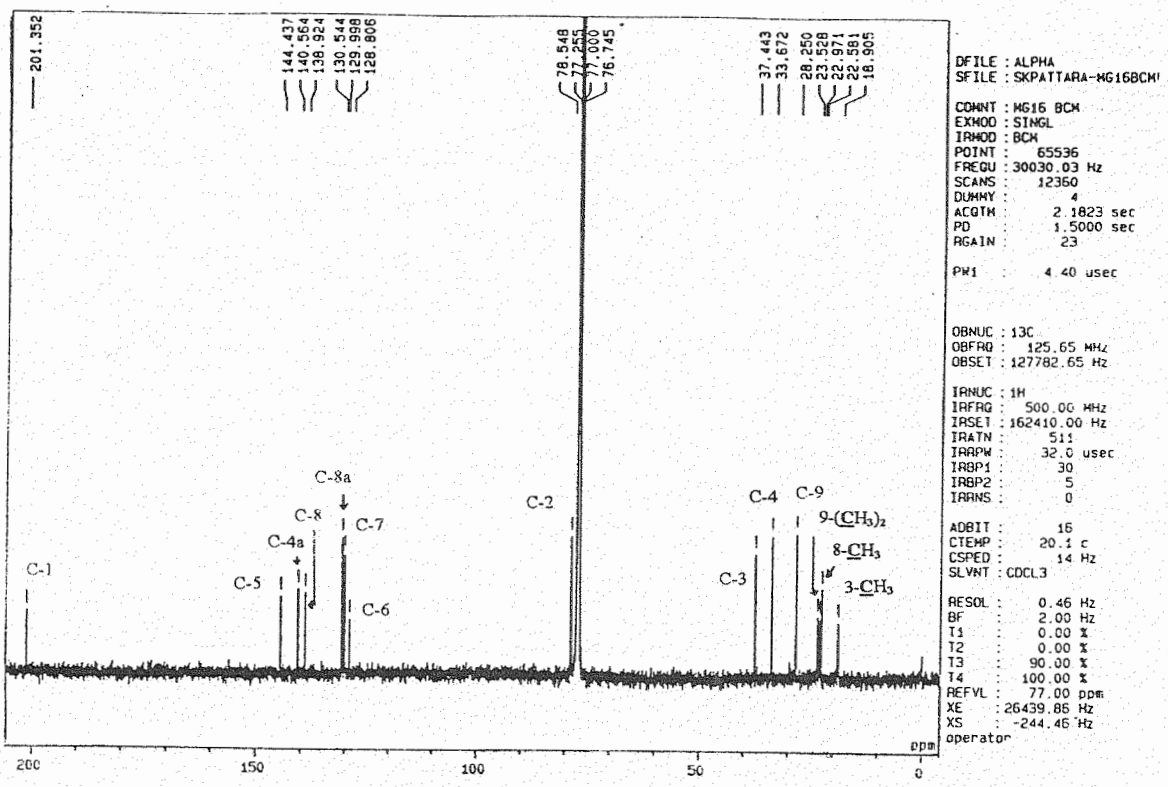


Fig 3.120 The ^{13}C-NMR spectrum of Compound 15

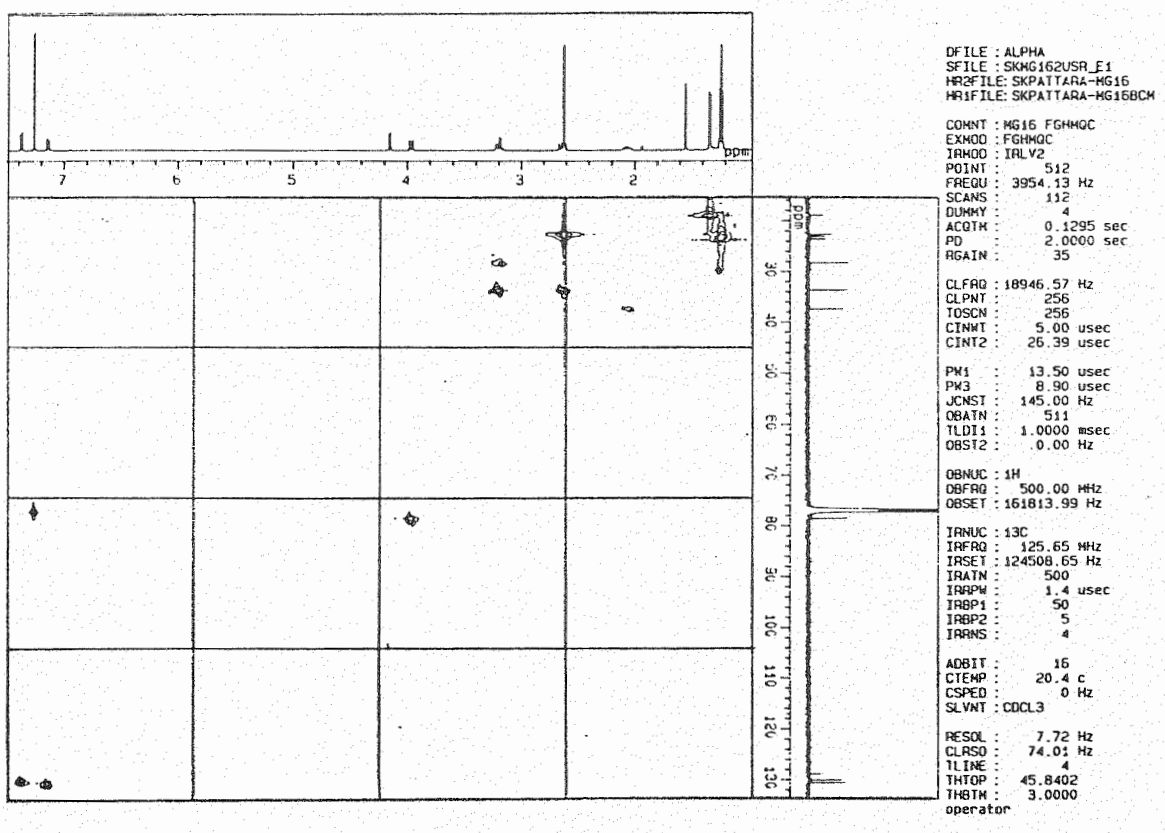


Fig 3.121 The HMQC spectrum of Compound 15

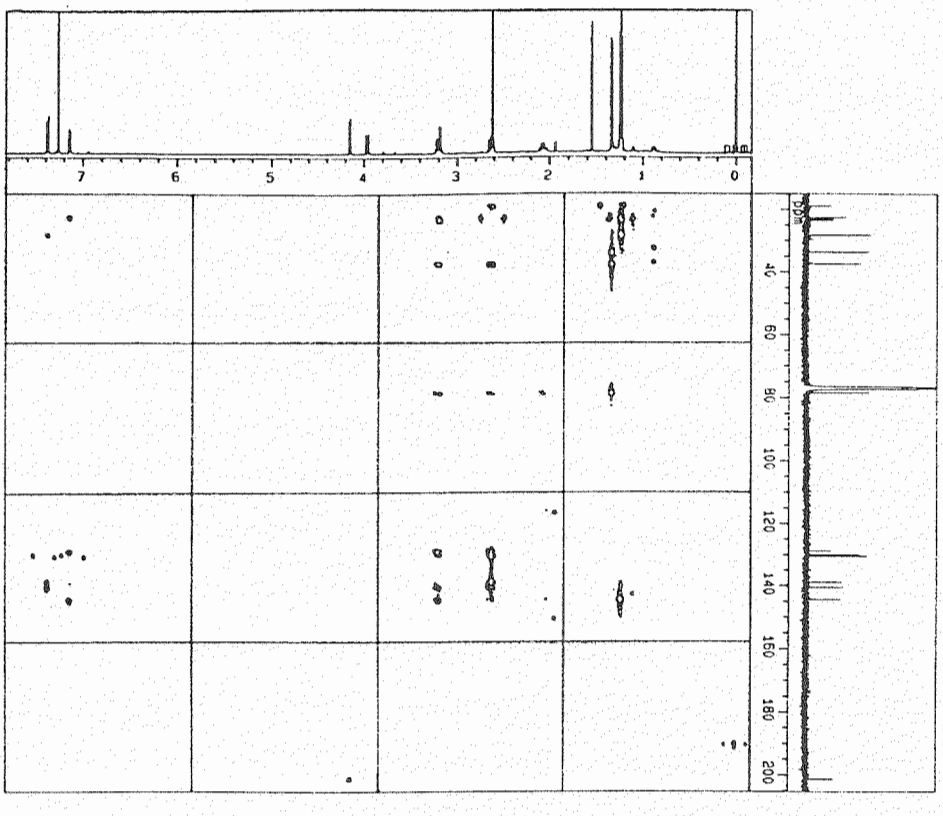


Fig 3.122 The HMBC spectrum of Compound 15

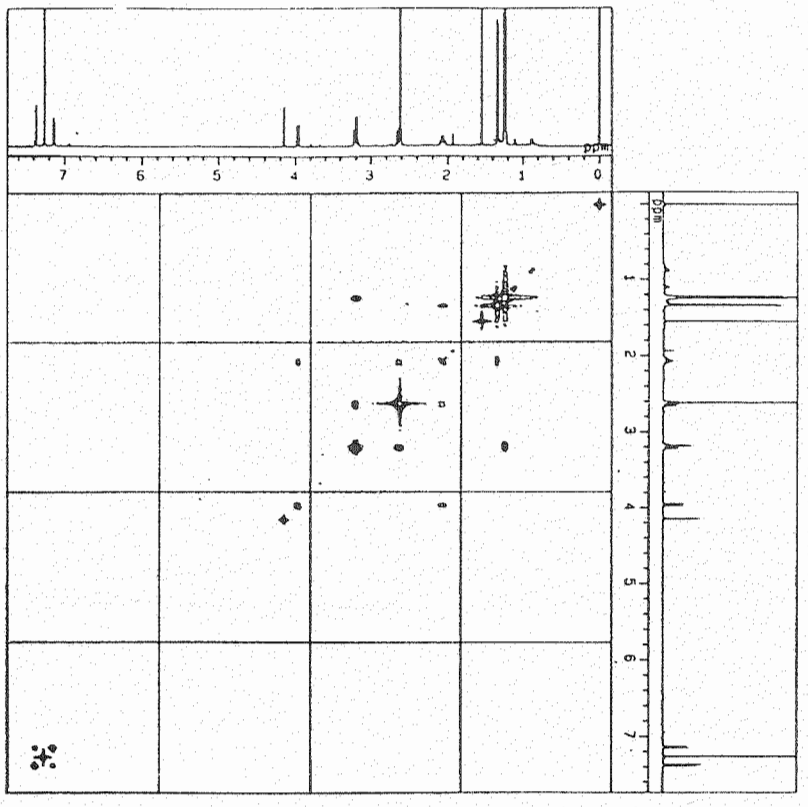
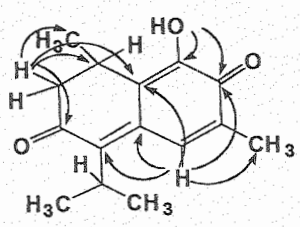
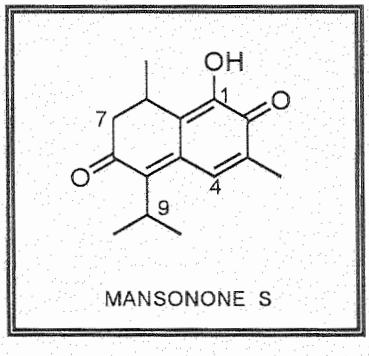


Fig 3.123 The COSY spectrum of Compound 15

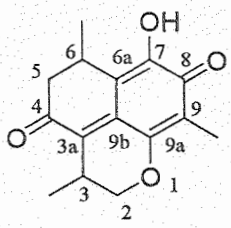
3.11.2 *Compound 16:* Mansonone S

Compound **16** was obtained as orange amorphous, and the molecular formula was determined to be $C_{15}H_{18}O_3$ from the molecular ion at m/e 247.1316 $[M+H]^+$ in the high-resolution FAB^+-mass spectrum (Fig 3.124). The ^{13}C-NMR spectrum (Fig 3.126) showed fourteen carbon signals. The signals at δ_C 180.8 (C-2) and 200.0 (C-6) were clearly ascribed to two carbonyl carbon signals. Comparison of the ^1H-NMR and ^{13}C-NMR spectral data of Compound **16** with those of mansonones in literature found that those data of Compound **16** were very similar to those of 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[1,8-b,c]pyran-4,8-dione,[16] (Table 3.29), except for the absence of the naphtho[1,8-b,c]pyran signals, and the presence of the additional isopropyl group signals at δ_H 1.28 (d, 3H, J = 7.0 Hz), 1.38 (d, 3H, J = 7.0 Hz) and 3.45 (m, 1H) in Compound **16**. The complete structure of Compound **16**, a new natural product named mansonone S, was well-confirmed by HMQC (Fig 3.127), HMBC (Fig 3.128) and COSY (Fig 3.129) spectra. The HMBC correlations of this compound are shown below.





MANSONONE S



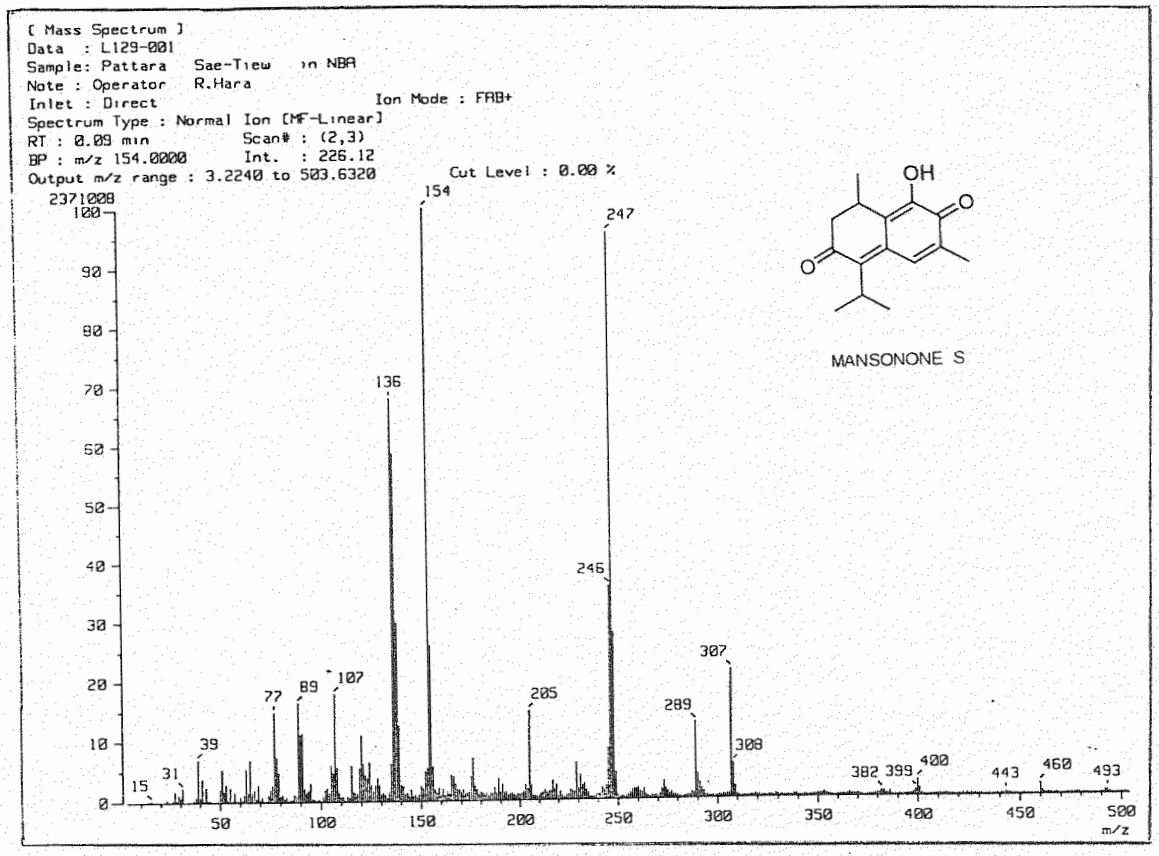
7-hydroxy-2,3,5,6-tetrahydro-

3,6,9-trimethylnaphtho[1,8-b,c]pyran-4,8-dione

Table 3.29 ^{1}H-NMR and ^{13}C-NMR spectral data of Compound **16** and 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[1,8-b,c]pyran-4,8-dione[16]

	Chemical Shift (ppm) of Compound **16**[a]			Chemical Shift (ppm) of 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[1,8-b,c] pyran-4,8-dione[b]	
Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1	-	144.6	7	-	143.6
2	-	180.8	8	-	181.3
3	-	136.1	9	-	115.0
4	7.55, s, 1H	132.4	9a	-	157.3
4a	-	135.3	9b	-	131.0
5	-	150.2	3a	-	139.4
6	-	200.0	4	-	197.1
7	2.50, d, 1H (15.0) 2.80, dd, 1H (6.4, 14.7)	46.0	5	2.60, dd, 1H (1.5, 16.3) 2.78, dd, 1H (6.6, 16.3)	44.5
8	3.55, m, 1H	28.0	6	3.55, m, 1H	27.5
8a	-	123.0	6a	-	115.1
9	3.45, m, 1H	28.5	3	3.12, dq, 1H (3.5, 7.1)	26.4
1-OH	6.86, s, 1H	-	2	-	71.9
3-CH_3	2.13, s, 3H	16.2	9-CH_3	1.94, s, 3H	8.0
8-CH_3	1.20, d, 3H (7.3)	20.5	6-CH_3	1.19, d, 3H (7.1)	20.6
9-$(CH_3)_2$	1.38, d, 3H (7.0) 1.28, d, 3H (7.0)	21.0 22.7	3-CH_3	1.16, d, 3H (7.1)	16.2

[a] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 400 and 100 MHz, respectively
[b] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 500 and 125.8 MHz, respectively



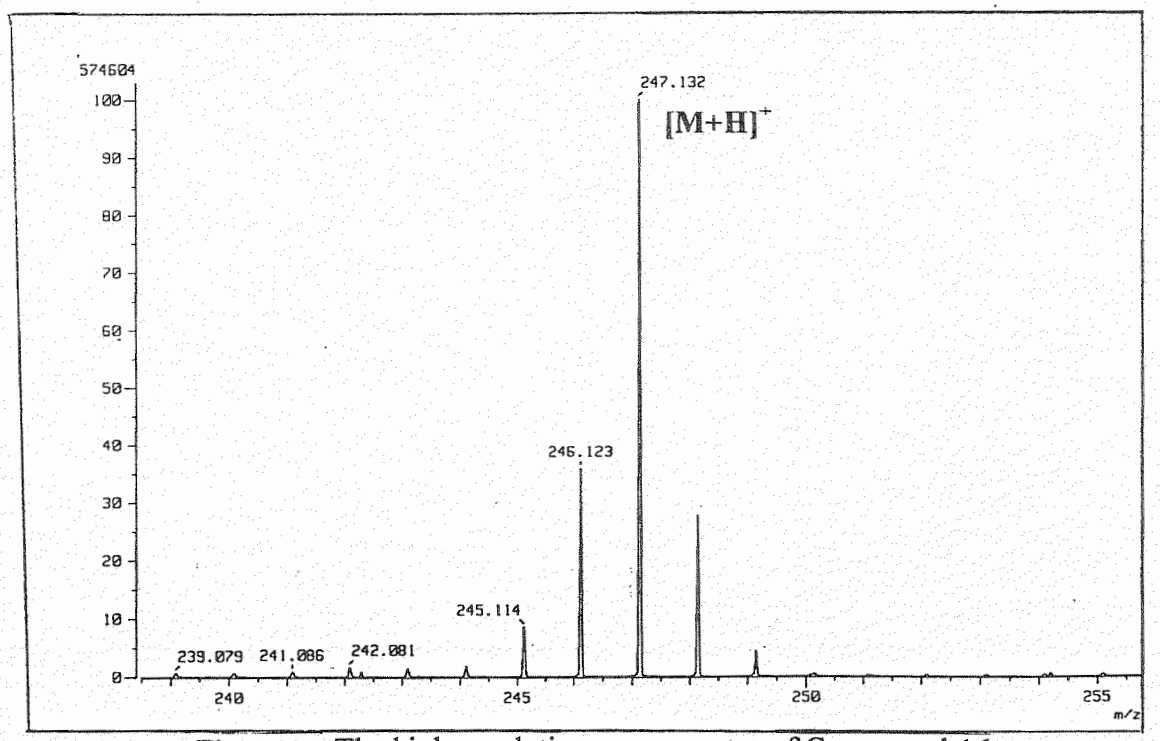


Fig 3.124 The high-resolution mass spectra of Compound **16**

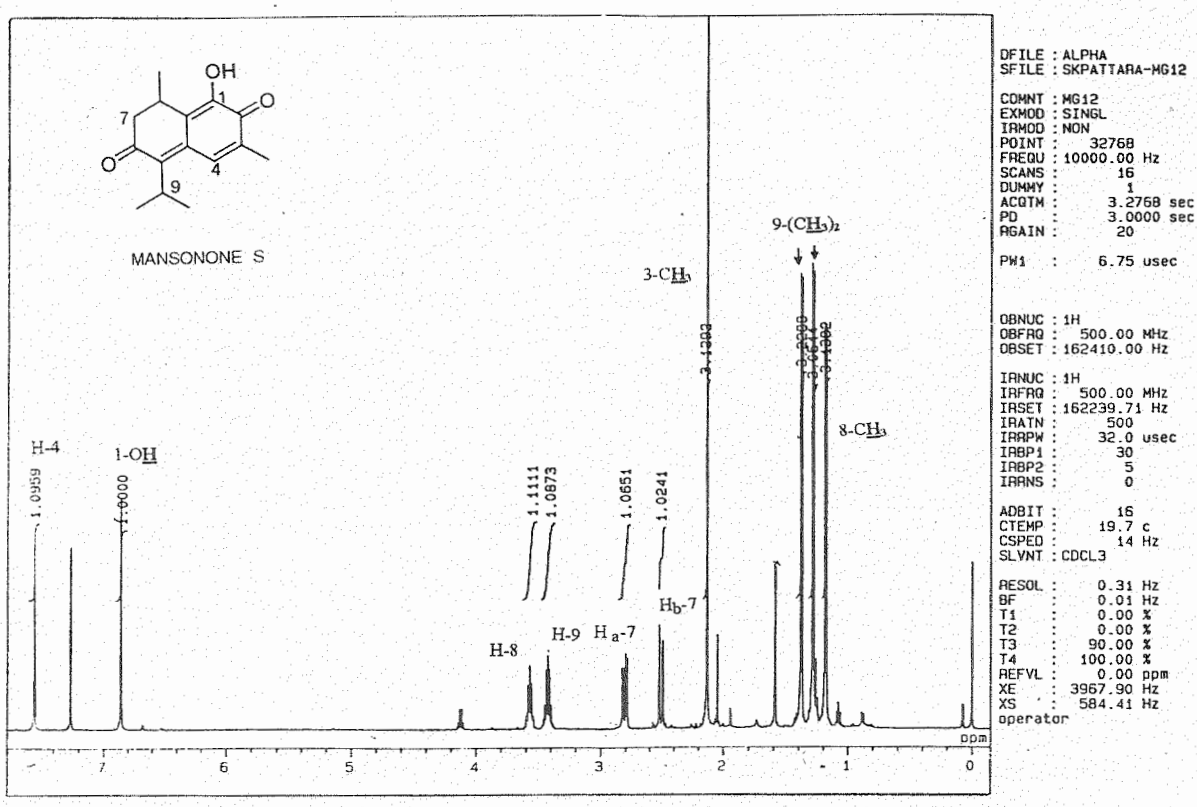


Fig 3.125 The ^{1}H-NMR spectrum of Compound **16**

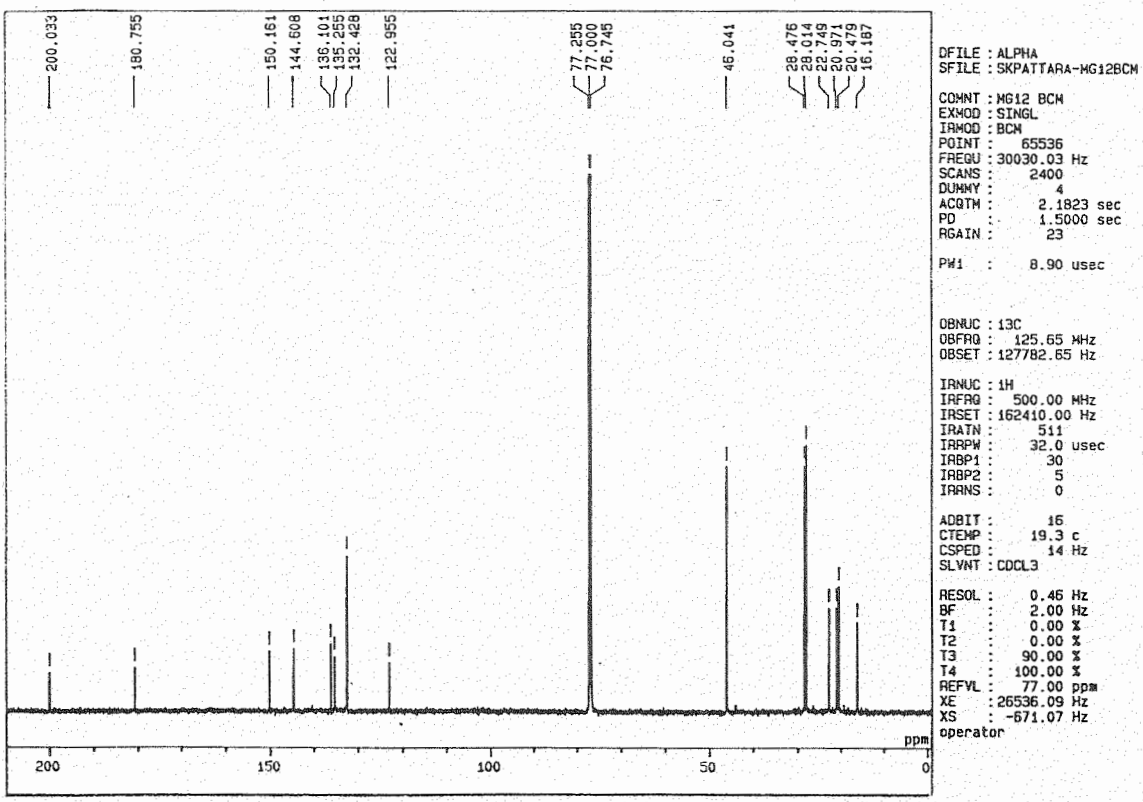


Fig 3.126 The ^{13}C-NMR spectrum of Compound **16**

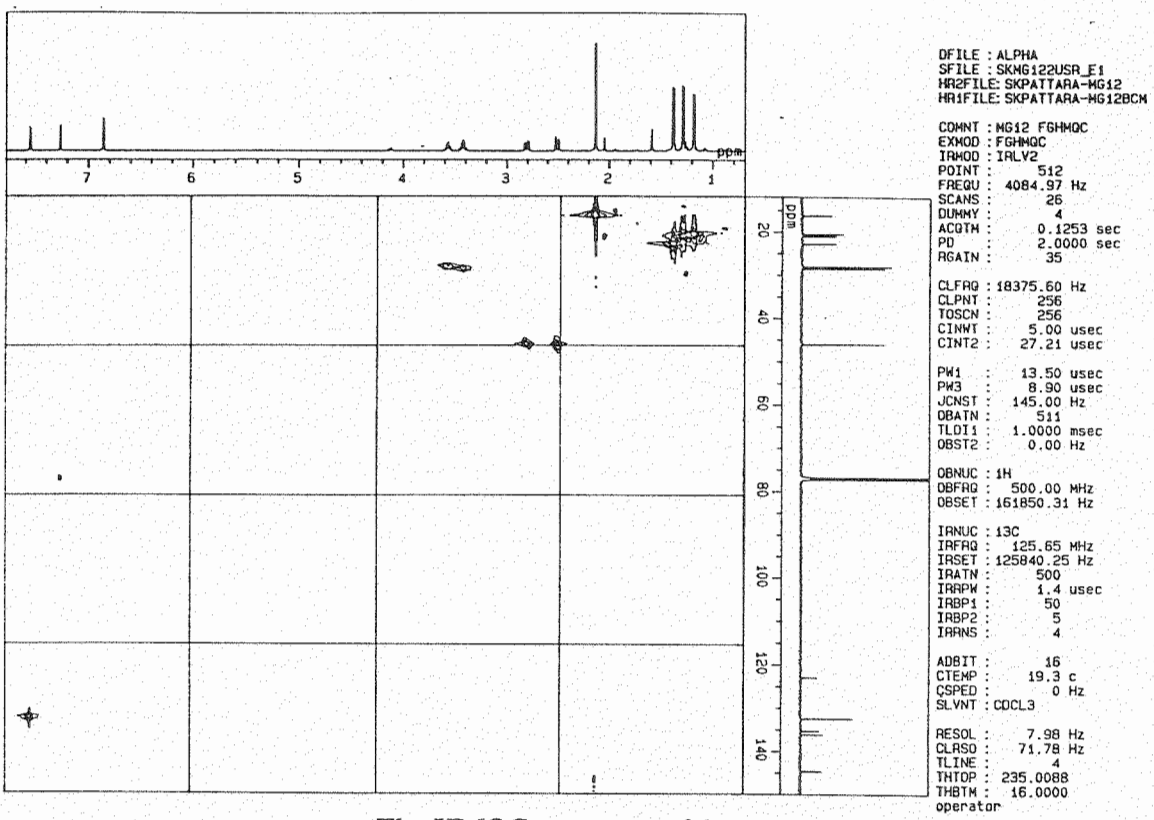


Fig 3.127 The HMQC spectrum of Compound 16

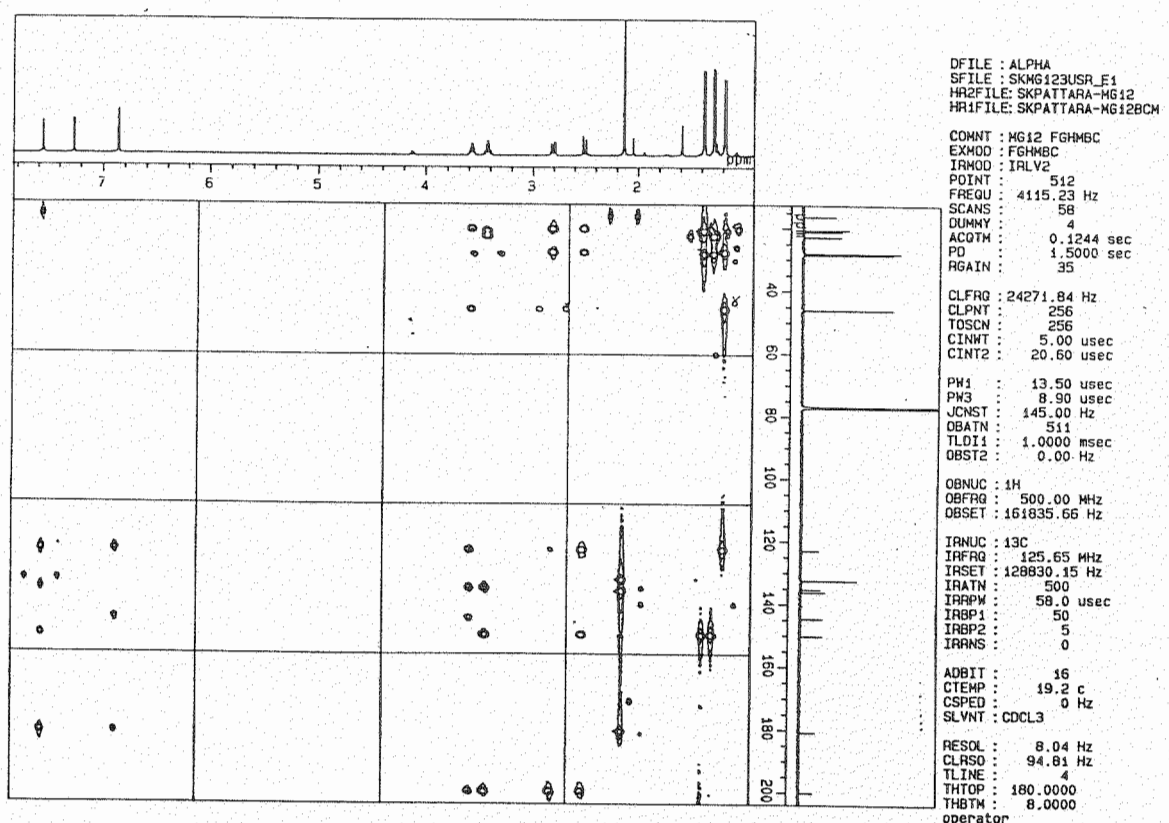
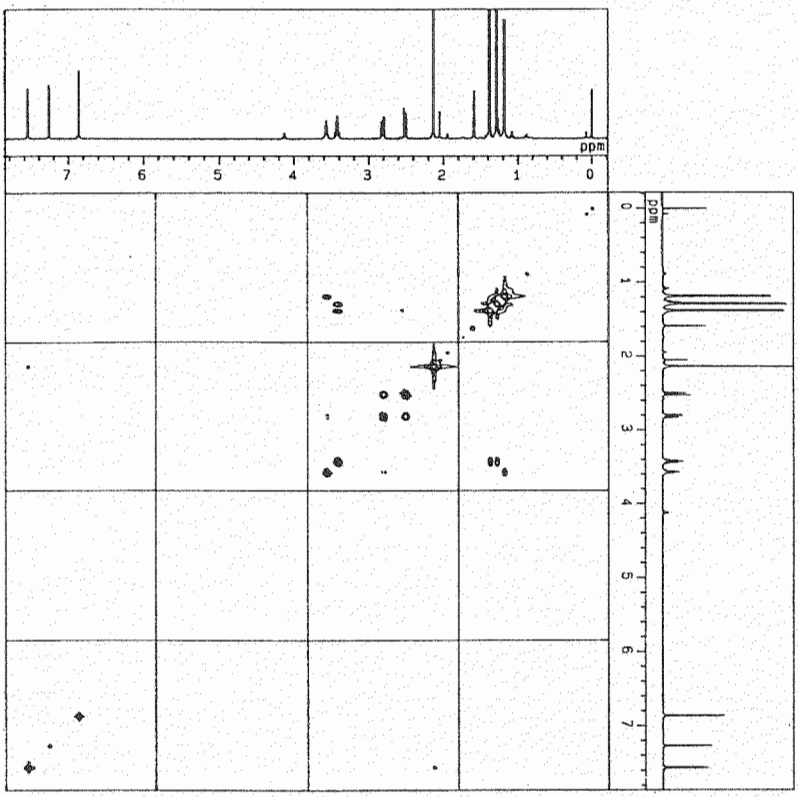


Fig 3.128 The HMBC spectrum of Compound 16

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HR2FILE: SKPATTARA-MG12
HR1FILE: SKPATTARA-MG12

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EXMOD : FGCOSYP
IRMOD : NON
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FREQU :  4042.04 Hz
SCANS :        4
DUMMY :        4
ACQTM :   0.1267 sec
PD    :   1.5000 sec
RGAIN :       21

CLFRQ :  4042.04 Hz
CLPNT :      512
TOSCN :      256
CINWT :    5.00 usec
CINTV :  247.40 usec

PW1   :   13.50 usec
PW2   :   27.00 usec
PI1   : 120.0000 msec
PI2   :   1.0000 msec


OBNUC : 1H
OBFRQ :   500.00 MHz
OBSET : 161828.64 Hz

IRNUC : 1H
IRFRQ :   500.00 MHz
IRSET : 162239.71 Hz
IRATN :      500
IRRPW :     32.0 usec
IRBP1 :       30
IRBP2 :        5
IRRNS :        0

ADBIT :       16
CTEMP :     19.5 c
CSPED :        0 Hz
SLVNT : CDCL3

RESOL :     7.89 Hz
CLRSO :     7.89 Hz
TLINE :        4
THTOP :  45.1612
THBTM :   3.0000
operator
```

Fig 3.129 The COSY spectrum of Compound **16** .

PART IV

3.12 Preliminary Bioassay Screening Results of the Extracts from the Roots, Barks and Leaves

According to the successful isolation of the active principles from the heartwoods of *M. gagei*, the other parts of this plant: roots, barks and leaves, were extracted with appropriate solvents following the procedure described in Chapter 2. All extracts, DCM-R, EtOAc-R, MeOH-R, DCM-B, EtOAc-B, MeOH-B, Hex-L, DCM-L, EtOAc-L and MeOH-L, were consequently preliminarily screened utilizing the methodology the same as those for the heartwood extracts. The results are shown in Table 3.30-3.32.

Table 3.30 Antifungal, larvicidal and radical scavenging activities of the extracts from the roots, barks and leaves of *M.gagei*.

Extract	*Cladosporium cucumerinum*[a]	*Candida albicans*[a]	*Aedes aegypti*[b]	DPPH[a]
DCM-R	+	+	-	-
EtOAc-R	+	+	-	-
MeOH-R	+	-	-	-
DCM-B	+	-	-	+
EtOAc-B	-	-	-	+
MeOH-B	-	-	-	-
Hex-L	-	-	-	+
DCM-L	-	-	-	-
EtOAc-L	-	-	-	-
MeOH-L	-	-	-	-

[a] : tested amount: 100 μg of crude extract
[b] : tested amount: 500 ppm of crude extract
+ : active
- : not active

Table 3.31 The antithrombin activity of the extracts from the roots, barks and leaves of *M. gagei*

Extract	% Inhibition
DCM-R	49.5
EtOAc-R	80.1
MeOH-R	57.61
DCM-B	86.2
EtOAc-B	74.4
MeOH-B	53.2
Hex-L	44.6
DCM-L	59.6
EtOAc-L	46.2
MeOH-L	67.9

Table 3.32 The results of anticellines test of the extracts from the roots, barks and leaves of *M. gagei*

Compounds	% Activities				
	Mouse Leukemia	Human Myeloma	Human Colon Adenocarcinoma	Human Leukemia	Human Breast Carcinoma
DCM-R	99.6	100	97.0	98.9	100
EtOAc-R	100	99.9	94.4	99.8	85.5
MeOH-R	n.t.	n.t.	n.t.	n.t.	n.t.
DCM-B	4.7	56.8	63.1	87.4	87.7
EtOAc-B	40.8	97.6	67.0	100.0	77.6
MeOH-B	0.0	17.7	45.2	36.4	0.0
Hex-L	n.t.	n.t.	n.t.	n.t.	n.t.
DCM-L	n.t.	n.t.	n.t.	n.t.	n.t.
EtOAc-L	94.1	81.2	18.1	92.3	79.4
MeOH-L	0.0	19.2	54.6	62.3	0.0

n.t = not test

From the preliminary screening test results, the ethyl acetate extract of the roots showed intriguing results. This fraction demonstrated strongly fungitoxicity against both *Cladosporium cucumerinum* and *Candida albicans*. In addition, it showed the antithrombin activity and inhibition a variety of cancer cell-lines. The dichloromethane extract of the roots exhibited the same tendency as the ethyl acetate extract of the roots except for lower antithrombin activity. Moreover, the dichloromethane extract of barks displayed antifungal activity against only *C. cucumerinum* as well as radical scavenging property toward DPPH and high inhibition against Human Leukemia and Human Breast Carcinoma cell-lines. According to these activities, the study on chemical constituents of roots, barks and leaves of this plant along with their bioactivities are still attractive to discover new therapeutic agents.

CHAPTER IV

CONCLUSION

Previous research of the heartwoods results in the isolation of three new coumarins, mansorin A-C, as well as three known mansonones, C, G and H. This woek involves continuing studies on the chemical constituents from *Mansonia gagei* and determination of their biological activities. Studies on the heartwood extracts have now led to the isolation of ten compounds that had not been isolated previously from this plant. Twelve compounds were obtained from the dichloromethane extract and their structures were established on the basis of physical properties and spectroscopic experiments as mansorin A (**1**), mansorin B (**2**), mansorin C (**3**), mansonone N (**4**), mansonone O (**5**), mansonone P (**6**), mansonone Q (**7**), mansonone C (**8**), mansonone E (**9**), mansonone G (**10**), mansonone H (**11**) and dehydrooxoperezinone (**12**). Among them, mansonones N, O, P and Q are new naturally occurring compounds. In addition, the ethyl acetate extract furnished 3-methoxy-4,5-dihydroxybenzaldehyde (**13**), mansoxetane (**14**) together with the same compounds as isolated from dichloromethane extract. Two additional new compounds, mansonones R (**15**) and S (**16**), were obtained from the methanolic extract.

All isolated substances from the heartwoods of *M. gagei* including their physical properties were summarized in Table 4.1.

Table 4.1 Structures of the isolated compounds from the heartwood extracts of *M. gagei* and their physical properties

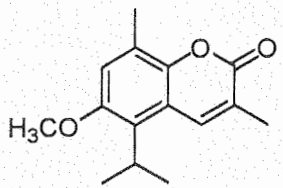
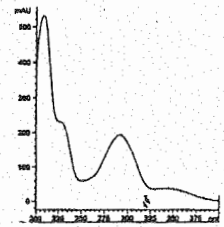
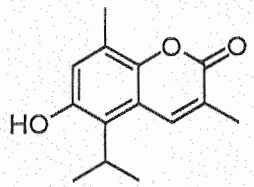
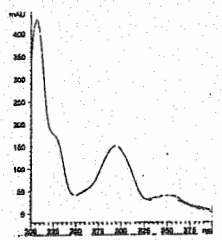
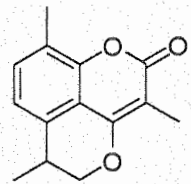
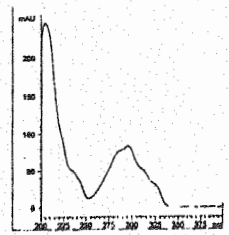
Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	UV spectrum	$[\alpha]_D$
Mansorin A **(1)**	yellow crystal	$C_{15}H_{18}O_3$		134-135		-
Mansorin B **(2)**	pale yellow powder	$C_{14}H_{16}O_3$		202-204		-
Mansorin C **(3)**	white cyrstal	$C_{14}H_{14}O_3$		151-153		+18.9° c=1.00, $CHCl_3$ Temp. 18°C

Table 4.1 (Cont.)

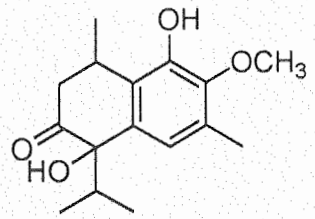
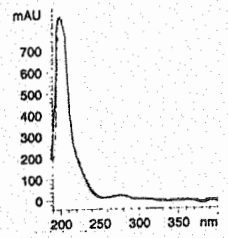
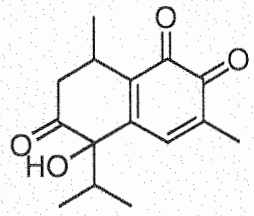
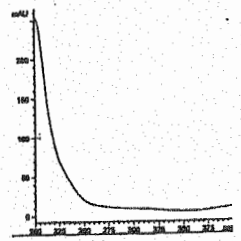
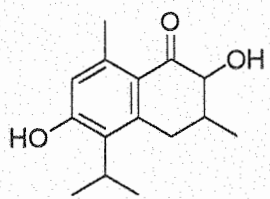
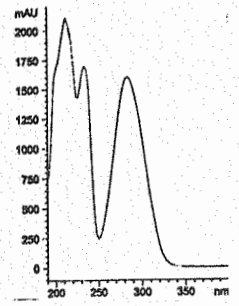
Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	UV spectrum	$[\alpha]_D$
Mansonone N **(4)**	colorless crystal	$C_{16}H_{22}O_4$		144-145		+30.0° c=0.20, $CHCl_3$ Temp. 20°C
Mansonone O **(5)**	violet orthorhombic	$C_{15}H_{18}O_4$		125-128		-76.0° c=0.10, $CHCl_3$ Temp. 20°C
Mansonone P **(6)**	pale yellow powder	$C_{15}H_{20}O_3$		175-176		+13.5° c=0.20, $CHCl_3$ Temp. 20°C

Table 4.1 (Cont.)

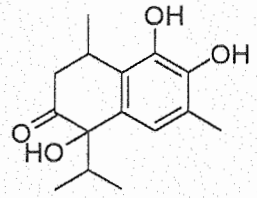
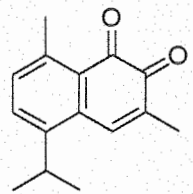
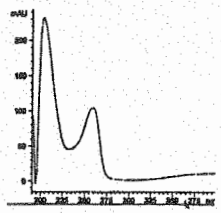
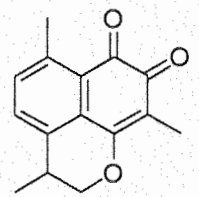
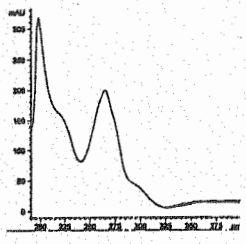
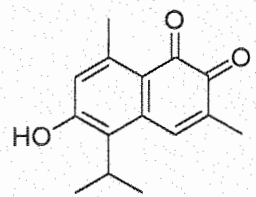
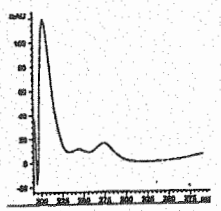
Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	UV spectrum	$[\alpha]_D$
Mansonone Q **(7)**	pale yellow powder	$C_{15}H_{20}O_4$		105-106	-	+9.5° c=0.10, $CHCl_3$ Temp. 20°C
Mansonone C **(8)**	Orange needle	$C_{15}H_{16}O_2$		113-115		-
Mansonone E **(9)**	orange solid	$C_{15}H_{14}O_3$		118-120		-
Mansonone G **(10)**	orange powder	$C_{15}H_{16}O_3$		204-207		-

Table 4.1 (Cont.)

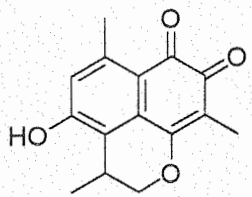
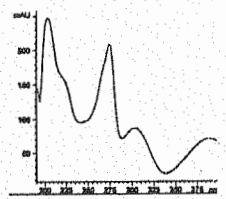
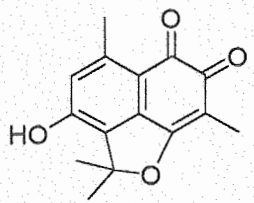
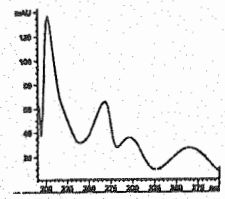
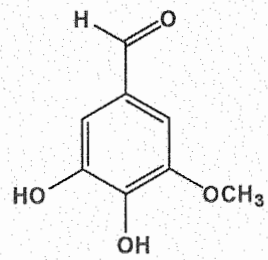
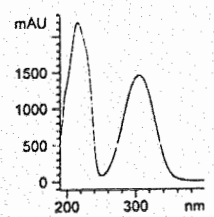
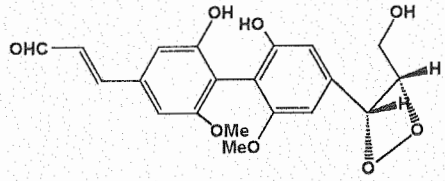
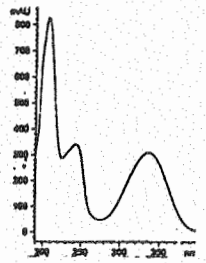
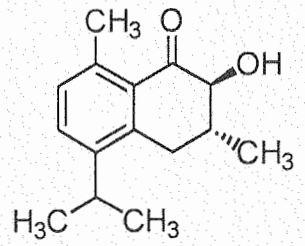
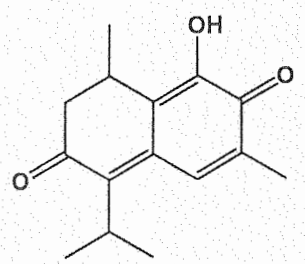
Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	UV spectrum	$[\alpha]_D$
Mansonone H **(11)**	red platelet	$C_{15}H_{14}O_4$		Decomposed at 270-272		-
Dehydrooxo-perezinone **(12)**	yellow powder	$C_{15}H_{14}O_4$		230-232		-
3-methoxy-4,5-dihydroxybenzaldehyde **(13)**	brown solid	$C_8H_8O_4$		-		-

Table 4.1 (Cont.)

Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	UV spectrum	$[\alpha]_D$
Mansoxetane **(14)**	pale brown solid	$C_{20}H_{20}O_8$		-		-12.8° c=0.27, $CHCl_3$ Temp. 15°C
Mansonone R **(15)**	pale yellow powder	$C_{15}H_{20}O_2$		-	-	-
Mansonone S **(16)**	orange amorphous	$C_{15}H_{18}O_3$		-	-	-

It can be seen from this research work that all isolated compounds contained the same basic skeleton belonging to cadinane type skeleton. Thus, the study of the role and biosynthesis of these compoounds would be an interesting research project for the future.

As part of on going investigation of biologically active compounds, some isolated compounds were tested in a variety of bioactivity assays. The results indicated that mansonones or 1,2-naphthoquinones showed higher activity than coumarins in Brine Shrimp Cytotoxicity test. The same trend was also visualized for anticancer test. Mansonones C **(8)** and G **(10)** showed high toxicity in Brine Shrimp Cytotoxicity test and high % inhibition value for a number of cancer cell lines whereas mansorinsA **(1)** and C **(3)** showed medium and low activities, respectively, for Brine Shrimp Cytotoxicity test and showed low %inhibition value for the same cancer cell lines except for mansorin A that had high %inhibition specifically for Human Myeloma. In addition, mansonones C **(8)** and E **(9)** showed fungitoxicity against *Cladosporium cucumerinum* and *Candida albicans* which were previously reported in the literature.[18-21] Mansorins A **(1)** and B **(2)** also contained antifungal activity against *C. cucumerinum*. This is the first report of larvicidal activity (*Aedes egypti*) of mansonone C **(8)**.

For the antioxidatnt properties of 1,2-naphthoquinones, it is very surprising that only mansonone N **(4)** including 3-methoxy-4,5-dihydroxybenzaldehyde **(13)** showed radical scavenging properties toward DPPH. Moreover, mansonones G **(10)**, H **(11)** and dehydrooxoperezinone **(12)** showed the potent tendency for antithrombin agent. According to a number of compounds obtained from the heartwood of *M. gagei* as well as those compounds showed the significant activities on a variety of bioassays, the root, bark and leaf of the same plant were subject to preliminary screening test. The root extracts possessed an interesting activity on antifungal and anticancer assays. In addition, the dichloromethane extract of bark had potential importance for antithrombin agent. These results call for further work on synthesizing additional mansonone-type derivatives including studying the chemical constituents in other parts of *M. gagei* with the aim of obtaining the lead compounds that are more potent and selective to above-mentioned bioactivities.

REFERENCES

1. Pongboonrod, S. *Mai Tet Muang Thai*, Bangkok: Kasembunnakich Press, **1976**, 162.
2. Puntumchai, A. *Chemical constituents of the heartwoods of Mansonia gagei and their biological activity*. Master's thesis, Department of Chemistry, Chulalongkorn University, **1997**.
3. Akavipak, K. *Pun Mai Ni Suan Pa Sirikit Pak Klang* (Ratchaburee), Chutima Press, **1992**, 40.
4. Santisuk, T. *Suan Tid Lad Da Puck Sa Pun*, Bangkok: Amarin Printing, **1995**, 223.
5. Marini Bettolo, G.B.; Casinovi, C. G.; Galeffi, C. A new class of quinones: sesquiterpenoid quinones of *Mansonia altissima* Chev., *Tetrahedron Lett.*, **1965**, *52*, 4857-4864.
6. Tanaka, N.; Yasue, M.; Imamura, H. The quinonoid pigments of *Mansonia altissima* wood, *Tetrahedron Lett.*, **1966**, *24*, 2767-2773.
7. Shimada, K.; Yasue, M.; Imamura, H. A new pigment from *Mansonia altissima* wood, *Mokuzai Gakkaishi*, **1967**, *13*, 126.
8. Galeffi, C.; Miranda delle Monache, E.; Casinovi, C. G.; Marini Bettolo, G. B. A new quinone from heartwood of *Mansonia altissima* Chev., *Tetrahedron Lett.*, **1969**, *40*, 3583-3584.
9. Allgeier, H.; Weiss, Ek.; Reichstein, T. Cardenolides from *Mansonia altissima* seeds, *Helv. Chim. Acta*, **1967**, *50*, 431-455.
10. Allgeier, H.; Weiss, Ek.; Reichstein, T. Cardenolides from *Mansonia altissima* seeds 2. Structure of mansonin and strophothevoside, *Helv. Chim. Acta*, **1967**, *50*, 456-462.
11. Tiew, P.; Puntumchai, A.; Kokpol, U.; Chavasiri, W. Coumarins from the heartwoods of *Mansonia gagei* Drumm., *Phytochemistry*, **2002**, *60*, 773-776.
12. Chen, C.–M.; Chen, Z.–T.; Hong, Y.–L. A mansonone from *Helicteres angustifolia*, *Phytochemistry*, **1990**, *29*, 980-982.
13. Kim, J.–P.; Kim, W.–G.; Koshino, H.; Jung, J.; Yoo, I.–D. Sesquiterpene *o*-naphthoquinones from the root bark of *Ulmus davidiana*, *Phytochemistry*, **1996**, *43*, 425-430.
14. Letcher, R. M.; Shirley, I. M. *o*-Naphthoquinones from the heartwood of *Azanza garckeana*, *Phytochemistry*, **1992**, *31*, 4171-4172.

15. Neelakantan, S.; Rajagopalan, V.; Raman, P. V. Thespesone and thespone, two new mansonones of heartwood of *Thespesia populnea* Sol. Ex Corr. (fam. Malvaceae), *Indian J. Chem.*, **1983**, *22B*, 95-98.

16. Milbrodt, M.; Konig, W. A.; Hausen, B. M. 7-Hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[1,8-b,c]pyran-4,8-dione from *Thespesia populnea*, *Phytochemistry*, **1997**, *45*, 1523-1525.

17. Kazuhito, O.; Zhao, J.; Higa, M.; Yogi, S. Studies on the constituents of *Aristolochia liukiuensis* II *Bull. Coll. Sci. Univ. Rynkyns*, **1992**, *54*, 17-28.

18. Overeem, J. C.; Elgersma, D. M. Accumulation of mansonones E and F in *Ulmus hollandica* infected with *Ceratocystis ulmi*, *Phytochemistry*, **1970**, *9*, 1949-1952.

19. Dumas, M. T.; Strunz, G. M.; Hubbes, M.; Jeng, R. S. Isolation and identification of six mansonones from *Ulmus americana* infected with *Ceratocystis ulmi*., *Experientia*, **1983**, *39*, 1089-1090.

20. Duchesne, L. C.; Jeng, R. S.; Hubbes, M. Accumulation of phytoalexins in *Ulmus americana* in response to infection by a nonaggressive and an aggressive strain of *Ophiostoma ulmi.*, *Can. J. Bot.*, **1985**, *63*, 678-680.

21. Duchesne, L.C.; Hubbes, M.; Jeng, R.S. Mansonones E and F accumulation in *Ulmus pumila* resistant to Dutch elm disease, *Can. J. For. Res.*, **1986**, *16*, 410-412.

22. Villamil, S. F.; Dubin, M.; Galeffi, C.; Stoppani, A. O. M. Effect of mansonones on lipid peroxidation, P450 monooxygenase activity, and superoxide anion generation by rat liver microsomes, *Biochem. Pharmacol.*, **1990**, *40*, 2343-2351.

23. Solis, P.N.; Wright, C.W.; Anderson, M.M.; Gupta, M.P.; Phillipson, J.D. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Med.*, **1993**, *59*, 250-252.

24. Meyer, B.N.; Ferrigni, N.R.; Putnam, J.E.; Jacobson, L.B.; Nichols, D.E.; McLoughlin, J.L. Brine shrimp: a convenient general bioassay for active plant constituents, *Planta Med.*, **1982**, *45*, 31-34.

25. Hufford, C.D.; Clark, A.M. Discovery and development of new drugs for systemic opportunistic infections. In *Studies in Natural Products Chemistry*, Atta-ur-Rahman, ed., Elsevier, Amsterdam, **1988**, *2*, 421-452.

26. Rios, J. L.; Recio, M. C; Villar, A. Screening methods for natural products with antimicrobial activity: a review of the literature, *J. Ethnopharmacol.*, **1988**, *23*, 127-149.

27. Homans, A. L.; Fuchs, A. Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances, *J. Chromaotgr.*, **1970**, *51*, 327-329.

28. Rahalison, L.; Hamburger, M.; Monod, M; Frenk, E.; Hostettmann, K. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants, *Phytochem. Anal.*, **1991**, *2*, 199-203.

29. Ioset, J.-R.; Marston, A.; Gupta, M.P.; Hostettmann, K. Antifungal and larvicidal cordiaquinones from the roots of *Cordia curassavica*, *Phytochemistry*, **2000**, *53*, 613-617.

30. Cepleanu, F. *Validation and application of three bench-top bioassays for screening of crude plant extracts and subsequent activity-guided isolation.* Ph.D's thesis, Institute of Pharmacognosy and Phytochemistry, University of Lausanne. Switzerland, **1993**.

31. Cuendet, M.; Hostettmann, K.; Potterat, O.; Dyatmiko, W.. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei.*, *Helv. Chim. Acta.*, **1997**, *80*, 1144-1151.

32. Medeiros, J.; Macedo, M.; Contacia, J.; Nguyen, C.; Cunningham, G.; Miles, H. Antithrombin activity of medicinal plants of the Azores, *J. Ethnopharmacol.*, **2000**, *72*, 157-165.

33. Cory, A. H.; Owen, T. C.; Bartrop, J. A.; Cory, J. G. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture, *Cancer Comm.*, **1991**, *3*, 207-212.

34. Macias, F. A.; Fronczek, F. R.; Fischer, N. H. Menthofurans from *Calamintha ashei* and the absolute configuration of desacetylcalaminthone, *Phytochemistry*, **1989**, *28*, 79-82.

35. Pretsch, E.; Simon, W.; Seibl, J.; Clere, T. Tables of spectral data for structure determination of organic compound, 2nd ed., Springer-Verlag, **1989**, I132.

36. Oliver, R. W. A.; Rashman, R. M. Study of distinguishing features found in the mass spectra of 1,2- and 1,4- naphthoquinones, *J. Chem. Soc.* B, **1971**, *2*, 341-345.

37. Mann, J. *Chemical Aspects of Biosynthesis,* Oxford University Press, Walton Street, Oxford, **1994**, 32-38.

38. Parker, W.; Roberts, J. S. Sesquiterpene biogenesis, *Quart.Rev.*, **1967**, *21*, 331-335.

39. Morita, H.; Kishi, E.; Takeya, Koichi.; Itokawa, H. Biphenylneolignans from wood of *Eurycoma longifolia*, *Phytochemistry*, **1992**, *11*, 3993-3995.

APPENDIX

Crystal structure of mansonone N (4) A hemisphere of Bragg intensities were measured, at 293 K, on a Stoe IPDS equipped with graphite monochromatized MoK α radiation (λ = 0.71073Å). 13375 reflections were integrated up to $\sin\theta/\lambda$=0.66 $Å^{-1}$ of which 3389 were unique (R_{int} = 0.034). The intensities were corrected for Lorentz and polarization effects, but no absorption correction was deemed necessary. The space group was $P2_12_12_1$ and the lattice constants were a=8.905(2) Å, b=11.963(2) Å and c=13.595(3) Å. The structure was solved and refined, using SHELXTL, to R_1=0.036. The structure owes its cohesion to rather strong hydrogen bonds between the alcohol groups O-1 and O-5 and the ester group O-2 and the keto groups O-6. There are two intermolecular (O-1 - H-1 ··· O-6 2.08(2) Å, 152.5(2)° and O-5 – H-5 ··· O-6 2.03(2) Å, 151.4(2)°) and two intramolecular hydrogen bonds per molecule. These hydrogen bonds result in endless chains of molecules along the ***a*** lattice vector.

Crystal structure of mansonone O (5) Space group $P2_12_12_1$, a = 6.420(0), b = 7.791 (2), c = 27.127(3), V = 1356.9(4), Dx 1.284g/cm3, Z= 4. X-ray diffraction data of an approximate 0.25 x 0.47 x 0.23 mm crystal was collected at room temperature on a Bruker SMART CCD area detector. The collected data was reduced using SAINT and empirical absorption correction was performed using SADABS. A total of 10,067 reflections were measured of which 3,899 ($R_{(int)}$ = 0.0313) reflections were unique. The structure was solved by direct methods using SHELXS. The non-hydrogen atoms were refined by full-matrix least-squares method with anisotropic displacement parameters using SHELX97. Hydrogen atoms were found from difference Fourier maps and included in the refinement with isotropic displacement parameters. The final refinement gave R_1 = 0.0782 and wR_2 = 0.1969 [$I > 2\sigma(I)$].

VITA

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