Cytotoxic Activity Against Tumour Cells and Free Radical Scavenging Activity of Zingiberaceous Rhizomes Used as Spices

Sariga Zaeoung

Master of Pharmacy Thesis in Pharmaceutical Sciences

Prince of Songkla University

Thesis Title	Cytotoxic Activity Against Tumour Cells and Free Radical Scavenging Activity of Zingiberaceous Rhizomes Used as Spices
Author	Miss Sariga Zaeoung
Major Program	Pharmaceutical Sciences
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ชื่อวิทยานิพนธ์ ฤทธิ์ต้านเซลล์มะเร็งและต้านอนุมูลอิสระของเหง้าพืชวงศ์ขิงที่ใช้เป็นเครื่องเทศ
ผู้เขียน สาริกา แซ่อึ้ง
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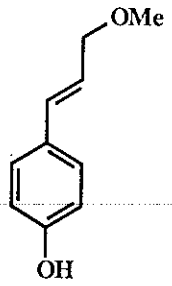
บทคัดย่อ

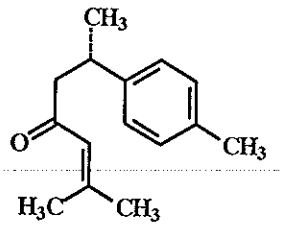
ได้นำสารสกัดเมธานอล สารสกัดน้ำและน้ำมันหอมระเหยของเหง้าพืชสดของข่า (*Alpinia galanga*) กระชาย (*Boesenbergia pandurata*) ขมิ้นชัน (*Curcuma longa*) เปราะหอม (*Kaempferia galanga*) และขิง (*Zingiber officinale*) มาทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay และทดสอบฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งเต้านม MCF7 (breast adenocarcinoma cell line) และเซลล์มะเร็งลำไส้ใหญ่ LS174T (colon adenocarcinoma cell line) พบว่าสารสกัดเมธานอลจากเหง้าขมิ้นชัน ขิง และข่า มีฤทธิ์ต้านอนุมูลอิสระที่ดีกว่าสารสกัดน้ำและน้ำมันหอมระเหย โดยมีค่าความเข้มข้นที่กำจัดอนุมูลอิสระได้ 50 % (EC_{50}) เป็น 9.7, 35.6 และ 57.7 ไมโครกรัม/มิลลิลิตร ตามลำดับ ส่วนการทดสอบฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง MCF7 และ LS174T ด้วยวิธี sulphorhodamine B (SRB) assay พบว่า น้ำมันหอมระเหยจากเหง้าพืชสดทั้ง 5 ชนิดและสารสกัดเมธานอลจากขมิ้นชันมีค่าความเข้มข้นที่ยับยั้งการเพิ่มจำนวนของเซลล์มะเร็ง 50 % (IC_{50}) น้อยกว่า 50 ไมโครกรัม/มิลลิลิตร ส่วนสารสกัดเมธานอลจากขิงมี IC_{50} อยู่ในช่วง 75.0-80.0 ไมโครกรัม/มิลลิลิตร ทั้งนี้ พบว่า น้ำมันหอมระเหยจากเหง้ากระชายมีค่า IC_{50} ต่อเซลล์ LS174T ดีที่สุด คือ 12.0 ไมโครกรัม/มิลลิลิตร และน้ำมันหอมระเหยจากเหง้าขิงมีค่า IC_{50} ต่อเซลล์ MCF7 ดีที่สุด คือ 14.2 ไมโครกรัม/มิลลิลิตร จากการวิเคราะห์องค์ประกอบทางเคมีด้วย GC/MS พบว่าน้ำมันหอมระเหยจากเหง้าข่า กระชาย ขมิ้นชัน เปราะหอม และขิง ประกอบด้วยสารอย่างน้อย 13, 4, 22, 12 และ 13 ชนิด ตามลำดับ สารหลักที่มีปริมาณมากที่สุดในน้ำมันหอมระเหยจากเหง้าข่า กระชาย ขมิ้นชัน เปราะหอมและขิง คือ *trans*-3-acetoxy-1,8-cineole, camphor, ar-turmerone, ethyl cinnamate และ geranial (*E*-citral) ตามลำดับ

จากการศึกษาทางพฤกษเคมีของสารสกัดเมธานอลจากข่า ขมิ้นชันและขิง สามารถแยกสารบริสุทธิ์ ชื่อ *p*-coumaryl-9-methyl ether ออกมาจากสารสกัดเมธานอลของข่า ซึ่งเป็นสารใหม่ที่ไม่เคยมีรายงานการตรวจพบในเหง้าข่าและพืชอื่นๆ มาก่อน ส่วนสารสกัดเมธานอลของขมิ้นชันสามารถแยกสารบริสุทธิ์ได้ 4 ชนิด คือ ar-turmerone, curcumin, demethoxycurcumin และ

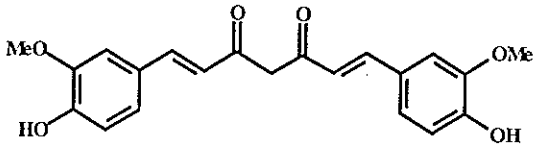
bisdemethoxycurcumin และจากสารสกัดเมธานอลของขิง สามารถแยกสารบริสุทธิ์ได้ 3 ชนิด คือ 6-shogaol, 6-dehydrogingerdione (หรือเรียกอีกชื่อหนึ่งว่า 1-dehydrogingerdione) และ 6-gingerol จากนั้นได้นำสารบริสุทธิ์ที่แยกได้ทั้งหมดมาทดสอบฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง LS174T และ MCF7 พบว่า curcumin มีฤทธิ์ต้านอนุมูลอิสระดีที่สุด โดยมีค่า EC_{50} เป็น 2.0 ไมโครกรัม/มิลลิลิตร ส่วน demethoxycurcumin มีฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง LS174T ดีที่สุด โดยมีค่า IC_{50} เท่ากับ 0.8 ไมโครกรัม/มิลลิลิตร ในขณะที่ 6-shogaol มีฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง MCF7 ดีที่สุด โดยมี IC_{50} เท่ากับ 1.7 ไมโครกรัม/มิลลิลิตร ข้อมูลที่ได้จากการศึกษาในครั้งนี้เป็นข้อมูลเบื้องต้นที่แสดงถึงศักยภาพทางยาของเหง้าพืชทั้ง 5 ชนิด ซึ่งควรจะได้นำไปศึกษาวิจัยขั้นสูงเพื่อใช้ป้องกันและรักษาโรคมะเร็งต่อไป



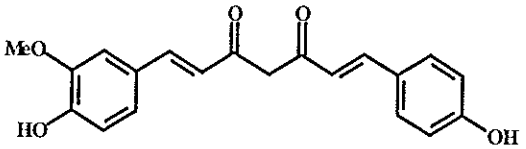
AGM1 (*p*-Coumaryl-9-methyl ether)



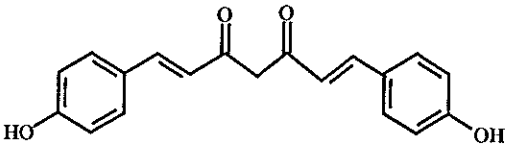
CLM01 (ar-Turmerone)



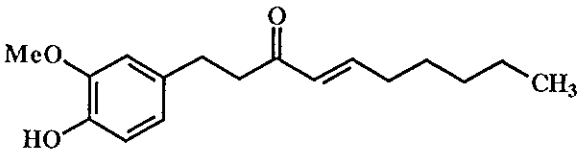
CLM02 (Curcumin)



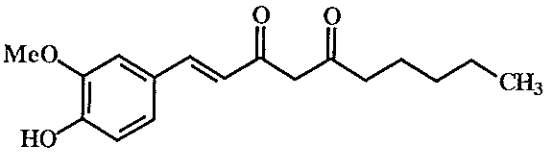
CLM03 (Demethoxycurcumin)



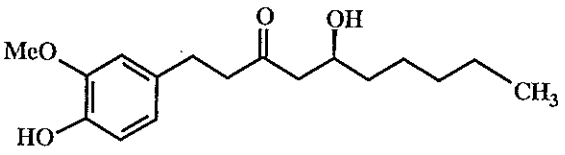
CLM06 (Bisdemethoxycurcumin)



ZOM0 (6-Shogaol)



ZOM1 (6-Dehydrogingerdione
or 1-Dehydrogingerdione)



ZOM3 (6-Gingerol)

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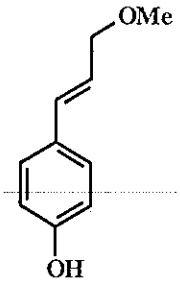
Abstract

Methanol extracts, water extracts and volatile oils of the fresh rhizomes of *Alpinia galanga* (greater galanga), *Boesenbergia pandurata* (fingerroot), *Curcuma longa* (turmeric), *Kaempferia galanga* (proh hom) and *Zingiber officinale* (ginger) have been assessed for free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and cytotoxic activity against MCF7 (breast adenocarcinoma cell line) and LS174T (colon adenocarcinoma cell line). Methanol extracts of the fresh rhizomes from *C. longa*, *Z. officinale* and *A. galanga* exhibited pronounced radical scavenging activity with EC_{50} values of 9.7, 35.6 and 57.7 μg/ml, respectively, whereas water extracts and volatile oils of the five plants showed weak activity. In cytotoxic activity assay against MCF7 and LS174T by SRB (sulforhodamine B) assay, it was found that the volatile oils of five fresh rhizomes and the methanol extract of *C. longa* were active against MCF7 and LS174T with IC_{50} values less than 50 μg/ml. The methanol extract of *Z. officinale* was active against the two cell lines with IC_{50} values in the range of 75.0-80.0 μg/ml. The volatile oil from *B. pandurata* was found to be the most active against LS174T with IC_{50} value of 12.0 μg/ml and the volatile oil from *Z. officinale* was the most active against MCF7 with IC_{50} value of 14.2 μg/ml. The results suggested that cytotoxic compounds resided in the volatile oils. Upon GC/MS analysis, the oils of *A. galanga* (AGV), *B. pandurata* (BPV), *C. longa* (CLV), *K. galanga* (KGV) and *Z. officinale* (ZOV) contained at least 13, 4, 22, 12 and 13 compounds, respectively. *Trans*-3-acetoxy-1,8-cineole, camphor, ar-turmerone, ethyl cinnamate and geranial (*E*-citral) were detected as main compounds in AGV, BPV, CLV, KGV and ZOV, respectively.

Phytochemical study of the methanol extract of *A. galanga* led to isolation of *p*-coumaryl-9-methyl ether. This is the first report of naturally occurring of this compound. Four compounds, ar-turmerone, curcumin, demethoxycurcumin and bisdemethoxycurcumin were isolated from the methanol extract of *C. longa*. 6-shogaol, 6-dehydrogingerdione (also known as 1-dehydrogingerdione) and 6-gingerol were isolated from the methanol extract of *Z. officinale*. These eight isolated compounds were further tested for free radical scavenging activity and tested against human cancer cell lines LS174T and MCF7. Curcumin was the most potent compound for free radical scavenging activity with EC_{50} value of 2.0 μg/ml. Demethoxycurcumin was the most active compound against LS174T with IC_{50} value of 0.8 μg/ml and 6-shogaol was the most potent compound against MCF7 with IC_{50} value of 1.7 μg/ml. The obtained results reveal medicinal efficacy of the rhizomes of these five Zingiberaceous spices and warrant further study to determine whether they could be of beneficial for the prevention and treatment of cancer.



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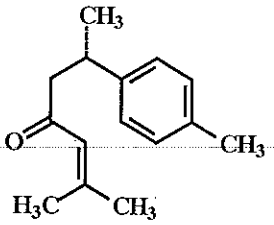

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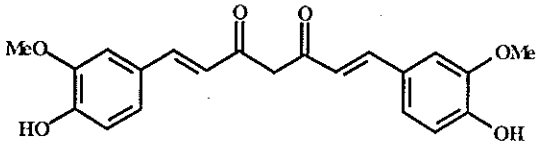
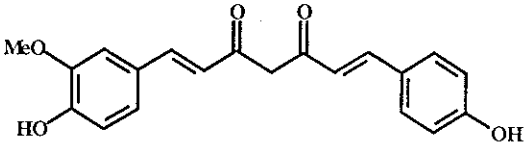


CLM02 (Curcumin)



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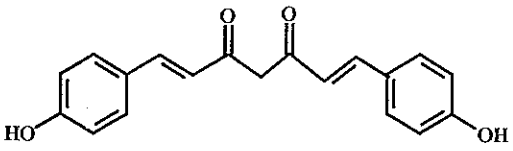

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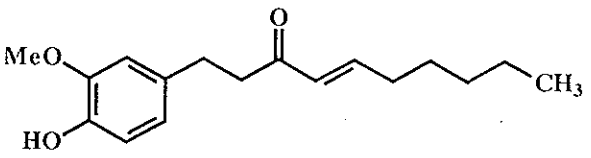

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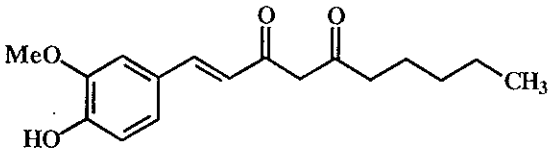
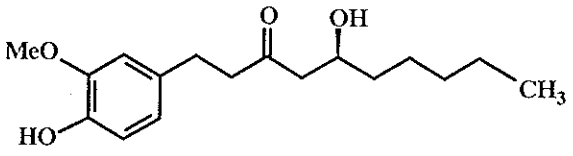


ZOM1 (6-Dehydrogingerdione
or 1-Dehydrogingerdione)



ZOM3 (6-Gingerol)

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All of these thoughts are deeply seated in my mind forever.

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ABBREVIATIONS AND SYMBOLS

amu	=	atomic mass unit
BHT	=	butylated hydroxytoluene
br.	=	broad (for NMR spectra)
br.d	=	broad doublet (for NMR spectra)
br.dq	=	broad doublet of quartets (for NMR spectra)
br.m	=	broad multiplet (for NMR spectra)
br.s	=	broad singlet (for NMR spectra)
br.t	=	broad triplet (for NMR spectra)
c	=	concentration (for optical rotations)
^{o}C	=	degree Celsius
$CDCl_3$	=	deuterochloroform
^{13}C NMR	=	carbon-13 nuclear magnetic resonance
cm	=	centimetre
COSY	=	correlated spectroscopy (^{1}H-^{1}H COSY: ^{1}H-^{1}H coupling)
d	=	doublet (for NMR spectra)
D.B.E.	=	double bond equivalence (degree of unsaturation)
dd	=	doublet of doublets (for NMR spectra)
dt	=	doublet of triplets (for NMR spectra)
DMSO	=	dimethyl sulphoxide
DPPH	=	1,1-diphenyl-2-picrylhydrazyl
EC_{50}	=	concentration causing 50 % effective activity
EDTA	=	ethylenediamine tetraacetic acid
EtOH	=	ethanol
FAB-MS	=	fast-atom bombardment mass spectroscopy
g	=	gram
GC/MS	=	gas chromatography/mass spectrometry
HMBC	=	heteronuclear multiple-bond correlation

ABBREVIATIONS AND SYMBOLS (continued)

HMQC	=	heteronuclear multiple-quantum correlation
^{1}H NMR	=	proton nuclear magnetic resonance
hr	=	hour
HR-FABMS	=	high resolution fast-atom bombardment mass spectrometry
Hz	=	hertz
IC_{50}	=	concentration causing 50 % inhibitory effect
IR	=	infrared
IU	=	international unit
J	=	nuclear spin-spin coupling constant (in Hz)
KBr	=	potassium bromide
kg	=	kilogram
M	=	molar (concentration)
m	=	metre
m	=	multiplet (for NMR spectra)
MeOH	=	methanol
mg	=	milligram
MHz	=	megahertz
µg	=	microgram
µl	=	microlitre
min	=	minute
ml	=	millilitre
mm	=	millimetre
mM	=	millimolar
mol	=	mole
MS	=	mass spectroscopy
MW	=	molecular weight
m/z	=	mass to charge ratio

ABBREVIATIONS AND SYMBOLS (continued)

nM	=	nanomolar
nm	=	nanometre
NMR	=	nuclear magnetic resonance
2D NMR	=	two dimentional nuclear magnetic resonance
NOE	=	nuclear Overhauser effect (change of signal intensities during decoupling experiments)
OD	=	optical density
PBS	=	phosphate buffered saline
ppm	=	parts per million
q	=	quartet (for NMR spectra)
s	=	singlet (for NMR spectra)
SD	=	standard deviation
sec	=	second
SEM	=	standard error of the mean
SRB	=	sulphorhodamine B
t	=	triplet (for NMR spectra)
TCA	=	trichloroacetic acid
TLC	=	thin-layer chromatography
TMS	=	tetramethylsilane
UV	=	ultraviolet
w/w	=	weight/weight
δ	=	chemical shift (in ppm, for NMR spectra)
ε	=	molar absorptivity (for UV spectra)
λ	=	wavelength (for UV spectra)
ν	=	wavenumber (for IR spectra)

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Cancer is perhaps the most progressive and devastating disease posing a threat of mortality to the entire world despite significant advances in medical technology for its diagnosis and treatment (Pal, *et al.*, 2001). Environmental chemicals may be involved in the etiology of a variety of human cancer. The cause for the majority of human tumours has been attributed to exposure to environmental carcinogens, pollutants, pesticides, drugs, UV-radiation, and tobacco products. A nutritional deficit or surplus or the absence of preventive micronutrients in the diet can further increase the susceptibility for developing cancers. In addition, tumours induced by environmental pollutants may be prevented by dietary strategies. It is therefore plausible that an imbalance between the exposure to cancer-causing environmental factors and the dietary intake of preventive nutrients facilitates the initiation and growth of tumours (Verma, Goldin and Lin, 1998). All cells are exposed to oxidative stress, and thus oxidation, and free radicals, may be important in carcinogenesis at multiple tumour sites (Sies, 1997). Metastasis of cancer cells to distant sites is one of the major deciding factors in cancer outcome. In fact, prognosis of cancer is mainly determined by the invasiveness of the tumour and its ability to metastasize. Although there are several drugs available to control cancer growth in humans, there are no drugs presently available to specifically inhibit the metastasis of cancer cells (Menon, Kuttan and Kuttan, 1999). Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the process of multistage carcinogenesis (Pal, *et al.*, 2001). Dietary antioxidants are known to decrease the risk of many chronic diseases such as cancer and cardiovascular disorders. The antioxidant activity may be a result of one of the followings: specific scavenging of reactive free radicals or scavenging of oxygen containing compounds such as hydrogen peroxide or chelation to metals (Priyadarsini,

1997). Wide arrays of phenolic substances, particularly those present in dietary and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic effects. The majority of these naturally occurring phenolics retain anti-oxidative and anti-inflammatory properties, which appear to contribute to their chemopreventive activity (Pal, *et al.*, 2001).

The use of medicinal plants is based on the experience of many generations of physicians and traditional systems of medicine from different ethnic societies. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most cases. However, today it is necessary to provide scientific proof as to whether or not it is justified to use a plant or its active principles (Ammon and Wahl, 1991). Natural products from some plants, fungi, bacteria and other organisms continue to be used in pharmaceutical preparations either as pure compounds or as extracts. There is a great variety of compounds that can be extracted and characterized from plants (Araújo and Leon, 2001).

The Zingiberaceae is a well-known plant family in Southeast Asia and numerous of its species are being used in traditional medicine, which is found to be quite effective in the treatment of several diseases. The five common Zingiberaceous spices used in Thailand, i.e. *Alpinia galanga* (Greater galanga), *Boesenbergia pandurata* (Fingerroot), *Curcuma longa* (Turmeric), *Kaempferia galanga* (Proh hom) and *Zingiber officinale* (Ginger), are perennial herbs widely cultivated in Thailand and tropical regions of Asia. Although there have been reports concerning chemical constituents and some biological activities of these five species, only a few reports focused on cytotoxic activity against human tumour cells and antioxidation against free radical. It is obvious that further study to evaluate the activities of the extracts and isolate compounds responsible for these two activities would provide additional useful data on biological activities of these five plants.

1.2 Review of Literatures

1.2.1 Plants of the family Zingiberaceae

According to a taxonomic survey of the family Zingiberaceae in the world, 52 genera and 1,100 species have been reported (Mabberley, 1997), among which 11 genera and 44 species have been found in southern Thailand (Sirirugsa, 1989). They are classified into four tribes as follows:

1.2.1.1 Tribe Alpineae

A. Genus *Alpinia*

(1) *Alpinia conchigera* Griff.
(2) *A. galanga* (L.) Willd.
(3) *A. javanica* Bl.
(4) *A. mutica* Roxb.
(5) *A. nigra* (Gaert.) B.L. Burtt
(6) *A. oxymitra* Schum.
(7) *A. purpurata* (Vieill.) Schum.

B. Genus *Amomum*

(8) *Amomum aculeatum* Roxb.
(9) *A. biflorum* Jack.
(10) *A. hastilabium* Ridl.
(11) *A. rivale* Ridl.
(12) *A. testaceum* Ridl.
(13) *A. uliginosum* Koenig

C. Genus *Elettariopsis*

(14) *Elettariopsis curtisii* Bak.
(15) *E. smithiae* Kam
(16) *E.* sp. (aff. *triloba* Gagnep.) Loes.

D. Genus *Etlingera*

(17) *Etlingera littoralis* (Koenig) Giseke

(18) *E. maingayi* (Bak.) Smith

(19) *E. venusta* (Ridl.) R.M. Smith

1.2.1.2 Tribe Globbeae

E. Genus *Globba*

(20) *Globba albiflora* Ridl.

(21) *G. fasciata* Ridl.

1.2.1.3 Tribe Hedychieae

F. Genus *Boesenbergia*

(22) *Boesenbergia basispicata* Larsen ex Sirirugsa

(23) *B. curtisii* (Bak.) Schltr.

(24) *B. longipes* (King & Prain) Schltr.

(25) *B. plicata* (Ridl.) Holtt.

(26) *B. rotunda* (L.) Mansf.

(syn: *B. pandurata* (Roxb.) Schltr.)

G. Genus *Curcuma*

(27) *Curcuma* aff. *colorata* Val.

(28) *C. longa* L.

(29) *C. viridiflora* Roxb.

H. Genus *Hedychium*

(30) *Hedychium coronarium* Koen.

I. Genus *Kaempferia*

(31) *Kaempferia angustifolia* Rosc.

(32) *K. galanga* L.

(33) *K. parviflora* Wall.

(34) *K. pulchra* Ridl.

J. Genus *Scaphochlamys*

(35) *Scaphochlamys biloba* (Ridl.) Holtt.

(36) *S.* sp.

1.2.1.4 Tribe Zingibereae

K. Genus *Zingiber*

(37) *Zingiber chrysostachys* Ridl.

(38) *Z. gracile* Jack

(39) *Z. officinale* Rosc.

(40) *Z. ottensii* Val.

(41) *Z. purpureum* Rosc.

(42) *Z. spectabile* Griff.

(43) *Z. zerumbet* Smith

(44) *Z.* sp.

Additionally, some other Zingiberaceous species such as *Amomum krervanh* Pierre, *Curcuma xanthorrhiza* Roxb., *C. parviflora* Wall. and *C. zedoaria* Rosc. have been found in Thailand (Smitinand, 1980).

1.2.2 Chemical constituents of the investigated species

The reported chemical constituents of *Alpinia galanga*, *Boesenbergia pandurata*, *Curcuma longa*, *Kaempferia galanga* and *Zingiber officinale* are shown in Tables 1, 2, 3, 4 and 5, respectively. Chemical structures of some constituents found in these five species are illustrated in Figures 1, 2, 3, 4 and 5, respectively.

1.2.2.1 *Alpinia galanga*

Table 1 Chemical constituents found in *A. galanga*

Botanical name	**Part of plant studied**	**Chemical constituents**	**References**
A. galanga	Essential oil (rhizome)	β-Bisabolene	De Pooter, *et al.*, 1985
		1,8-Cineole	De Pooter, *et al.*, 1985
		ar-Curcumene	De Pooter, *et al.*, 1985
		trans-β-Farnesene	De Pooter, *et al.*, 1985
		β-Sesquiphellandrene	De Pooter, *et al.*, 1985
	Fresh fruit	1′-Acetoxyeugenol acetate	Itokawa, *et al.*, 1987
	Leaf	Alanine	Yeoh, Wee and Watson, 1986
		Arginine	Yeoh, Wee and Watson, 1986

Table 1 Chemical constituents found in *A. galanga* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
A. galanga	Leaf	Aspartic acid	Yeoh, Wee and Watson, 1986
		Galanga	Janssen and Scheffer, 1985
		Glutamic acid	Yeoh, Wee and Watson, 1986
		Glycine	Yeoh, Wee and Watson, 1986
		Histidine	Yeoh, Wee and Watson, 1986
		Tyrosine	Yeoh, Wee and Watson, 1986
		Valine	Yeoh, Wee and Watson, 1986
	Rhizome	Acetoxy-1,8-cineole	Kubota, Nakamura and Kobayashi, 1998; Someya, Kobayashi and Kubota, 2001
		1′-Acetoxychavicol acetate	Janssen and Scheffer, 1985; Itokawa, *et al.*, 1987; Kondo, *et al.*, 1993; Murakami, Ohigashi and Koshimizu, 1994; Yang and Eilerman, 1999; Murakami, *et al.*, 2000; Moffatt, *et al.*, 2002
		1′-Acetoxyeugenol acetate	Janssen and Scheffer, 1985

Table 1 Chemical constituents found in *A. galanga* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
A. galanga	Rhizome	4-Hydroxybenzaldehyde	Nori, *et al.*, 1988
		α-Bergamotene	De Pooter, *et al.*, 1985
		Borneol	De Pooter, *et al.*, 1985
		Camphene	De Pooter, *et al.*, 1985
		Carveol I	De Pooter, *et al.*, 1985
		Carveol II	De Pooter, *et al.*, 1985
		cis-2-Acetoxy-1,8-cineol	Kubota, *et al.*, 1999
		trans-2-Acetoxy-1,8-cineol	Kubota, *et al.*, 1999
		Citronellol acetate	De Pooter, *et al.*, 1985
		ar-Curcumene	De Pooter, *et al.*, 1985
		Galanga	Kondo, *et al.*, 1993
		Galanga acetate	Tanaka, *et al.*, 1997
		1′-Hydroxychavicol acetate	Janssen and Scheffer, 1985
		p-Hydroxycinnamaldehyde	Barik, kundu and Dey, 1987
		Limonene	De Pooter, *et al.*, 1985

Table 1 Chemical constituents found in *A. galanga* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
A. galanga	Rhizome	Linalool	De Pooter, *et al.*, 1985
		Nerol acetate	De Pooter, *et al.*, 1985
		Pentadecane	De Pooter, *et al.*, 1985
		Sabinene	De Pooter, *et al.*, 1985
		Santalene	De Pooter, *et al.*, 1985
		Terpinen-4-ol	Janssen and Scheffer, 1985
		Terpinolene	De Pooter, *et al.*, 1985
	Seed	Aframodial	Ayafor, *et al.*, 1994
		Astragalin	Qiao, *et al.*, 2000
		Caryophyllene I	Mitsui, *et al.*, 1976
		Caryophyllenol II	Mitsui, *et al.*, 1976
		1′-Acetoxy-eugenol acetate	Mitsui, *et al.*, 1976
		Galanga	Tanaka, *et al.*, 1997
		Galanal A	Morita and Itokawa, 1986
		Galanal B	Morita and Itokawa, 1986

1.2.2.2 *Boesenbergia pandurata*

Table 2 Chemical constituents found in *B. pandurata*

Botanical name	Part of plant studied	Chemical constituents	References
B. pandurata	Entire plant	Alpinetin	Suphat, 1964
		Boesenbergin A	Tuntiwachwuttikul, *et al.*, 1980
		2′-6′-Dihydroxy-4′-methoxy chalcone	Tuntiwachwuttikul, *et al.*, 1980
		Pinocembrin	Tuntiwachwuttikul, *et al.*, 1980
		Pinostrobin	Suphat, 1964
	Essential oil (rhizome)	Camphene	Jantan, *et al.*, 2001
		Camphor	Jantan, *et al.*, 2001
		1,8-Cineole	Jantan, *et al.*, 2001
		Geraniol	Jantan, *et al.*, 2001
		Methyl cinnamate	Jantan, *et al.*, 2001
		(*E*)-β-Ocimene	Jantan, *et al.*, 2001
	Rhizome	Alpinetin	Supat, 1961; Jaipetch, *et al.*, 1982; Pandji, *et al.*, 1993
		Boesenbergin A	Jaipetch, *et al.*, 1982

Table 2 Chemical constituents found in *B. pandurata* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
B. pandurata	Rhizome	Boesenbergin A	Mahidol, *et al.*, 1984
		(*dl*)-Boesenbergin B	Mahidol, *et al.*, 1984
		Cardamonin	Jaipetch, *et al.*, 1982; Murakami, *et al.*, 1993; Trakoontivakorn, *et al.*, 2001
		Chalcone cardamonin	Pandji, *et al.*, 1993
		2′-4′-Dihydroxy-6′-methoxy chalcone	Jaipetch, *et al.*, 1982
		2′-6′-Dihydroxy-4′-methoxy chalcone	Jaipetch, *et al.*, 1982
		Chrysin dimethyl ether	Pathong, *et al.*, 1989
		Geranial	Pandji, *et al.*, 1993
		Neral	Pandji, *et al.*, 1993
		Panduratin A	Mahidol, *et al.*, 1984; Trakoontivakorn, *et al.*, 2001
		4-Hydroxy panduratin A	Trakoontivakorn, *et al.*, 2001
		Panduratin B-1	Pancharoen, *et al.*, 1987
		Panduratin B-2	Pancharoen, *et al.*, 1987

Table 2 Chemical constituents found in *B. pandurata* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
B. pandurata	Rhizome	Pinocembrin	Jaipetch, *et al.*, 1982; Pandji, *et al.*, 1993; Trakoontivakorn, *et al.*, 2001
		Pinocembrin chalcone	Trakoontivakorn, *et al.*, 2001
		Pinostrobin	Supat, 1961; Jaipetch, *et al.*, 1982; Pandji, *et al.*, 1993; Trakoontivakorn, *et al.*, 2001
	Root	Cardamonin	Tiwawech, *et al.*, 2000

1.2.2.3 *Curcuma longa*

Table 3 Chemical constituents found in *C. longa*

Botanical name	Part of plant studied	Chemical constituents	References
C. longa	Essential oil (rhizome)	1-(3-Cyclopentyl-formyl)-2-benzene	Hu, Du and Tang, 1997
		Borneol	Mitra, 1975
		Camphene	Gopalan, *et al.*, 2000
		Camphor	Fang, *et al.*, 1982
		Car-3-ene	Mccarron, *et al.*, 1995
		Citronellal	Gopalan, *et al.*, 2000
		Cineol	Yasuda, *et al.*, 1988
		Curcumene	Fang, *et al.*, 1982; Gopalan, *et al.*, 2000
		p-Cymene	Nguyen, Nguyen and Leclercq, 1995
		Limonene	Fang, *et al.*, 1982
		Myrcene	Nguyen, Nguyen and Leclercq, 1995
		Palmitic acid	Richmond and Pombo-Villar, 1997
		α-Phellandrene	Mccarron, *et al.*, 1995
		α-Pinene	Nguyen, Nguyen and Leclercq, 1995

Table 3 Chemical constituents found in *C. longa* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
C. longa	Essential oil (rhizome)	β-Pinene	Nguyen, Nguyen and Leclercq, 1995
		(+)-Sabinene	Mitra, 1975
		Terpinene	Fang, *et al.*, 1982
		Terpinolene	Mccarron, *et al.*, 1995
		ar-Turmerone	Nigam and Ahmed, 1991; Martins, *et al.*, 2001; Gopalan, *et al.*, 2000
		α-Turmerone	Martins, *et al.*, 2001; Gopalan, *et al.*, 2000
		β-Turmerone	Martins, *et al.*, 2001
		Zerumbone	Richmond and Pombo-Villar, 1997
		Zingiberene	Mitra, 1975
	Powdered turmeric	Curcumin, Bisdemethoxycurcumin, Demethoxycurcumin	Simon, *et al.*, 1998
	Rhizome	β-Bisabolene	Richmond and Pombo-Villar, 1997
		Bisabolone	Richmond and Pombo-Villar, 1997

Table 3 Chemical constituents found in *C. longa* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
C. longa	Rhizome	Bisacumol	Ohshiro, Kuroyanagi and Ueno, 1990
		Bisacurone	Ohshiro, Kuroyanagi and Ueno, 1990
		Borneol	Chen, Yu and Fang, 1983
		Caffeic acid	Schultz and Herrmann, 1980
		Campesterol	Moon, Park and Koh, 1976
		Camphor	Chen, Yu and Fang, 1983
		Cholesterol	Moon, Park and Koh, 1976
		Cineol	Yasuda, *et al.*, 1988
		Coumarin	Hiserodt, *et al.*, 1996
		Curcumene	Hiserodt, *et al.*, 1996
		Curcumenol	Ohshiro, Kuroyanagi and Ueno, 1990
		Curcumenone	Ohshiro, Kuroyanagi and Ueno, 1990
		Curcumin	Jentzsch, Spiegl and Kamitz, 1970; Rasmussen, *et al.*, 2000
		Bisdemethoxycurcumin	Taylor and Mcdowell, 1992; Rasmussen, *et al.*, 2000
		Demethoxycurcumin	Jentzsch, Spiegl and Kamitz, 1970;

Table 3 Chemical constituents found in *C. longa* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
C. longa	Rhizome	Demethoxycurcumin	Rasmussen, *et al.*, 2000
		Curlone	Kiso, *et al.*, 1983
		Diferuloyl-methane	Gupta and Ghosh, 1999
		Eugenol	Chen, Yu and Fang, 1983
		Germacron-13-al	Ohshiro, Kuroyanagi and Ueno, 1990
		Germacrone	Uehara, *et al.*, 1992a
		Linalool	Fang, *et al.*, 1982
		β-Pinene	Chen, Yu and Fang, 1983
		β-Sitosterol	Moon, Park and Koh, 1976
		Stigmasterol	Moon, Park and Koh, 1976
		Tolyl-methyl-carbinol	Supniewski and Hano, 1935
		ar-Tumerol	Hiserodt, *et al.*, 1996
		Turmerin	Cohly, *et al.*, 1998a
		ar-Turmerone	Suzuki, Murata and Yasuda, 2000
		Zedoarondiol	Ohshiro, Kuroyanagi and Ueno, 1990
		Zingiberene	Uehara, *et al.*, 1992b

Table 3 Chemical constituents found in *C. longa* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
C. longa	Root	α-Atlantone	Su, Horvat and Jilani, 1982
		γ-Atlantone	Su, Horvat and Jilani, 1982
		Bisabolene	Su, Horvat and Jilani, 1982
		Borneol	Su, Horvat and Jilani, 1982
		Cineol	Su, Horvat and Jilani, 1982
		Curcumin	Choiu and Chang, 1983
		Bisdemethoxycurcumin	Choiu and Chang, 1983
		Demethoxycurcumin	Choiu and Chang, 1983
		(+)-α-Phellandrene	Mitra, 1975
		(+)-Sabinene	Mitra, 1975
		ar-Turmerone	Su, Horvat and Jilani, 1982
		Zingiberene	Su, Horvat and Jilani, 1982

1.2.2.4 *Kaempferia galanga*

Table 4 Chemical constituents found in *K. galanga*

Botanical name	Part of plant studied	Chemical constituents	References
K. galanga	Essential oil (rhizome)	*p*-Methoxy-*trans*-cinnamic acid	Liu and Jinag, 1993
	Leaf	4-Hydroxy benzoic acid	Merh, Daniel and Sabnis, 1986
		Chlorogenic acid	Merh, Daniel and Sabnis, 1986
		Vanillic acid	Merh, Daniel and Sabnis, 1986
	Rhizome	Car-3-en-5-one	Kiuchi, Nakamura and Tsuda, 1987
		Cinnamic acid	Pandji, *et al.*, 1993
		Cinnamic acid ethyl ester	Kosuge, *et al.*, 1985; Kiuchi, *et al.*, 1988
		p-Methoxy-*trans*-cinnamic acid	Noro, *et al.*, 1983
		Deoxypodophyllotoxin	Kosuge, *et al.*, 1985
		Ethyl cinnamate	Othman, *et al.*, 2002
		p-Methoxycinnamate	Pandji, *et al.*, 1993
		Ethyl *p*-methoxy-*trans*-cinnamate	Kosuge, *et al.*, 1985
	Root	*p*-Methoxy-*trans*-cinnamic acid	Chau, Hong and Quy, 1979

1.2.2.5 *Zingiber officinale*

Table 5 Chemical constituents found in *Z. officinale*

Botanical name	**Part of plant studied**	**Chemical constituents**	**References**
Z. officinale	Aerial parts	Cysteine	Takahashi, *et al.*, 1982
	Essential oil (rhizome)	ar-Curcumene	Variyar, Gholap and Thomas, 1997
		Calamenene	Miyazawa and Kameoka, 1988
		Camphor	Miyazawa and Kameoka, 1988
		Citral-1,8-cineole	Menut, *et al.*, 1994
		Geranial	Sakamura, 1987;
			Onyenekwe and Hashimoto, 1999
		Geraniol	Sakamura, 1987
		Geranyl acetate	Sakamura, 1987
		Neral	Sakamura, 1987;
			Onyenekwe and Hashimoto, 1999
		β-Sesquiphellandrene	Variyar, Gholap and Thomas, 1997
		Zingiberin	Variyar, Gholap and Thomas, 1997
	Leaf	Shikimic acid	Yoshida, Tazaki and Minamikawa, 1975

Table 5 Chemical constituents found in *Z. officinale* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
Z. officinale	Rhizome	Asparagine	Murakami, *et al.*, 1965
		Benzaldehyde	Wu, Kuo and Ho, 1990
		Bisabolene	Wu, Kuo and Ho, 1990
		Borneol	Van-Beek, *et al.*, 1987
		Caffeic acid	Schultz and Herrmann, 1980
		Camphene	Tanabe, *et al.*, 1991
		Chrysanthemin	Fu, *et al.*, 1993
		Cineol	Nishimura, 1995
		Citronellal	Nishimura, 1995
		p-Coumaric acid	Schultz and Herrmann, 1980
		ar-Curcumene	Yoshikawa, *et al.*, 1993a
		Curcumin	Kikuzaki and Nakatani, 1993
		6-Dehydrogingerdione	Charles,Garg and Kumar, 2000
		(or 1-Dehydrogingerdione)	
		6, 8, 10-Dehydroshogaol	Wu, *et al.*, 1998
		Diethyl sulfide	Kami, Nakayama and Hayashi, 1972

Table 5 Chemical constituents found in *Z. officinale* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
Z. officinale	Rhizome	Furanogermenone	Shiba, *et al.*, 1986
		Galanolactone	Yoshikawa, *et al.*, 1993b
		Geranial	Tanabe, *et al.*, 1991; Sekiwa-Iijima, Aizawa and Kubota, 2001
		Geraniol	Sakamura, *et al.*, 1986; Sekiwa-Iijima, Aizawa and Kubota, 2001
		6-Gingerdiol	Kikuzaki, Tsai and Nakatani,1992; He, *et al.*, 1998; Sekiwa, Kubota and Kobayashi, 2000
		10-Gingerdione	Kiuchi, Shibuya and Sankawa, 1982
		Gingerenone A, Gingerenone B, Isogingerenone B, Gingerenone C	Endo, Kanno and Oshima, 1990
		Gingerol	Sane, *et al.*, 1998
		6, 8,10-Gingerol	Shoji, *et al.*, 1982; Balladin, *et al.*, 1998; Hiserodt, Franzblau and Rosen, 1998; He, *et al.*, 1998

Table 5 Chemical constituents found in *Z. officinale* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
Z. officinale	Rhizome	6, 8, 10, 12, 14-Gingerol	Chen, Rosen and Ho, 1986a
		8, 10-Gingerol	Yamada, kikuzaki and Nakatani, 1992
		Limonene	Kami, Nakayama and Hayashi, 1972
		Linalool	Sakamura, *et al.*, 1986
		Myrcene	Sakamura, *et al.*, 1986
		Neral	Tanabe, *et al.*, 1991
		Nerolidol	Wu, Kuo and Ho, 1990
		6-Paradol	Chung, *et al.*, 2001
		8-Paradol	Tjendraputra, *et al.*, 2001
		6-Shogaol	Suekawa, *et al.*, 1988
		8-Shogaol	Tjendraputra, *et al.*, 2001
		6, 8, 10, 12-Shogaol	Chen, Rosen and Ho, 1986b
		6, 8, 10-Shogaol	Balladin, *et al.*, 1998
			He, *et al.*, 1998
		Terpinen-4-ol	Nishimura, 1995
		Zerumbodienone	Fujimoto, Maruno and Made, 1989
		Zingerone	Chen, *et al.*, 1986

Table 5 Chemical constituents found in *Z. officinale* (continued)

Botanical name	Part of plant studied	Chemical constituents	References
Z. officinale	Rhizome	Zingiberene	Millar, 1998
		Zingiberenol	Terhune, *et al.*, 1975
		Zingiberone	Nomura, 1917
	Root	Bisabolene	Mcgaw, Yen and Dyal,1984
		Citral	Mcgaw, Yen and Dyal, 1984
		6-Dehydrogingerdione	Kiuchi, Shibuya and Sankawa, 1982
		(or 1-Dehydrogingerdione)	
		10-Dehydrogingerdione	Kiuchi, Shibuya and Sankawa, 1982
		6, 10-Gingerdione	Kiuchi, Shibuya and Sankawa, 1982
		6-Gingerol	Kiuchi, Shibuya and Sankawa, 1982
	Seed	Aframodial	Ayafor, *et al.*, 1994
	Tuber	Arginine	Takahashi, *et al.*, 1982
		Leucine	Takahashi, *et al.*, 1982

1.2.3 Biological activities of the investigated species

Previous investigations on biological activity of *Alpinia galanga, Boesenbergia pandurata, Curcuma longa, Kaempferia galanga* and *Zingiber officinale* are shown in Tables 6, 7, 8, 9 and 10, respectively.

1.2.3.1 *Alpinia galanga*

Table 6 Biological activities of *A. galanga*

Plant	**Part of plant used**	**Activity**	**References**
A. galanga	Dried entire plant	Aphrodisiac activity	Islam, *et al.*, 2000
	Dried rhizome	Antifungal activity	Chinsiriwong and Hirankarn,1983
		Antimicrobial activity	Janssen and Scheffer, 1985
		Antiyeast activity	Chinsiriwong and Hirankarn,1983
	Dried roots	Antifungal activity	Haraguchi, *et al.*, 1996
	Dried stem	Antibacterial activity	George and Pandalai, 1949
	Essential oil (rhizome)	Antiamebic activity	Chopra, Khajuria and Chopra, 1957
		Antibacterial activity	Janssen and Scheffer, 1985
		Antifungal activity	Janssen and Scheffer, 1985

Table 6 Biological activities of *A. galanga* (continued)

Plant	Part of plant used	Activity	References
A. galanga	Essential oil	Antimycobacterial activity	Chopra, Khajuria and Chopra, 1957
	(rhizome)	Antiprotozoan activity	Chopra, Khajuria and Chopra, 1957
		Insecticide activity	Chopra, Khajuria and Chopra, 1957
	Fresh rhizome	Antitumour against sarcoma 180	Itokawa, *et al.*, 1987
	Rhizome	Antiascariasis activity	Kaleysaraj, 1975
		Antibacterial activity	Ross, *et al.*, 1980
		Antimutagenic activity	Ruangchom and Vinitketkummuen, 1993
		Antioxidative activity by thiocyanate and TBA method	Jitoe, *et al.*, 1992
		Antitumour activity	Itokawa, *et al.*, 1987
		Antiulcer activity	Al-yahya, *et al.*, 1990
		Cytotoxic activity	Murakami, *et al.*, 1993
		Diuretic activity	Dhawan, *et al.*, 1977
		Inhibition of tumour promoter-induced Epstein-Barr virus activation	Kondo, *et al.*, 1993; Murakami, *et al.*, 2000
	Root and seed	Antifungal activity	Haraguchi, *et al.*, 1996
		Antimicrobial activity	Haraguchi, *et al.*, 1996
	Seed	Antiulcer activity	Mitsui, *et al.*, 1976

1.2.3.2 *Boesenbergia pandurata*

Table 7 Biological activities of *B. pandurata*

Plant	Part of plant used	Activity	References
B. pandurata	Dried entire plant	Smooth muscle relaxant activity	Apisariyakul, 1984
	Dried rhizome	Anticholinergic activity	Apisariyakul and Anantasarn, 1984
		Antifungal activity	Achararit, Panyayong and Ruchatakomut, 1983
		Antispasmodic activity	Apisariyakul and Anantusarn, 1984
		Antitumour-promoting activity	Maurakami, *et al.*, 1997
		Smooth muscle relaxant activity	Apisariyakul and Anantasarn, 1984
	Dried root	Cocarcinogenic activity	Tiwawech, *et al.*, 2000
	Fresh rhizome	Antispasmodic activity	Mahidol, 1985
		Antitumour-promoting activity	Murakami, *et al.*, 1993
		Inhibition of tumour promoter-induced Epstein-Barr virus (EBV) activation	Murakami, *et al.*, 1993
	Fresh root	Antitumour-promoting activity	Tiwawech, *et al.*, 2000

1.2.3.3 *Curcuma longa*

Table 8 Biological activities of *C. longa*

Plant	Part of plant used	Activity	References
C. longa	Aqueous extracts (rhizome)	Antidepressant activity	Yu, Kong and Chen, 2002
	Dried bulb	Antibacterial activity	Alkofahi, *et al.*, 1997
		Cytotoxic activity	Alkofahi, *et al.*, 1997
	Dried leaf	Antioxidative activity	Maulik, *et al.*, 1997
		Radical scavenging effect	Maulik, *et al.*, 1997
	Dried rhizome	Anticoagulant activity	Kosuge, *et al.*, 1984
		Antifungal activity	Roth, Chandra and Nair, 1998
		Antiinflammatory activity	Kinoshita, Nakamura and Maruyama, 1986
		Antitumour activity	Kinoshita, Nakamura and Maruyama, 1986
	Dried rhizome	Antiviral activity	Cai, *et al.*, 1988
		Carcinogenesis inhibition	Soni, *et al.*, 1997
	Dried root	Antioxidative activity	Lee, *et al.*, 1998

Table 8 Biological activities of *C. longa* (continued)

Plant	Part of plant used	Activity	References
C. longa	Dried root	Radical scavenging effect	Lee, *et al.*, 1998
	Essential oil (rhizome)	Antibacterial activity	Rath, *et al.*, 1999; Negi, *et al.*, 1999
		Antiinflammatory activity	Gupta, Chandra and Mishra, 1972
		Antifungal activity	Apisariyakul, Vanittanakom and Buddhasukh, 1995
		Antimicrobial activity	Martins, *et al.*, 2001
	Extract	Antiinflammatory activity	Ammon and Wahl, 1991
		Antioxidative activity	Selvam, *et al.*, 1995
	Fresh tuber	Induction of chromosome aberrations	Abraham, Abraham and Radhamony, 1976
	Rhizome	Antiamoebic activity	Dhar, *et al.*, 1968
		Antibacterial activity	Bhavani-Shankar and Murthy, 1979
		Antiinflammatory activity	Okuyama, *et al.*, 1995
		Antioxidative activity	Selvam, *et al.*, 1995
		Antioxidative activity by thiobarbituric acid (TBA) assay and free radical scavenging activity by DPPH	Kim, *et al.*, 1997

Table 8 Biological activities of *C. longa* (continued)

Plant	Part of plant used	Activity	References
C. longa	Rhizome	Carcinogenesis inhibition	Deshpande, Ingle and Maru, 1997
		Cytotoxic activity	Lee, Kang and Ahn, 1986
		Lipid peroxide formation inhibition	Cohly, *et al.*, 1998b
		Nematocidal activity	Kiuchi, 1995
	Turmeric powder (rhizome)	Antioxidative activity	Srinivas, Shalini and Shylaja,1992
	ar-Turmerone (pure compound)	Antivenom effect	Ferreira, *et al.*, 1992
	Curcumin (pure compound)	Antiinflammatory in *in vivo* animal models	Ammon, *et al.*, 1993
		Antioxidant against lipid peroxidation	Noguchi, *et al.*, 1994
		Antispasmodic activity	Ammon and Wahl, 1991
		Antitumour activity in AK-5 tumour cell	Khar, *et al.*, 1999
	Curcumin (pure compound)	Inhibit lipid autoxidation by coupling with peroxyl radicals	Masuda, *et al.*, 2001

Table 8 Biological activities of *C. longa* (continued)

Plant	Part of plant used	Activity	References
C. longa	Curcumin (pure compound)	Chemopreventive action during the promotion/progression stage of colon cancer	Kawamori, *et al.*, 1999
		Cytotoxic activity against urinary bladder cancer	Sindhwani, *et al.*, 2000
		Free radical reactions	Priyadarsini, 1997
		Free radical scavenging ability and antioxidant efficiency	Khopde, *et al.*, 1999
		Induce HL-60 cells death (promyelocytic leukemia)	Kuo, Huang and Lin, 1996
	Curcumin (pure compound)	Inhibit the process of carcinogenesis	Limtrakul, *et al.*, 1997
		Inhibit tumourigenesis during both initiation and promotion period in several experimental animal models	Huang, Newmark and Frenkel, 1997
		Inhibition of cyclooxygenase-2 (COX-2) in HT-29 human colon cancer cells	Goel, Boland and Chauhan, 2001

Table 8 Biological activities of *C. longa* (continued)

Plant	Part of plant used	Activity	References
C. longa	Curcumin (pure compound)	Inhibition of HIV-1 integrase	Barthelemy, *et al.*, 1998
		Inhibitory effects on the growth of human breast cancer MCF7 cells	Verma, Salamone and Goldin, 1997
	Curcumin (pure compound)	Telomerase inhibitor through human telomerase reverse transcritpase in MCF7 breast cancer cell line	Ramachandran, *et al.*, 2002
	Curcumin, Bisdemethoxycurcumin, Demethoxycurcumin (pure compound)	Antiprotozoal activity	Rasmussen, *et al.*, 2000
	Curcumin, Bisdemethoxycurcumin, Demethoxycurcumin (pure compound)	Antitumour and antioxidative activity	Ruby, *et al.*, 1995
	Curcuminoids (mixture)	Inhibit MCF7 cell proliferation	Simon, *et al.*, 1998

1.2.3.4 *Kaempferia galanga*

Table 9 Biological activities of *K. galanga*

Plant	Part of plant used	Activity	References
K. galanga	Dried rhizome	Antibacterial activity	Inada, *et al.*, 1998
		Antispasmodic activity	Itokawa, *et al.*, 1983
		Antitumour-promoting activity	Murakami, *et al.*, 1993
		Colony formation inhibition	Kosuge, *et al.*, 1985
		Nematocidal activity	Ali, *et al.*, 1991
		Smooth muscle stimulant activity	Mokkhasmit, *et al.*, 1971
	Dried root	Antibacterial activity	George and Pandalai, 1949
	Fresh rhizome	Cytotoxic activity on Hela cells	Mackeen, *et al.*, 1997
	Rhizome	Antiascariasis activity	Kaleysaraj, 1975
		Cytotoxic activity against Hela cell	Kosuge, *et al.*, 1985
		Insecticide activity	Insun, *et al.*, 1999
		Monoamine oxidase inhibition	Noro, *et al.*, 1983
		Vasorelaxant effect	Othman, *et al.*, 2002
	Essential oil (rhizome)	Glutathione-*S*-transferase induction	Lam and Zheng, 1991

Table 9 Biological activities of *K. galanga* (continued)

Plant	Part of plant used	Activity	References
K. galanga	Tuber	Anticancer	Kosuge, *et al.*, 1985
		Larvacidal	Kiuchi, Nakamura and Tsuda, 1987

1.2.3.5 *Zingiber officinale*

Table 10 Biological activities of *Z. officinale*

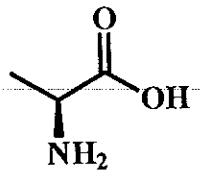
Plant	Part of plant used	Activity	References
Z. officinale	Dried aerial parts	Diuretic activity	Aswal, *et al.*, 1984
		Hypoglycemic activity	Aswal, *et al.*, 1984
	Dried entire plant	Hyperglycemic activity	Singhal and Joshi, 1983
	Dried rhizome	Antibacterial activity	Alkofahi, *et al.*, 1997
		Cytotoxic activity	Alkofahi, *et al.*, 1997
	Essential oil (rhizome)	Antibacterial activity	Meena and Sethi, 1994
		Antifungal activity	Meena and Sethi, 1994
		Antiinflammatory activity	Sharma, Srivastava and Gan, 1994
		Antimicrobial activity	Martins, *et al.*, 2001
		Antimutagenic activity	Hashim, *et al.*, 1994
		Antioxidative activity	Kawamura and Okada, 1992
	Fresh aerial parts	Tumour promotion inhibition	Koshimizu, *et al.*, 1988
	Fresh rhizome	Antiemetic activity	Yamahara, *et al.*, 1989
		Antimutagenic activity	Sakai, *et al.*, 1988

Table 10 Biological activities of *Z. officinale* (continued)

Plant	Part of plant used	Activity	References
Z. officinale	Fresh rhizome	Antimycobacterial activity	Hiserodt, Franzblau and Rosen, 1998
		Antitumour promoting activity	Vimala, Norhanom and Yadav, 1999
	Immature rhizome	Antitumour promoting activity	Murakami, *et al.*, 1995
	Plant extract	Antiviral activity	Roy, Sinha and Gupta, 1979
	Rhizome	Antibacterial activity	Alzoreky and Nakahara, 2002
		Anticonvulsant activity	Sugaya, *et al.*, 1978
		Anaesthetic activity	Sugaya, *et al.*, 1979
		Antihepatotoxic effect	Hikino, *et al.*, 1985
		Antioxidative activity	Lee, Kim and Ashmore, 1986
		Antioxidative activity by DPPH	Sekiwa, Kubota and Kobayashi, 2000
		Antiulcer activity	Yamahara, *et al.*, 1992
	Root	Antimicrobial activity	Cheeptham and Towers, 2002
	6-Dehydrogingerdione (or 1-Dehydrogingerdione) 10-Dehydrogingerdione (pure compound)	Inhibitors of prostaglandin biosynthesis	Kiuchi, Shibuya and Sankwa, 1982

Table 10 Biological activities of *Z. officinale* (continued)

Plant	Part of plant used	Activity	References
Z. officinale	6,10-Gingerdione (pure compound)	Inhibitors of prostaglandin biosynthesis	Kiuchi, Shibuya and Sankwa, 1982
	Gingerenone A (pure compound)	Antifungal activity and anticoccidium activity	Endo, Kanno and Oshima, 1990
	6-Gingerol (pure compound)	Antitumour promoting activity	Park, *et al.*, 1998
		Antitumour promoting activity on HL-60 cells	Surh, *et al.*, 1999
		Cytotoxic activity on HL-60 cells	Lee and Surh, 1998
	6, 8,10-Gingerol (pure compound)	Antimicrobial effects against *B.subtilis* and *E. coli* K-12	Yamada, Kikuzaki and Nakatani, 1992
		Inhibition of *Mycobacterium avium* and *Mycobacterium tuberculosis*	Hiserodt, Franzblau and Rosen, 1998
	6-Paradol (pure compound)	Antioxidative and antitumour promoting activity	Chung, *et al.*, 2001
		Cytotoxic activity on HL-60 cells	Lee and Surh, 1998



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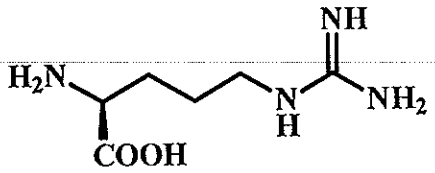

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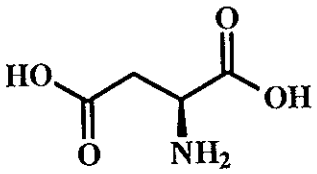
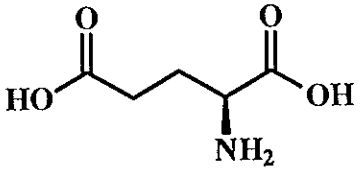
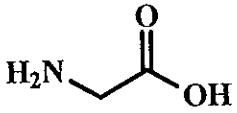


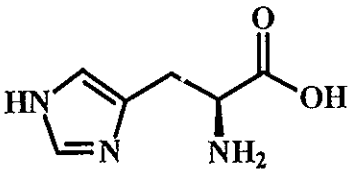
Aspartic acid



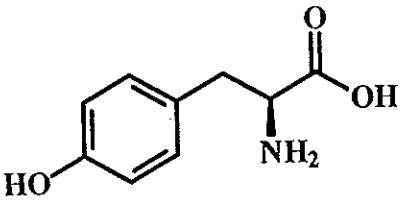
Glutamic acid



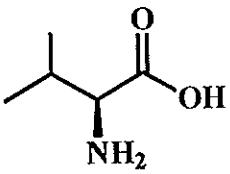
Glycine



Histidine

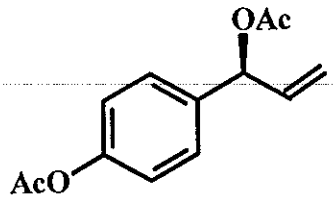


Tyrosine

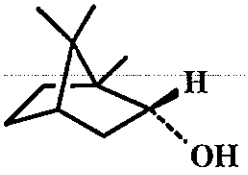


Valine

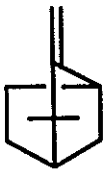
Figure 1 Structures of some chemical constituents found in *A. galanga*



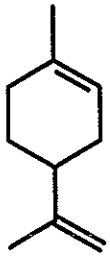
1′-Acetoxychavicol acetate



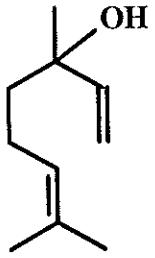
Borneol



Camphene

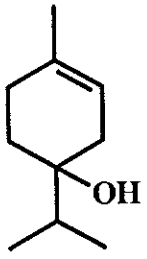


Limonene



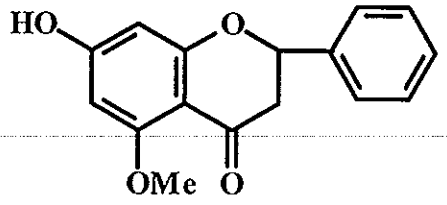
Linalool

Sabinene

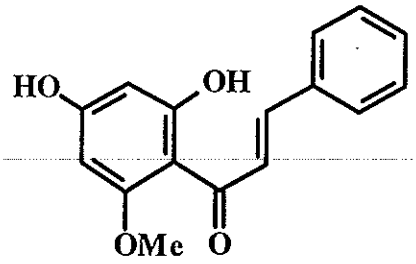


Terpinen-4-ol

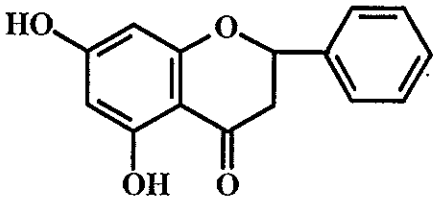
Figure 1 Structures of some chemical constituents found in *A. galanga* (continued)



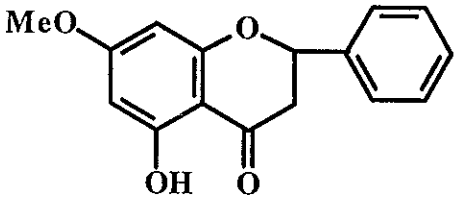
Alpinetin



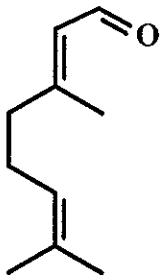
Cardamonin



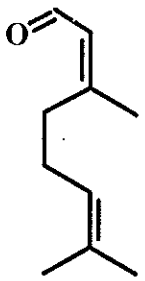
Pinocembrin



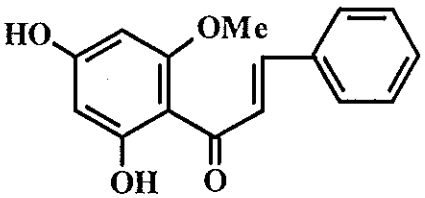
Pinostrobin



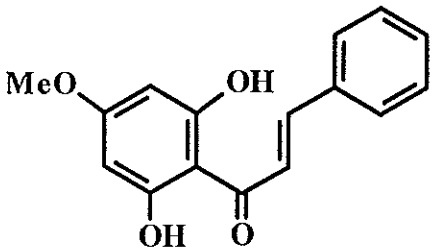
Geranial (*E*-Citral)



Neral (*Z*-Citral)

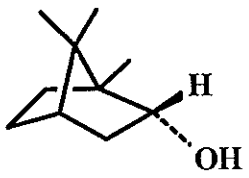


Chalcone cardamonin

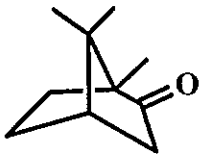


2′-6′-Dihydroxy-4′-methoxy chalcone

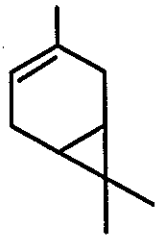
Figure 2 Structures of some chemical constituents found in *B. pandurata*



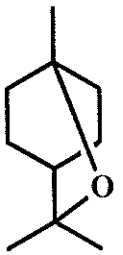
Borneol



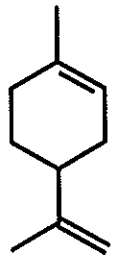
Camphor



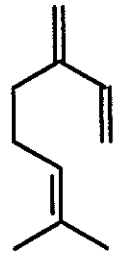
Car-3-ene



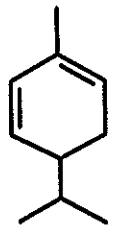
Cineol



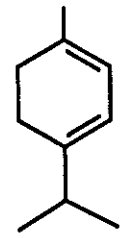
Limonene



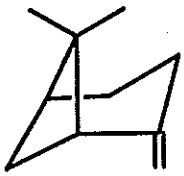
Myrcene



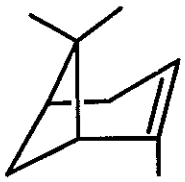
α-Phellandrene



Terpinene

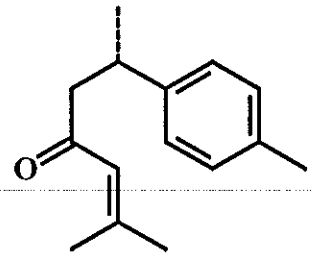


β-Pinene

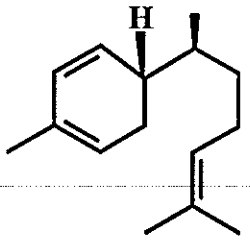


α-Pinene

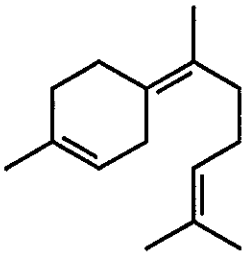
Figure 3 Structures of some chemical constituents found in *C. longa*



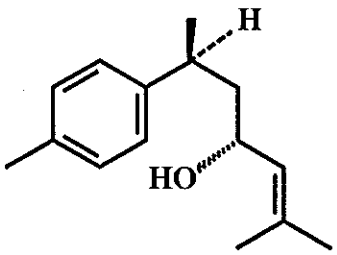
ar-Turmerone



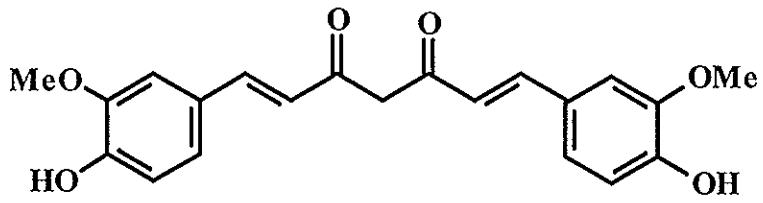
Zingiberene



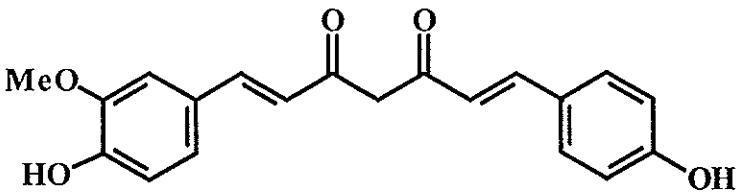
Bisabolene



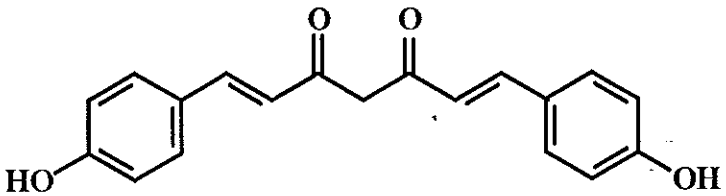
Bisacumol



Curcumin

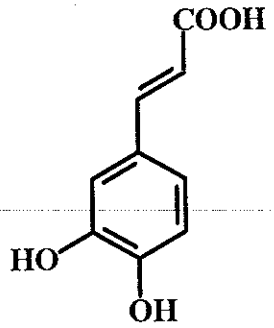


Demethoxycurcumin

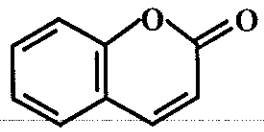


Bisdemethoxycurcumin

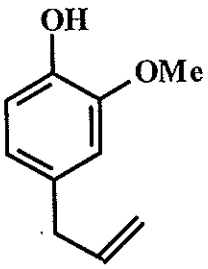
Figure 3 Structures of some chemical constituents found in *C. longa* (continued)



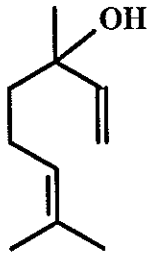
Caffeic acid



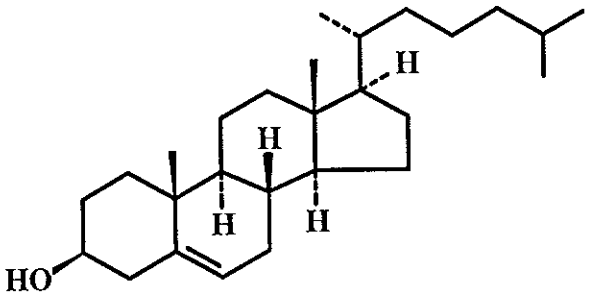
Coumarin



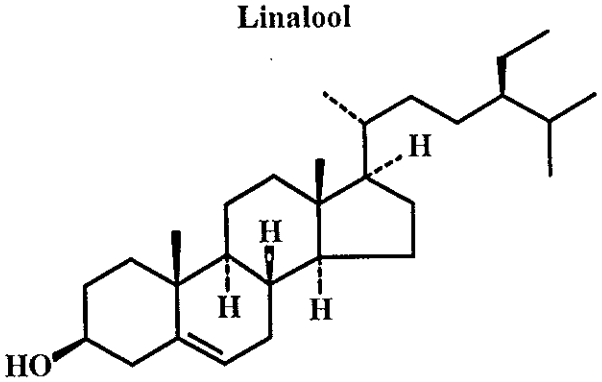
Eugenol



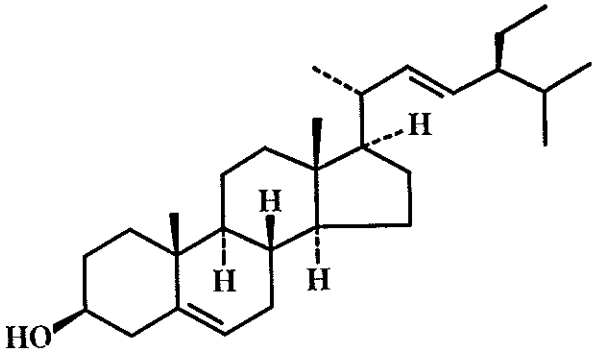
Linalool



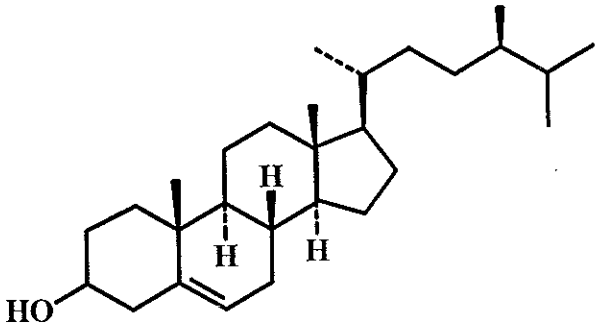
Cholesterol



β-Sitosterol

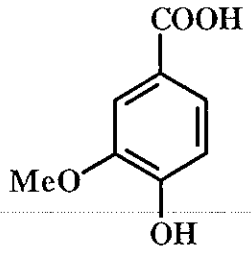


Stigmasterol

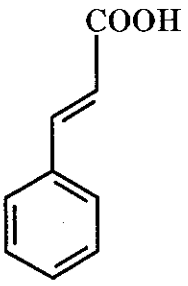


Campesterol

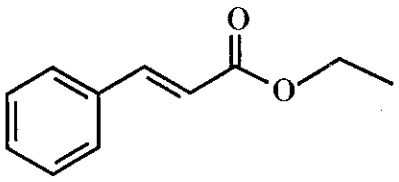
Figure 3 Structures of some chemical constituents found in *C. longa* (continued)



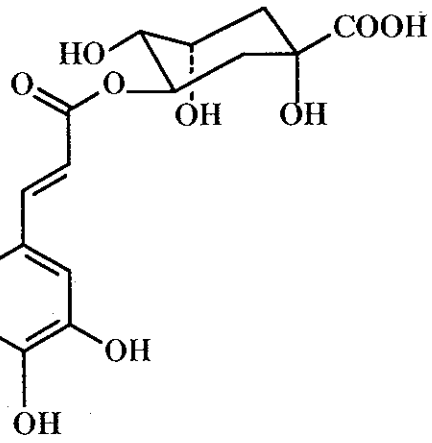
Vanillic acid



Cinnamic acid

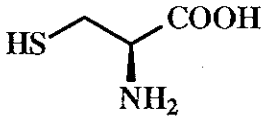


Ethyl cinnamate

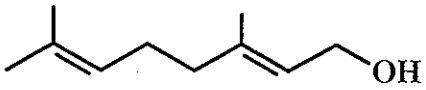


Chlorogenic acid

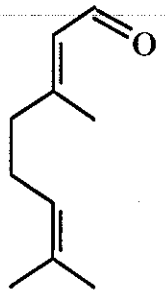
Figure 4 Structures of some chemical constituents found in *K. galanga*



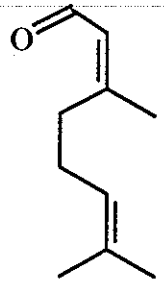
Cysteine



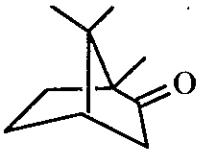
Geraniol



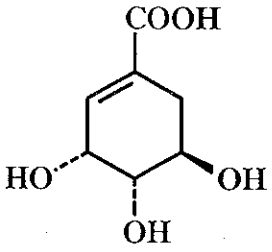
Geranial (*E*-Citral)



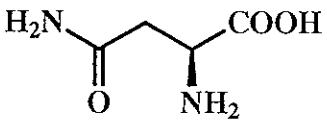
Neral (Z-Citral)



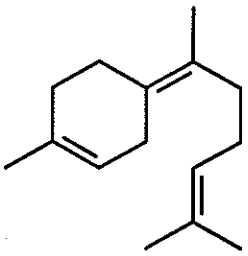
Camphor



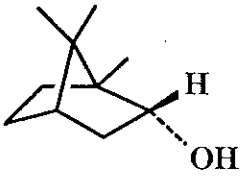
Shikimic acid



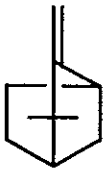
Asparagine



Bisabolene

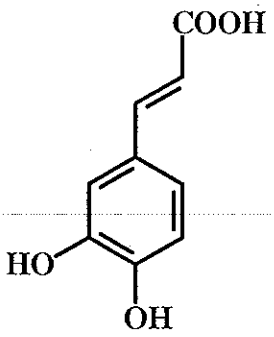


Borneol

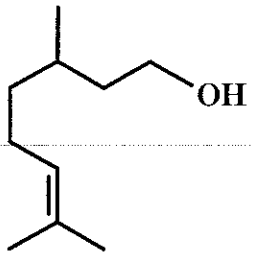


Camphene

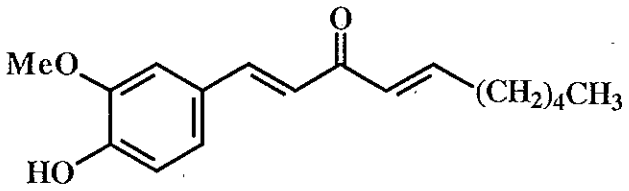
Figure 5 Structures of some chemical constituents found in *Z. officinale*



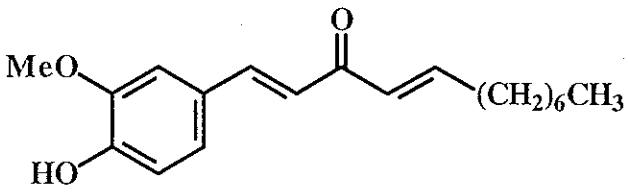
Caffeic acid



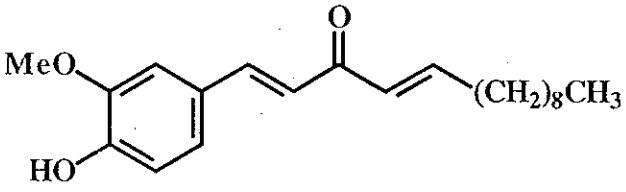
Citronellal



6-Dehydroshogaol

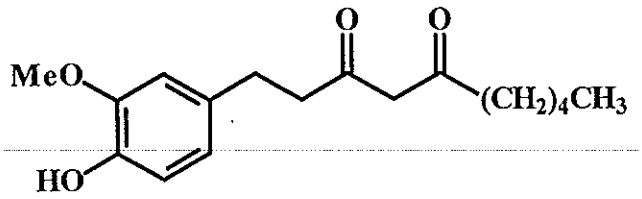


8-Dehydroshogaol

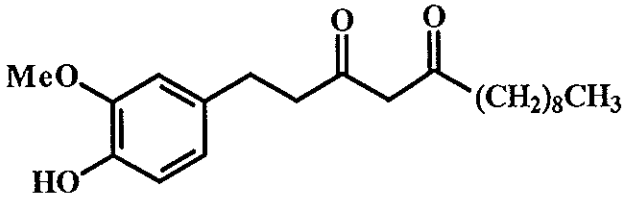


10-Dehydroshogaol

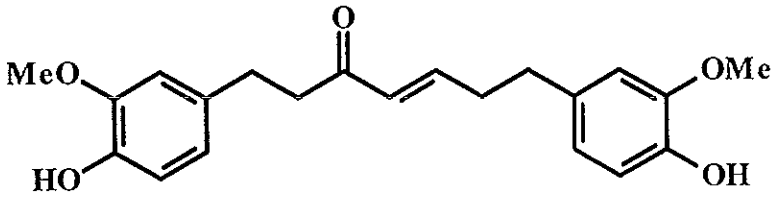
Figure 5 Structures of some chemical constituents found in *Z. officinale* (continued)



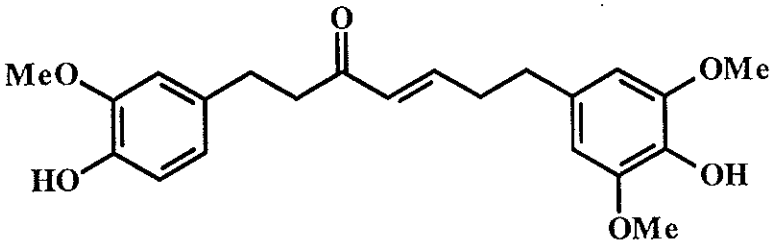
6-Gingerdione



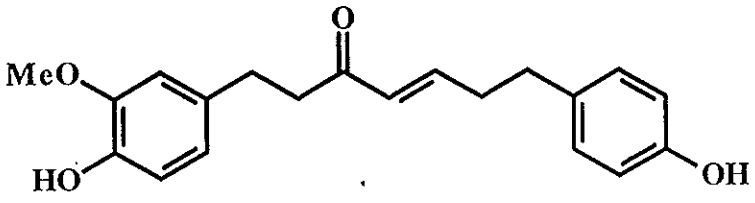
10-Gingerdione



Gingerenone A

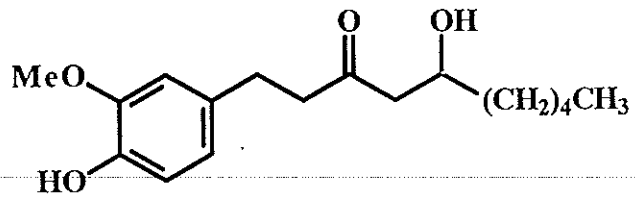


Gingerenone B

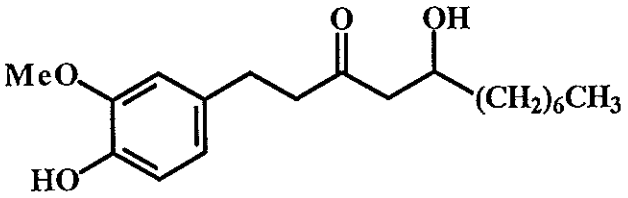


Gingerenone C

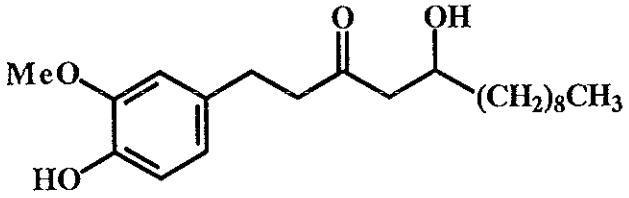
Figure 5 Structures of some chemical constituents found in *Z. officinale* (continued)



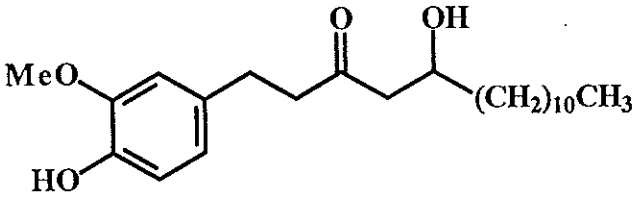
6-Gingerol



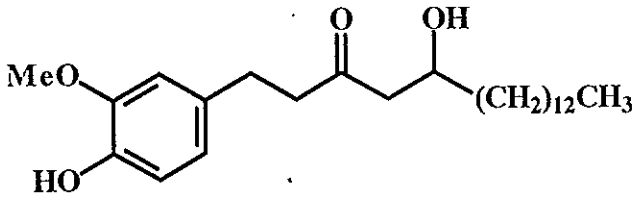
8-Gingerol



10-Gingerol

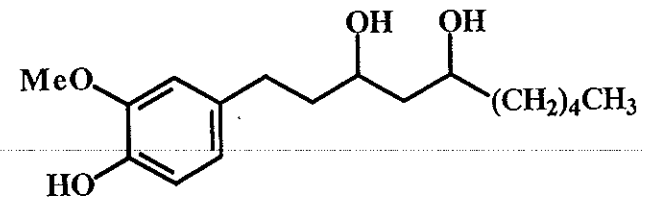


12-Gingerol

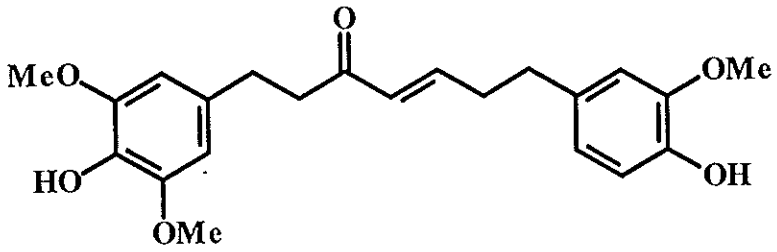


14-Gingerol

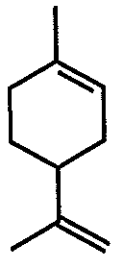
Figure 5 Structures of some chemical constituents found in *Z. officinale* (continued)



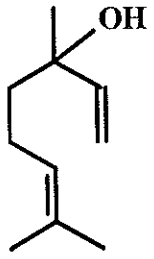
6-Gingerdiol



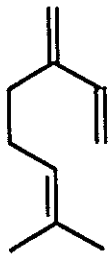
Isogingerenone B



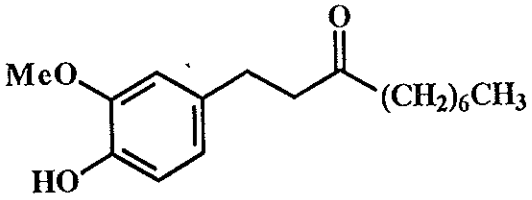
Limonene



Linalool

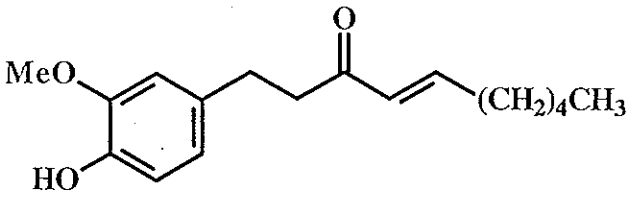


Myrcene

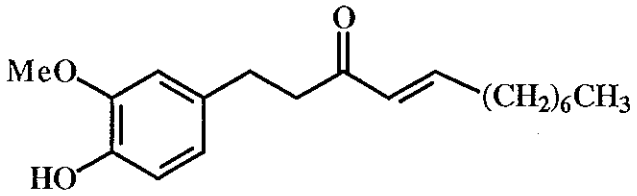


6-Paradol

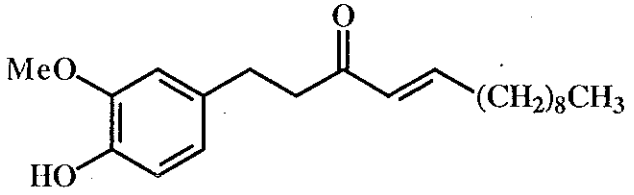
Figure 5 Structures of some chemical constituents found in *Z. officinale* (continued)



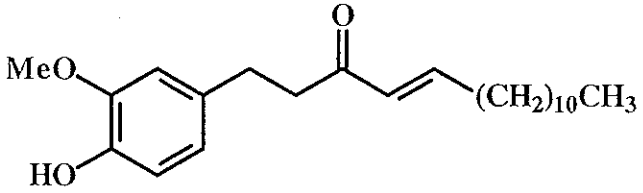
6-Shogaol



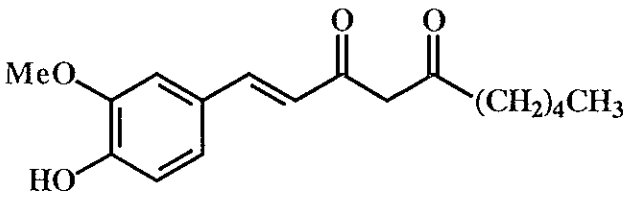
8-Shogaol



10-Shogaol

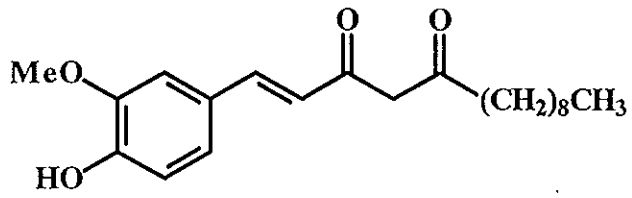


12-Shogaol

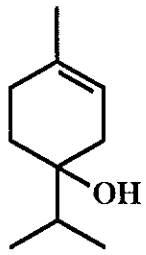


6-Dehydrogingerdione (or 1-Dehydrogingerdione)

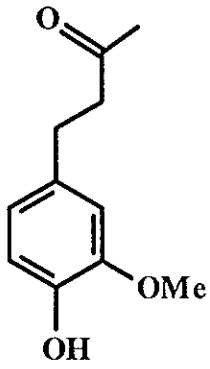
Figure 5 Structures of some chemical constituents found in *Z. officinale* (continued)



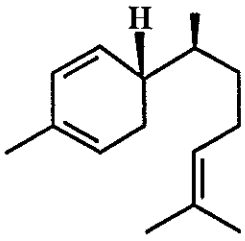
10-Dehydrogingerdione



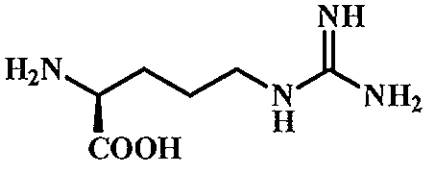
Terpinen-4-ol



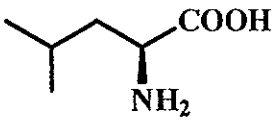
Zingerone



Zingiberene



Arginine



Leucine

Figure 5 Structures of some chemical constituents found in *Z. officinale* (continued)

The above data revealed that the five plants possessed diverse biological activities, for instance, antimicrobial activity, antiinflammatory activity, antioxidant activity, cytotoxic activity and antispasmodic activity. However, plant materials used in the previous studies were mainly crude extracts with exceptions of curcuminoids from *C. longa* and some gingerols from *Z. officinale*. A few data for cytotoxic activity were reported but the activity against human colon adenocarcinoma cell line LS174T of the five plants have not previously been reported. In addition, pure compounds responsible for antioxidant activity have not yet been identified from *A. galanga, B. pandurata* and *K. galanga*. Apparently, further work on biological evaluation and chemical investigation of these five plants is needed.

1.3 Objectives

1. To study free radical scavenging activity and cytotoxic activity against tumour cells of the extracts and volatile oils from the fresh rhizomes of *Alpinia galanga*, *Boesenbergia pandurata, Curcuma longa, Kaemferia galanga* and *Zingiber officinale*.
2. To study chemical constituents of the extracts and volatile oils which show free radical scavenging activity and/or cytotoxic activity against tumour cells.
3. To assess free radical scavenging activity and/or cytotoxic activity against tumour cells of the isolated compounds.

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and Instruments

IR spectra were recorded on a Jasco IR-810 Spectrophotometer (KBr) of Japan Spectroscopic Co., Ltd. and Perkin Elmer 1600 series FT-IR of Perkin Elmer Co., Ltd. ^{1}H and ^{13}C-Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a FTNMR, Varian UNITY INOVA 500 MHz using either operating solvent or tetramethylsilane (TMS) as an internal standard. Spectra were recorded as chemical shift parameter (δ) value in ppm unit. A 90°-t_1-45° pulse sequence (COSY 45) was used for ^{1}H-^{1}H COSY experiments. Standard program for the library $^{n}J_{CH}$ = 8 Hz was used for the HMBC (heteronuclear multiple bond coherence) experiment. EI-MS data were recorded on a Hewlett-Packard HP 5890 Series II Plus GC-HP 5972 Mass Selective Detector (EI mode with mass range of 35-700 amu). Analysis of volatile oil was carried out by Gas chromatography/Mass spectrometry with a Hewlett-Packard HP 5890 Series II Plus GC-HP 5972 Mass Selective Detector. The operating conditions were as follows: inlet temperature 250 °C, initial temperature 70 °C, detector temperature 280 °C and final temperature 280 °C (hold for 5 min). It was used with column HP-5 length 30 m, film thickness 0.25 μm and ID 0.25 mm. Carrier gas was ultra high purity helium (UHP He). FAB-MS data were recorded by MAT 95 XL Mass Spectrometer which run high and low resolution techniques with solid probe (FAB probe). Ultraviolet spectra (UV) were measured (scanning mode) in the wave length 200-400 nm with Hewlett Packard 8452A Diode Array Spectrometer. The absorbance for free radical scavenging activity was measured at 520 nm with UV spectrometer of Milton Roy Company (Spectronic®Genesys). The absorbance (OD) of each well in cytotoxic activity assay was read on a Power Wave X plate reader (Bio-TEK Instruments Inc.) at 492 nm. Optical rotations were measured by POLAX-L Polarimeter. Silica gel 60 (Merck, 0.040-0.063 mm) was used for vacuum liquid chromatography (VLC) and column chromatography (CC). Sephadex® LH-20 of Amersham Biosciences AB was used for

size-exclusion chromatography. Preparative TLC was performed on silica gel 60 GF_{254} (Merck), 0.5 mm thick, activated at 105 °C for 60 min before use. The zones were detected using UV at 254 nm, scraped off, eluted with chloroform:methanol (3:1) and evaporated to dryness under reduced pressure. Analytical TLC was performed on precoated plates of silica gel $60F_{254}$ (Merck, 0.20 mm thick).

2.2 Plant Materials

Fresh rhizomes of *Alpinia galanga* (L.) Willd., *Boesenbergia pandurata* (Roxb.) Schltr., *Curcuma longa* L., *Kaempferia galanga* L. and *Zingiber officinale* Rosc. were purchased from a local market in Songkhla in April, 2001. They were identified by Assistant Professor Dr. Niwat Keawpradub. Authentication of plant materials were carried out at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, where the herbarium specimens have been kept.

2.3 Extraction and Isolation

Fresh rhizomes of *A. galanga, B. pandurata, C. longa, K. galanga* and *Z. officinale* were washed with water to remove the remaining sand and to reduce the microbial load. The cleaned rhizomes were cut into small pieces and then were divided into two portions.

2.3.1 Volatile oils

The first portion (1 kg of fresh rhizomes of each plant) was subjected to water distillation for 3 hr. After allowed the system cooling down overnight, the volatile oil of each plant was collected.

2.3.2 Water extracts

Marc and water from water distillation in 2.3.1 was filtered by filter paper and evaporated on a water bath (60 °C) to obtain water extract of each plant.

2.3.3 Methanol extracts

The second portion of fresh rhizomes of each plant was blended with methanol by electrical blender, soaked for 72 hr, filtered and the marc was then extracted with methanol repeatedly 2 times. The filtrates were combined and evaporated to dryness to yield methanol extract of each plant.

Volatile oils, water extracts and methanol extracts from the five fresh rhizomes were subjected to preliminary assays for free radical scavenging activity (section 2.5) and cytotoxic activity (section 2.6).

Results from the preliminary assays for free radical scavenging activity (section 3.1.1) and cytotoxic activity (section 3.1.2) of the methanol extracts, water extracts and volatile oils from the fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* gave evidences of the presence of active constituents in the volatile oils of the five fresh rhizomes, the methanol extracts of *A. galanga, C. longa*, and *Z. officinale*. Thus, separation of the active extracts were undertaken and the volatile oils were subjected to chemical analysis (GC/MS).

2.3.4 Isolation of chemical constituents from *Alpinia galanga*

An aliquot (50 g) of the methanol extract of fresh *A. galanga* rhizomes (AGM) was suspended in chloroform:methanol (9:1) overnight. Then it was sonicated 10 min and filtered by filter paper. It was repeatedly dissolved 3 times with 800 ml of chloroform:methanol (9:1). The combined filtrates were evaporated to dryness under reduced pressure to obtain 4 g of dark brown oily gum. Further separation was carried out on a silica gel column chromatograghy using gradient of solvents

chloroform:methanol (19:1, 9:1, 4:1) and finally being washed with methanol to afford thirty-nine fractions (50 ml each). Fractions with the similar TLC chromatogram characteristics were combined and evaporated to dryness under reduced pressure.

Fractions 9-12 were combined and obtained as green-yellow oil (0.666 g). Further separation by preparative TLC on silica gel plates using chloroform:methanol (19:1) as a mobile phase afforded three bands. Further separation of the middle band by preparative TLC on silica gel plates with chloroform:methanol (19:1) as a mobile phase gave **AGM1** (a pure component) as yellow oil (0.039 g).

2.3.5 Isolation of chemical constituents from *Curcuma longa*

An aliquot (30 g) of the methanol extract of fresh *C. longa* rhizomes (CLM) was chromatographed over a silica gel column chromatography using gradient of solvents chloroform:methanol (19:1, 9:1, 4:1) and finally being washed with methanol to obtain seventy-five fractions (50 ml each). Fractions with the similar TLC chromatogram characteristics were combined and evaporated to dryness under reduced pressure to obtain fractions 1-20, 21-23, 24-27, 28-53, 54-74 and 75.

Fractions 1-20 were obtained as pale yellow oil (2.137 g). Chromatogram characteristics on normal phase TLC with toluene:chloroform (2:1) indicated the presence of **CLM01** as a pure component. Fractions 21-23 obtained only few milligrams of residue, thus no further work have been carried out.

Fractions 24-27 were obtained as dark orange liquid (1.546 g) with orange crystals (0.267 g, mixture **CLM02+CLM03**). The total dark orange liquid was further separated by silica gel column chromatography using gradient of solvents chloroform:methanol (19:1, 9:1, 4:1) to obtain thirty-nine fractions (50 ml each). Fractions with the similar TLC chromatogram characteristics were combined and evaporated to dryness under reduced pressure to obtain fractions 13a-15a. Further separation of fractions 13a-15a by preparative TLC on silica gel plates with chloroform:methanol (19:1) as a mobile phase afforded four bands. The top band and

the lowest band yielded **CLM02** as orange crystals (0.161 g) and **CLM03** as reddish orange powder (0.167 g), respectively.

Fractions 28-53 was obtained as dark brown liquid with dark orange crystals. Further separation by preparative TLC on silica gel plates with chloroform:methanol (19:1) as a mobile phase afforded three bands. In the top band and the middle band yielded **CLM02** (0.025 g) and **CLM03** (0.025 g), respectively.

The lowest band was obtained as dark orange liquid. It was further separated by preparative TLC on silica gel plates with chloroform:methanol (19:1) as a mobile phase to afford two bands. The lower band was separated by preparative TLC on silica gel plates with ethyl acetate:chloroform:methanol (10:9.5:0.5) as a mobile phase to afford **CLM06** as reddish orange powder (0.002 g).

2.3.6 Isolation of chemical constituents from *Zingiber officinale*

An aliquot (50 g) of the methanol extract of fresh *Z. officinale* rhizomes (ZOM) was dissolved with methanol (10 ml) and mixed with silica gel to obtain dried sandy sample for vacuum liquid chromatography. Mobile phases used in vacuum liquid chromatography were chloroform:methanol (9:1, 4:1) and methanol, respectively. Three fractions were obtained and evaporated to dryness under reduced pressure.

Fraction 1 (chloroform:methanol; 9:1), upon chromatographic separation of fraction 1 using preparative TLC on silica gel plates and hexane:ethyl acetate (3:1) as a mobile phase, five bands were obtained (band1-5). Further separation of band 3 by sephadex column chromatography eluting with methanol, sixteen fractions were obtained. Fractions with the similar TLC chromatogram characteristics were combined and evaporated to dryness under reduced pressure to obtain fractions 8a-10a and fraction 13a.

Fractions 8a-10a were obtained as orange-yellow oil (0.131 g). Further separation by preparative TLC on silica gel plates with hexane:ethyl acetate (5:1) as a mobile phase gave **ZOM0** as yellow oil (0.042 g).

Fraction 13a, upon standing at room temperature, afforded yellow crystals (0.032 g) which was designated to be **ZOM1**. It gave violet colour by spraying with anisaldehyde reagent.

Band 4 of Fraction 1 was obtained as brown liquid. Upon separation by sephadex column chromatography eluting with methanol, fourteen fractions were afforded. Fractions with the similar TLC chromatogram characteristics were combined and evaporated to dryness under reduced pressure to afford fractions 7b-8b as pale yellow oil (0.154 g). Further separation was carried out by preparative TLC on silica gel plates with hexane:ethyl acetate (3:1) as a mobile phase to afford three bands. The lowest band was obtained as yellow oil (0.078 g) which was designated to be **ZOM3**.

Attempts had been made on purification of **Fraction 2** (chloroform:methanol; 4:1) and **Fraction 3** (methanol) of ZOM but none of pure compounds were obtained.

2.4 Physical and Spectral Properties of the Isolated Compounds

AGM1 (*p*-Coumaryl-9-methyl ether): $C_{10}H_{12}O_2$ (0.039 g); yellow oil; UV (MeOH) λ_{max} (log ε) 264 (4.01) nm; IR (KBr disc) ν_{max} 3350, 2920, 1610, 1520 cm^{-1}; HR-FABMS *m/z* 164.0840 (calc. for $C_{10}H_{12}O_2$ 164.0837); GC/MS (Electron Ionization) *m/z* (% relative intensity) 165 (M+H, 11), 164 (68), 163 (19), 137 (18), 131 (100), 121 (42), 115 (23), 103 (43), 91 (40), 77 (81). ^1H (500 MHz; $CDCl_3$) and ^{13}C NMR (125 MHz; $CDCl_3$) see Table 22; page 83

CLM01 (ar-Turmerone): $C_{15}H_{20}O$ (2.137 g); pale yellow oil; UV (MeOH) λ_{max} (log ε) 238 (3.94) nm; IR (KBr disc) ν_{max} 3000, 1700, 1600 cm^{-1}; GC/MS (Electron Ionization) *m/z* (% relative intensity) 217 (M+H, 4), 216 (23), 201 (14), 132 (17), 119

(59), 83 (100), 55 (25); $[\alpha]_D$ +64.3° (c 0.7, $CHCl_3$); ^1H (500 MHz; $CDCl_3$) and ^{13}C NMR (125 MHz; $CDCl_3$) see Table 23; page 86

CLM02 (Curcumin): $C_{21}H_{20}O_6$ (0.186 g); orange crystals; UV (MeOH) λ_{max} (log ε) 236 (4.93), 424 (4.75) nm; IR (KBr disc) ν_{max} 3500, 1630, 1510, 1430 cm^{-1}; FAB-MS (low resolution) *m/z* (% relative intensity) 369 (M+H, 96), 350 (6), 285 (6), 219 (17), 177 (57), 137 (25), 133 (100); ^1H (500 MHz; $CDCl_3$) and ^{13}C NMR (125 MHz; $CDCl_3$) see Table 24; page 89

CLM03 (Demethoxycurcumin): $C_{20}H_{18}O_5$ (0.192 g); reddish orange powder; UV (MeOH) λ_{max} (log ε) 240 (4.31), 420 (4.77) nm; IR (KBr disc) ν_{max} 3450, 1650, 1600, 1500 cm^{-1}; FAB-MS (low resolution) *m/z* (% relative intensity) 339 (M+H, 25), 316 (5), 277 (13), 224 (21), 185 (100), 147 (10), 132 (21), 93 (85), 75 (18); ^1H (500 MHz; $CDCl_3$) and ^{13}C NMR (125 MHz; $CDCl_3$) see Table 25; page 92

CLM06 (Bisdemethoxycurcumin): $C_{19}H_{16}O_4$ (0.002 g); reddish orange powder; UV (MeOH) λ_{max} (log ε) 246 (4.06), 414 (4.57) nm; IR (KBr disc) ν_{max} 3500-3200 (broad) and 1600 cm^{-1}; FAB-MS (low resolution) *m/z* (% relative intensity) 309 (M+H, 63), 271 (8), 225 (16), 223 (61), 167 (86), 147 (100), 107(55); ^1H (500 MHz; DMSO-d_6) and ^{13}C NMR (125 MHz; DMSO-d_6) see Table 26; page 94

ZOM0 (6-Shogaol): $C_{17}H_{24}O_3$ (0.042 g); yellow oil; UV (MeOH) λ_{max} (log ε) 226 (4.28) nm; IR (KBr disc) ν_{max} 3400, 2960, 1700, 1500 cm^{-1}; FAB-MS (low resolution) *m/z* (% relative intensity) 277 (M+H, 29), 276 (35), 271 (3), 205 (5), 151 (7), 138 (10), 137(100); ^1H (500 MHz; $CDCl_3$) and ^{13}C NMR (125 MHz; $CDCl_3$) see Table 27; page 97

ZOM1 (6-Dehydrogingerdione also known as 1-Dehydrogingerdione): $C_{17}H_{22}O_4$ (0.032 g); yellow crystals; UV (MeOH) λ_{max} (log ε) 370 (4.29) nm; IR (KBr disc) ν_{max} 3350, 2960, 1600, 1500 cm^{-1}; FAB-MS (low resolution) *m/z* (% relative

intensity) 291 (M+H, 80), 289 (6), 276 (4), 219 (14), 191 (18), 177 (83), 137(100); ^{1}H (500 MHz; $CDCl_3$) and ^{13}C NMR (125 MHz; $CDCl_3$) see Table 28; page 100

ZOM3 (6-Gingerol): $C_{17}H_{26}O_4$ (0.078 g); yellow oil; UV (MeOH) λ_{max} (log ε) 282 (3.49) nm; IR (KBr disc) ν_{max} 3450, 2960, 1700, 1550 cm^{-1}; FAB-MS (low resolution) *m/z* (% relative intensity) 295 (M+H, 5), 294 (25), 277 (4), 179 (7), 151 (11), 137 (100); $[\alpha]_D$ +29.3° (c 0.478, $CHCl_3$); ^{1}H (500 MHz; $CDCl_3$) and ^{13}C NMR (125 MHz; $CDCl_3$) see Table 29; page 103

2.5 Assay for Free Radical Scavenging Activity

The antioxidative activity of these rhizomes was evaluated by DPPH radical scavenging assay which was originally described by Blois (1958).

DPPH (1,1-diphenyl-2-picrylhydrazyl) is considered as a stable radical because of the paramagnetism confered by its odd electron (delocalization of the spare electron over the molecule as a whole). The solution (in absolute ethanol) appears as a deep violet colour and shows a strong absorption band at 520 nm. DPPH radical can accept an electron or hydrogen radical to become a stable diamagnetic molecule and has pale violet. If substance for testing antioxidative activity is mixed with DPPH solution and gives rise to pale violet, it suggests that this substance has antioxidative effect by mechanism of free radical scavenging activity. The following assay procedure was modified from those described by Blois (1958) and Yamasaki, *et al.* (1994).

1. Dissolved samples for testing in absolute ethanol (for volatile oils), distilled water (for water extracts) and methanol (for methanol extracts).
2. Diluted each sample for at least 5 concentrations (two-fold dilutions). Each concentration was tested in triplicate.
3. Prepared $6x10^{-5}$ M of DPPH in absolute ethanol.
4. Pipeted 500 µl of sample solution into an eppendorf tube. Each concentration was tested in triplicate.

5. Pipeted 500 µl of DPPH solution to mix with sample solution.

6. Shaked and left at room temperature for 20 min.

7. Measured absorbance at 520 nm by comparing with blank solution of each concentration (sample solution 500 µl + absolute ethanol 500 µl).

8. Prepared standard solution and control in each experiment as follows:

-Control ethanol: 500 µl of absolute ethanol + 500 µl of $6x10^{-5}$ M of DPPH in absolute ethanol; **blank:** 1,000 µl of absolute ethanol.

-Control methanol: 500 µl of methanol + 500 µl of $6x10^{-5}$ M of DPPH in absolute ethanol; **blank:** 500 µl of methanol + 500 µl of absolute ethanol.

-Control water: 500 µl of distilled water + 500 µl of $6x10^{-5}$ M of DPPH in absolute ethanol; **blank:** 500 µl of distilled water + 500 µl of absolute ethanol.

9. Calculation of % inhibition.

$$\text{\% inhibition} = \frac{\text{OD control-OD sample}}{\text{OD control}} \times 100$$

10. Plotted dose-response curve between % inhibition and concentrations.

11. Linear regression analysis was carried out for calculating the effective concentration of sample required to scavenge DPPH radical by 50 % (EC_{50} value).

12. In each experiment BHT (butylated hydroxytoluene, a well known synthetic antioxidant) and caffeic acid were tested as positive standards.

2.6 Assay for Cytotoxic Activity

Volatile oils, methanol extracts, water extracts from the five fresh rhizomes and the isolated compounds were assessed for cytotoxic activity by the Sulphorhodamine B (SRB) assay (Skehan, *et al.*, 1990). SRB is a pink aminoxanthene dye. It is an anionic protein stain containing two sulphonic groups that bind electrostatically to basic amino acid residues of cellular protein under mildly acidic conditions. The bound dye can be quantitatively extracted from cells and solubilized for spectrophotometry by weak bases (Skehan, *et al.*, 1990). This colorimetric assay therefore can be used to estimate

cell number indirectly (for cell monolayer) by providing a sensitive index of total cellular protein content which is linear to cell density.

2.6.1 Human tumour cell lines

The human colon adenocarcinoma cell line LS174T was obtained from King's College London, University of London and the human breast adenocarcinoma cell line MCF7 was obtained from The National Cancer Institute, Bangkok, Thailand. The cells were cultured in Minimum Essestial Media (MEM) with Earle's salt, supplemented with 10 % heat-inactivated newborn calf serum, 2 mM L-glutamine, 50 IU/ml penicillin G sodium, 50 µg/ml streptomycin sulphate and 0.125 µg/ml amphotericin B. The cell were maintained at 37 °C in a 5 % CO_2 atmosphere with 95 % humidity.

2.6.2 Testing procedure

According to growth profile, the optimal plating density of the cell line MCF7 and LS174T were determined to be 2,000 and 1,000 cells/well, respectively to ensure the exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at 492 nm and cell number when analysed by SRB assay (Skehan, *et al.,* 1990). Cells growing as monolayer in a 25 cm^3 flask were washed with phosphate buffered saline (PBS) pH 7.4 and trypsinized with 0.1 % trypsin-EDTA to make a single-cell suspension. The viable cells were counted by trypan blue exclusion in a haemocytometer (Freshney, 1994) and diluted with the medium to give a final concentration of $2x10^4$ cells/ml for cell line MCF7 (2,000 cells/well) and $1x10^4$ cells/ml for cell line LS174T (1,000 cells/well), respectively. 100 µl/well of these cell suspensions were seeded in 96-well microtiter plates and incubated at 37 °C to allow for cell attachment. After 24 hr the cells were treated with the extracts or pure compounds. Each sample was initially dissolved in DMSO for the methanol extracts, or sterile distilled water for the water extracts and vinblastine sulphate, or absolute ethanol for the volatile oil and isolated compounds. They were further diluted in the culture medium to produce the required concentrations. Vinblastine sulphate (anticancer drug, Sigma, MW 909.1) and berberine were used as

positive controls. 100 µl per well of each concentration was added to the plates in 6 replicates. The final mixture used for treating the cells contained not more than 0.5 % of the solvent, the same as in solvent control wells. The 96-well microtiter plates were incubated for the exposure time of 72 hr. At the end of exposure time, the medium was removed. Then 200 µl of fresh medium was added to each well. The plates were further incubated for 72 hr. On the seventh day of culture period, cells were fixed by 100 µl of ice-cold 40 % trichloroacetic acid (TCA) per well, left in the refrigerator at 4 °C for 1 hr and washed 5 times with tap water. Non viable cells were washed and viable cells were fixed as monolayer in each well. 50 µl of SRB solution (0.4 % w/v in 1 % acetic acid) was added to each well and left in contact with the cells for 30 min. The plates were washed 5 times with 1 % acetic acid and dried overnight. On the day of reading plates, bound dye was dissloved with 100 µl of 10 mM Tris base (Tris [hydroxymethyl] aminomethane), shaked on a gyratory shaker 20 min. The absorbance (OD) of each well was read on a plate reader at 492 nm. The intensity of the colour formed in the wells is an indication of the viable cell number. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells). The IC_{50} values (concentrations required to inhibit cell growth by 50 %) were calculated from the dose-response curves obtained by plotting the percentage of survival versus the concentrations. Based on probit analysis (Finney, 1971), computer program was used to determine the IC_{50} values.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Screening of Biological Activity of Crude Extracts and Volatile Oils

The methanol extracts, water extracts and volatile oils of the fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* were prepared as described in section 2.3. Yields and characteristics of the extracts and volatile oils were shown in Table 11. Each volatile oil has characteristic aroma with the yield of about 0.1w/w (fresh weight). The water extracts were obtained as gummy residues with mild odour while the methanol extracts were obtained as oily gums with strong odour.

Table 11 Yields and characteristics of the extracts and volatile oils from the fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale*

Fresh rhizomes	Weight (kg)	Extracts/volatile oils	Physical appearance	Weight (g)	% Yield (w/w)
A. galanga	1	water extract (AGW)	yellowish brown gum	44.2	4.4
		volatile oil (AGV)	yellow oil	0.7	0.1
	2.2	methanol extract (AGM)	black oily gum	113.0	5.1
B. pandurata	1	water extract (BPW)	black gum	24.9	2.5
		volatile oil (BPV)	pale yellow oil	0.9	0.1
	3.6	methanol extract (BPM)	dark brown oily gum	59.8	1.7
C. longa	1	water extract (CLW)	black gum	18.0	1.8
		volatile oil (CLV)	pale yellow oil	0.6	0.1
	1.6	methanol extract (CLM)	blackish orange oily gum	67.2	4.2
K. galanga	1	water extract (KGW)	black gum	12.7	1.3
		volatile oil (KGV)	pale yellow oil	0.7	0.1
	3.1	methanol extract (KGM)	black oily gum	61.1	2.0
Z. officinale	1	water extract (ZOW)	blackish brown gum	19.2	1.9
		volatile oil (ZOV)	dark yellow oil	0.7	0.1
	4.4	methanol extract (ZOM)	black oily gum	115.1	2.6

3.1.1 Free radical scavenging activity

The antioxidative activity of the methanol extracts, water extracts and volatile oils of the five plants was evaluated by DPPH radical scavenging assay as described in section 2.5. The results were shown in Table 12.

Table 12 Percent inhibition of the extracts and volatile oils of the fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* tested by DPPH radical scavenging assay (screening) at the final concentration of 100 µg/ml.

Fresh rhizomes	Extracts/volatile oils	% Inhibition±SD
A. galanga	water extract (AGW)	18.7±1.7 (n=3)
	volatile oil (AGV)	2.7±1.3 (n=3)
	methanol extract (AGM)	92.5±1.3 (n=3)
B. pandurata	water extract (BPW)	11.3±1.6 (n=3)
	volatile oil (BPV)	5.4±1.2 (n=3)
	methanol extract (BPM)	47.4±3.5 (n=3)
C. longa	water extract (CLW)	49.2±0.0 (n=3)
	volatile oil (CLV)	6.5±1.1 (n=3)
	methanol extract (CLM)	91.6±0.2 (n=3)
K. galanga	water extract (KGW)	33.5±0.6 (n=3)
	volatile oil (KGV)	2.2±1.8 (n=3)
	methanol extract (KGM)	34.0±3.8 (n=3)
Z. officinale	water extract (ZOW)	61.5±0.4 (n=3)
	volatile oil (ZOV)	4.1±0.8 (n=3)
	methanol extract (ZOM)	86.6±0.0 (n=3)

n = number of samples tested (triplicate)

The results depicted in Table 12 revealed that the methanol extracts of the fresh rhizomes of *A. galanga, C. longa* and *Z. officinale* possessed strong antioxidative activity against DPPH radical with % inhibition in the range of 86.6-92.5 %. On the contrary, the five volatile oils of the fresh rhizomes showed very weak activity (% inhibition less than 7

%). It is notable that in each rhizome the methanol extract was more active than the corresponding water extract and volatile oil. Subsequently, the methanol extracts of *A. galanga* (AGM), *C. longa* (CLM) and *Z. officinale* (ZOM) were further evaluated for EC_{50} values by DPPH radical scavenging assay as shown in Table 13.

Table 13 EC_{50} values of the methanol extracts of *A. galanga* (AGM), *C. longa* (CLM) and *Z. officinale* (ZOM) against DPPH radical.

Methanol extracts[a]	**EC_{50} (μg/ml) (mean±SD)**
AGM	**57.0±3.7 (n=6)**
CLM	**9.7±0.3 (n=6)**
ZOM	**35.6±1.0 (n=6)**
BHT (positive standard)	**8.2±0.2 (n=6)**
Caffeic acid (positive standard)	**0.9±0.05 (n=6)**

BHT = Butylated hydroxytoluene

[a] See the isolation procedure

n = number of samples tested (6 replicates)

The results of antioxidative activity using the DPPH assay of the methanol extracts of the three fresh rhizomes exhibited that the methanol extract of *C. longa* (CLM) was the most active extract with an EC_{50} value of 9.7 μg/ml.

3.1.2 Cytotoxic activity

The cytotoxic activity of the five rhizomes was evaluated by the Sulphorhodamine B (SRB) assay. The results of cytotoxic activity of the extracts and volatile oils (screening) were shown in Tables 14 and 15.

Table 14 Percent survival of LS174T tested with the extracts and volatile oils of the fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* by SRB assay (screening) at final concentration of 100 μg/ml.

Fresh rhizomes	Extracts/volatile oils	% Survival±SD
A. galanga	water extract (AGW)	51.5±19.0 (n=6)
	volatile oil (AGV)	7.5±1.6 (n=6)
	methanol extract (AGM)	45.6±10.9 (n=6)
B. pandurata	water extract (BPW)	92.6±14.3 (n=6)
	volatile oil (BPV)	0.6±0.7 (n=6)
	methanol extract (BPM)	20.6±7.7 (n=6)
C. longa	water extract (CLW)	97.2±18.9 (n=6)
	volatile oil (CLV)	0.9±0.7 (n=6)
	methanol extract (CLM)	0.0±0.0 (n=6)
K. galanga	water extract (KGW)	52.4±13.2 (n=6)
	volatile oil (KGV)	0.9±0.6 (n=6)
	methanol extract (KGM)	88.4±3.8 (n=6)
Z. officinale	water extract (ZOW)	97.2±29.2 (n=6)
	volatile oil (ZOV)	0.4±0.3 (n=6)
	methanol extract (ZOM)	12.0±6.3 (n=6)

n = number of samples tested (6 replicates)

Table 15 Percent survival of MCF7 tested with the extracts and volatile oils of the fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* by SRB assay (screening) at final concentration of 100 μg/ml

Fresh rhizomes	Extracts/volatile oils	% Survival±SD
A. galanga	water extract (AGW)	59.8±7.3 (n=6)
	volatile oil (AGV)	4.8±0.6 (n=6)
	methanol extract (AGM)	95.2±11.6 (n=6)
B. pandurata	water extract (BPW)	79.4±6.0 (n=6)
	volatile oil (BPV)	5.2±0.5 (n=6)
	methanol extract (BPM)	89.9±2.4 (n=6)
C. longa	water extract (CLW)	66.4±7.1 (n=6)
	volatile oil (CLV)	5.2±0.9 (n=6)
	methanol extract (CLM)	1.3±0.4 (n=6)
K. galanga	water extract (KGW)	76.6±14.7 (n=6)
	volatile oil (KGV)	4.3±0.9 (n=6)
	methanol extract (KGM)	73.0±2.3 (n=6)
Z. officinale	water extract (ZOW)	94.3±11.7 (n=6)
	volatile oil (ZOV)	2.3±0.6 (n=6)
	methanol extract (ZOM)	41.0±7.3 (n=6)

n = number of samples tested (6 replicates)

In Tables 14 and 15, the five volatile oils were found to be active against the cell lines LS174T and MCF7, which showed percent survival in the range of 0.4-7.5 %. The methanol extract of *C. longa* showed pronounced cytotoxic activity against the two cell lines with percent survival in the range of 0-1.3 % at the final concentration of 100 μg/ml.

On the other hand, the methanol extract of *Z. officinale* exhibited moderate cytotoxic activity with percent survival in the range of 12.0-41.0 %. In contrast, the water extracts of the five plants exhibited slight cytotoxic activity (% survival more than 50 %) on both cell lines at 100 µg/ml. It is interesting to note that the volatile oils and methanol extracts of the five plants showed tendency to be more cytotoxic against LS174T rather than MCF7.

So, the methanol extracts of *C. longa* (CLM), *Z. officinale* (ZOM) and the five volatile oils of fresh rhizomes were evaluated for IC_{50} values by Sulphorhodamine B (SRB) assay as shown in Table 16.

Table 16 IC_{50} values of the active methanol extracts and volatile oils tested against LS174T and MCF7 (mean±SEM) by Sulphorhodamine B (SRB) assay

Extracts/volatile oils[a]	IC_{50} against LS174T (µg/ml)[b] (mean±SEM)	IC_{50} against MCF7 (µg/ml)[b] (mean±SEM)
AGV	47.8±6.0 (N =2)	30.5±6.8 (N =2)
BPV	12.0±1.6 (N =2)	31.7±5.4 (N =2)
CLV	20.3±1.4 (N =2)	20.9±0.0 (N =2)
KGV	15.9±1.1 (N =2)	15.4±4.4 (N =2)
ZOV	15.9±0.7 (N =2)	14.2±1.9 (N =2)
CLM	6.4±1.6 (N =2)	14.2±2.1 (N =2)
ZOM	80.0±13.3 (N =3)	75.0+10.5 (N =3)
Berberine	0.8±0.0 (N =2)	0.6±0.0 (N =2)
Vinblastine sulphate (nM)	0.011±0.004 (N =2)	0.008±0.004 (N=2)

[a] See the isolation procedure

[b] Assays for cytotoxic activities were performed in 6 replicates

N = number of independent experiments

The results of cytotoxic activity in Table 16 revealed that the methanol extract of *C. longa* was the most active extract against LS174T and MCF7 with IC_{50} values of 6.4 and 14.2 μg/ml, respectively.

The above results indicated that the five volatile oils were active against LS174T and MCF7 with IC_{50} values in the range of 12.0-47.8 μg/ml but lacked of antioxidative activity. The methanol extracts of the five rhizomes were more active against DPPH radical than their corresponding water extracts. In the antioxidative assay against DPPH radical at final concentration of 100 μg/ml, the methanol extracts of *A. galanga*, *C. longa* and *Z. officinale* exhibited strong antioxidative activity. Their EC_{50} values were less than 100 μg/ml of which the methanol extract of *C. longa* was the most active extract with an EC_{50} value of 9.7 μg/ml. In the cytotoxic activity assay against LS174T and MCF7, the methanol extract of *C. longa* possessed IC_{50} value less than 20 μg/ml (IC_{50} against LS174T was 6.4 μg/ml and that against MCF7 was 14.2 μg/ml). The criteria of cytotoxic activity for the crude extracts, as established by the American National Cancer Institute (NCI), is an IC_{50} value of less than 20 μg/ml in the preliminary assay (Suffness and Pezzuto, 1991).

It is evident that the methanol extracts of *A. galanga*, *C. longa* and *Z. officinale* were of interest for further isolation for pure components (sections 2.3.4, 2.3.5 and 2.3.6) and volatile oils of the five rhizomes, which showed cytotoxic activity, were further analysed to determine chemical constituents by Gas chromatography/Mass spectrometry (section 3.2.1).

3.2 Analysis of Chemical Composition of the Volatile Oils and Structure Determination of the Isolated Compounds

3.2.1 Characterization of component of the volatile oils

The cleaned fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* were prepared for volatile oils (section 2.3.1). Chemical constituents of these volatile oils were analysed by Gas chromatography/Mass spectrometry with a Hewlett-Packard HP 5890 series II plus GC-HP 5972 Mass Selective Detector. Chemical constituents of the volatile oils from *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* were shown in Tables 17, 18, 19, 20 and 21, respectively.

Table 17 Chemical constituents of the volatile oil from *Alpinia galanga* (GC/MS analysis)

Retention time (min)	**% Area**	**Compound**	**Molecular weight**	**Molecular formula**
3.53	Minor compound	Unable to be identified	-	-
3.73	11.59	Terpinene-4-ol	154.14	$C_{10}H_{18}O$
3.88	5.54	1-α-Terpineol	154.14	$C_{10}H_{18}O$
4.74	6.91	4-Allylphenol	134.07	$C_9H_{10}O$
6.06	11.44	4-Allylphenyl acetate	176.08	$C_{11}H_{12}O_2$
6.16	58.47	*trans*-3-Acetoxy-1,8-cineole	212.00	$C_{12}H_{20}O_3$
7.01	Minor compound	*trans*-Methyl isoeugenol	178.10	$C_{11}H_{14}O_2$
7.57	6.05	Unable to be identified	-	-
8.09	Minor compound	α-Humulene	204.19	$C_{15}H_{24}$
8.58	Minor compound	Unable to be identified	-	-
9.05	Minor compound	Unable to be identified	-	-
9.66	Minor compound	Unable to be identified	-	-
11.00	Minor compound	Unable to be identified	-	-

Table 18 Chemical constituents of the volatile oil from *Boesenbergia pandurata* (GC/MS analysis)

Retention time (min)	**% Area**	**Compound**	**Molecular weight**	**Molecular formula**
3.19	81.43	Camphor	152.12	$C_{10}H_{16}O$
4.85	5.99	Geraniol	154.14	$C_{10}H_{18}O$
4.95	5.37	Geranial (*E*-Citral)	152.12	$C_{10}H_{16}O$
6.57	7.21	Methyl cinnamate	162.07	$C_{10}H_{10}O_2$

Table 19 Chemical constituents of the volatile oil from *Curcuma longa* (GC/MS analysis)

Retention time (min)	% Area	Compound	Molecular weight	Molecular formula
3.70	2.07	4-Terpineol	154.14	$C_{10}H_{18}O$
3.85	2.11	1-Methyl-4-(2-propanol-2-yl)-cyclohexene	154.14	$C_{10}H_{18}O$
4.48	5.20	Unable to be identified	-	-
5.15	1.00	Unable to be identified	-	-
5.57	0.92	Unable to be identified	-	-
5.65	1.24	Unable to be identified	-	-
8.52	1.98	ar-Curcumene	202.17	$C_{15}H_{22}$
9.21	1.48	β-Sesquiphellandrene	204.19	$C_{15}H_{24}$
9.88	2.66	Unable to be identified	-	-
10.17	1.06	Unable to be identified	-	-
10.77	1.95	Unable to be identified	-	-
11.13	38.00	ar-Turmerone	216.17	$C_{15}H_{20}O$
11.23	7.41	α-Turmerone	218.17	$C_{15}H_{22}O$
11.69	11.43	β-Turmerone	218.17	$C_{15}H_{22}O$
12.54	1.43	Unable to be identified	-	-
12.96	1.43	Unable to be identified	-	-
13.65	4.79	Unable to be identified	-	-
13.79	4.36	Unable to be identified	-	-
13.85	2.12	Unable to be identified	-	-
14.03	1.53	Unable to be identified	-	-
14.73	5.24	Unable to be identified	-	-
15.38	0.62	Unable to be identified	-	-

Table 20 Chemical constituents of the volatile oil from *Kaempferia galanga* (GC/MS analysis)

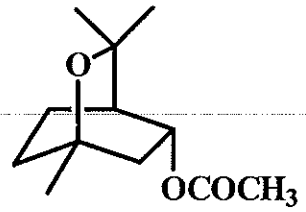
Retention time (min)	**% Area**	**Compound**	**Molecular weight**	**Molecular formula**
3.29	0.66	Unable to be identified	-	-
3.43	2.31	Unable to be identified	-	-
3.55	7.58	*l*-Borneol	154.14	$C_{10}H_{18}O$
3.67	1.11	*p*-Cymen-8-ol	150.10	$C_{10}H_{14}O$
3.72	1.95	Terpinene-4-ol	154.14	$C_{10}H_{18}O$
3.87	1.23	β-Fenchyl alcohol	154.14	$C_{10}H_{18}O$
3.97	0.69	Unable to be identified	-	-
4.20	0.87	Unable to be identified	-	-
4.55	0.86	Unable to be identified	-	-
8.09	61.81	Ethyl cinnamate	176.08	$C_{11}H_{12}O_2$
9.16	2.60	Pentadecane	212.25	$C_{15}H_{32}$
12.33	18.33	3-(4-Methoxyphenyl)-2-propenoic acid ethyl ester	206.09	$C_{12}H_{14}O_3$

Table 21 Chemical constituents of the volatile oil from *Zingiber officinale* (GC/MS analysis)

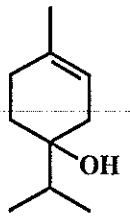
Retention time (min)	**% Area**	**Compound**	**Molecular weight**	**Molecular formula**
3.35	1.87	Citronellal	154.14	$C_{10}H_{18}O$
3.53	4.26	*l*-Borneol	154.14	$C_{10}H_{18}O$
3.62	3.18	Cryptone	138.10	$C_9H_{14}O$
3.87	2.82	1-α-Terpineol	154.14	$C_{10}H_{18}O$
4.54	26.59	Neral (*Z*-Citral)	152.12	$C_{10}H_{16}O$
4.97	33.02	Geranial (*E*-Citral)	152.12	$C_{10}H_{16}O$
5.44	1.99	2-Undecanone	170.17	$C_{11}H_{22}O$
5.83	5.17	Unable to be identified	-	-
6.39	6.87	Unable to be identified	-	-
6.73	8.32	Unable to be identified	-	-
8.08	1.52	Unable to be identified	-	-
8.54	3.07	ar-Curcumene	202.17	$C_{15}H_{22}$
10.56	1.34	Unable to be identified	-	-

Chemical compositions of the volatile oils, analyzed by GC/MS technique, were shown in Tables 17-21. Volatile oil of *A. galanga* (AGV) was composed of at least 13 compounds having *trans*-3-acetoxy-1,8-cineole (% area 58.47) and terpinene-4-ol (% area 11.59) as major components. This was partly similar to the previous data reported by De Pooter, *et al.* (1985) of which α-pinene, 1,8-cineole, bornyl acetate, geranyl acetate, α-bergamotene, *trans*-β-farnesene and β-bisabolene were identified as major compounds of the volatile oil of *A. galanga.* The oil of *B. pandurata* (BPV) contained at least 4 compounds including camphor (% area 81.43) and methyl cinnamate (% area 7.21) which were the main constituents. This was in accordance with the result reported by Jantan, *et al.* (2001) of which the major constituents of the rhizome oil of *B. pandurata* were detected as camphor (16.1-32.1 %), geraniol (16.2-26.0 %), (*E*)-β-ocimene (19.0-23.7 %), 1,8-cineole (7.5-13.9 %), camphene (5.4-6.0 %) and methyl cinnamate (2.2-5.8 %). The oil of *C. longa* (CLV) was composed of at least 22 compounds, characterized by high proportion of ar-turmerone (% area 38.00) and β-turmerone (% area 11.43). Analysis of the oil of *K. galanga* (KGV) revealed the existence of at least 12 compounds, in which the main constituents were identified as ethyl cinnamate (% area 61.81) and 3-(4-methoxyphenyl)-2-propenoic acid ethyl ester (% area 18.33). The volatile oil of *Z. officinale* (ZOV) were composed of at least 13 compounds with geranial (% area 33.02) and neral (% area 26.59) being main compounds. It is notable that volatile oils of the five spices were mainly composed of monoterpenes, sesquiterpenes and phenylpropanoids. The major component in the volatile oil from *C. longa* rhizomes was identified as ar-turmerone which was in good agreement with the previous reports (Gopalan, *et al.*, 2000; Negi, *et al.*, 1999; Martins, *et al.*, 2001). The volatile oil from the fresh rhizome of *Z. officinale* was characterized by presence of acyclic oxygenated monoterpenes mainly composed of geranial (*E*-citral) and neral (*Z*-citral), which was in accordance with the result reported by Sakamura (1987). These major and minor compounds in the volatile oils could be responsible for the observed cytotoxic activity of the volatile oils. For example, growth of V-79 cells (lung fibroblasts of Chinese hamster) was completely inhibited on treatment with camphor at 0.3 % for 24-48 hr (Toshihiko, 1987). A

sesquiterpene β-sesquiphellandrene, one of the minor compounds detected in CLV, was reported to be cytotoxic against mouse lymphocytic leukaemia cells L1210 (Ahn and Lee, 1989). Citral, one of the major compounds of ZOV, was found to be cytotoxic against P388 mouse leukaemia cells (Dubey, Takeya and Itokawa, 1997). Geraniol (at 400 μM), one of the minor compounds of BPV, inhibited the growth of Caco-2 (human colon cancer cells) by 70 % (Carnesecchi, *et al.*, 2001). These studies gave support to the pronounced cytotoxic activity against tumour cells of the five volatile oils observed in the present work. It is obvious that the volatile oils are accounted for one of the cytotoxic constituents against tumour cells of the five rhizomes, which is of interest for medicinal purposes.



trans-3-Acetoxy-1,8-cineole



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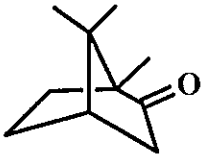

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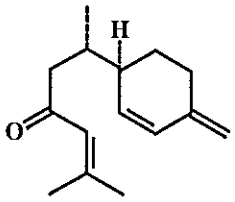
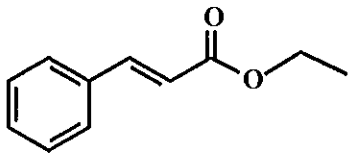
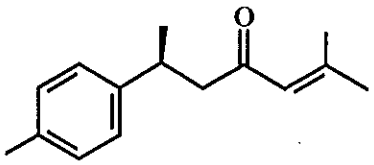


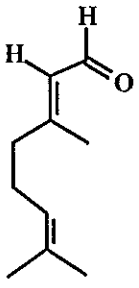
β-Turmerone



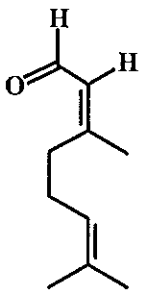
Ethyl cinnamate



ar-Turmerone



Geranial (*E*-Citral)



Neral (*Z*-Citral)

Figure 6 Structures of some major compounds detected in the volatile oils of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale*.

3.2.2 Structure elucidation of the isolated compounds

Results from the preliminary assays for free radical scavenging activity in section 2.5 and cytotoxic activity in section 2.6 of the methanol extracts, water extracts and volatile oils of the fresh rhizomes of *A. galanga*, *B. pandurata*, *C. longa*, *K. galanga* and *Z. officinale* were shown in section 3.1. They gave evidences of the presence of active constituents in the volatile oils of the five fresh rhizomes and the methanol extracts of *A. galanga*, *C. longa*, and *Z. officinale*. Thus, a separation of the active extracts was carried out as shown in section 2.3 to give the pure compounds as follows:

AGM1 from the methanol extract of the fresh rhizome of *A. galanga*
CLM01 from the methanol extract of the fresh rhizome of *C. longa*
CLM02 from the methanol extract of the fresh rhizome of *C. longa*
CLM03 from the methanol extract of the fresh rhizome of *C. longa*
CLM06 from the methanol extract of the fresh rhizome of *C. longa*
ZOM0 from the methanol extract of the fresh rhizome of *Z. officinale*
ZOM1 from the methanol extract of the fresh rhizome of *Z. officinale*
ZOM3 from the methanol extract of the fresh rhizome of *Z. officinale*

3.2.2.1 AGM1

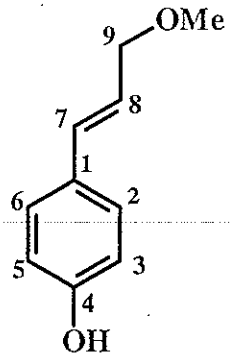
AGM1 was obtained as yellow oil. Its UV spectrum showed maximum absorption at 264 nm. The IR spectrum (KBr disc) showed the presence of hydroxyl function at 3350, C-H stretching at 2920 and olefinic carbon stretching at 1610 and 1520 cm^{-1}.

The molecular formula of AGM1 was proposed to be $C_{10}H_{12}O_2$ (MW = 164, D.B.E. = 5) as deduced from EIMS spectrum. The ^{13}C NMR (125 MHz, $CDCl_3$) revealed 10 carbons, 8 of which corresponded with 11 protons as observed from HMQC spectrum. This corresponded well with the observed molecular mass from HR-FABMS at *m/z* 164.0840. In the ^{1}H NMR spectrum of AGM1 (500 MHz; $CDCl_3$),

the 3-H singlet signal of a methoxyl group was detected at δ 3.41 and 2 aromatic spin systems (integrated two protons each) were observed. They are in *ortho* position of benzene ring (δ 6.78, d, J = 9.0 Hz, H-3, H-5 and δ 7.25, d, J = 9.0 Hz, H-2, H-6). This suggested that the benzene ring had substituted groups in *para* position. The upfield shift of proton signal at δ 6.78 (δ_C 115.6) was due to the shielding effect of the hydroxyl group at C-4. The existence of OH function was confirmed with the IR absorption band at 3350 cm^{-1}. The two quaternary carbons at δ 129.3 and 155.8 were attributed to C-1 and C-4 respectively, based on hydroxyl substitution at C-4. Two proton signals were observed at δ 6.55 (br.d, J = 16.0 Hz) and δ 6.13 (dt, J = 16.0, 6.0 Hz), which were characteristic of two *trans* olefinic protons and subsequently assigned for H-7 and H-8, respectively. Their corresponding carbons C-7 and C-8 were respectively observed at δ 132.8 and 123.1 in the HMQC spectrum.

Chemical shifts of the remaining H-bonded carbons were allocated by HMQC experiment as shown in Table 22. The position of methoxyl group was allocated by analysis of nuclear Overhauser effect (nOe) difference spectra. Upon irradiation of the signal at δ 3.41 (methoxyl group) resulted in strong enhancement to signal at δ 4.10 (H-9), indicating that these methylene protons (-CH_2-) were adjacent to the methoxyl group. In addition, H-8 and H-9 showed vicinal (3-bond) coupling with coupling constant of 6.0 Hz, suggesting the presence of a propenoid side chain (C-7, C-8 and C-9).

These spectral features suggested that AGM1 was a derivative of coumaric acid, which has been modified through reduction and methylation in shikimate pathway in plant. Thus, AGM1 was characterized as a phenylpropanoid and named to be *p*-coumaryl-9-methyl ether. Some phenylpropanoids, e.g. *p*-coumaryl alcohol, *p*-coumaric acid and cinnamic acid were isolated from the rhizome of *A. galanga* (Matsuda *et al.*, 2003) but this compound has not previously been reported from *A. galanga*. This is the first report of naturally occurring *p*-coumaryl-9-methyl ether with complete ^1H and ^{13}C NMR assignments.



p-Coumaryl-9-methyl ether (AGM1)

Table 22 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of AGM 1[a]

Position	δ_H (mult., *J* in Hz)[b]	δ_C
1	-	129.3
2, 6	7.25 (d, 9.0, 2H)	127.9
3, 5	6.78 (d, 9.0, 2H)	115.6
4	-	155.8
7	6.55 (br.d, 16.0)	132.8
8	6.13 (dt, 16.0, 6.0)	123.1
9	4.10 (dd, 6.0, 1.5, 2H)	73.4
OMe	3.41 (s, 3H)	57.8

Note; [a]In $CDCl_3$

[b]If not indicated, the integration of each proton signal is equal to 1 proton.

3.2.2.2 CLM01

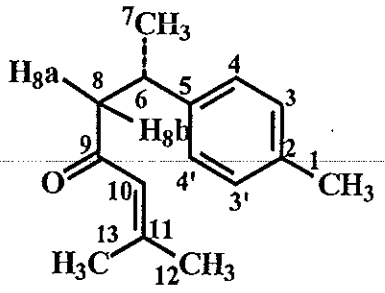
Phytochemical investigation of the methanol extract of *C. longa* led to the isolation of pure compound CLM01 as pale yellow oil. The EI mass spectrum of CLM01 showed a protonated molecular ion peak at *m/z* 217 consistent with the molecular formula $C_{15}H_{20}O$ (MW = 216, D.B.E. = 6). The IR spectrum (KBr disc) of CLM01 showed C-H stretching at 3000, carbonyl stretching at 1700 and double bond stretching at 1600 cm^{-1}. CLM01 showed UV absorption at 238 nm and specific optical rotation of +64.3° (c 0.7, $CHCl_3$).

The ^1H and ^{13}C NMR, ^1H-^1H COSY, HMQC and HMBC spectra of CLM01 showed the existence of one benzene ring, one olefinic function, four methyl groups, one methylene group and four quaternary carbons (including a carbonyl function). Chemical shifts of all protonated carbons were assigned by HMQC experiment. The quaternary carbon at δ 199.9 was attributed to a carbonyl function, which showed two-bond cross peaks with protons at δ 2.60 (H-8a), δ 2.70 (H-8b) and δ 6.02 (H-10) in the HMBC spectrum. This resulted in the assignments of carbon signals at δ 52.6 and 124.0 to be C-8 and C-10, respectively, according to the HMQC correlation with their corresponding proton signals. Having allocated the chemical shift of olefinic carbon (C-10) at δ 124.0, the two methyl functions at δ 1.85 (3H, d, 1.3 Hz) and δ 2.10 (3H, d, 1.3 Hz), which showed 3-bond correlation with C-10 in the HMBC spectrum, were assigned to be 12-CH_3 and 13-CH_3, respectively. The downfield shift of 13-CH_3, comparing with 12-CH_3, was due to deshielding effect of the carbonyl function. The quaternary carbon at δ 155.2 was attributed to C-11 due to the correlation with the two methyl functions at δ 1.85 and δ 2.10 in the HMBC spectrum. The methine carbon at δ 35.2 (δ_H 3.28) was attributed to C-6 according to two-bond correlation with H-8a and H-8b in the HMBC spectrum. The methyl function at δ 1.23 (3H, d, 7.1 Hz), which showed cross peaks with C-6 (δ 35.2) and C-8 (δ 52.6) in the HMBC spectrum, was therefore assigned to be 7-CH_3. Furthermore, H-6 (δ 3.28) also showed cross peaks with quaternary carbon at δ 143.6 and protonated carbon at δ 126.6 leading to

assignments of these two signals as C-5 and C-4/C-4′ (equivalent carbons), respectively.

The correlation of H-4/H-4′ (δ 7.09) with protonated carbon signal at δ 129.0 and quaternary carbon at δ 135.5 in the HMBC spectrum resulted in assignments of these two signals to be C-3/C-3′ (equivalent carbons) and C-2, respectively. The spin coupling between H-3/H-3′ and H-4/H-4′ as doublet with coupling constant (*J* value) of 8.5 Hz (*ortho* coupling) and the presence of two quaternary carbons at δ 135.5 (C-2) and δ 143.6 (C-5) were indicative of a 1,4-disubstitued benzene ring. The remaining methyl function at δ 2.30 (3H, s), which showed correlation with carbon signals at δ 135.5 (C-2) and δ 129.0 (C-3/C-3′) in the HMBC spectrum, was attributed to 1-CH_3.

These spectral features of CLM01 were in good agreement with those of the sesquiterpene ar-turmerone previously isolated from the rhizome of *Curcuma longa* (Ferreira *et al.*, 1992). However, the published chemical shift assignments of C-5 (δ 154.6) and C-11 (δ 143.5) in the previous work should be reassigned on the other way round (C-11 should be more downfield than C-5) according to the data obtained in this present work. The stereochemistry at C-6 was assumed to be *S*-configuration based on the similar profiles of optical rotation (+64.3°; literatures: +82.7° and +68°) and proton NMR characteristics of H-6 and 7-CH_3 of CLM01 to those of the published data (Honwad and Rao, 1964; Li *et al.*, 2003). The unambiguous ^{1}H and ^{13}C NMR assignments of CLM01 obtained from the present work were shown in Table 23.



ar-Turmerone (CLM01)

Table 23 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of CLM01[a]

Position	δ_H (mult., *J* in Hz)[b]	δ_C	HMBC $^{13}C \longrightarrow {}^{1}H$
1	2.30 (s, 3H)	20.9	-
2	-	135.5	H-1, H-3, H-3′, H-4, H-4′
3, 3′	7.11 (d, 8.5, 2H)	129.0	H-1, H-4, H-4′
4, 4′	7.09 (d, 8.5, 2H)	126.6	H-3, H-3′, H-6
5	-	143.6	H-3, H-3′, H-4, H-4′, H-6, H-7, H-8a, H-8b
6	3.28 (br.dq, 8.4, 7.1)	35.2	H-7, H-8a, H-8b
7	1.23 (d, 7.1, 3H)	21.9	H-6, H-8a, H-8b
8	2.60 (dd, 15.8, 8.4, H-8a)	52.6	H-6, H-7
	2.70 (dd, 15.8, 6.2, H-8b)	52.6	H-6, H-7
9	-	199.9	H-8a, H-8b, H-10
10	6.02 (septet, 1.3)	124.0	H-12, H-13
11	-	155.2	H-12, H-13
12	1.85 (d, 1.3, 3H)	27.6	H-10, H-13
13	2.10 (d, 1.3, 3H)	20.7	H-10, H-12

Note; [a]In $CDCl_3$

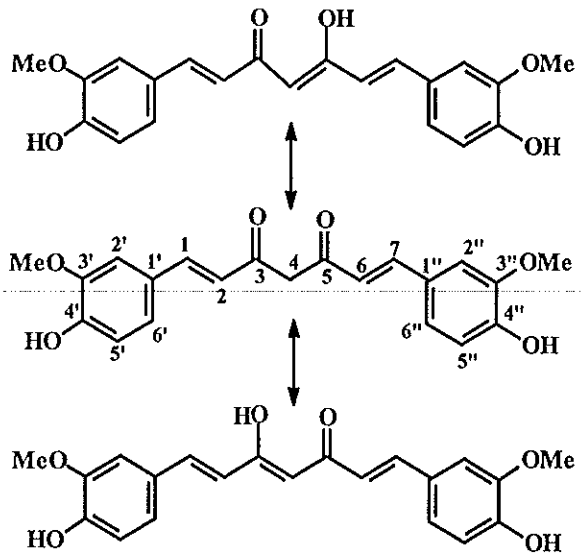
[b]If not indicated, the integration of each proton signal is equal to 1 proton.

3.2.2.3 CLM02

CLM02 was the major compound of the methanol extract of *C. longa*, obtained as orange crystals and showed protonated molecular ion peak in FAB mass spectrum at *m/z* 369, corresponding with a molecular formula of $C_{21}H_{20}O_6$ (MW = 368, D.B.E. = 12). The IR (KBr disc) spectrum indicated the presence of hydroxy group at 3500, carbonyl function at 1630 and olefinic carbon stretching at 1510 cm^{-1}. Its UV (MeOH) spectrum showed absorption maxima at 236 and 424 nm.

^{1}H and ^{13}C NMR spectra of CLM02 revealed the existence of aromatic, olefinic, methoxyl, hydroxyl and carbonyl functions. Analysis of chemical shifts, integration and spin coupling patterns of these functional groups gave evidence that CLM02 was a diarylheptanoid. The signal of carbonyl function at δ 183.2, which showed correlation with *trans*-olefinic protons at δ 7.60 (2H, d, 15.5 Hz) and δ 6.49 (2H, d, 15.5 Hz) in the HMBC spectrum, was attributed to C-3/C-5 (equivalent carbons). The substantial downfield shift of signal at δ 7.60, comparing with the signal at δ 6.49, was typical for the β-olefinic proton of the α,β-unsaturated ketone. Hence the signals at δ 7.60 and δ 6.49 were assignable for H-1/H-7 and H-2/H-6, respectively. The corresponding C-1/C-7 and C-2/C-6 were assigned by HMQC experiment at δ 140.5 and 121.8, respectively. The cross peaks of C-1/C-7 with proton signals at δ 7.06 (H-2′ and H-2″) and δ 7.14 (H-6′ and H-6″) in the HMBC spectrum yielded additional support for the presence of 1,6-heptadiene-3,5-dione moiety with two equivalent phenyl groups attached to C-1 and C-7 of the molecule. It was notable that the signal of H-4 was assignable as an enol form at δ 5.81 (δ_C 101.2). The spin coupling patterns of H-2′/H-2″, H-5′/H-5″ and H-6′/H-6″, which were doublet (2.0 Hz), doublet (8.3 Hz) and doublet of doublets (8.3 and 2.0 Hz), respectively, indicated the presence of 1,3,4-trisubstituted benzene ring. The 3-bond cross peaks of quaternary carbon signal at δ 146.8 with proton signals at δ 6.92 (H-5′/H-5″) and δ 3.96 (3′-OCH_3/3″-OCH_3) in the HMBC spectrum enabled us to assign this signal as C-3′/C-3″. The quaternary carbon signal at δ 147.8 was attributed to C-4′/C-4″ due to the 3-bond cross peaks with proton signals at δ 7.06 (H-2′/H-2″) and δ 7.14 (H-6′/H-6″) in the HMBC spectrum.

These spectral features of CLM02 was in accordance with those of published data of curcumin or 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (Kosuge, Ishida and Yamazaki, 1985; Uehara *et al.*, 1987; Masuda *et al.*, 1992). Curcumin has been identified as the major constituent in Turmeric (*Curcuma longa*) and also has been isolated from some other *Curcuma* species, e.g. *C. xanthorrhiza* and *C. aromatica* (Kosuge, Ishida and Yamazaki, 1985; Ishida *et al.*, 2002). The complete ^{1}H and ^{13}C NMR assignments of curcumin (CLM02) obtained from the present work were shown in Table 24.



Curcumin (CLM02)

Table 24 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of CLM02[a]

Position	δ_H (mult., *J* in Hz)[b]	δ_C	HMBC $^{13}C \longrightarrow {}^{1}H$
1,7	7.60 (d, 15.5, 2H)	140.5	H-6′, H-6″, H-2′, H-2″
2, 6	6.49 (d, 15.5, 2H)	121.8	H-4
3, 5	-	183.2	H-1, H-2, H-4, H-6, H-7
4	5.81 (s)[c]	101.2	H-2, H-6
1′, 1″	-	127.7	H-2, H-6, H-5′, H-5″
2′, 2″	7.06 (d, 2.0, 2H)	109.6	H-1, H-7, H-6′, H-6″
3′, 3″	-	146.8	H-5′, H-5″, OMe
4′, 4″	-	147.8	H-2′, H-2″, H-6′, H-6″
5′, 5″	6.92 (d, 8.3, 2H)	114.8	H-6′, H-6″
6′, 6″	7.14 (dd, 8.3, 2.0, 2H)	122.9	H-1, H-7, H-2′, H-2″
3′-OMe, 3″-OMe	3.96 (s, 6H)	55.9	-
4′-OH, 4″-OH	5.88 (br.s, 2H)	-	-

Note; [a]In $CDCl_3$

[b]If not indicated, the integration of each proton signal is equal to 1 proton.

[c]Detected as an enol form.

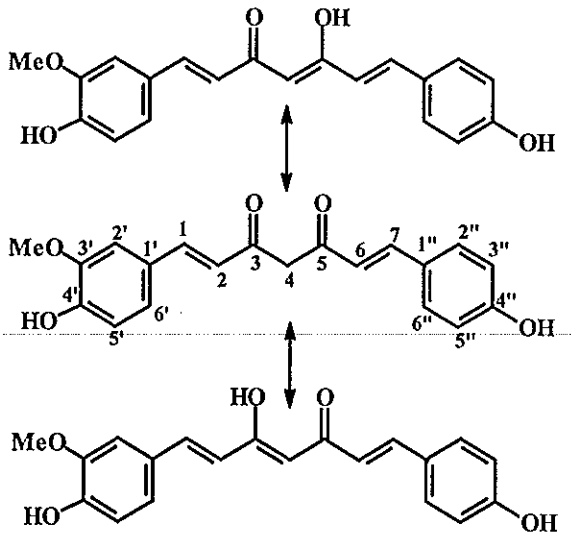
3.2.2.4 CLM03

CLM03 was isolated from the methanol extract of *C. longa* as reddish orange powder. The FAB mass spectrum (low resolution) spectrum of CLM03 showed protonated molecular ion peak at *m/z* 339, consistent with the molecular formula $C_{20}H_{18}O_5$ (MW = 338, D.B.E. = 12). The IR (KBr disc) spectrum indicated the presence of hydroxy group at 3450, carbonyl function at 1650 and olefinic carbon stretching at 1500 cm^{-1}. Its UV (MeOH) spectrum showed absorption maxima at 240 and 420 nm.

^{1}H and ^{13}C NMR spectra of CLM03 showed the existence of functional groups almost similar to those of CLM02 (curcumin), suggesting that CLM03 should be a diarylheptanoid. This was strongly supported by the presence of two carbonyl signals at δ 183.3 and δ 183.4 and four signals of *trans*-olefinic protons at δ 7.60 (1H, d, 15.8 Hz), δ 6.50 (1H, d, 15.8 Hz), δ 6.49 (1H, d, 15.8 Hz) and δ 7.62 (1H, d, 15.8 Hz), which indicated the characteristics of 1,6-heptadiene-3,5-dione skeleton. Furthermore, signals of two aromatic systems, which showed different substitution patterns, were observed. The first system was composed of three signals at δ 7.06 (1H, d, 1.8 Hz), δ 6.95 (1H, d, 8.4 Hz) and δ 7.13 (1H, dd, 8.4 and 1.8 Hz) suggesting the presence of 1,3,4-trisubstituted benzene ring according to the spin couplings. These three signals were attributed to H-2′, H-5′ and H-6′, respectively, based on the 3-bond correlation of the olefinic carbon signal at δ 140.6 (C-1) with signals at δ 7.06 (H-2′) and δ 7.13 (H-6′) in the HMBC spectrum (Table 25). The signal of methoxyl group at δ 3.96 (3H, s) was assigned as 3′-OCH_3 due to the cross peaks with C-3′ (δ 146.8) in the HMBC spectrum. Having allocated the position of C-1, the signal of carbonyl function at δ 183.3 was assignable to C-3 due to the cross peaks between C-3 and proton signals at δ 7.60 (H-1) and δ 6.50 (H-2). The second aromatic system was composed of two proton signals at δ 7.45 (2H, d, 8.7 Hz) and δ 6.87 (2H, d, 8.7 Hz), indicating the existence of 1,4-disubstituted benzene ring due to the *ortho* couplings. These two signals were assigned to be H-2″/H-6″ and H-3″/H-5″, respectively, according to the correlation with carbon signals at δ 128.0 (C-1″) and δ 157.5 (C-4″), and the

correlation between H-2″/H-6″ and the olefinic carbon at δ 140.0 (C-7) in the HMBC spectrum. The noticeable upfield shift of H-3″/H-5″, comparing with that of H-2″/H-6″, was due to the shielding effect of the hydroxyl substitution at C-4″. The carbonyl signal at δ 183.4 was attributed to C-5 based on the HMBC correlation with proton signals at δ 7.62 (H-7), δ 6.49 (H-6) and δ 5.80 (H-4) in the HMBC spectrum. All protonated carbons were assigned by HMQC experiment including C-4 (δ 101.3), which was assignable to an enol form.

The obtained spectral information indicated that the structure of CLM03 related to that of curcumin (CLM02) with an absence of one methoxyl group. This was also supported by the difference of molecular weight of the two compounds, of which CLM03 was 30 amu less than that of curcumin. Hence, CLM03 was concluded to be demethoxycurcumin or 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione. Demethoxycurcumin was previously isolated from the rhizome of *C. longa* and some other *Curcuma* species (Kosuge, Ishida and Yamazaki, 1985; Masuda *et al.*, 1992). However, the chemical shifts of carbons and protons of demethoxycurcumin have only been partially assigned in the previous publications. The unambiguous ^{1}H and ^{13}C NMR assignments of demethoxycurcumin (CLM03) was achieved in the present work through 2D-NMR experiments as shown in Table 25.



Demethoxycurcumin (CLM03)

Table 25 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of CLM03[a]

Position	δ_H (mult., *J* in Hz)[b]	δ_C	HMBC $^{13}C \longrightarrow {}^{1}H$
1	7.60 (d, 15.8)	140.6	H-2′, H-6′
2	6.50 (d, 15.8)	121.8	H-4
3	-	183.3	H-1, H-2, H-4
4	5.80 (s)[c]	101.3	H-2, H-6
5	-	183.4	H-4, H-6, H-7
6	6.49 (d, 15.8)	121.7	H-4
7	7.62 (d, 15.8)	140.0	H-2″, H-6″
1′	-	127.4	H-5′
2′	7.06 (d, 1.8)	109.6	H-1, H-6′
3′	-	146.8	H-5′, 3′-OMe
4′	-	147.8	H-2′, H-6′
5′	6.95 (d, 8.4)	114.8	H-6′
6′	7.13 (dd, 8.4, 1.8)	122.9	H-1, H-2′
1″	-	128.0	H-6, H-3″, H-5″
2″, 6″	7.45 (d, 8.7, 2H)	130.0	H-3″,H-5″, H-7
3″, 5″	6.87 (d, 8.7, 2H)	115.9	H-2″, H-6″
4″	-	157.5	H-2″, H-3″, H-5″,H-6″
3′-OMe	3.96 (s, 3H)	56.0	-
4′-OH	5.88 (br.s)	-	-
4″-OH	not detected	-	-

Note; [a]In $CDCl_3$,

[b]If not indicated, the integration of each proton signal is equal to 1 proton.

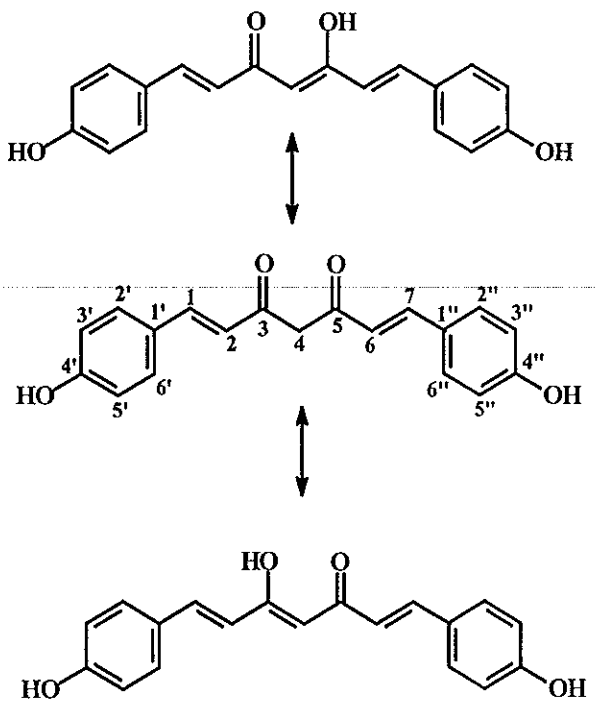
[c]Detected as an enol form.

3.2.2.5 CLM06

CLM06 was isolated from the methanol extract of *C. longa*. The FAB mass spectrum (low resolution) of CLM06 showed protonated molecular ion peak at *m/z* 309 (M+H) consistent with the molecular formula $C_{19}H_{16}O_4$ (MW = 308, D.B.E. = 12). The IR spectrum indicated the presence of hydroxy group and carbonyl function at 3500-3200 (broad) and 1600 cm^{-1}, respectively. Its UV spectrum showed absorption with λ_{max} at 246 and 414 nm.

^{1}H and ^{13}C NMR spectra of CLM06 exhibited the 1,6-heptadiene-3,5-dione skeleton, according to the appearance of *trans*-olefinic proton signals at δ 7.54 (H-1/H-7) and δ 6.68 (H-2/H-6) together with the carbonyl signal at δ 183.4 (C-3/C-5). The two signals at δ 7.55 (4H, d, 8.6 Hz) and δ 6.81 (4H, d, 8.6 Hz) were assignable to protons of two 1,4-disubstituted benzene rings. The proton signal at δ 6.00 (1H, s) and its corresponding carbon signal at δ 101.1 were attributed to H-4 and C-4, respectively (detected as enol form). These spectral features suggested that CLM06 had a highly symmetrical structure, regarding to the molecular weight of 308 amu (60 amu less than that of curcumin). The absence of methoxyl signal in NMR spectra of CLM06 gave additional evidence to propose the assumption that CLM06 should be a bisdemethoxy derivative of curcumin. The ion peak at *m/z* 147 (base peak) in the mass spectrum of CLM06, which was assignable to a $[HO\text{-}C_6H_4\text{-}CH{=}CH\text{-}\underset{|}{C}{=}O]^+$ fragment, gave strong support to the assignment. Furthermore, the quaternary carbon signal at δ 160.0 (C-4′/C-4″) suggested the hydroxyl substitution on C-4′ and C-4″.

Comparison of ^{1}H and ^{13}C NMR assignments of CLM06 with the published data (Kosuge, Ishida and Yamazaki, 1985; Masuda *et al.*, 1992) resulted in determination of CLM06 as bisdemethoxycurcumin or 1,7-bis (4-hydroxyphenyl)-1,6-heptadiene-3,5-dione, which was previously isolated from the rhizome of *C. longa* and *C. xanthorrhiza* (Kosuge, Ishida and Yamazaki, 1985; Uehara *et al.*, 1987).



Bisdemethoxycurcumin (CLM06)

Table 26 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of CLM06[a]

Atom	δ_H (mult., *J* in Hz)[b]	δ_C
1, 7	7.54 (d, 15.4, 2H)	140.5
2, 6	6.68 (d, 15.4, 2H)	121.0
3, 5	-	183.4
4	6.00 (s)[c]	101.1
1′, 1″	-	126.0
2′, 2″, 6′, 6″	7.55 (d, 8.6, 4H)	130.5
3′, 3″, 5′, 5″	6.81 (d, 8.6, 4H)	116.1
4′, 4″	-	160.0
4′-OH, 4″-OH	not detected	-

Note; [a]In DMSO-d_6

[b]If not indicated, the integration of each proton signal is equal to 1 proton.

[c]Detected as an enol form.

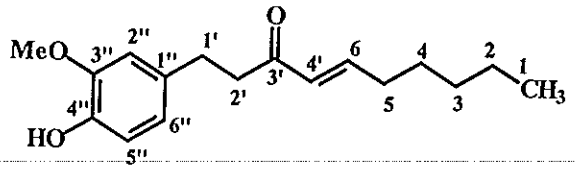
3.2.2.6 ZOM0

Phytochemical investigation of the methanol extract of *Z. officinale* led to the isolation of pure compound ZOM0 as yellow oil. Its FAB mass spectrum (low resolution) established a molecular formula of $C_{17}H_{24}O_3$ (MW = 276, D.B.E. = 6) according to the protonated ion peak at *m/z* 277 (M+H). Its UV (MeOH) spectrum showed absorption with λ_{max} at 226 nm. The IR (KBr disc) spectrum indicated the presence of hydroxy group, C-H, carbonyl function and olefinic carbon stretching at 3400, 2960, 1700 and 1500 cm^{-1}, respectively.

The ^1H, ^{13}C NMR and HMQC spectra of ZOM0 exhibited a carbonyl function, a methoxyl group, a methyl group, five olefinic methines and six methylene functions. The chemical shifts and spin couplings of proton signals at δ 6.71 (1H, d, 2.0 Hz), δ 6.82 (1H, d, 8.0 Hz) and δ 6.68 (1H, dd, 8.0 and 2.0 Hz) suggested the existence of 1,3,4-trisubstituted benzene ring. These aromatic protons and their corresponding carbons were attributed to H-2″ (δ_C 111.1), H-5″ (δ_C 114.3) and H-6″ (δ_C 120.8), respectively, base on the correlation observed in the ^1H-^1H COSY and HMQC spectra. The HMBC correlation between methoxyl signal at δ 3.87 (3H, s) and quaternary carbon signal at δ 146.3 helped allocating the two signals as 3″-OCH_3 and C-3″, respectively. The HMBC cross peaks of C-3″ and proton signals at δ 6.71 (H-2″) and δ 6.82 (H-5″) confirmed the assignment of signal at δ 146.3 as C-3″. As a consequence, the hydroxyl substitution was allocated to be at C-4″ (δ 143.8), the quaternary carbon which showed HMBC cross peaks with H-2″, H-5″ and H-6″. The signal of quaternary carbon at δ 133.2, which correlated with methylene signal at δ 2.85 (H-1′) and signals of aromatic protons at δ 6.71 (H-2″) and δ 6.82 (H-5″) in the HMBC spectrum, was attributed to C-1″. The assignment of carbon signal at δ 29.8 (δ_H 2.85) as C-1′ was deduced from the HMBC cross peaks with H-2′, H-2″ and H-6″. The assignments of methyl signal at δ 0.88 (1-CH_3) and four methylene signals at δ 1.29 (H-2), δ 1.31 (H-3), δ 1.44 (H-4) and δ 2.20 (H-5) were deduced from ^1H-^1H COSY, HMQC and HMBC spectra. The chemical shifts and spin couplings of these five signals suggested the existence of –CH_2-CH_2-CH_2-CH_2-CH_3 side chain. The

downfield shift of methylene signal at δ 2.20 (2H, tdd, 7.2, 7.0 and 1.4 Hz), which was attributed to H-5, suggested the proximity to the olefinic function. This was confirmed by the cross peaks of H-5 with two *trans*-olefinic protons at δ 6.83 (1H, dt, 16.0 and 7.0 Hz) and δ 6.09 (1H, dt, 16.0 and 1.4 Hz) in the ^{1}H-^{1}H COSY spectrum. The signal at δ 6.83 (δ_C 147.9) was attributed to H-6 due to the HMBC correlation of C-6 with H-5 (δ 2.20) and H-4 (δ 1.44). The other signal at δ 6.09 was, therefore, assigned as H-4′. The assignments of all protonated carbons were deduced from the HMQC and ^{1}H-^{1}H COSY spectra. The HMBC cross peaks of carbonyl signal at δ 199.8 (C-3′) with proton signals at δ 6.83 (H-6), δ 6.09 (H-4′) and δ 2.84 (H-2′) allowed the aliphatic moiety and the aromatic moiety to be connected.

Comparison the spectral data of ZOM0 with the published assignments led to the determination of ZOM0 as 6-shogaol [or 1-(4-hydroxy-3-methoxyphenyl)-4-decen-3-one] one of the pungent principles previously isolated from the rhizome of *Z. officinale* (Chen, Rosen and Ho, 1986b). The unambiguous ^{1}H and ^{13}C NMR assignments of 6-shogaol (ZOM0) obtained from the present work were shown in Table 27.



6-Shogaol (ZOM0)

Table 27 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of ZOM0[a]

Atom	δ_H (mult., *J* in Hz)[b]	δ_C	HMBC $^{13}C \longrightarrow {}^{1}H$
1	0.88 (t, 7.0, 3H)	13.9	H-3
2	1.29 (m, 2H)	22.4	H-1, H-4
3	1.31 (m, 2H)	31.3	H-1, H-5
4	1.44 (quintet, 7.2, 2H)	27.7	H-5, H-6
5	2.20 (tdd, 7.2, 7.0, 1.4, 2H)	32.4	H-4, H-6, H-4′
6	6.83 (dt, 16.0, 7.0)	147.9	H-4, H-5
1′	2.85 (m, 2H)	29.8	H-2′, H-2″, H-6″
2′	2.84 (m, 2H)	41.9	H-1′
3′	-	199.8	H-6, H-2′, H-4′
4′	6.09 (dt, 16.0, 1.4)	130.3	H-5
1″	-	133.2	H-1′, H-2″, H-5″
2″	6.71 (d, 2.0)	111.1	H-1′
3″	-	146.3	H-2″, H-5″, OMe
4″	-	143.8	H-2″, H-5″, H-6″
5″	6.82 (d, 8.0)	114.3	H-6″
6″	6.68 (dd, 8.0, 2.0)	120.8	H-1′, H-2″, H-5″
OMe	3.87 (s, 3H)	55.8	-

Note; [a]In $CDCl_3$

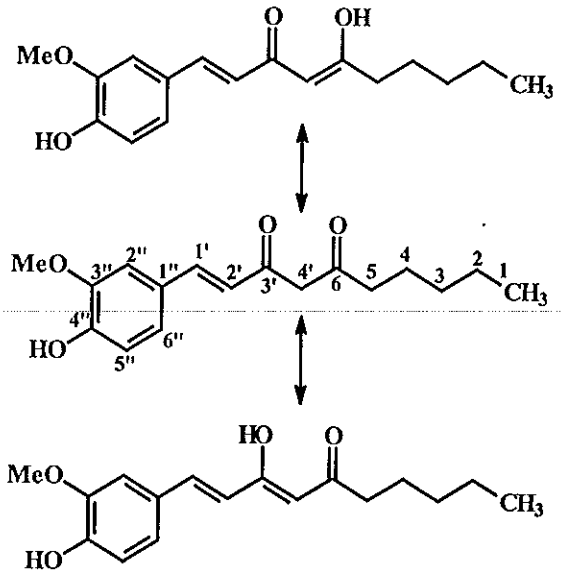
[b]If not indicated, the integration of each proton signal is equal to 1 proton.

3.2.2.7 ZOM1

ZOM1 was obtained as yellow crystals. Its FAB mass spectrum showed the protonated ion peak at *m/z* 291 corresponding with the molecular formula of $C_{17}H_{22}O_4$ (MW = 290, D.B.E. = 7). From the ^{13}C NMR spectrum, seventeen carbon signals were found including two carbonyl functions at δ 200.2 (C-6) and δ 178.0 (C-3′). The correlation between C-3′ and *trans*-olefinic proton signals at δ 7.53 (1H, d, 16.0 Hz) and δ 6.34 (1H, d, 16.0 Hz) in the HMBC spectrum suggested the existence of the α,β-unsaturated ketone moiety, resulting in assignments of the two signals as H-1′ and H-2′, respectively. Their corresponding carbons were observed in the HMQC spectrum at δ 139.8 (C-1′) and 120.6 (C-2′). The chemical shifts and spin coupling patterns of three proton signals at δ 7.02 (1H, d, 1.8 Hz), δ 6.92 (1H, d, 8.3 Hz) and δ 7.09 (1H, dd, 8.3 and 1.8 Hz) indicated the presence of the 1,3,4-trisubstituted benzene ring. The 3-bond cross peaks between C-1′ (δ 139.8) and signals at δ 7.02 and δ 7.09 in the HMBC spectrum gave evidence for conectivity and assignments of these two aromatic protons as H-2″ and H-6″, respectively. As a consequence, the signal at δ 6.92 was attributed to H-5″. The methoxyl signal at δ 3.93 (3H, s), which showed cross peak with quaternary carbon signal at δ 147.6 in the HMBC spectrum, was assigned to 3″-OCH_3. The allocation of C-3″ at δ 147.6 was supported by the cross peaks with proton signals at δ 7.02 (H-2″) and δ 6.92 (H-5″). The quaternary carbon at δ 146.7, which correlated with signals of H-5″ in the HMBC spectrum, was attributed to C-4″. The hydroxyl signal at δ 5.84, which was the characteristic for the hydroxyl function of the 4-hydroxy-3-methoxyphenyl moiety as that of curcumin (CLM02), was assigned to be 4″-OH. This was further supported by its HMBC cross peaks with carbon signals at δ 147.6 (C-3″) and δ 114.8 (C-5″). The presence of hydroxyl group was also supported by the absorption peak at 3350 cm^{-1} in the IR spectrum. The methyl signal at δ 0.88 (3H, t, 6.8 Hz) and four signals of methylene groups at δ 1.31 (H-2), 1.32 (H-3), 1.65 (H-4) and 2.37 (H-5) showed typical spin coupling patterns of $-CH_2$-CH_2-CH_2-CH_2-CH_3 chain, as deduced from ^1H-^1H COSY, HMQC and HMBC spectra. Assignment of the aliphatic side chain was also supported by the HMBC cross peak between C-6 (δ 200.2) and methylene signal at δ 2.37 (H-5). The proton signal at δ 5.62 (δ_C 100.1),

which correlated with carbon signals at δ 200.2 (C-6), δ 178.0 (C-3′) and δ 120.6 (C-2′), was attributed to H-4′ (detected as an enol form).

The spectral data mentioned above were in good agreement with those of 6-dehydrogingerdione, which was previously isolated from *Z. officinale* (Kiuchi, Shibuya and Sankawa, 1982). ZOM1 was, therefore, concluded to be 6-dehydrogingerdione, which has also been known as 1-dehydrogingerdione (Charles, Garg and Kumar, 2000). The unambiguous ^{1}H and ^{13}C NMR assignments of ZOM1 obtained from the present work were shown in Table 28.



6-Dehydrogingerdione (ZOM1) (also known as 1-dehydrogingerdione)

Table 28 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of ZOM1[a]

Atom	δ_H (mult., *J* in Hz)[b]	δ_C	HMBC $^{13}C \longrightarrow {}^{1}H$
1	0.88 (t, 6.8, 3H)	13.9	-
2	1.31 (m, 2H)	22.4	H-1, H-3
3	1.32 (m, 2H)	31.5	H-1, H-2, H-4, H-5
4	1.65 (quintet, 7.5, 2H)	25.3	H-5
5	2.37 (br.t, 7.5, 2H)	40.1	H-4
6	-	200.2	H-5, H-4′
1′	7.53 (d, 16.0)	139.8	H-2″, H-6″
2′	6.34 (d, 16.0)	120.6	H-4′
3′	-	178.0	H-1′, H-2′, H-4′
4′	5.62 (s)[c]	100.1	H-5, H-2′
1″	-	127.7	H-2′, H-5″
2″	7.02 (d, 1.8)	109.4	H-1′, H-6″
3″	-	147.6	H-2″, H-5″, OMe, 4″-OH
4″	-	146.7	H-5″
5″	6.92 (d, 8.3)	114.8	H-6″, 4″-OH
6″	7.09 (dd, 8.3, 1.8)	122.6	H-1′, H-2″, H-5″
OMe	3.93 (s, 3H)	55.9	-
4″-OH	5.84 (br.s)	-	-

Note; [a]In $CDCl_3$

[b]If not indicated, the integration of each proton signal is equal to 1 proton.

[c]Detected as an enol form.

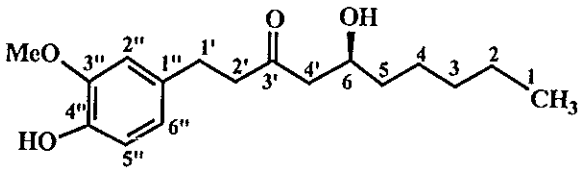
3.2.2.8 ZOM3

Phytochemical investigation of the methanol extract of *Z. officinale* led to the isolation of pure compound ZOM3 as yellow oil. Its FAB mass spectrum (low resolution) established a molecular formula of $C_{17}H_{26}O_4$ (MW = 294, D.B.E. = 5) according to the protonated ion peak at *m/z* 295 (M+H). Its UV (MeOH) spectrum showed absorption with λ_{max} at 282 nm and specific optical rotation of +29.3° (c 0.478, $CHCl_3$). The IR (KBr disc) spectrum indicated the presence of hydroxy group, C-H and carbonyl function at 3450, 2960 and 1700 cm^{-1}, respectively.

The majority of ^1H and ^{13}C NMR profiles of ZOM3 were in accordance with those of ZOM0 (6-shogaol) and ZOM1 (6-dehydrogingerdione). The exceptions were the absence of *trans*-olefinic function and the presence of oxygen-bearing methine carbon at δ 67.6 (δ_H 4.03) in the HMQC spectrum of ZOM3. The chemical shifts and spin couplings of proton signals at δ 6.69 (1H, d, 1.8 Hz), δ 6.83 (1H, d, 7.7 Hz) and δ 6.66 (1H, dd, 7.7 and 1.8 Hz) revealed the existence of 1,3,4-trisubstituted benzene ring, which were assigned as H-2″, H-5″ and H-6″, respectively. Their corresponding carbons, as deduced from the HMQC spectrum, were allocated at δ 110.9 (C-2″), 114.4 (C-5″) and 120.7 (C-6″). Allocation of the methoxyl function at C-3″ was confirmed by the HMBC correlation between the signal of quaternary carbon at δ 146.4 (C-3″) and the methoxyl signal at δ 3.87. The assignment of signal at δ 146.4 as C-3″ was supported by the HMBC cross peaks with H-2″ and H-5″. The hydroxyl signal at δ 5.59 was assigned as 4″-OH due to its HMBC cross peaks with carbon signals at δ 146.4 (C-3″), δ 143.9 (C-4″) and δ 114.4 (C-5″). The assignment of C-4″ was confirmed by the cross peaks with H-2″, H-5″ and H-6″ in the HMBC spectrum. The signal of quaternary carbon at δ 132.6, which correlated with two methylene functions at δ 2.84 (1′-CH_2) and δ 2.73 (2′-CH_2), H-2″ and H-5″ in the HMBC spectrum, was attributed to C-1″. The assignment of signal at δ 29.2 (δ_H 2.84) as C-1′ was confirmed by the HMBC correlation with H-2″ and H-6″. The assignments of 2′-CH_2 and 4′-CH_2 was deduced from the HMBC correlation with the carbonyl signal at δ 211.5 (C-3′) and the cross peaks between H-1′ and H-2′ in the ^1H-^1H COSY spectrum. The

methylene carbon at δ 49.3 was attributed to C-4′ due to the HMQC correlation with two proton signals at δ 2.57, 2.49 and HMBC correlation with H-2′. The downfield proton signal at δ 4.03 (1H, br.m) was assigned to be H-6 according to the HMQC cross peak with carbon signal at δ 67.6 (C-6), and the couplings with H-4′ and H-5 (δ 1.43) in the ^{1}H-^{1}H COSY spectrum. The NMR features of H-6 and C-6 suggested the presence of hydroxyl function as secondary alcohol at C-6, which was allocated at δ 3.00 (6-OH). The assignments of methyl signal at δ 0.88 (H-1) and four methylene signals at δ 1.28 (H-2), 1.29 (H-3), 1.30 (H-4) and 1.43 (H-5) were deduced from the ^{1}H-^{1}H COSY, HMQC and HMBC spectra.

The mentioned spectral features of ZOM3 were consistent with those of 6-gingerol (Shoji, *et al.*, 1982; Yamada, Kikuzaki and Nakatani, 1992). The relative configuration at C-6 of ZOM3 (6-gingerol) was assumed to be (*S*)-configuration based on the similarity of specific optical rotation and NMR profiles of H-6 (δ 4.03) and H-4′ (δ 2.57 and δ 2.49) of ZOM3 to those of published (*S*)-6-gingerol previously isolated from the rhizome of *Z. officinale* (Shoji, *et al.*, 1982; Yamada, Kikuzaki and Nakatani, 1992). It is of interest to note that although the chemical shifts of protons and carbons of 6-gingerol were reported, but they have not yet been completely assigned. The present work contributed unambiguous ^{1}H and ^{13}C NMR assignments of ZOM3 (6-gingerol) as shown in Table 29.



6-Gingerol (ZOM3)

Table 29 NMR spectral data (500 MHz for ^{1}H and 125 MHz for ^{13}C) of ZOM3[a]

Atom	δ_H (mult., *J* in Hz)[b]	δ_C	HMBC $^{13}C \longrightarrow ^{1}H$
1	0.88 (t, 7.3, 3H)	14.0	H-2
2	1.28 (m, 2H)	22.6	H-1
3	1.29 (m, 2H)	25.1	H-4
4	1.30 (m, 2H)	31.7	H-5
5	1.43 (m, 2H)	36.4	H-4′
6	4.03 (br.m)	67.6	H-4′
1′	2.84 (ddd, 7.6, 7.6, 1.4, 2H)	29.2	H-2′, H-2″, H-6″
2′	2.73 (ddd, 7.6, 7.6, 1.4, 2H)	45.4	H-1′, H-4′
3′	-	211.5	H-1′, H-2′, H-4′
4′	2.57 (dd, 17.6, 3.0)	49.3	H-2′
	2.49 (dd, 17.6, 8.9)		
1″	-	132.6	H-1′, H-2′, H-2″, H-5″
2″	6.69 (d, 1.8)	110.9	H-1′, H-6″
3″	-	146.4	H-2″, H-5″, OMe, 4″-OH
4″	-	143.9	H-2″, H-5″, H-6″, 4″-OH
5″	6.83 (d, 7.7)	114.4	H-6″, 4″-OH
6″	6.66 (dd, 7.7, 1.8)	120.7	H-1′, H-2″, H-5″
OMe	3.87 (s, 3H)	55.8	-
4″-OH	5.59 (br.s)	-	-
6-OH	3.00 (br.s)	-	-

Note; [a]In $CDCl_3$

[b]If not indicated, the integration of each proton signal is equal to 1 proton.

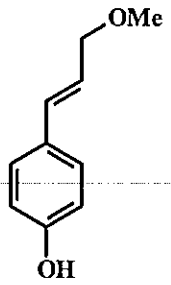
3.3 Activity of the Isolated Compounds

The eight isolated compounds (AGM1, CLM01, CLM02, CLM03, CLM06, ZOM0, ZOM1 and ZOM3) were assessed for free radical scavenging activity against DPPH radical and cytotoxic activity against the tumour cell lines LS174T and MCF7. The chemical structures are shown in Figure 7 and the results are depicted in Table 30. BHT (butylated hydroxytoluene) and caffeic acid were used as positive standards for antioxidative assay while vinblastine sulphate and the cytotoxic alkaloid berberine were used as standard drugs in the cytotoxicity assay. The strong radical scavenging activity was exhibited by CLM02 (curcumin), CLM03 (demethoxycurcumin), ZOM0 (6-shogaol), ZOM1 (6-dehydrogingerdione) and ZOM3 (6-gingerol) with EC_{50} values ranging from 2.0-4.7 μg/ml. This was about 2-4 times more active than that of BHT, the well-known synthetic antioxidant. By contrast, CLM01 (ar-turmerone) showed very slight antioxidant effect (EC_{50} > 100 μg/ml). AGM1 (*p*-coumaryl-9-methyl ether) and CLM06 (bisdemethoxycurcumin) were found to possess moderate activity with EC_{50} values of 73.9 and 40.9 μg/ml, respectively. Rao (1996) reported that antioxidative activity of the curcuminoids from turmeric is in the following order: curcumin > demethoxycurcumin > bisdemethoxycurcumin, which is consistent with the results obtained from the present work. The strong antioxidant activity of CLM02 (curcumin) could be due to its capability to stabilize the two aroxyl radicals, which occurred after donating two hydrogen atoms of the hydroxyl functions to DPPH radicals, through aromatic system, conjugated double bonds and carbonyl functions. This, however, the two phenolic moieties of CLM06 are more exchangeable than that of CLM02, suggesting some degree of reversibility of reaction between CLM06 and DPPH radicals, hence being less active. Furthermore, it has been reported that the antioxidant mechanism of curcumin in polyunsaturated lipid (ethyl linoleate) was proposed to be an oxidative coupling reaction at the phenolic moiety of the curcumin with the peroxyl radical (Masuda, *et al.*, 2001). In general, these three curcuminoids (CLM02, CLM03 and CLM06) are regarded as main antioxidative constituents of *C. longa* rhizome. Radical scavenging activity of the three gingerol

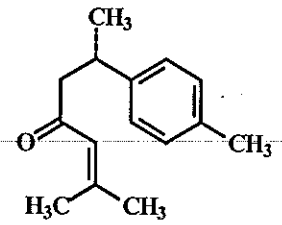
derivatives (ZOM0, ZOM1 and ZOM3) could be attributed to the hydroxyl group of the 4-hydroxy-3-methoxyphenyl moiety, which is almost comparable to that of CLM03 (demethoxycurcumin). The present results gave additional evidence to support the assumption that gingerols are responsible for antioxidant activity of *Z. officinale* rhizome (Sekiwa, Kubota and Kobayashi, 2000; Chung, *et al.*, 2001). The extract of *A. galanga* rhizome has been known to possess antioxidative effect (Cheah and Gan, 2000) but the active constituents has not yet been identified. The radical scavenging activity against DPPH of AGM1 with EC_{50} value of 73.9 μg/ml, although less active than its corresponding methanol extract (EC_{50} = 57.0 μg/ml), provided new evidence of active compound responsible for antioxidative activity of *A. galanga* rhizome. It is likely that some other antioxidative compounds remain to be identified in the rhizome of *A. galanga*. Natural antioxidants have been known to be capable of reducing toxic from oxygen species or free radicals thus inhibiting formation of carcinogens from precursor substances such as lipid peroxidation. They may also improve the resistance of tissue to oxidative damage and enhance immune system. These suggested that natural antioxidants could provide preventive effect against cancer (Gordon, 1996). The antioxidative activity of the active compounds obtained from the present work give strong evidence of chemopreventive potential of the rhizomes of *C. longa*, *Z. officinale* and *A. galanga*.

Pronounced cytotoxic activity against the two tumour cell lines were observed for CLM03 (demethoxycurcumin) and ZOM0 (6-shogaol) with IC_{50} values in the range of 0.8-2.8 μg/ml. These two compounds were considered to be significantly cytotoxic according to the criteria for cytotoxic activity of pure compounds established by the American National Cancer Institute (IC_{50} < 4 μg/ml) (Suffness and Pezzuto, 1991). CLM02 (curcumin) and AGM1 (*p*-coumaryl-9-methyl ether) were slightly less active than CLM03 and ZOM0 with IC_{50} values on LS174T and MCF7 in the range of 5-10 μg/ml. On the other hand, ZOM1, ZOM3 and CLM01 were considerably less active than CLM03 and ZOM0 with IC_{50} values against both cell lines in the range of 11-32 μg/ml. The strong cytotoxic activity of CLM03 (demethoxycurcumin) observed in the present work

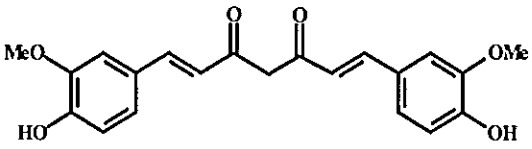
is in accordance with the previous study of Simon, *et al.* (1998) of which demethoxycurcumin showed stronger cytotoxic activity than curcumin and bisdemethoxycurcumin. Furthermore, the activity of CLM02 (curcumin) against MCF7 cells with IC_{50} value of 8.3 μg/ml was in the similar range with the previous report as 10 μg/ml (Kuo, Huang and Lin, 1996). According to the chemical structures, it could be proposed that the hydroxylated benzene ring and/or the α,β-unsaturated ketone moieties are essential for cytotoxic activity of the eight isolated compounds. However, further study is needed to determine the exact essential structure and mechanism of action of these cytotoxic compounds. So far, curcumin was reported to be active through inhibition of telomerase activity in human breast cancer cells (Ramachandran, *et al.*, 2002). Huang *et al.* (1997) pointed out that this effect of curcumin may be linked to its strong inhibitory action on DNA and RNA synthesis that has been previously shown on cultured Hela cells. It is also of interest to note that, for each compound investigated in the present work, there was no significant cell-type selectivity. Under the same test conditions, the positive standard berberine showed cytotoxic activity with IC_{50} values in the same range as those of CLM03 (demethoxycurcumin) and ZOM0 (6-shogaol). However, the anticancer drug vinblastine sulphate was found to be far more active than all of the isolated compounds. The eight isolated compounds were mainly responsible for the cytotoxic activity against LS174T and MCF7 tumour cell lines observed in their corresponding methanol extracts. The finding of AGM1 (*p*-coumaryl-9-methyl ether) as a new cytotoxic compound in the present work provided additional evidence of the presence of antitumour principles in the rhizome of *A. galanga* of which 1′-acetoxychavicol acetate and 1′-acetoxyeugenol acetate were previously identified (Itokawa, *et al.*, 1987). The obtained results confirm therapeutic potential against tumour cells of the rhizomes of *A. galanga*, *C. longa* and *Z. officinale.*



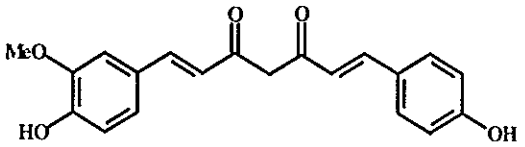
AGM1 (*p*-Coumaryl-9-methyl ether)



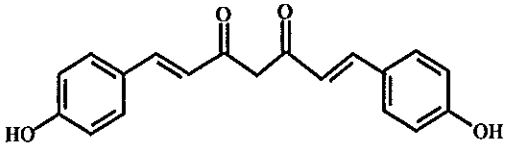
CLM01 (ar-Turmerone)



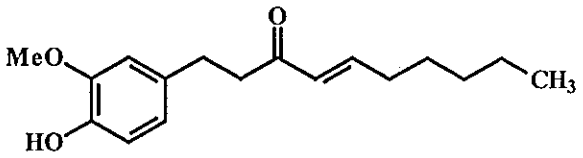
CLM02 (Curcumin)



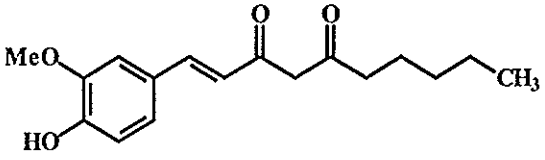
CLM03 (Demethoxycurcumin)



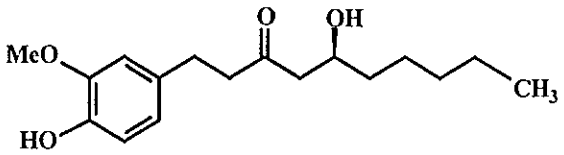
CLM06 (Bisdemethoxycurcumin)



ZOM0 (6-Shogaol)



ZOM1 (6-Dehydrogingerdione or 1-Dehydrogingerdione)



ZOM3 (6-Gingerol)

Figure 7 Structure of the isolated compounds from the rhizome of *Alpinia galanga*, *Curcuma longa* and *Zingiber officinale*.

Table 30 EC_{50} values against DPPH radical and IC_{50} values against LS174T and MCF7 cells of the isolated compounds from *A. galanga*, *C. longa* and *Z. officinale*.

Compounds	EC_{50} (μg/ml) (DPPH) (mean±SD)	IC_{50} against LS174T (μg/ml) (mean±SEM)	IC_{50} against MCF7 (μg/ml) (mean±SEM)
AGM1 (*p*-Coumaryl-9-methyl ether)	73.9±2.1 (n=6) (450.3±13.1 μM)	7.5±0.7 (N=2) (45.8±4.3 μM)	7.4±0.3 (N=2) (44.8±2.1 μM)
CLM01 (ar-Turmerone)	>100 (n=6) (>463.0 μM)	19.3±0.6 (N=2) (89.6±3.0 μM)	14.6±2.1(N=2) (67.8±13.4 μM)
CLM02 (Curcumin)	2.0±0.2 (n=6) (5.4±0.6 μM)	5.9±0.6 (N=2) (16.2±1.8 μM)	8.3±1.4 (N=2) (22.7±5.2 μM)
CLM03 (Demethoxycurcumin)	2.8±0.1 (n=6) (8.4±0.4 μM)	0.8±0.0 (N=2) (2.3±0.0 μM)	2.8±0.3 (N=2) (8.5±1.1 μM)
CLM06 (Bisdemethoxycurcumin)	40.9±4.8 (n=6) (132.4±16.2 μM)	*	*
ZOM0 (6-Shogaol)	4.0±0.1 (n=6) (14.5±0.5 μM)	1.2±0.1 (N=2) (4.2±0.2 μM)	1.7±0.1 (N=2) (6.0±0.8 μM)
ZOM1 (6-Dehydrogingerdione) (or 1-Dehydrogingerdione)	4.7±0.1 (n=6) (16.2±0.3 μM)	11.3±3.2 (N=2) (39.2±11.0 μM)	13.9±0.9 (N=2) (47.8±3.2 μM)
ZOM3 (6-Gingerol)	4.4±0.1 (n=6) (14.8±0.4 μM)	30.6±7.5 (N=2) (104.1±25.5 μM)	31.6±0.3 (N=2) (107.5±1.4 μM)
Butylated hydroxytoluene (BHT) (positive standard)	8.2±0.2 (n=6) (37.3±0.9 μM)	N/A	N/A
Caffeic acid (positive standard)	0.9±0.1 (n=6) (5.2±0.3 μM)	N/A	N/A
Berberine (positive standard)	N/A	0.8±0.0 (N=2) (2.4±0.0 μM)	0.6±0.0 (N=2) (1.8±0.0 μM)
Vinblastine sulphate (positive standard)	N/A	0.011±0.004 nM (N=2)	0.008±0.00 nM (N=2)

* CLM06 was obtained only 2 mg, not sufficient for cytotoxic test.

n = number of samples tested

N = number of independent experiments (6 replicates in each experiment)

CHAPTER 4

CONCLUSIONS

The investigation on antioxidative and cytotoxic activities of the fresh rhizomes of *Alpinia galanga*, *Boesenbergia pandurata*, *Curcuma longa*, *Kaempferia galanga* and *Zingiber officinale* were achieved in the present work. The methanol extracts of the rhizomes of *A. galanga*, *C. longa* and *Z. officinale* showed strong antioxidative activity against DPPH radical whereas the water extracts exhibited rather weak activity. The five volatile oils, although lacked of antioxidative effect, showed pronounced cytotoxic activity against LS174T and MCF7 cell lines with IC_{50} values ranging from 12.0-47.8 μg/ml. The methanol extract of *C. longa* was found to be the most cytotoxic against LS174T and MCF7 with IC_{50} values of 6.4 and 14.2 μg/ml, respectively.

AGM1 (*p*-coumaryl-9-methyl ether) was isolated as novel antioxidative and cytotoxic compound from the methanol extract of *A. galanga*. Four compounds, ar-turmerone (CLM01), curcumin (CLM02), demethoxycurcumin (CLM03) and bisdemethoxycurcumin (CLM06), were isolated from the methanol extract of *C. longa*, among which demethoxycurcumin showed significant cytotoxic activity on both tumour cell lines (IC_{50} < 4 μg/ml). Curcumin and demethoxycurcumin exhibited strong radical scavenging activity against DPPH with EC_{50} values of 2.0 and 2.8 μg/ml, respectively, which considerably more active than those of bisdemethoxycurcumin and ar-turmerone. Three compounds, 6-shogaol (ZOM0), 6-dehydrogingerdione (ZOM1; also known as 1-dehydrogingerdione) and 6-gingerol (ZOM3), were isolated as antioxidative and cytotoxic constituents from the methanol extract of *Z. officinale*, among which 6-shogaol showed pronounced activities on both assay systems. Chemical structures of the eight isolated compounds were determined by spectroscopic methods, particularly 2D-NMR techniques. The unambiguous ^{1}H and ^{13}C NMR assignments of *p*-coumaryl-9-methyl ether (AGM1) and 6-gingerol (ZOM3) were described for the first time in the present work.

Beside ar-turmerone and the volatile oils, the other seven isolated compounds possess both antioxidative and cytotoxic activities suggesting that these compounds could act as both preventive and cytotoxic agents as far as the cancer is concerned. The obtained results from the present work suggest that the rhizomes of the five Zingiberaceous plants commonly used as spices in Thailand are potential sources of antioxidants (by acting as free radical scavengers) and/or cytotoxic agents against tumour cells, particularly *A. galanga*, *C. longa* and *Z. officinale.* Further study in animal model is strongly recommended in order to evaluate whether these medicinal plants are of promising for clinical trials.

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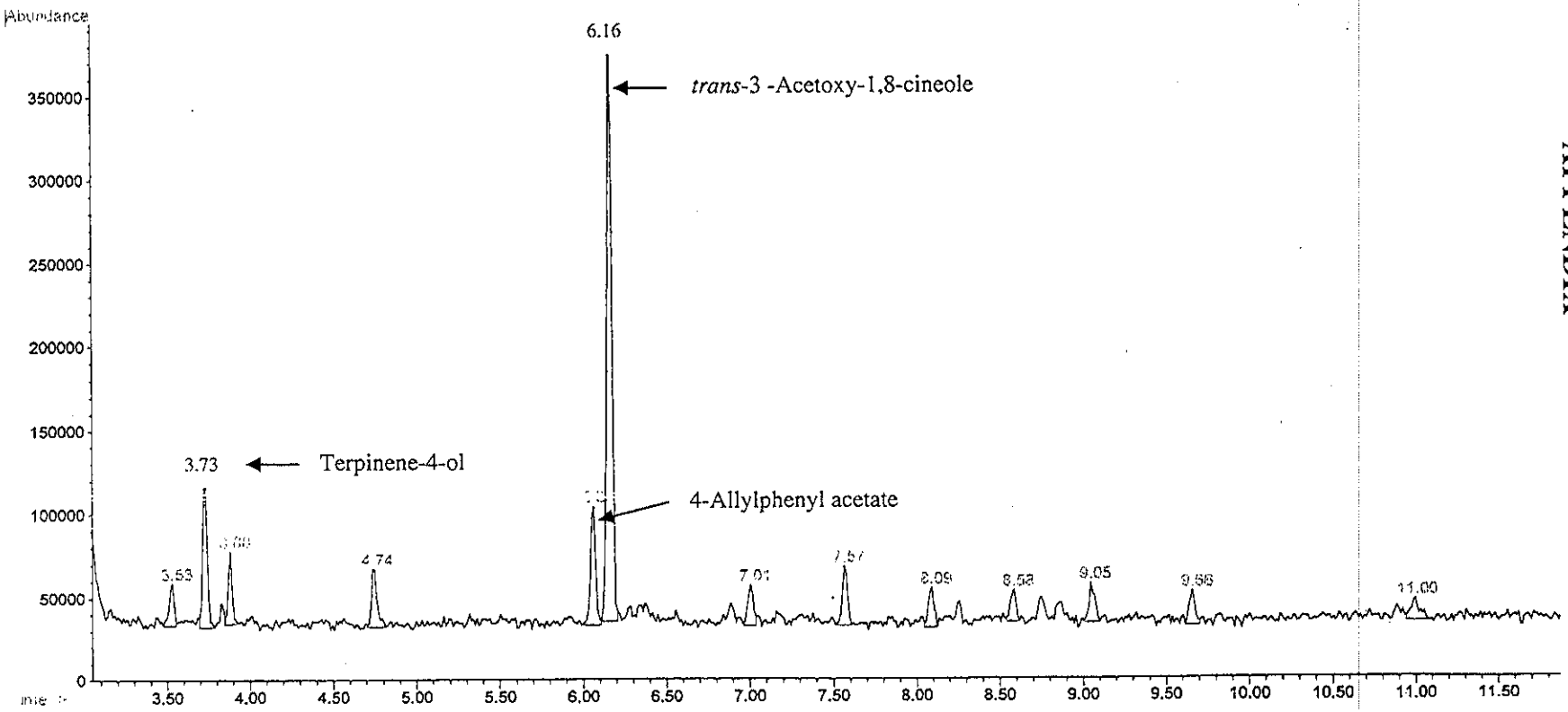

Figure 8 GC chromatogram of volatile oil from *Alpinia galanga* (water distillation)

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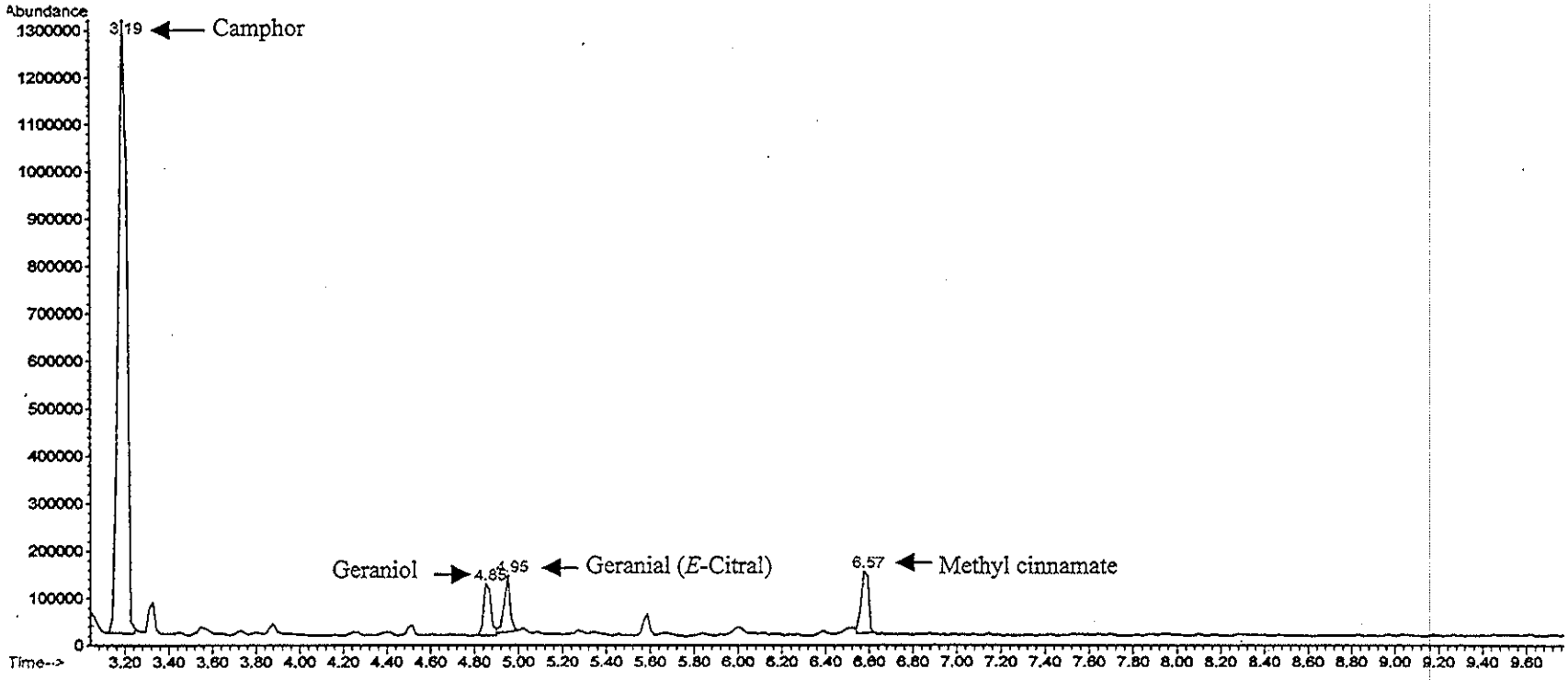

Figure 9 GC chromatogram of volatile oil from *Boesenbergia pandurata* (water distillation)

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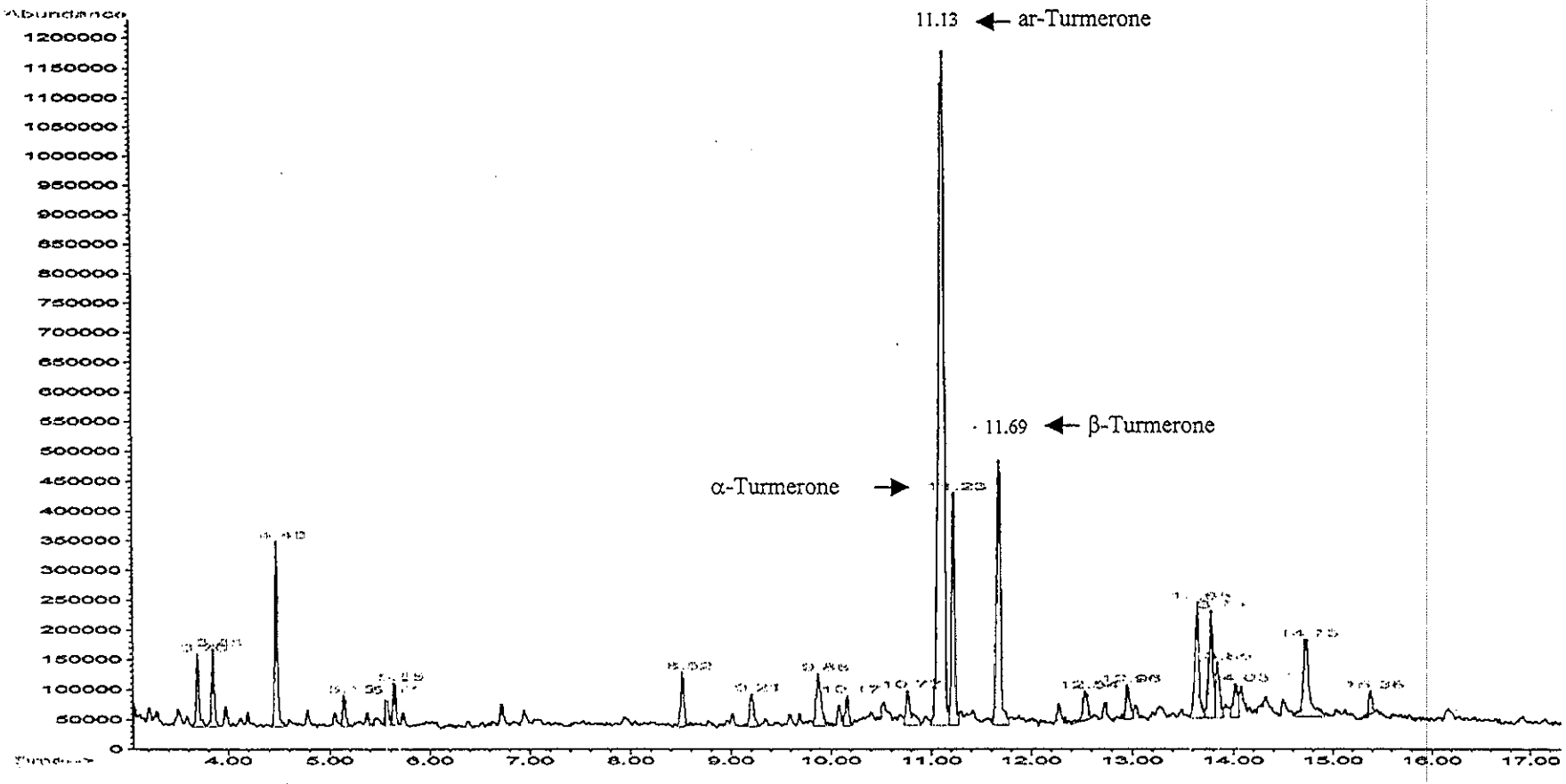

Figure 10 GC chromatogram of volatile oil from *Curcuma longa* (water distillation)

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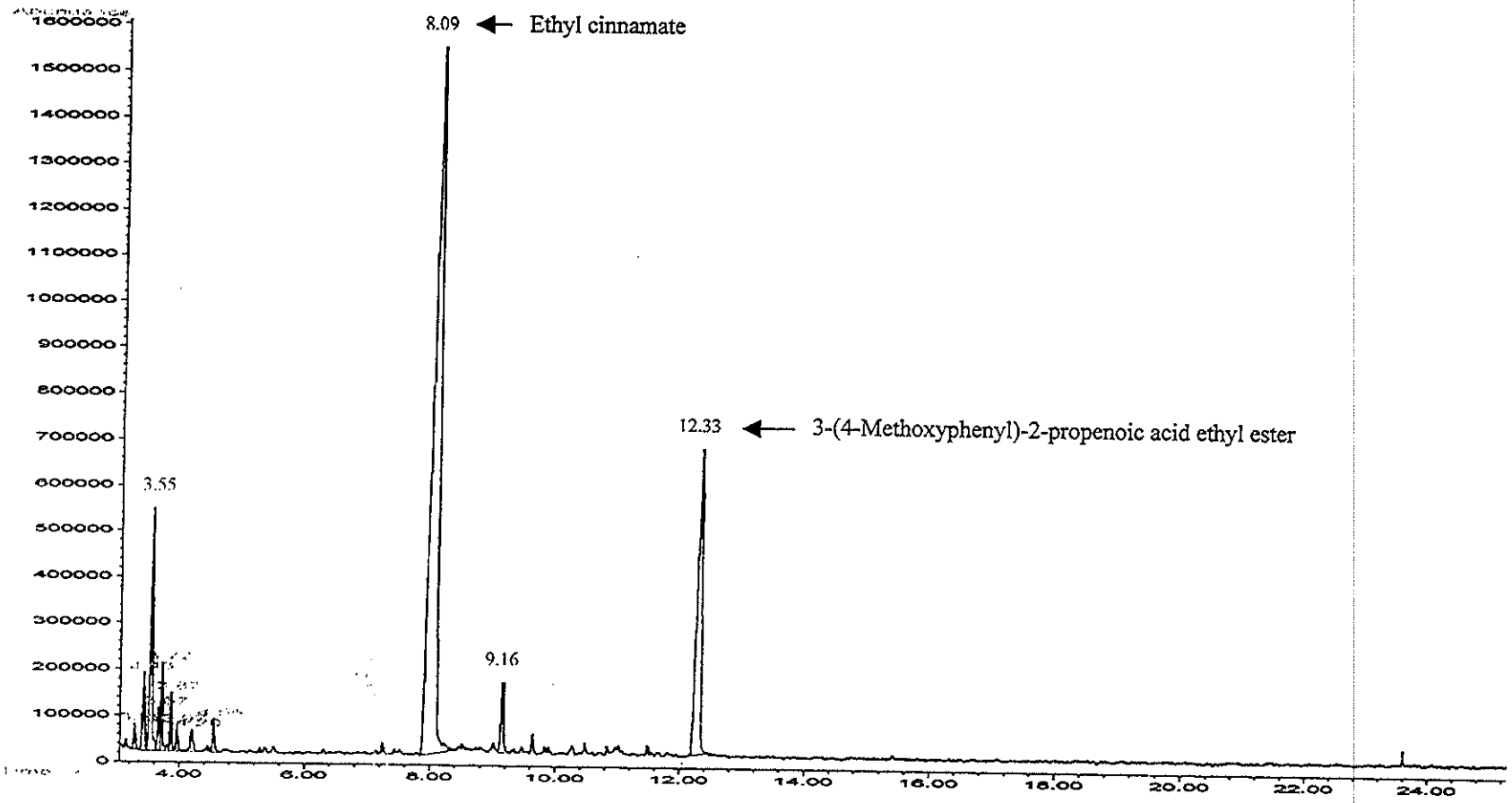

Figure 11 GC chromatogram of volatile oil from *Kaempferia galanga* (water distillation)

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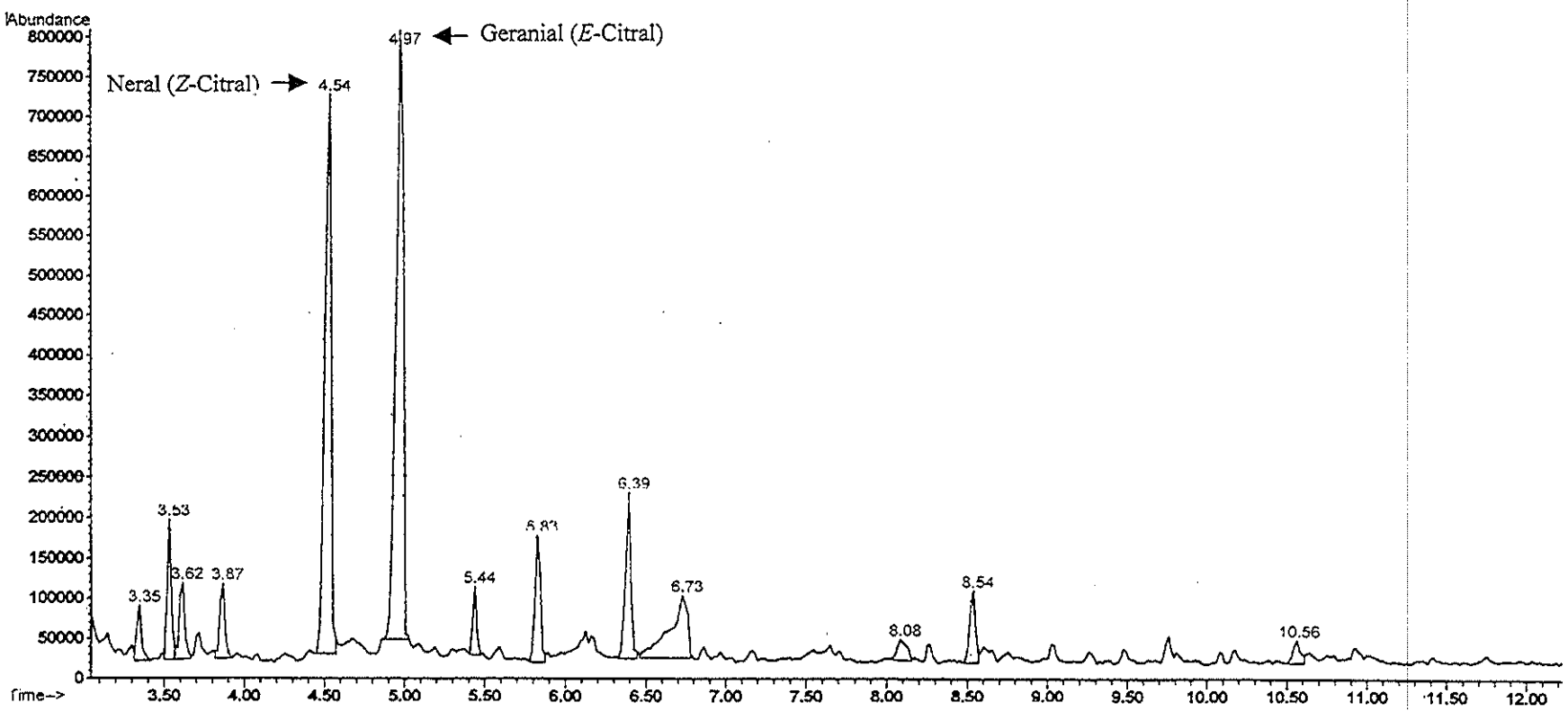
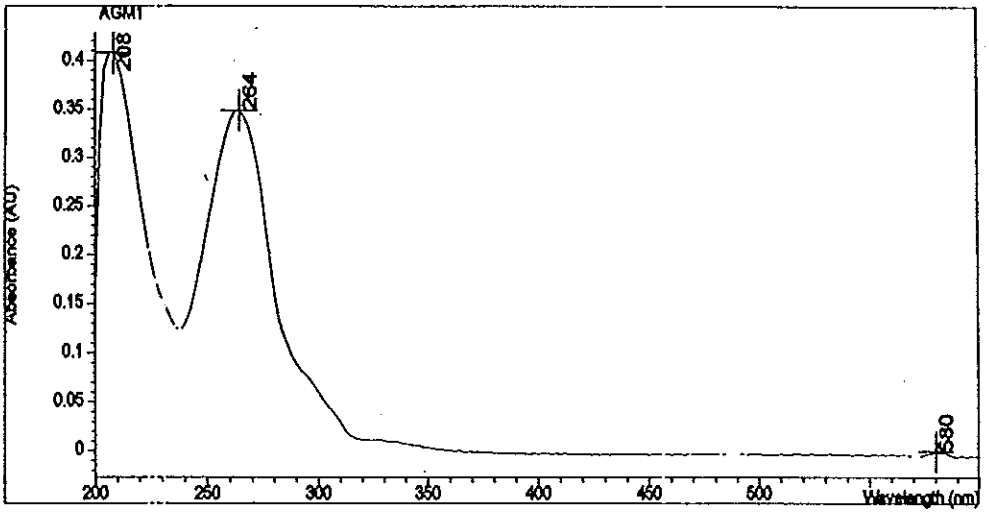


Figure 12 GC chromatogram of volatile oil from *Zingiber officinale* (water distillation)

Spectrum/Peak Report Date 06/12/03 Time 17:34:49 Page 1 of 1

Overlaid Spectra:



#	Name	Peaks(nm)	Abs(AU)	Valleys(nm)	Abs(AU)
1	AGM1	208.0	0.40775	570.0	-5.7068E-3
1		264.0	0.34889	486.0	-4.4250E-3
1		580.0	-2.7466E-4	238.0	0.12393

Figure 13 Ultraviolet spectrum of AGM1 (*p*-coumaryl-9-methyl ether)

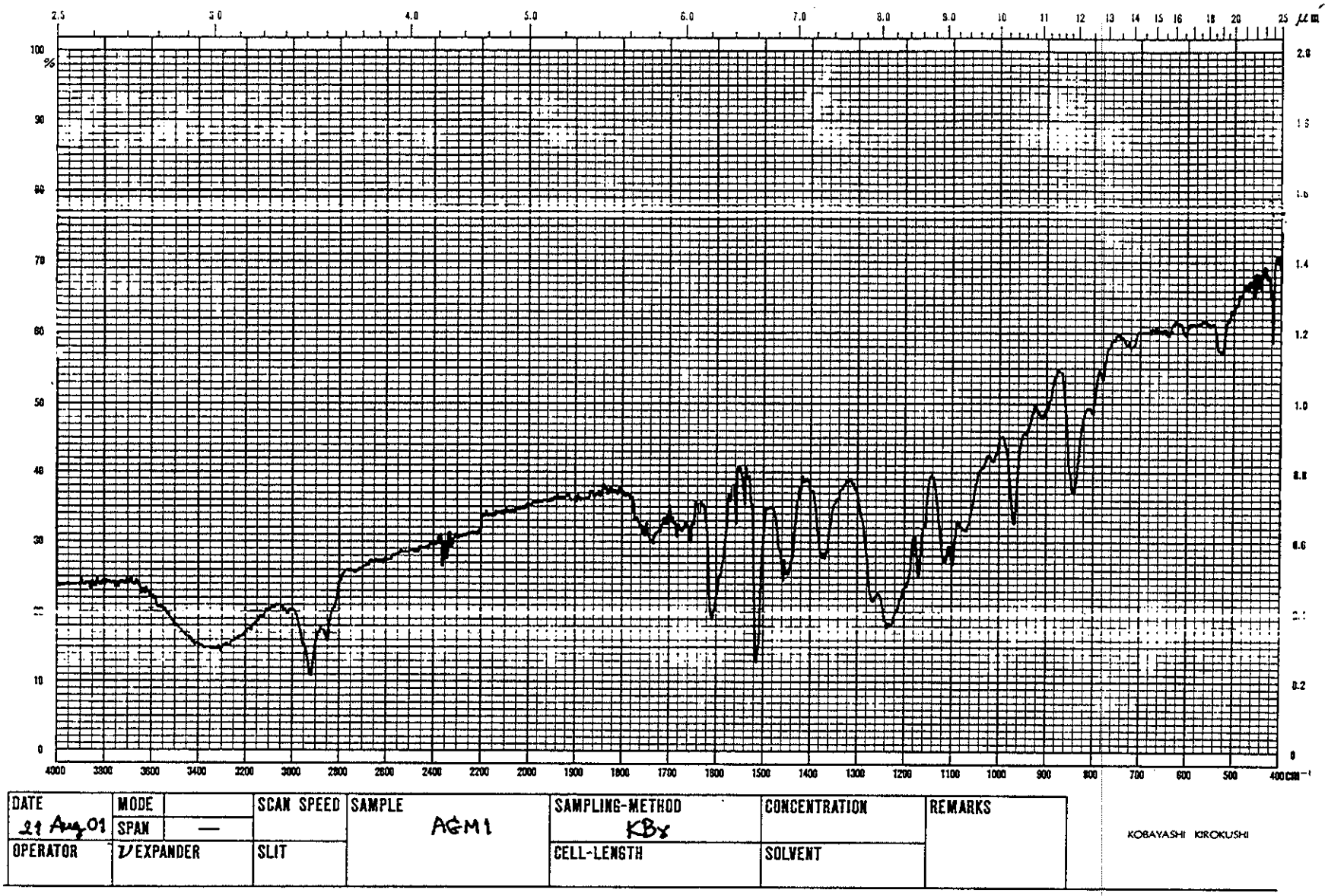


Figure 14 IR spectrum of AGM1 (*p*-coumaryl-9-methyl ether)

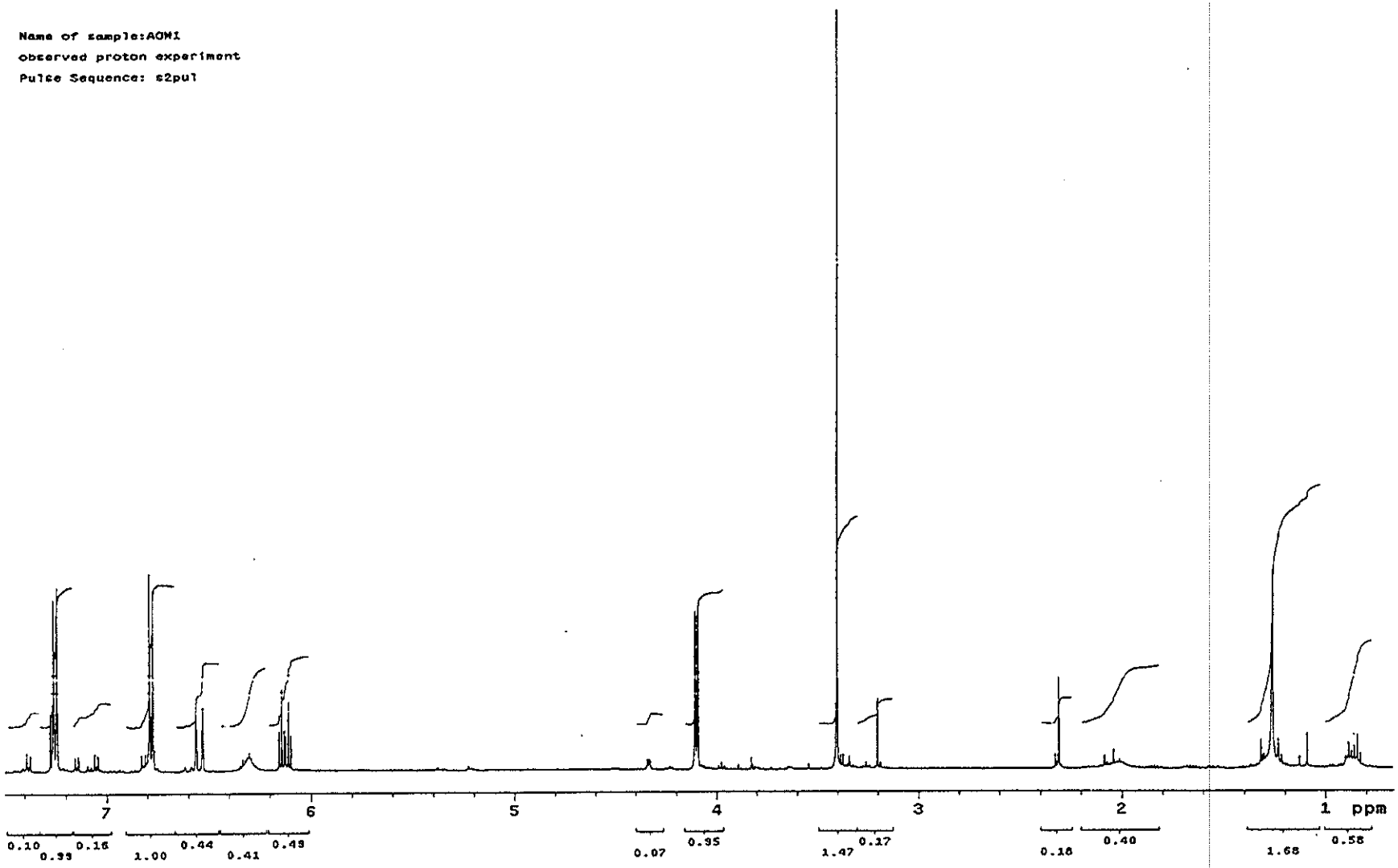


Figure 15 ^{1}H-NMR spectrum of AGM1 (*p*-coumaryl-9-methyl ether)

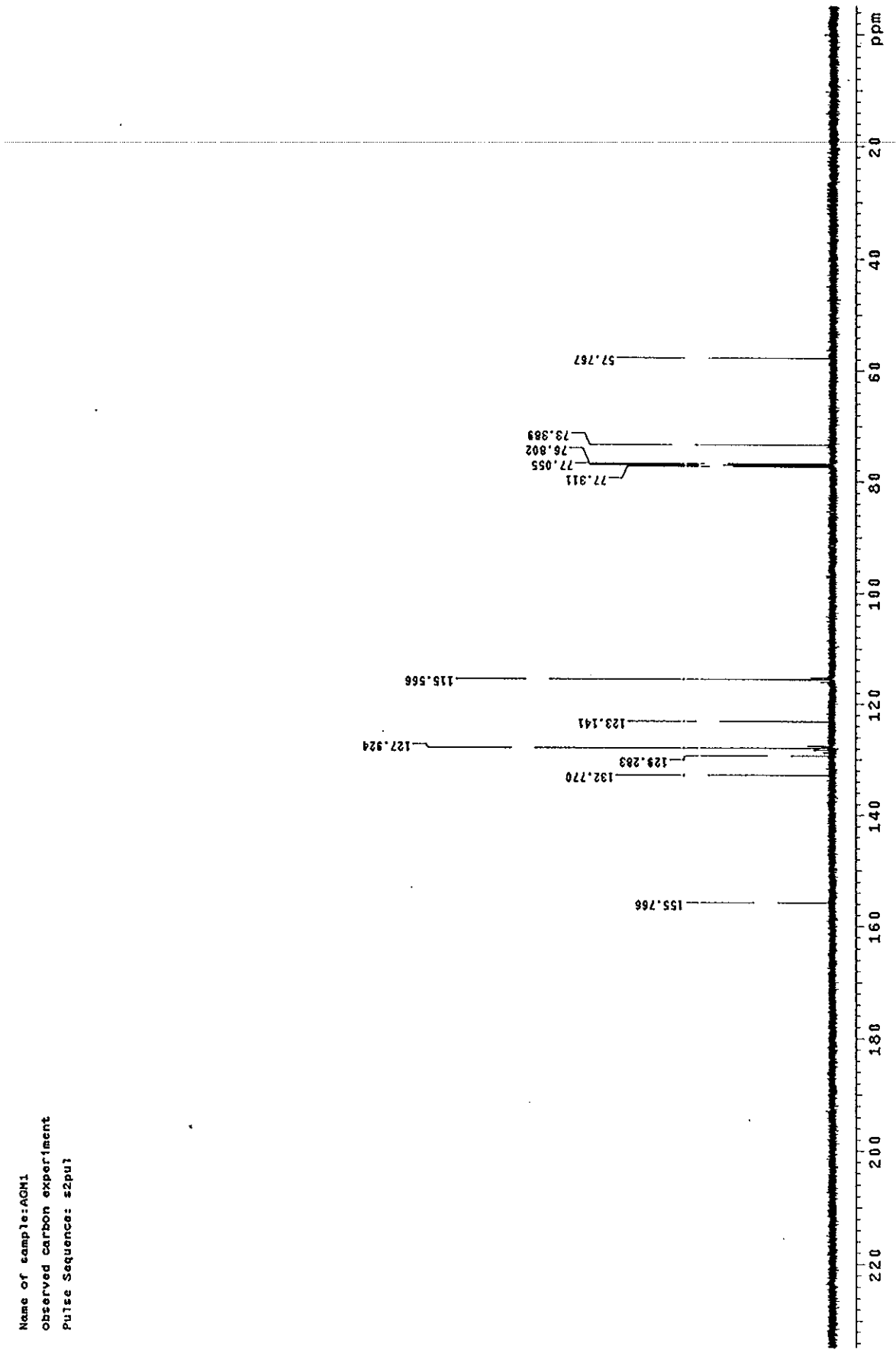


Figure 16 ^{13}C-NMR spectrum of AGM1 (*p*-coumaryl-9-methyl ether)

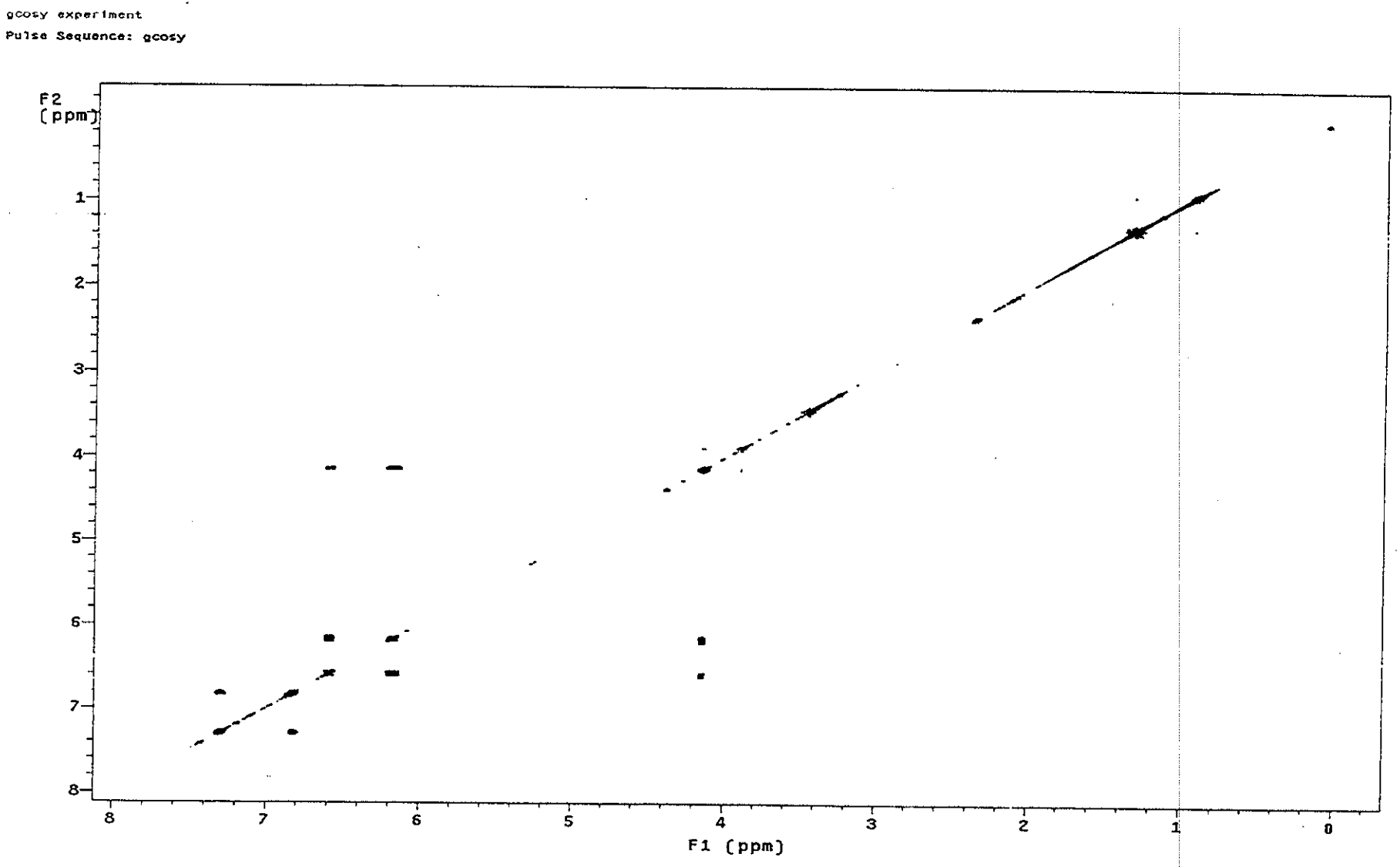


Figure 17 ^{1}H-^{1}H COSY spectrum of AGM1 (*p*-coumaryl-9-methyl ether)

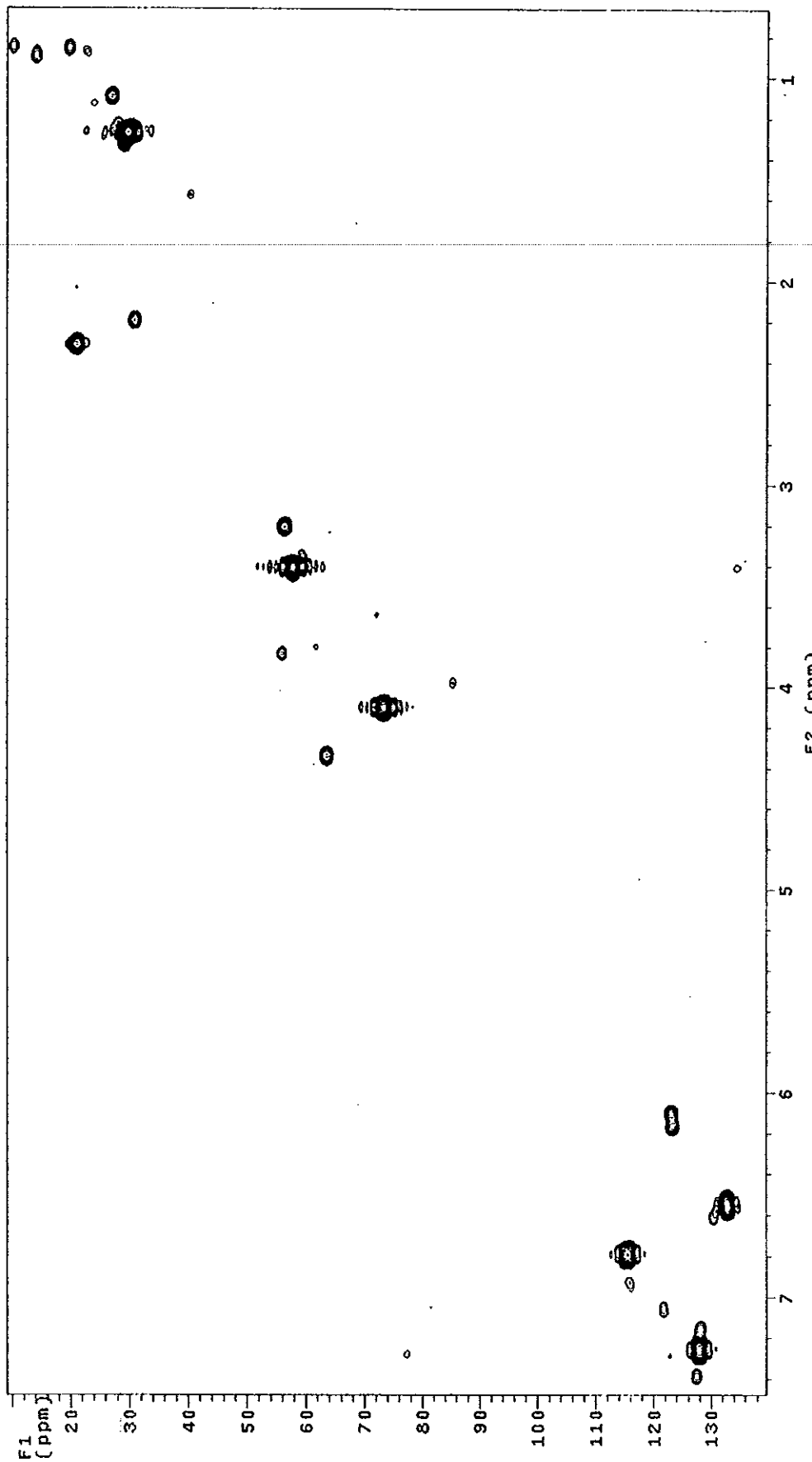


Figure 18 HMQC spectrum of AGM1 (*p*-coumaryl-9-methyl ether)

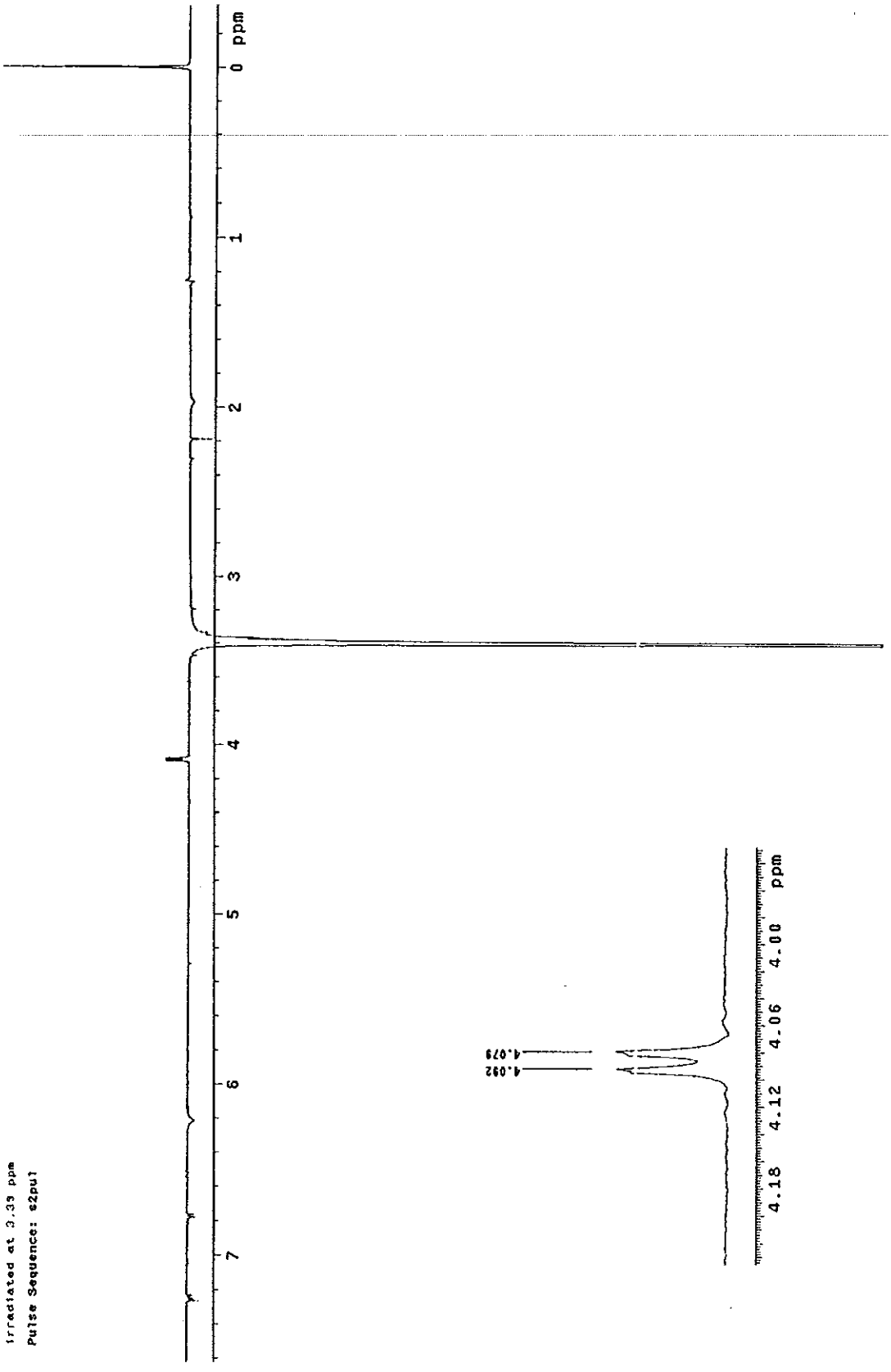


Figure 19 NOE spectrum of AGM1 (*p*-coumaryl-9-methyl ether)

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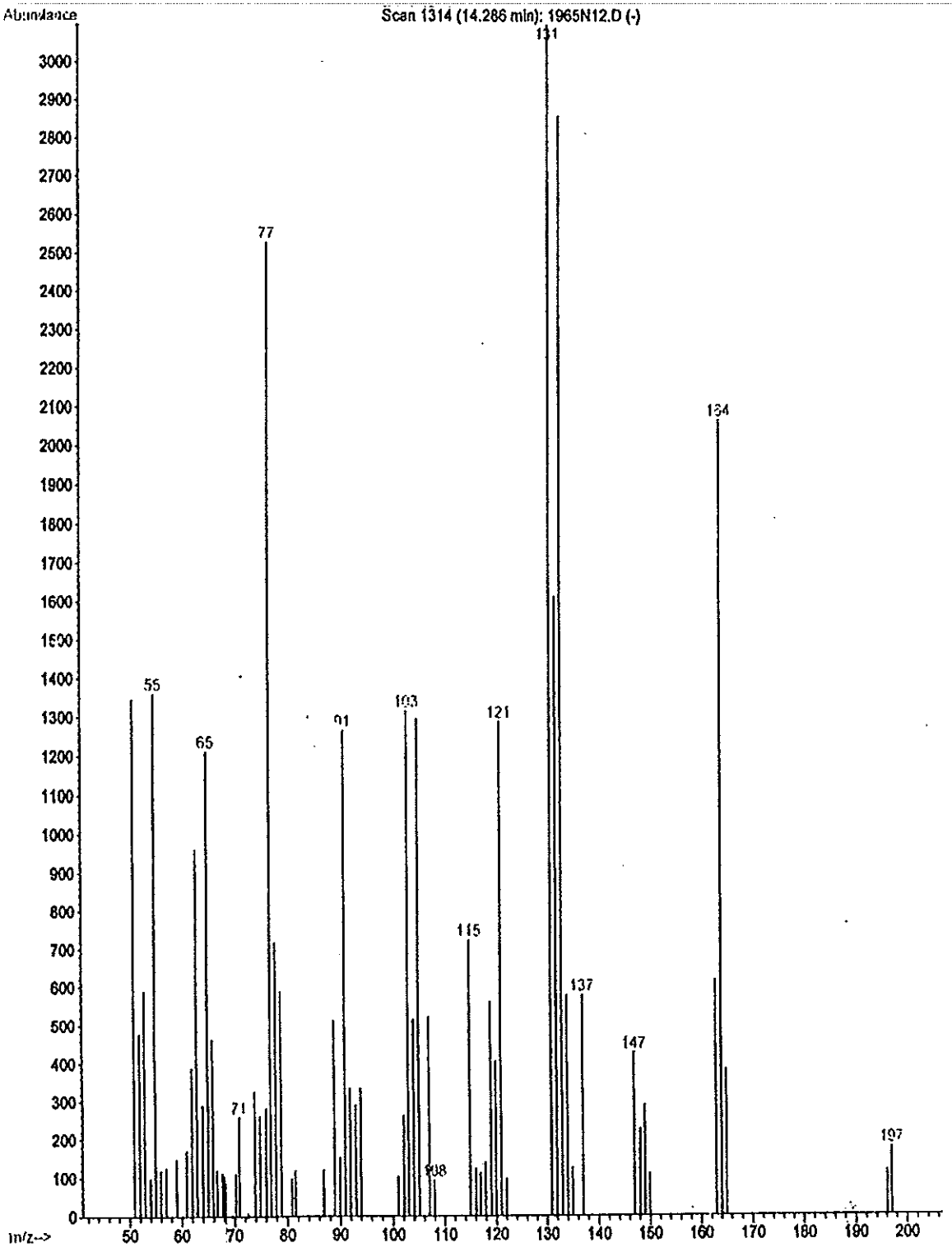
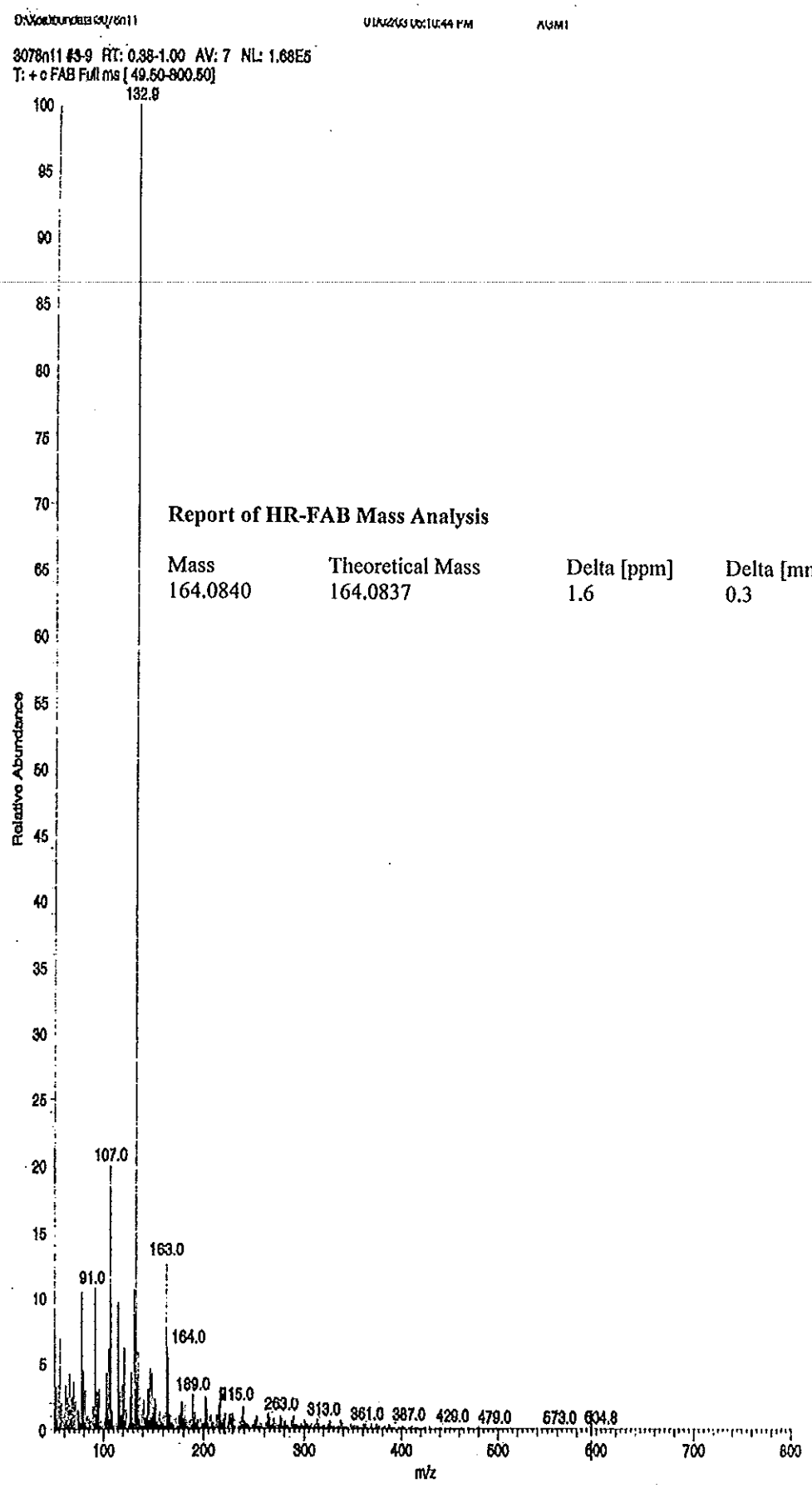


Figure 20 Mass spectrum (EI) of AGM1 (*p*-coumaryl-9-methyl ether)



Report of HR-FAB Mass Analysis

Mass	Theoretical Mass	Delta [ppm]	Delta [mmu]	Composition
164.0840	164.0837	1.6	0.3	$C_{10}H_{12}O_2$

Figure 21 Mass spectrum (FAB) of AGM1 (*p*-coumaryl-9-methyl ether)

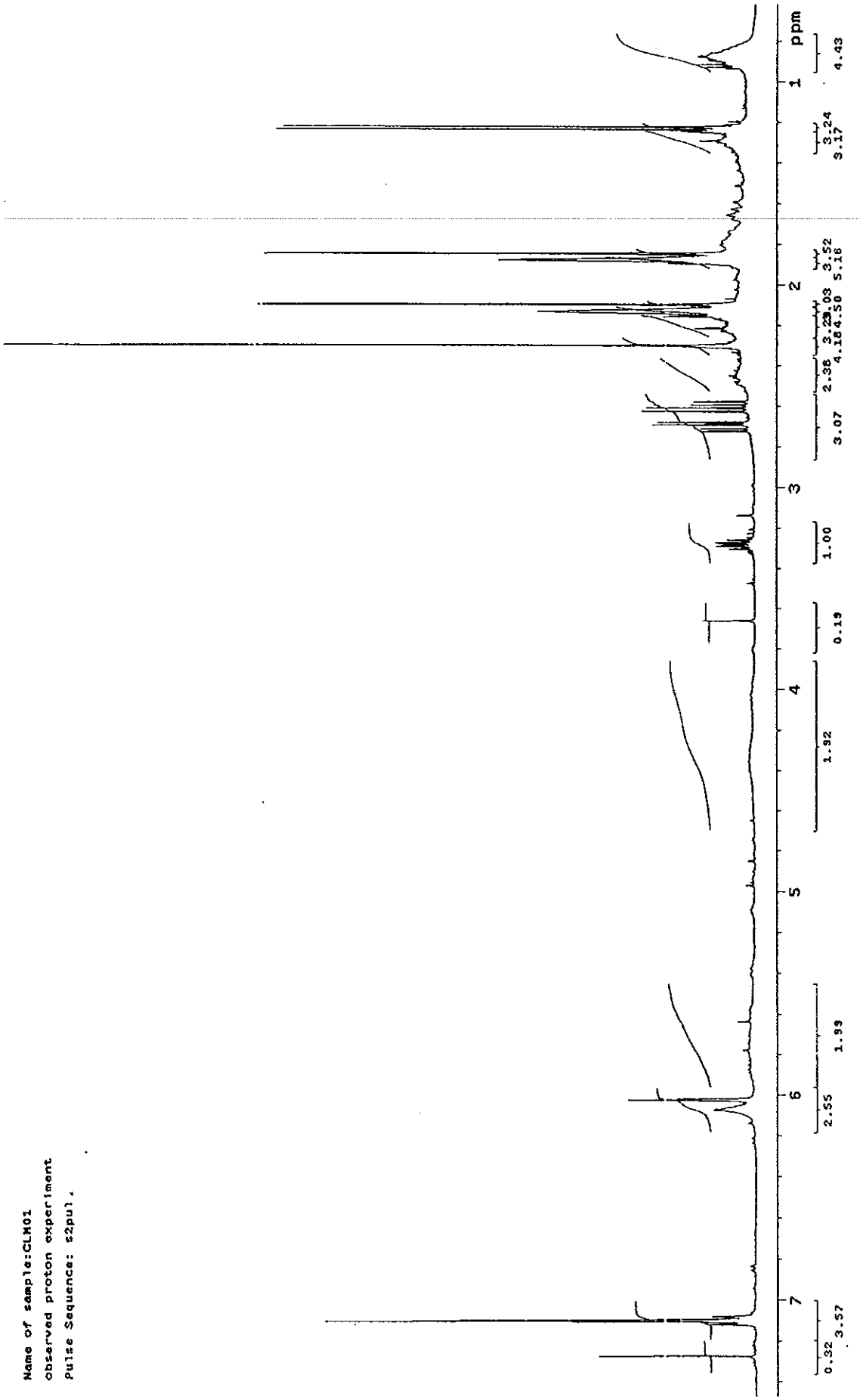


Figure 22 ^{1}H-NMR spectrum of CLM01 (ar-turmerone)

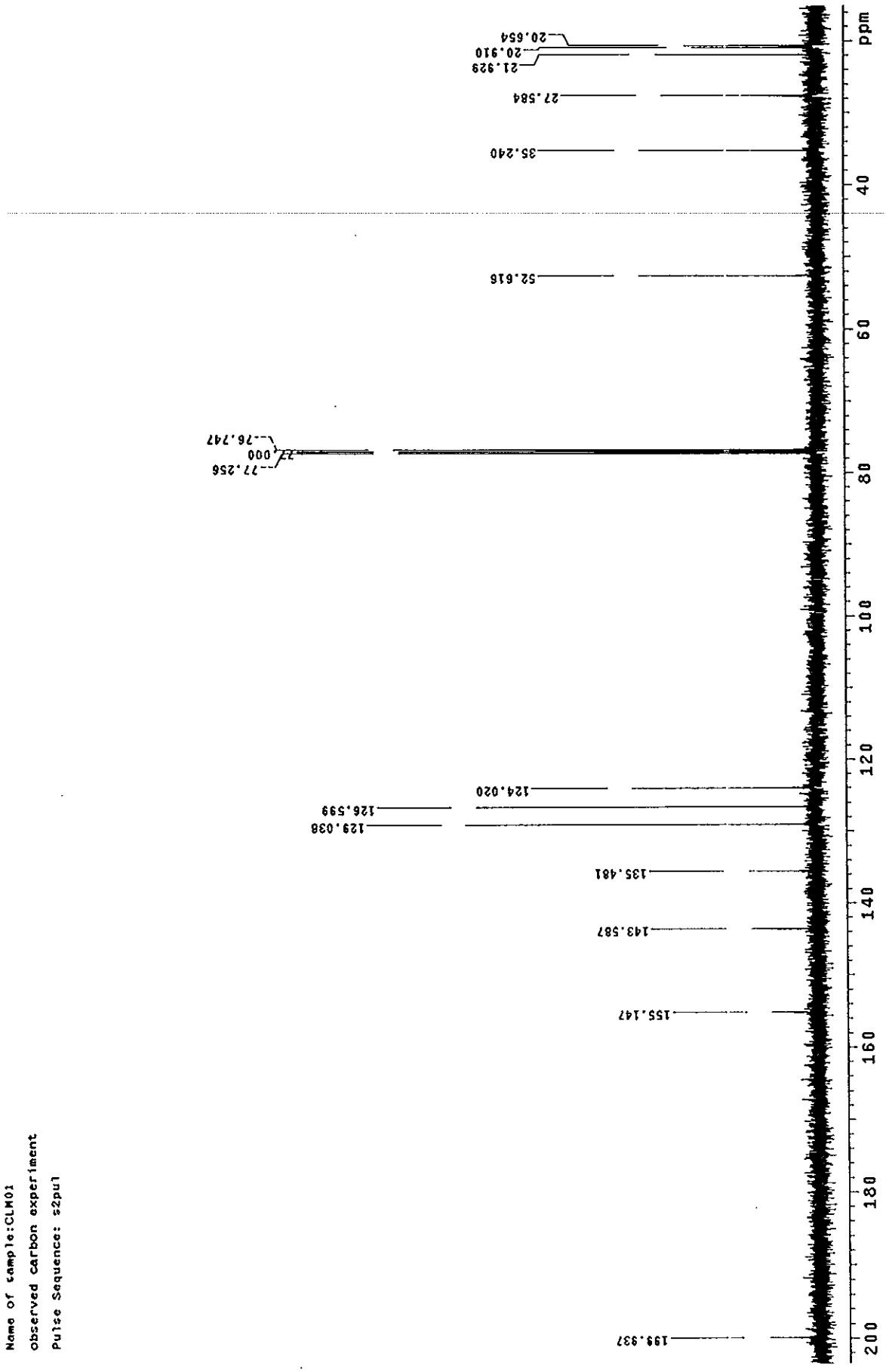


Figure 23 ^{13}C-NMR spectrum of CLM01 (ar-turmerone)

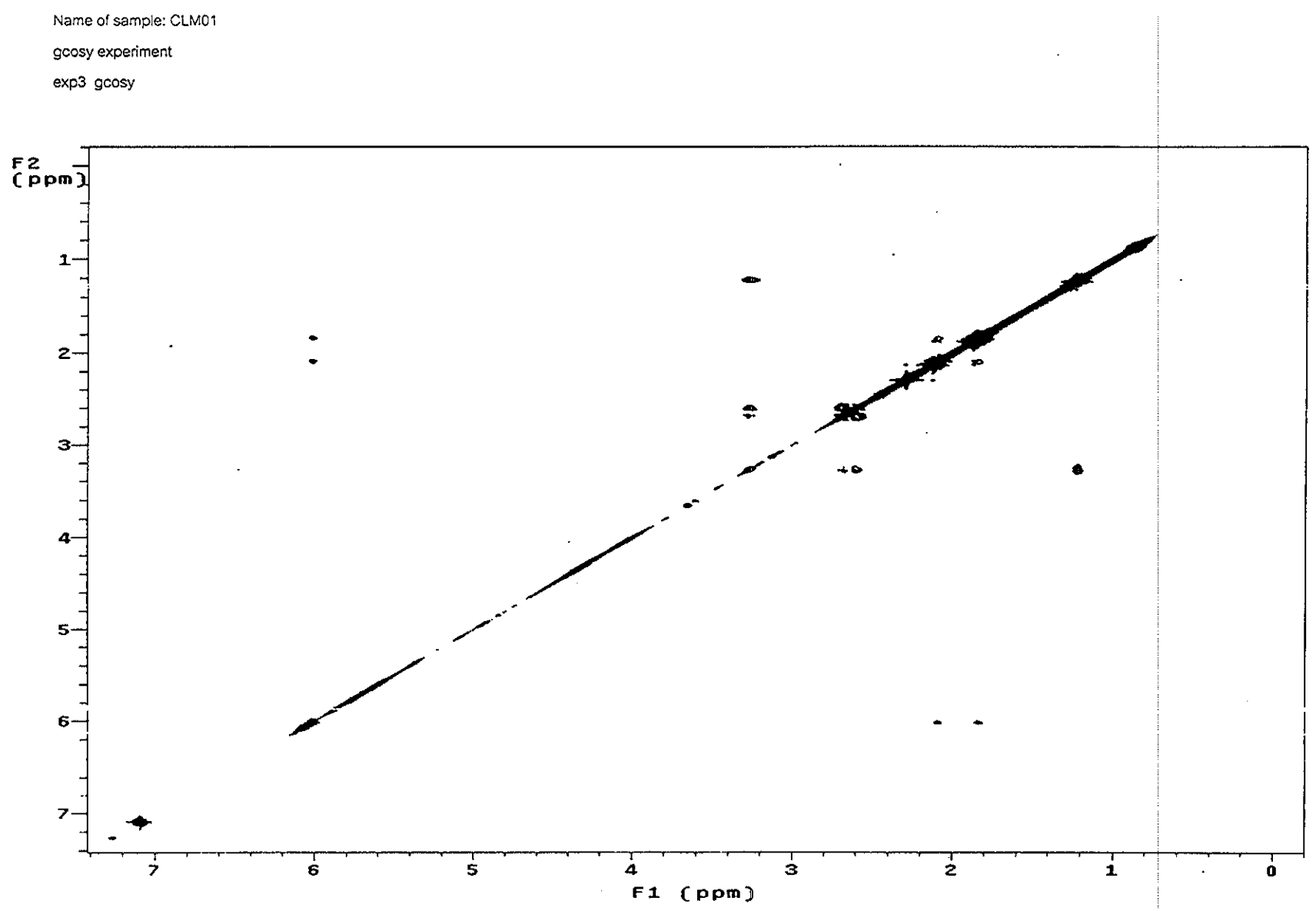


Figure 24 ^{1}H-^{1}H COSY spectrum of CLM01 (ar-turmerone)

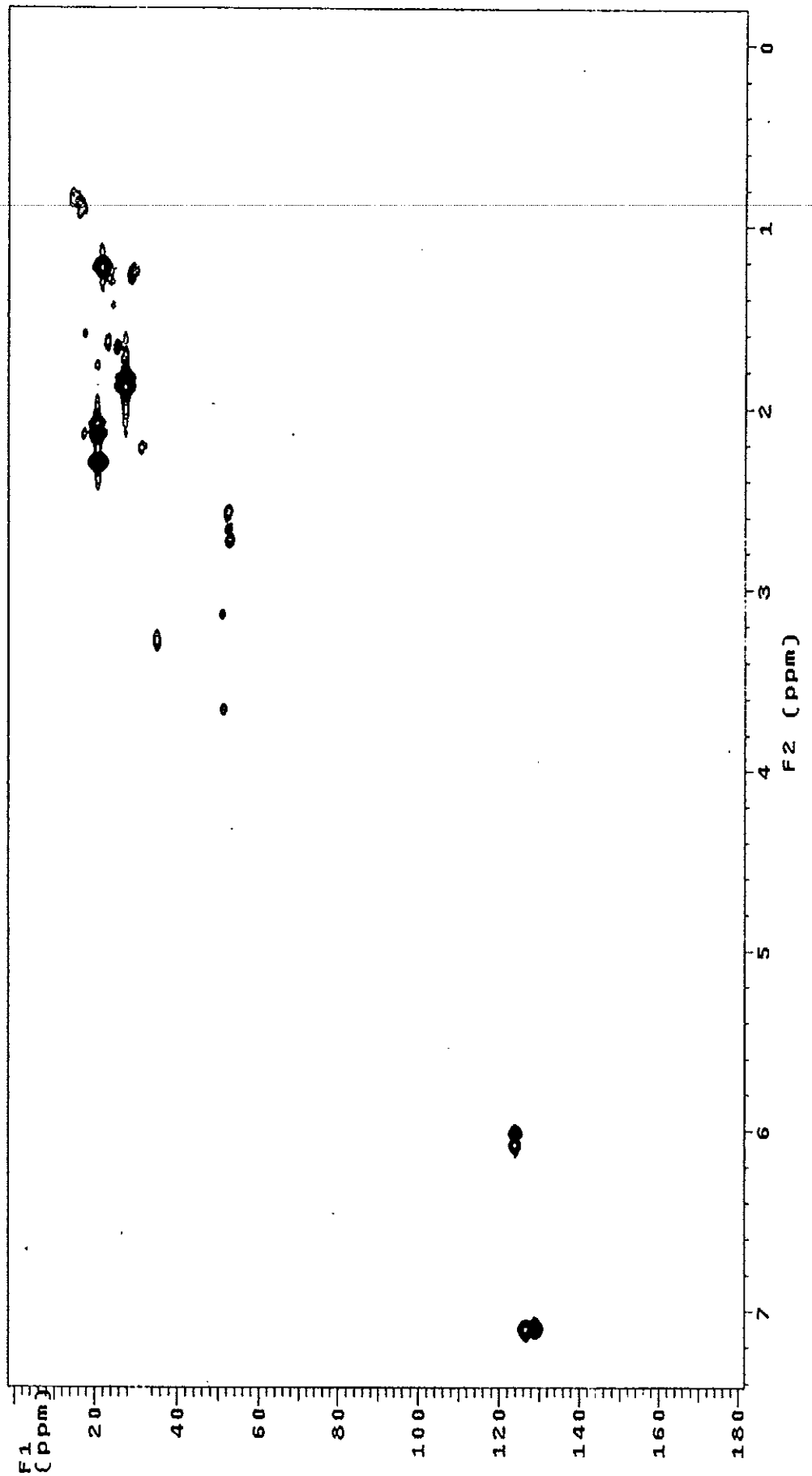


Figure 25 HMQC spectrum of CLM01 (ar-turmerone)

ghmqc experiment
exp1 ghmqc-da

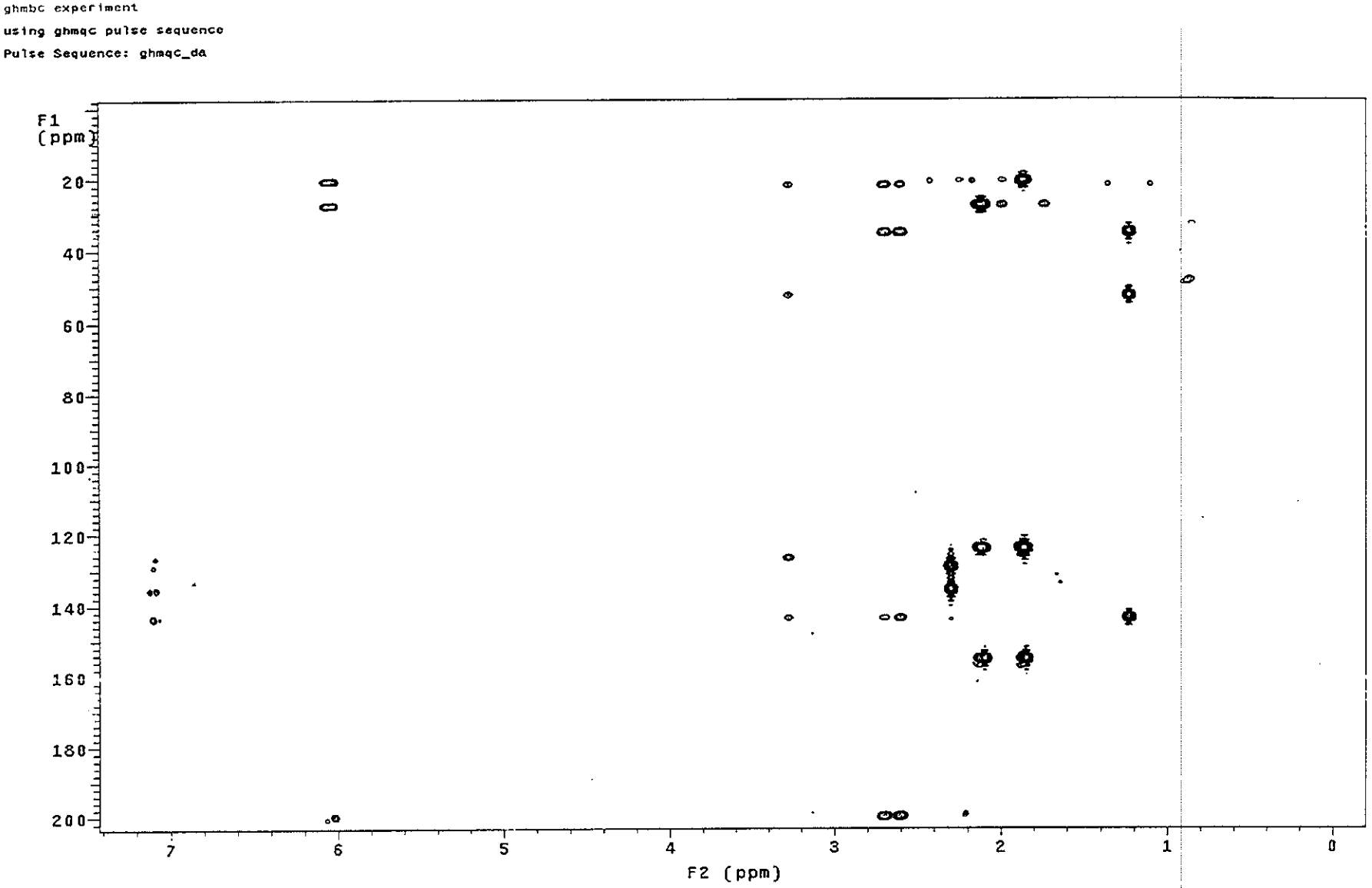


Figure 26 HMBC spectrum of CLM01 (ar-turmerone)

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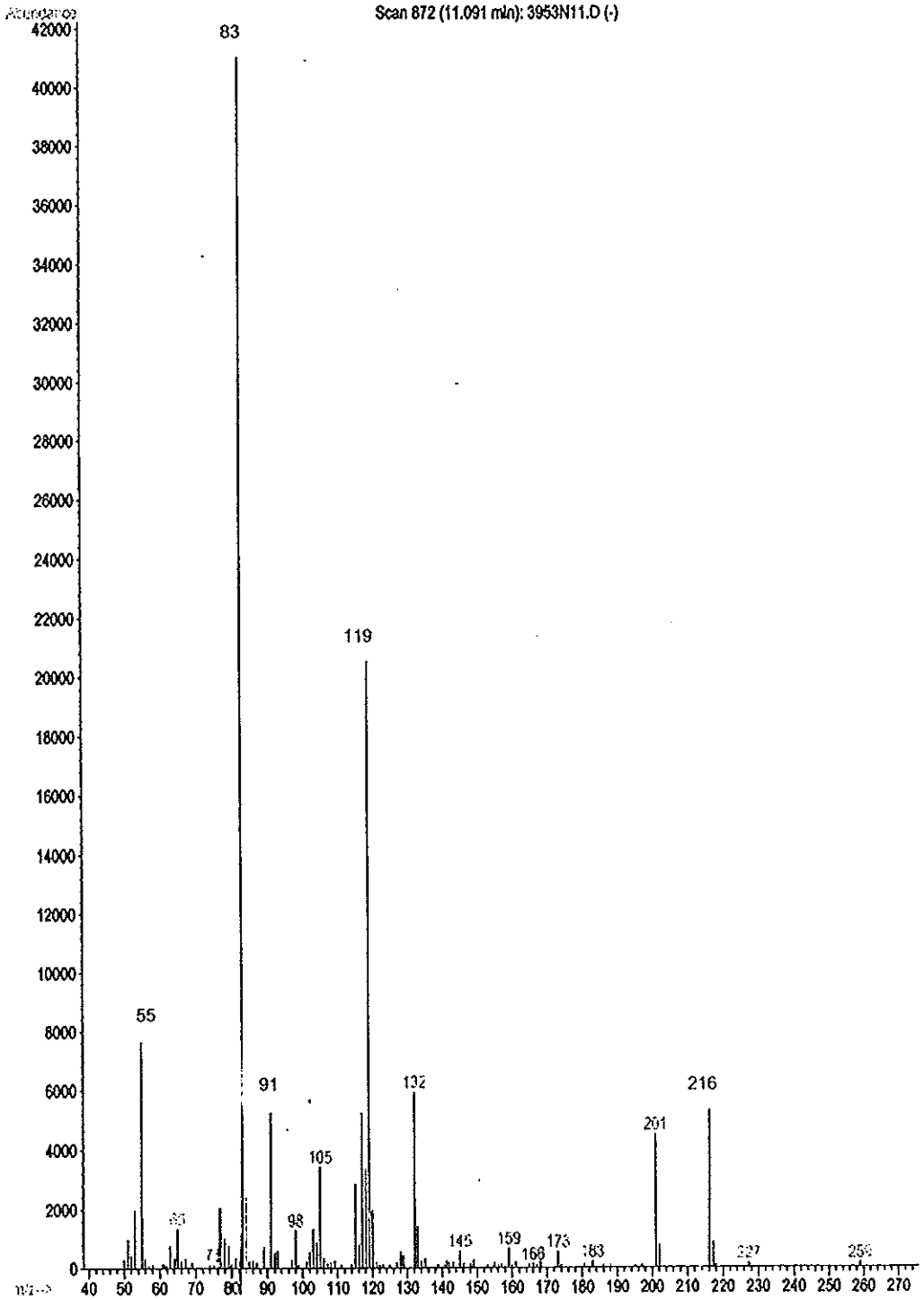

Figure 27 Mass spectrum (EI) of CLM01 (ar-turmerone) (GC/MS)

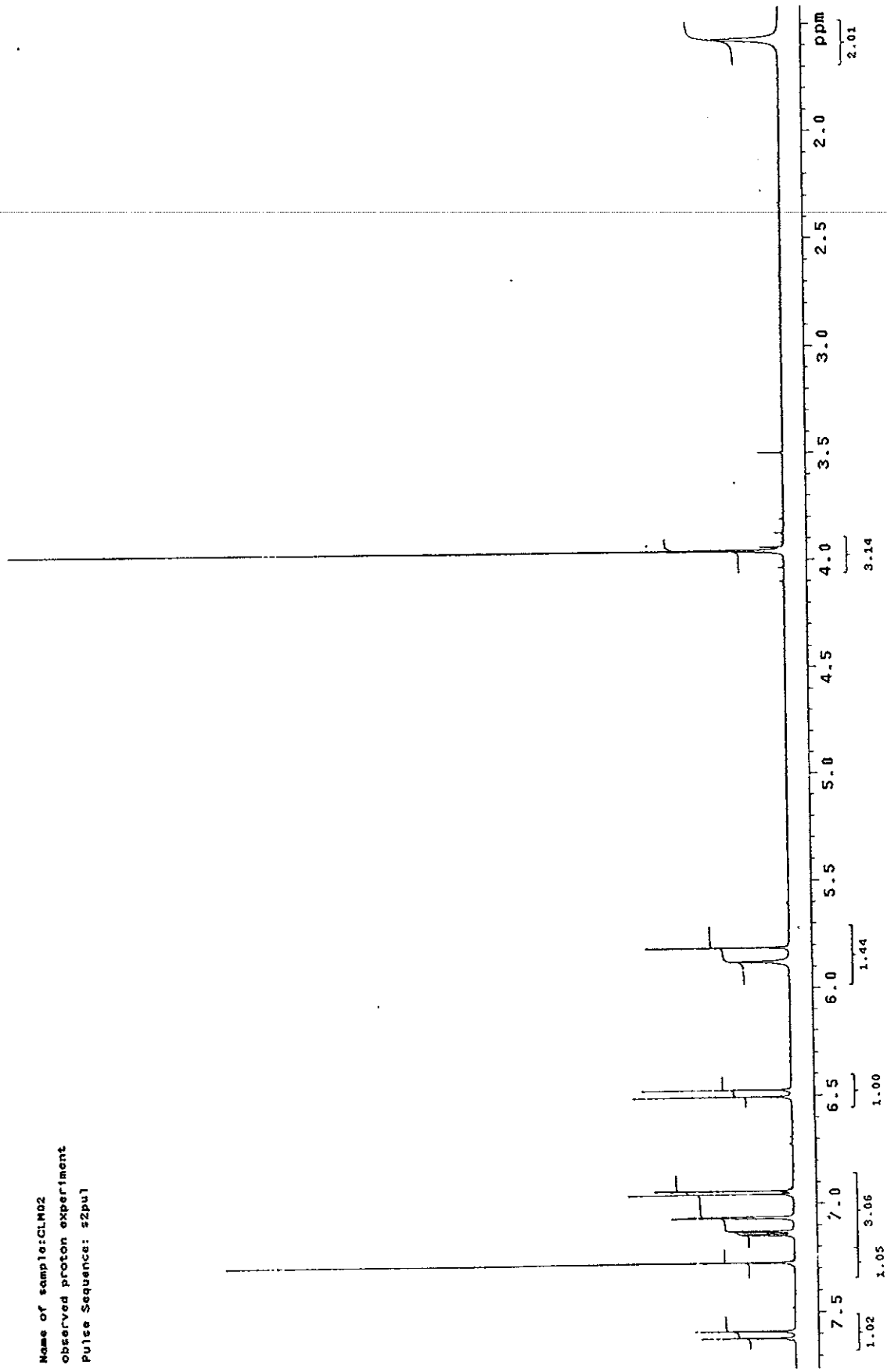

Figure 28 ^{1}H-NMR spectrum of CLM02 (curcumin)

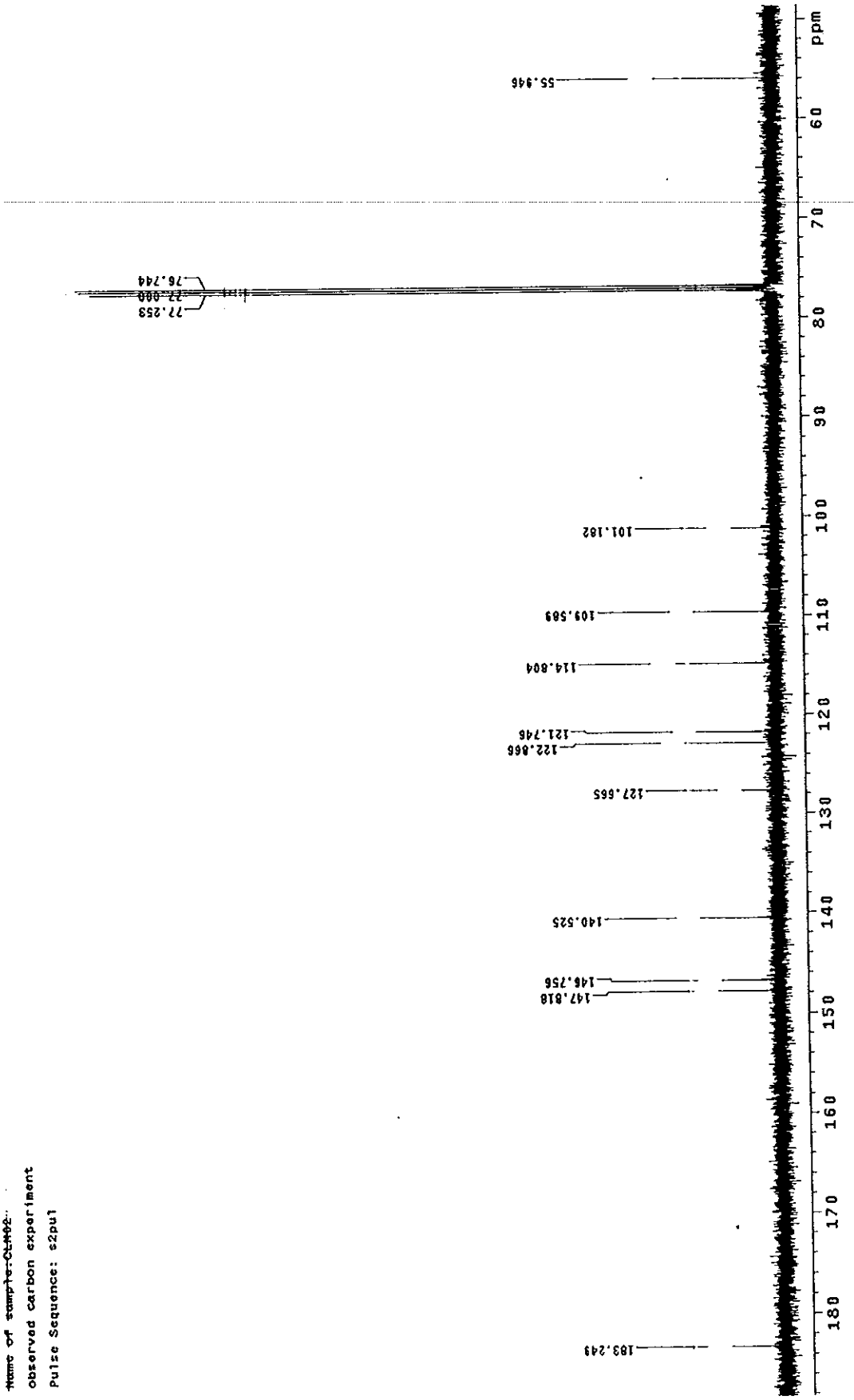

Figure 29 ^{13}C-NMR spectrum of CLM02 (curcumin)

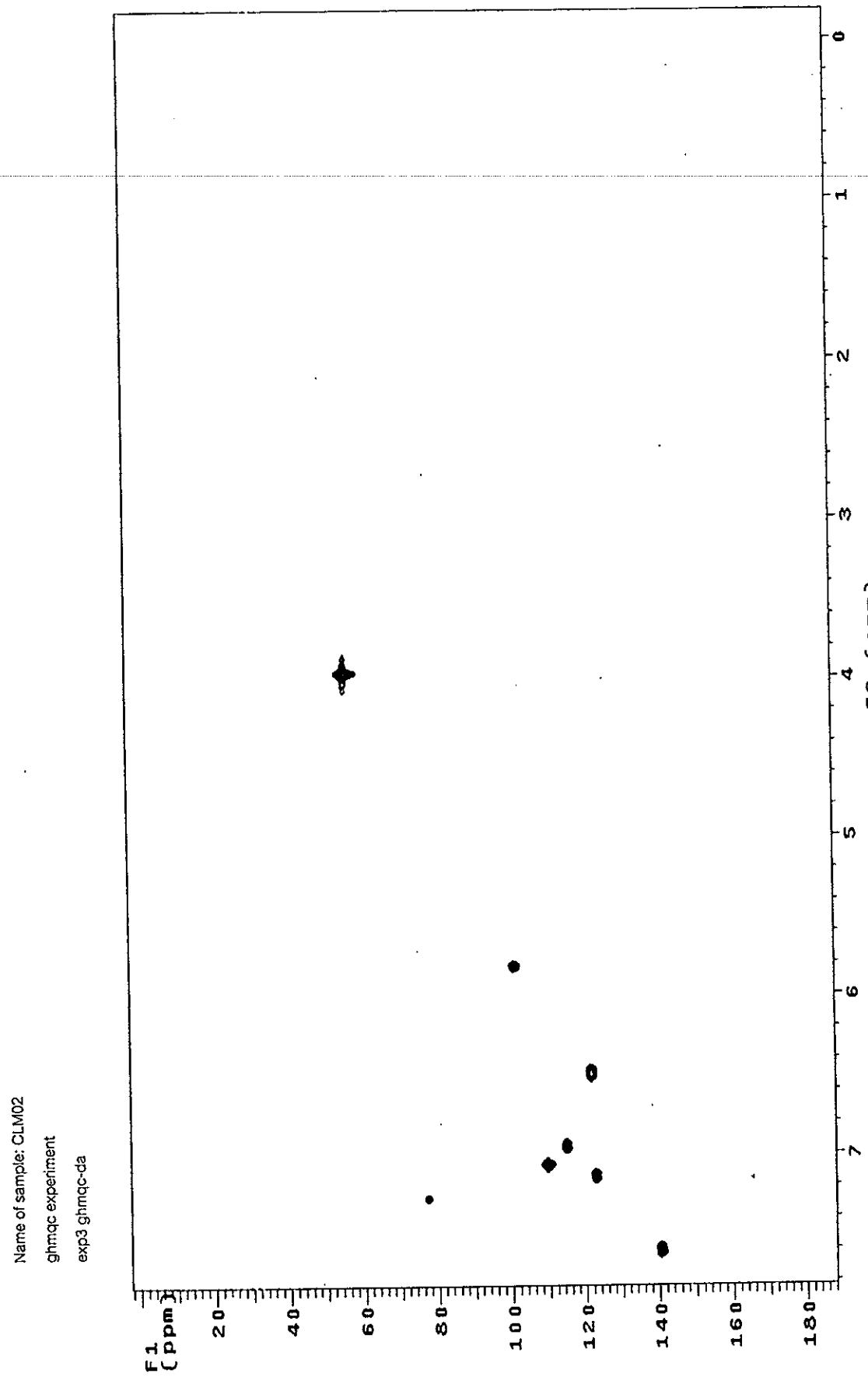

Figure 30 HMQC spectrum of CLM02 (curcumin)

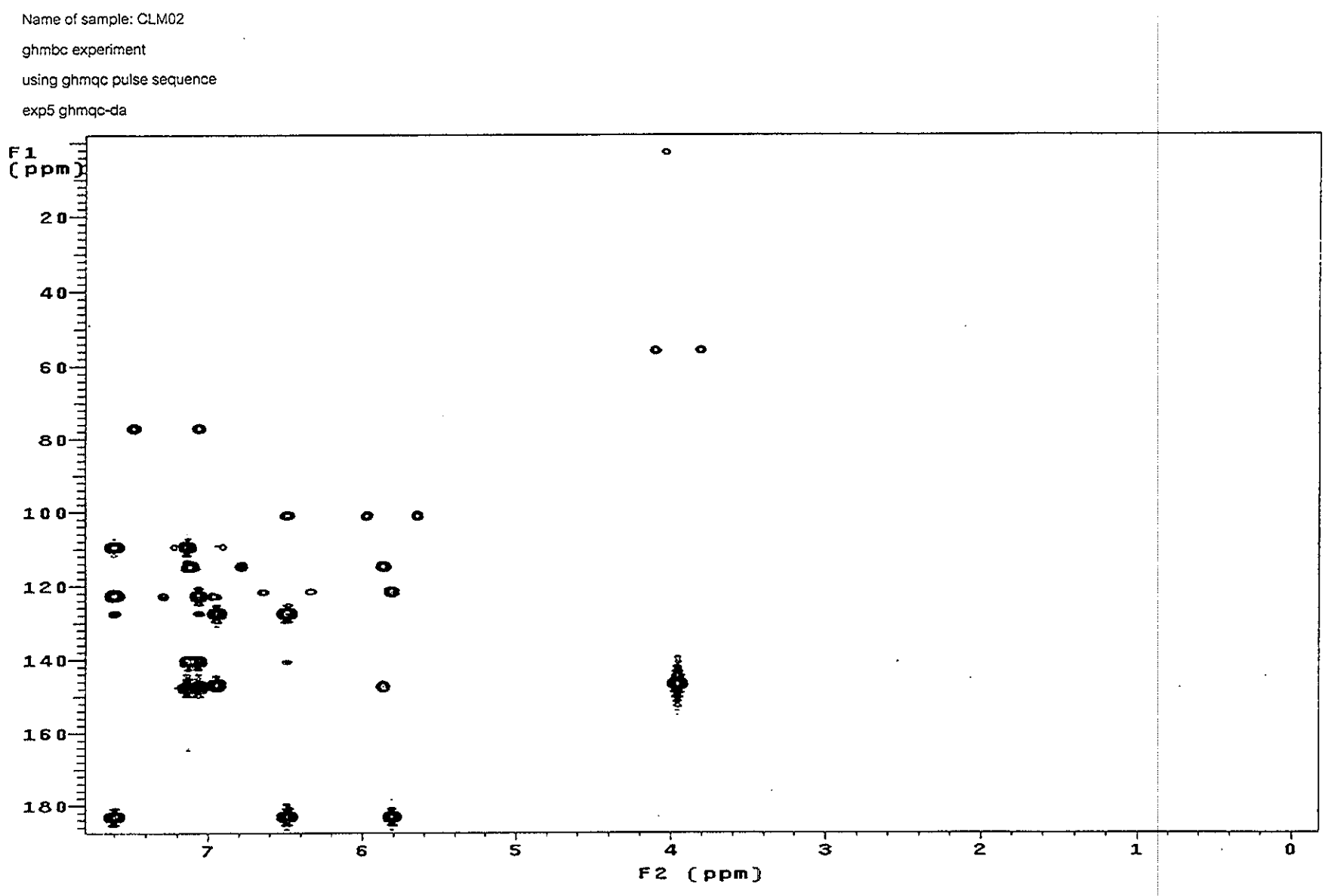

Figure 31 HMBC spectrum of CLM02 (curcumin)

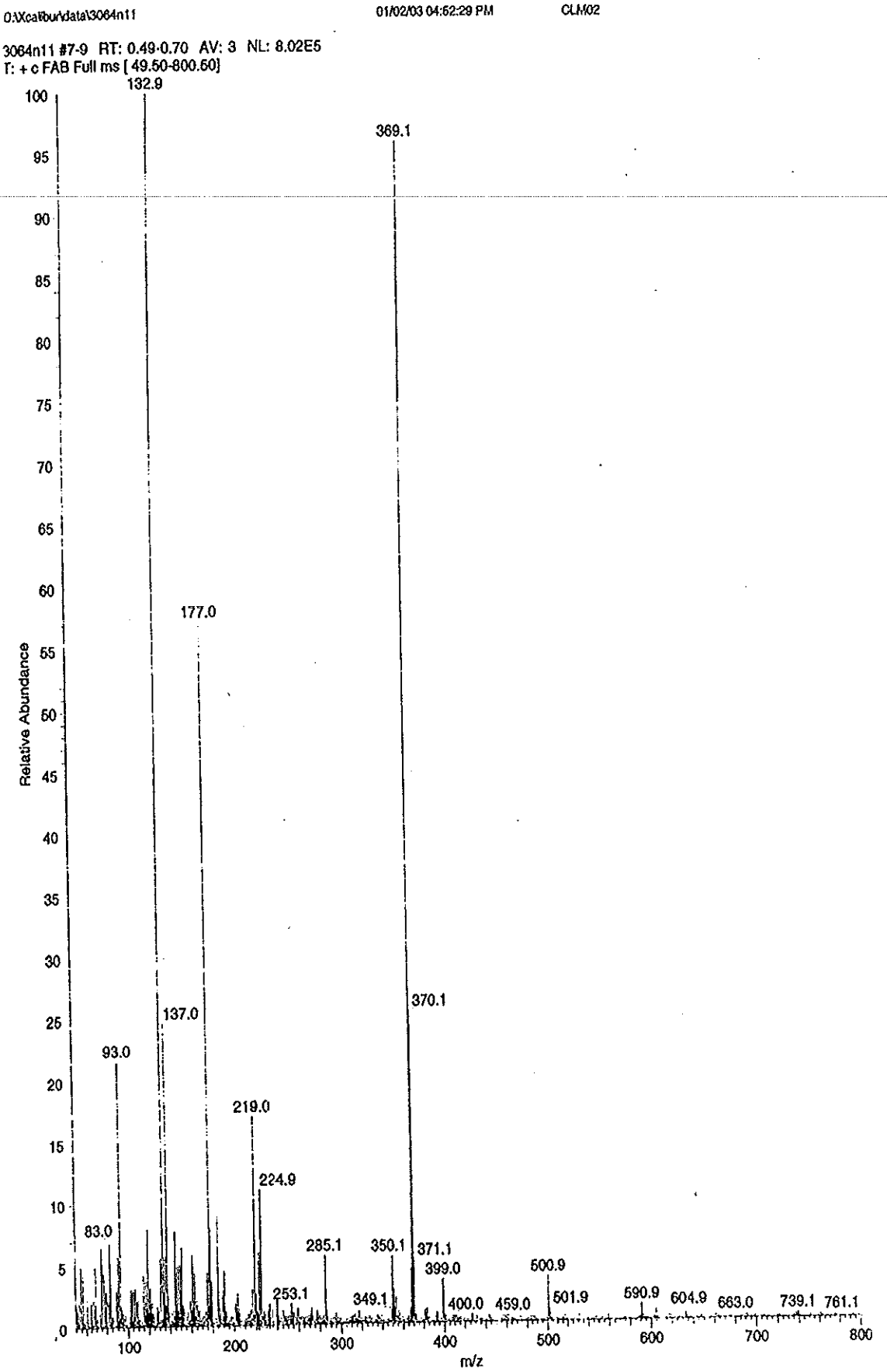

Figure 32 Mass spectrum (FAB) of CLM02 (curcumin)

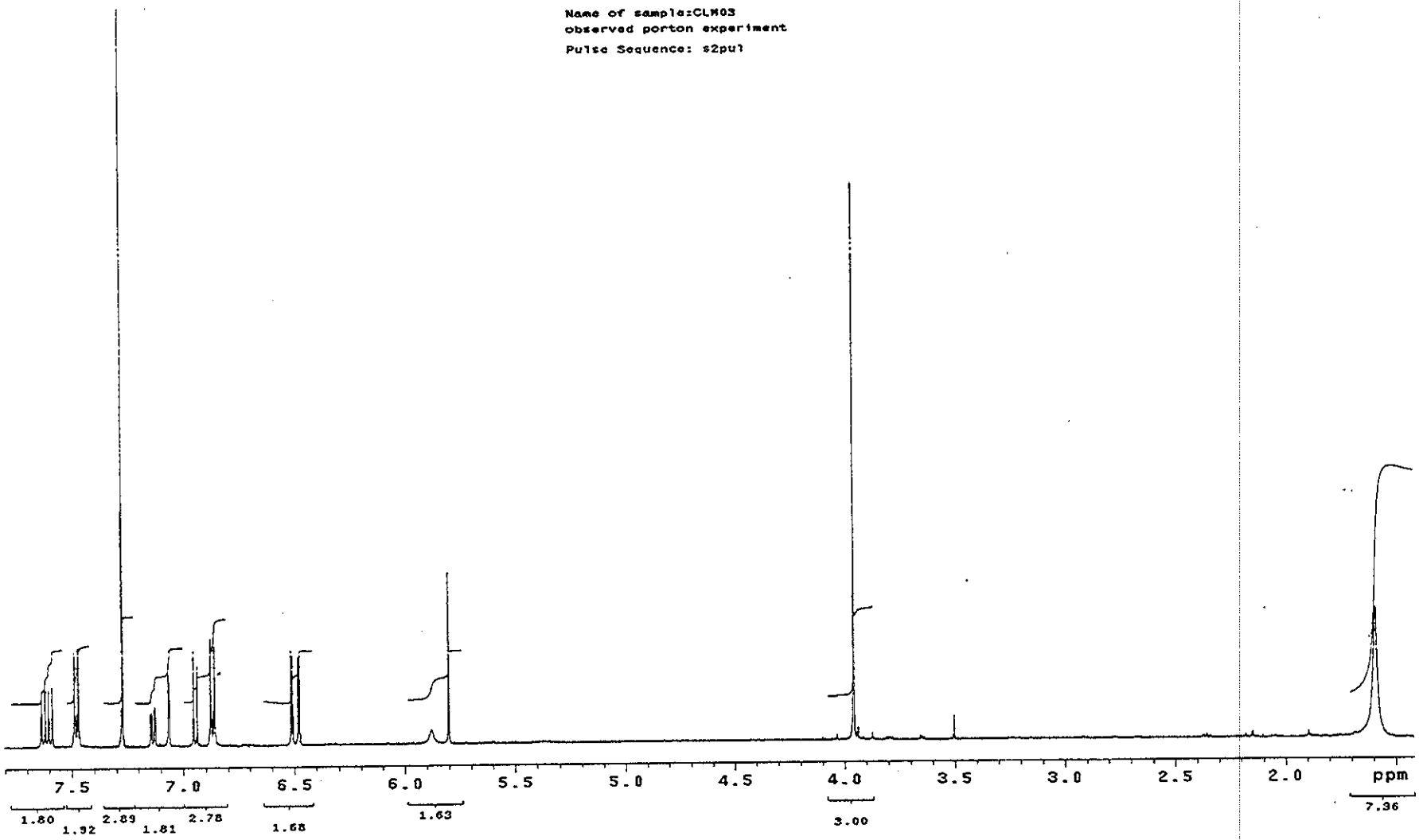

Figure 33 ^{1}H-NMR spectrum of CLM03 (demethoxycurcumin)

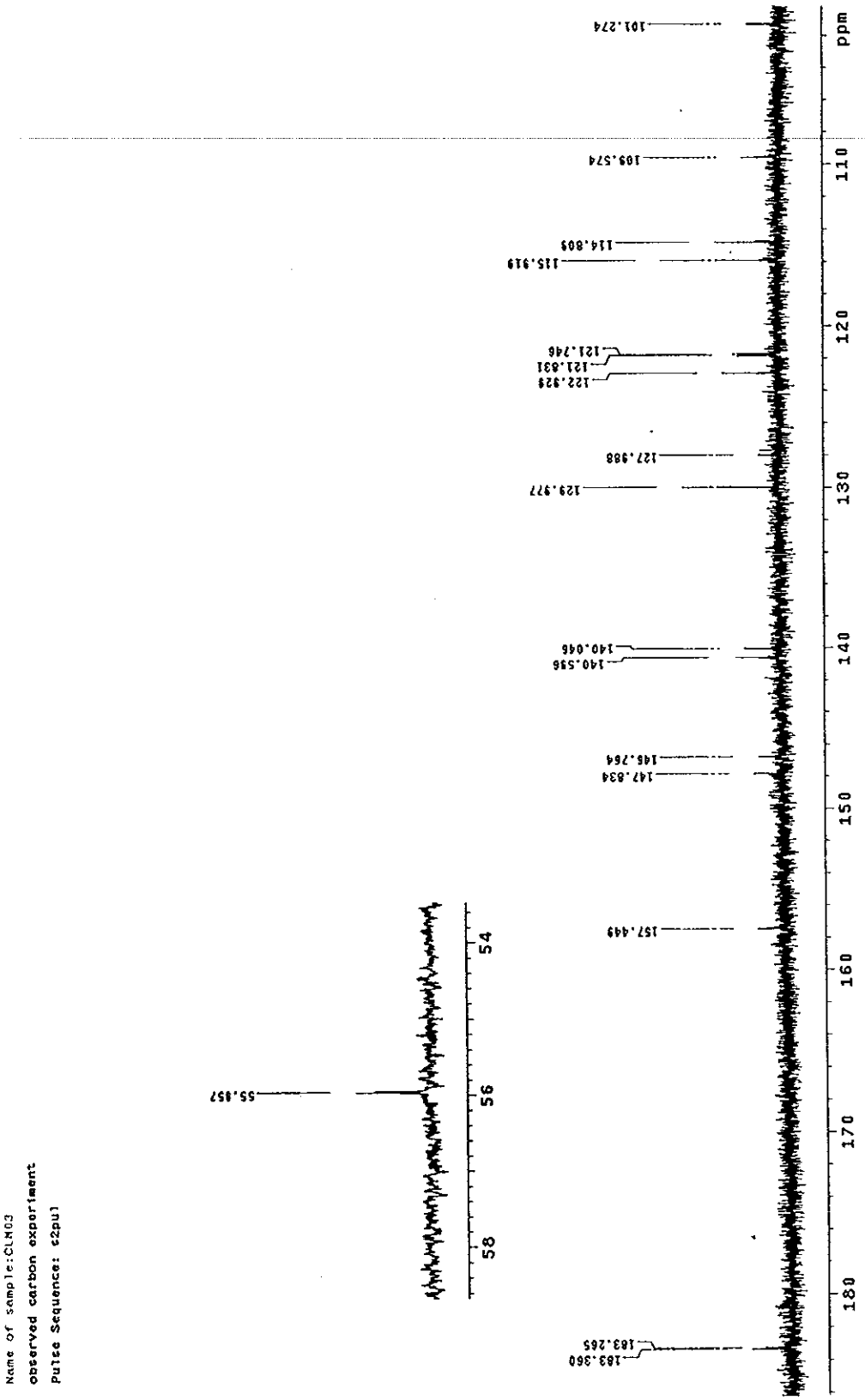

Figure 34 ^{13}C-NMR spectrum of CLM03 (demethoxycurcumin)

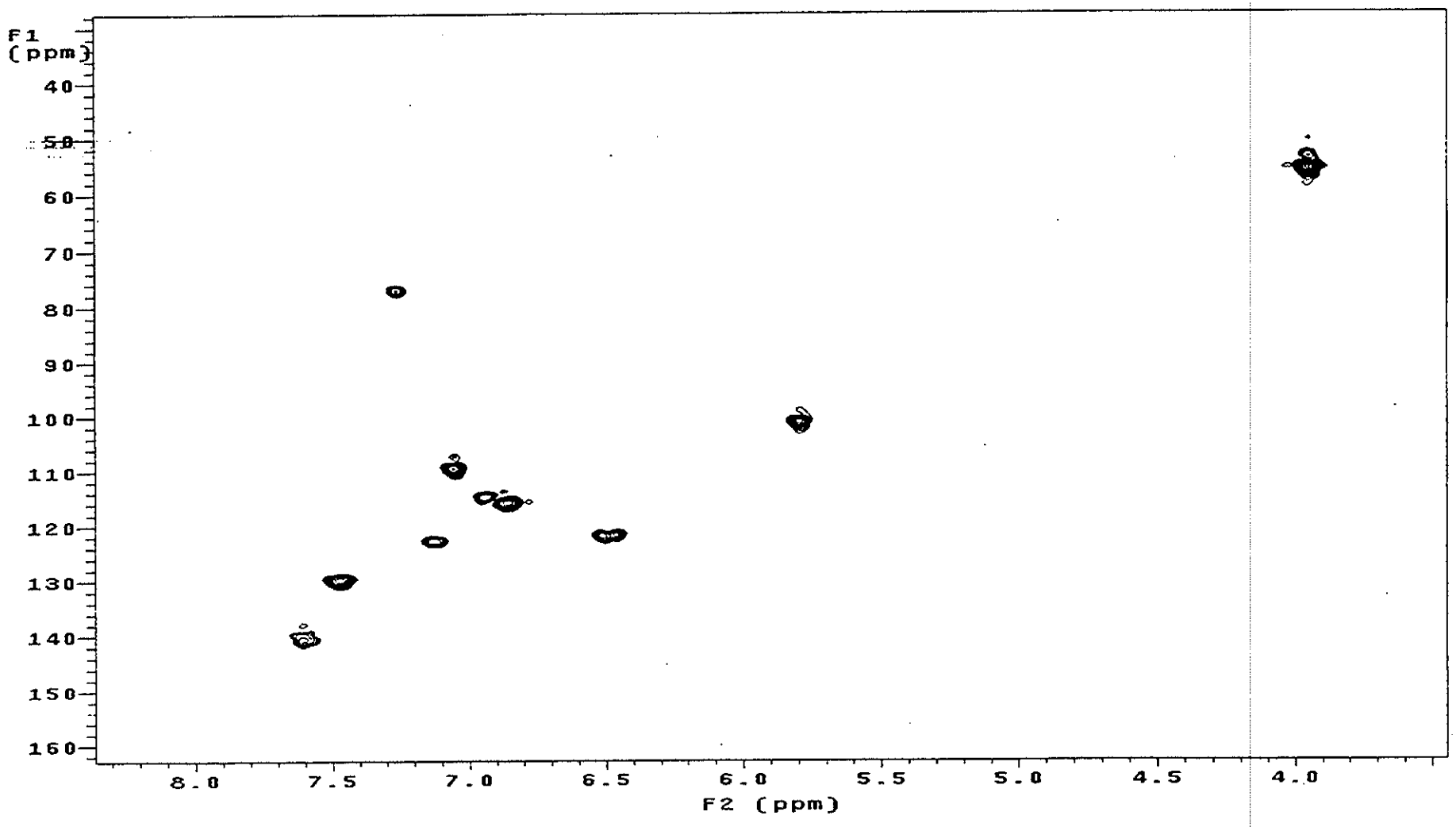

Figure 35 HMQC spectrum of CLM03 (demethoxycurcumin)

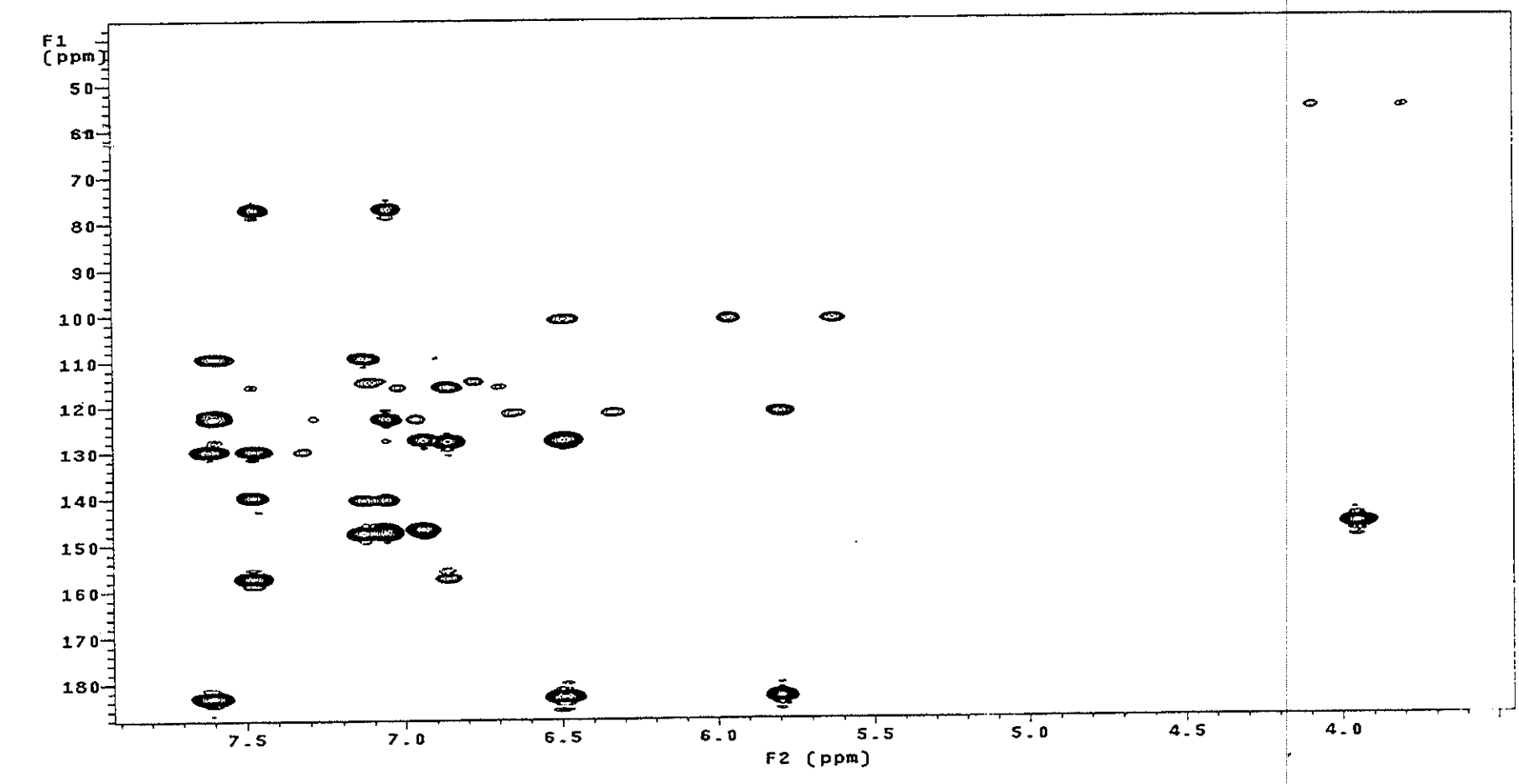

Figure 36 HMBC spectrum of CLM03 (demethoxycurcumin)

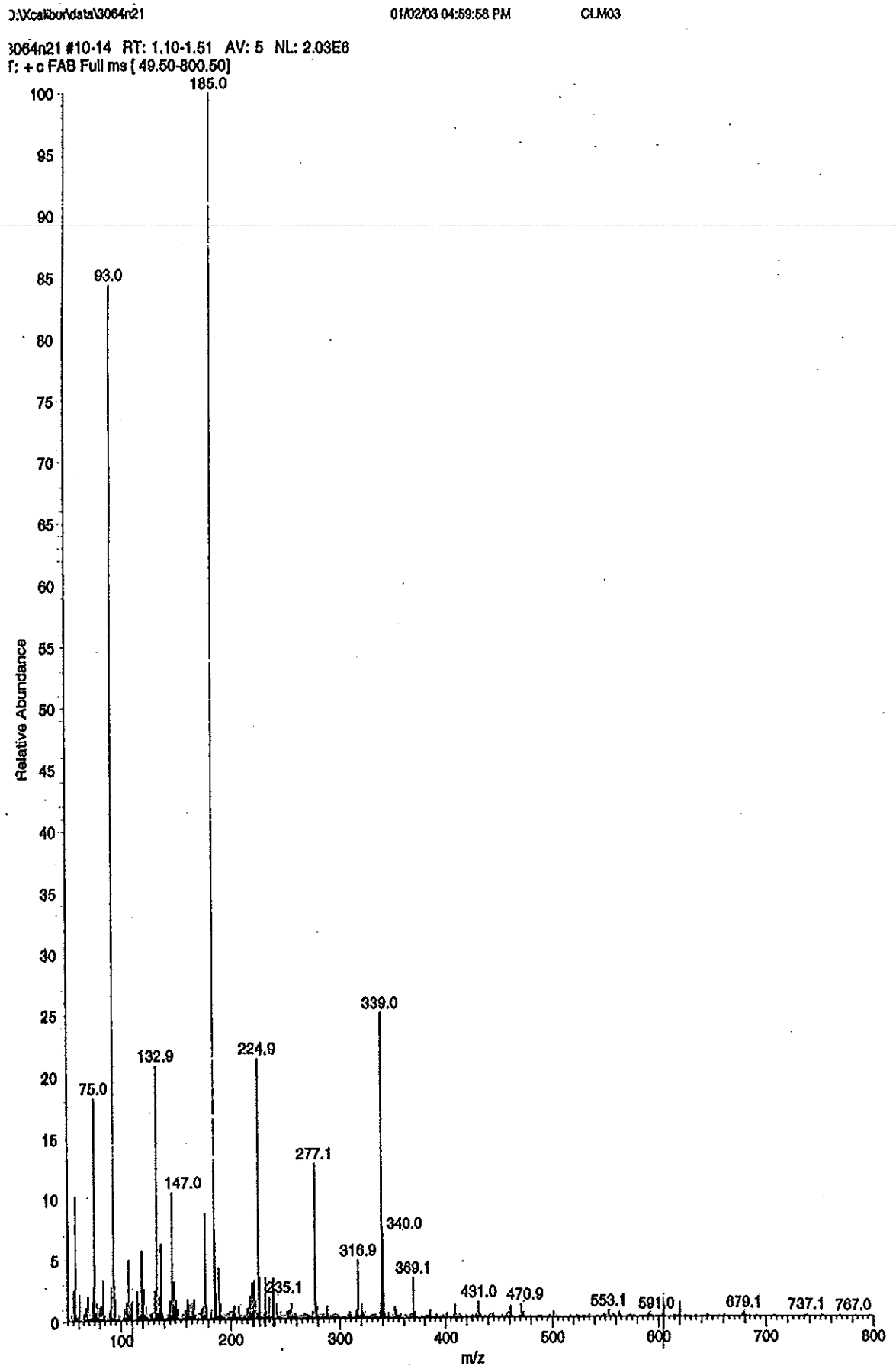

Figure 37 Mass spectrum (FAB) of CLM03 (demethoxycurcumin)

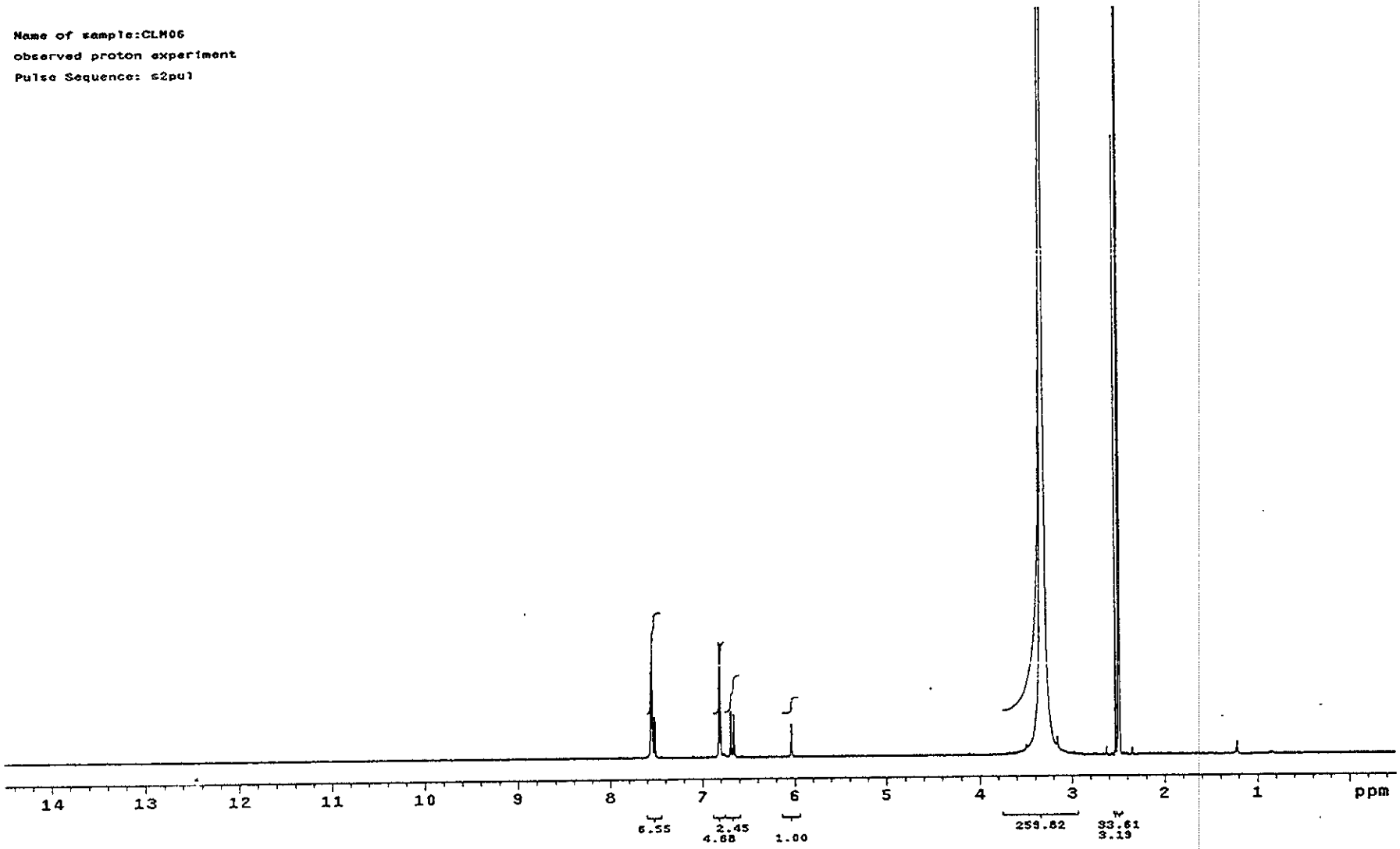

Figure 38 ^{1}H-NMR spectrum of CLM06 (bisdemethoxycurcumin)

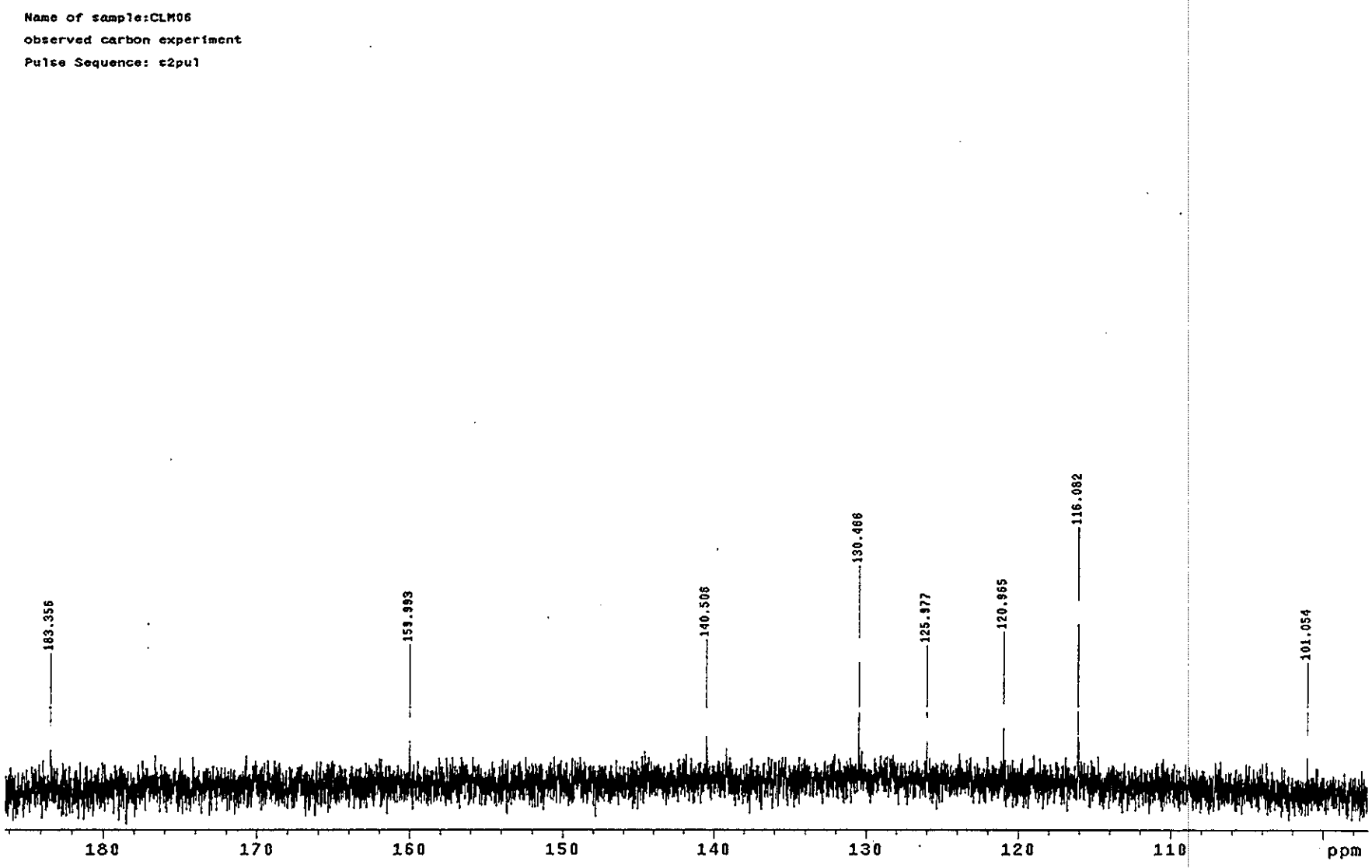

Figure 39 ^{13}C-NMR spectrum of CLM06 (bisdemethoxycurcumin)

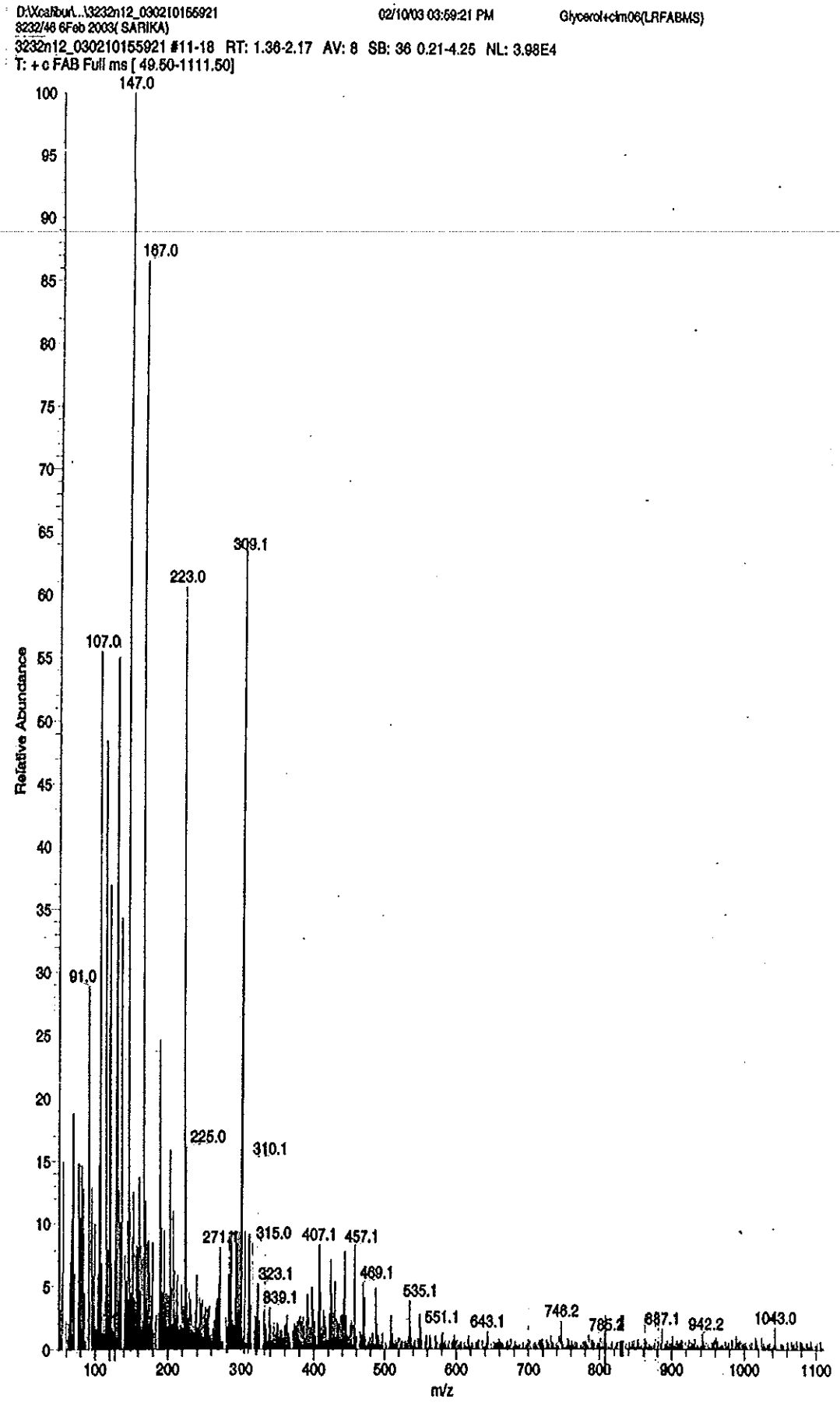

Figure 40 Mass spectrum (FAB) of CLM06 (bisdemethoxycurcumin)

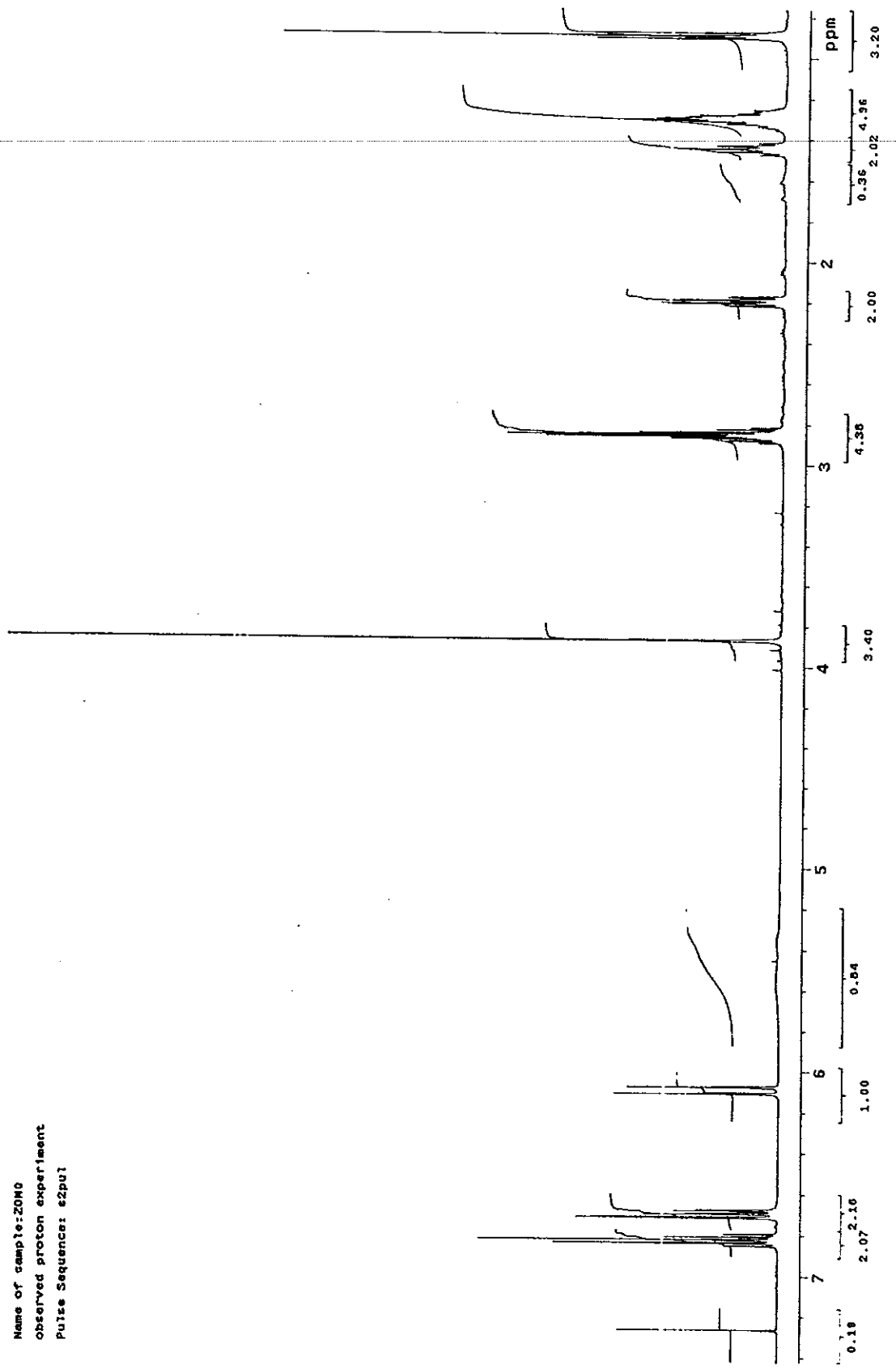

Figure 41 ^{1}H-NMR spectrum of ZOM0 (6-shogaol)

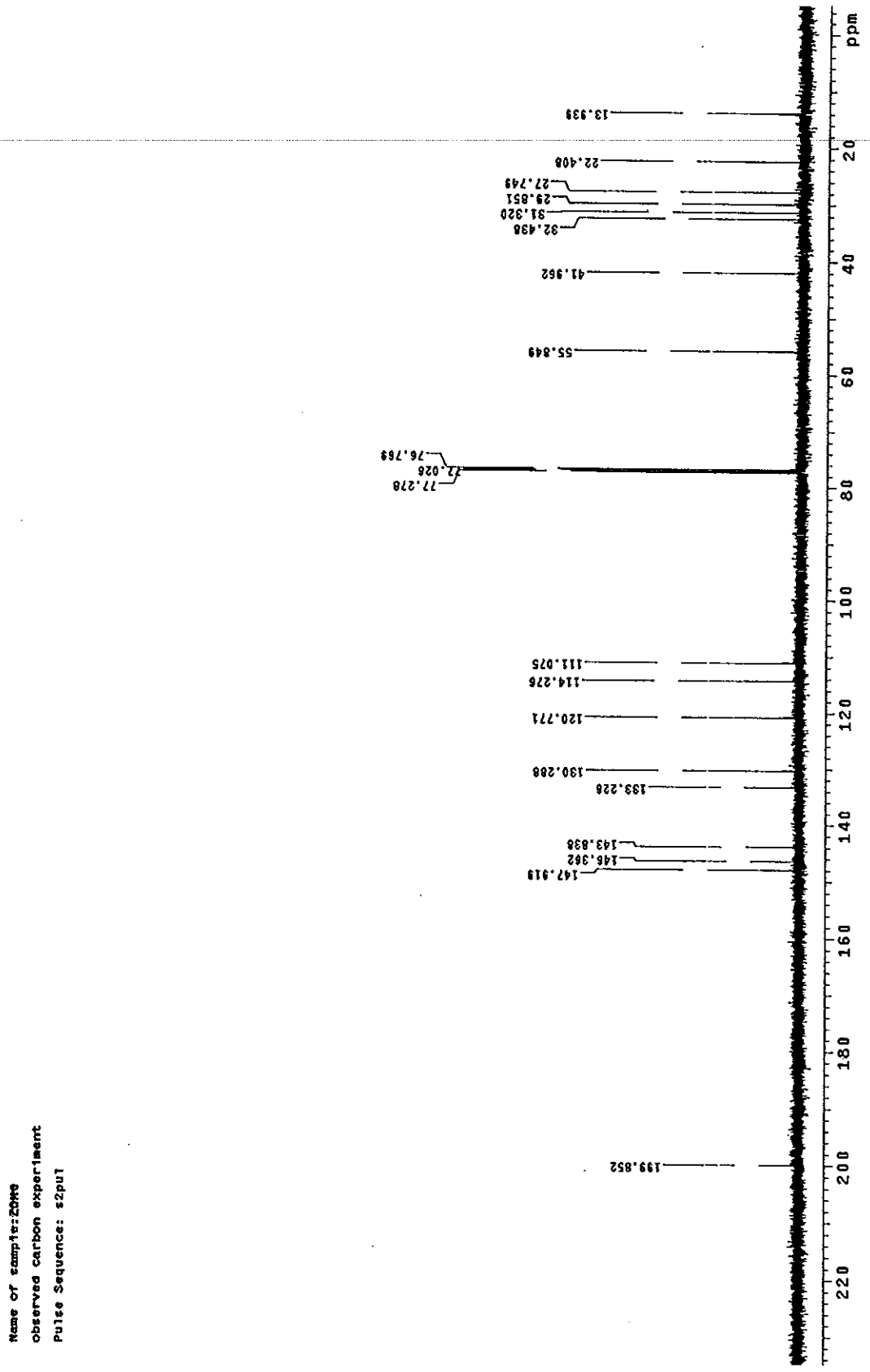

Figure 42 ^{13}C-NMR spectrum of ZOM0 (6-shogaol)

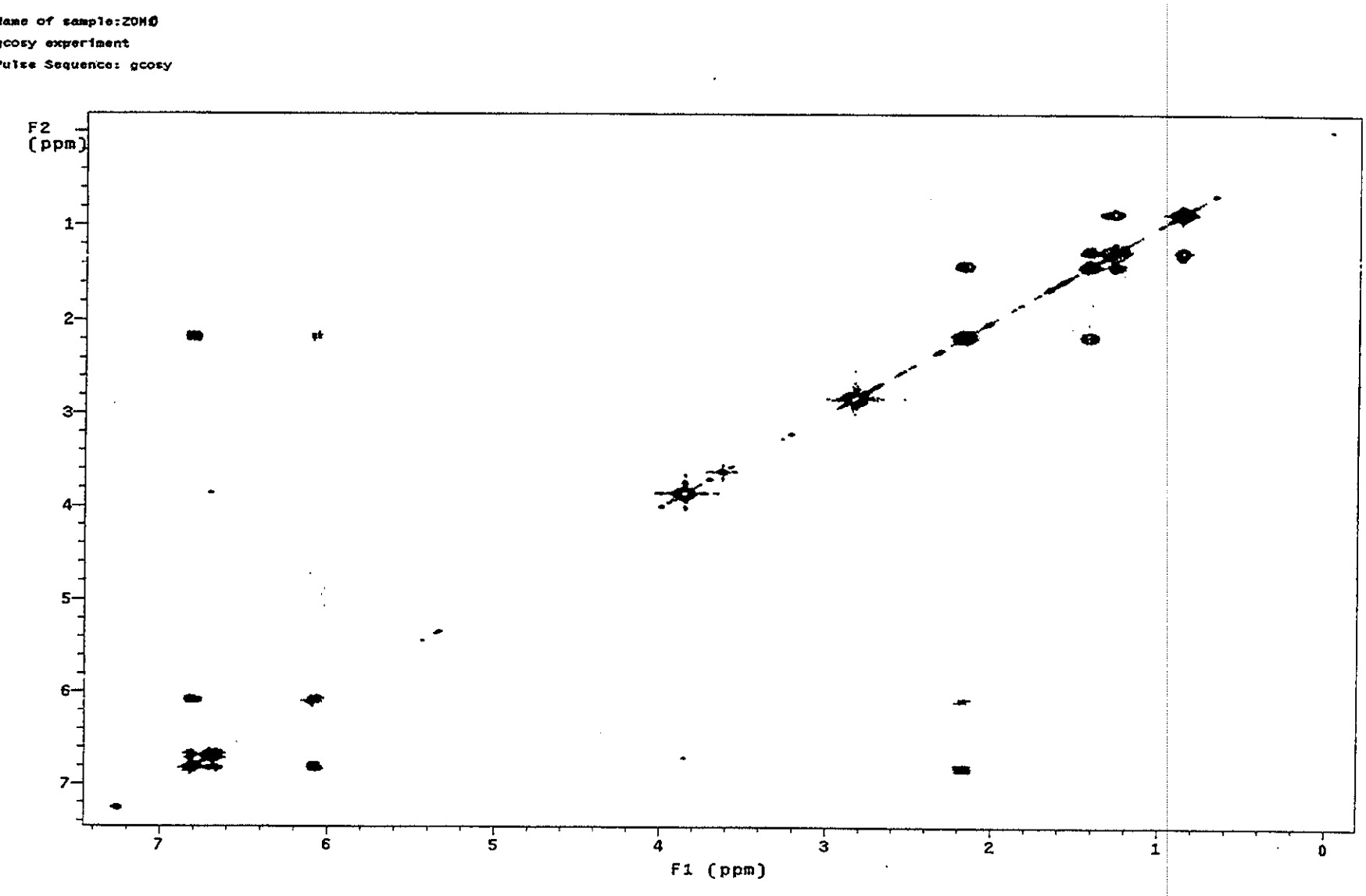

Figure 43 ^{1}H-^{1}H COSY spectrum of ZOM0 (6-shogaol)

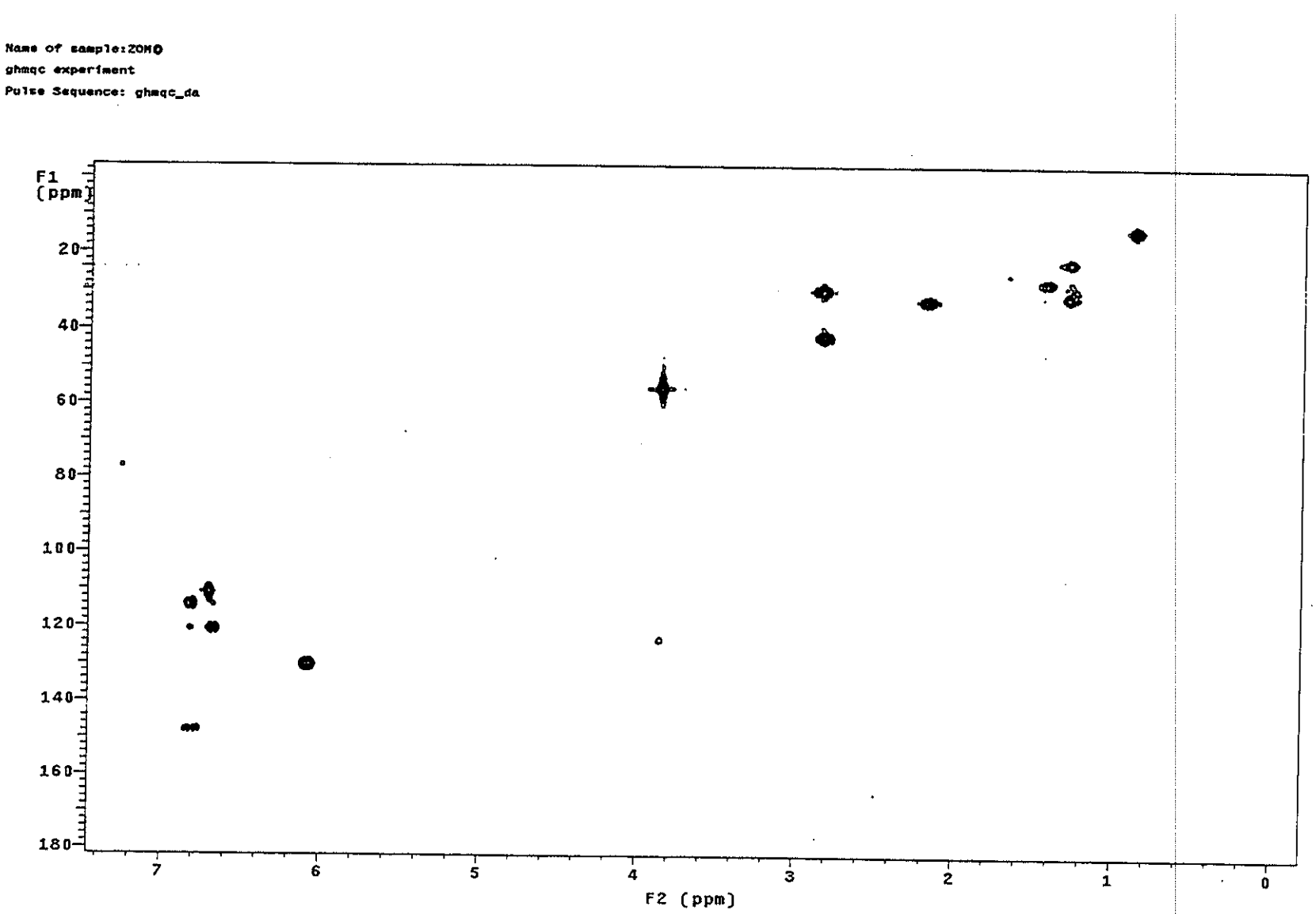

Figure 44 HMQC spectrum of ZOM0 (6-shogaol)

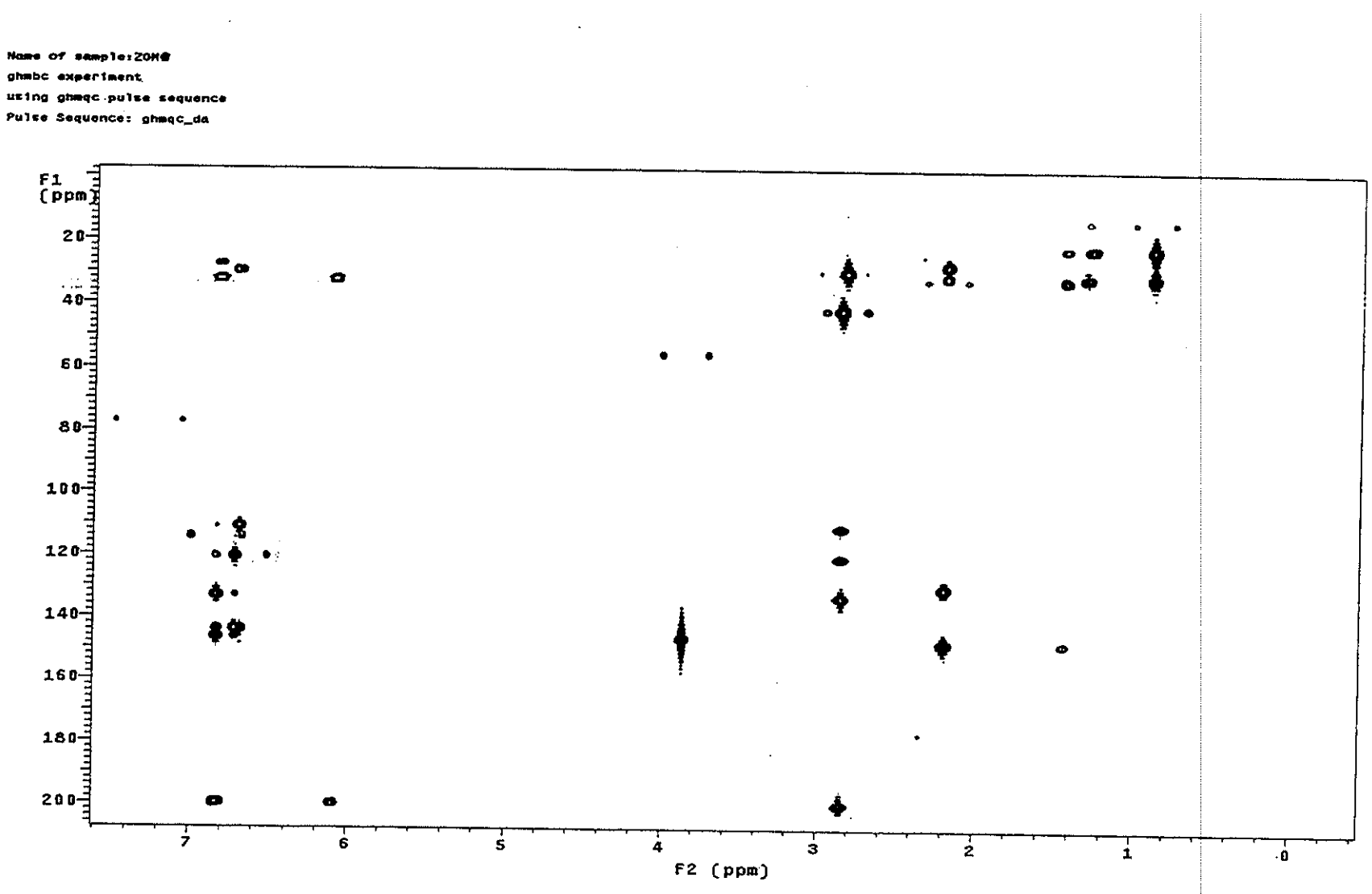

Figure 45 HMBC spectrum of ZOM0 (6-shogaol)

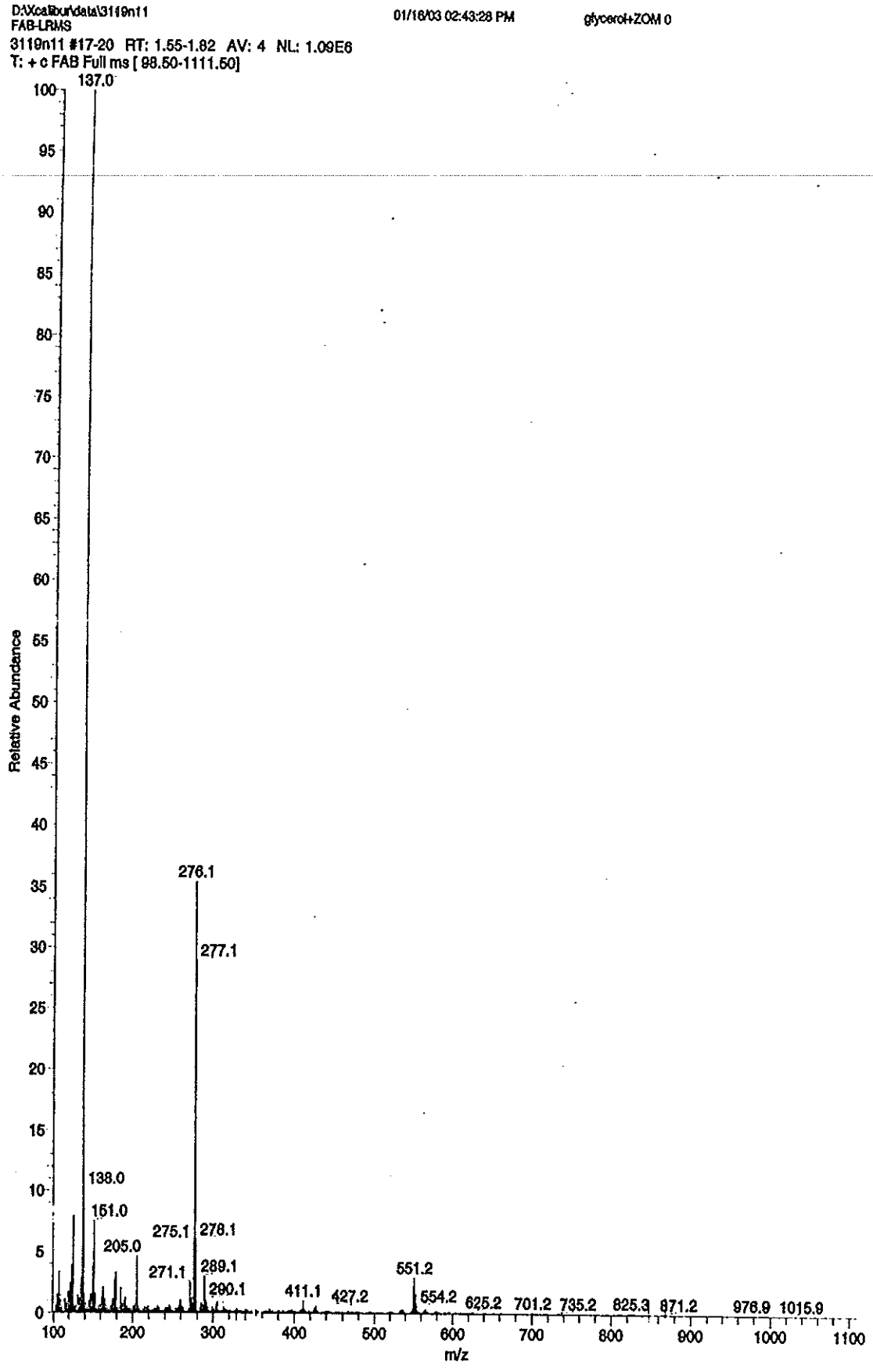

Figure 46 Mass spectrum (FAB) of ZOM0 (6-shogaol)

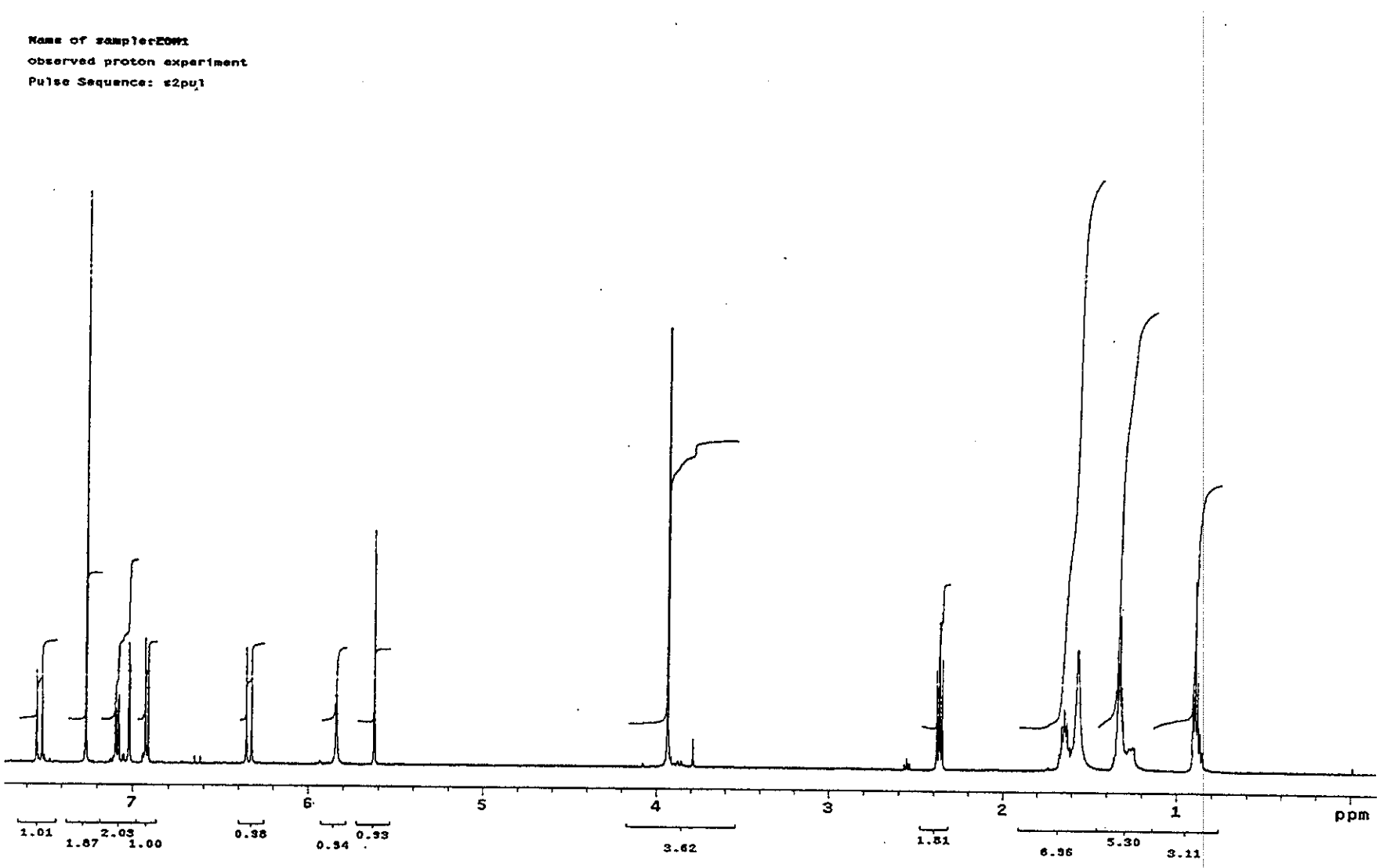

Figure 47 ^{1}H-NMR spectrum of ZOM1 (6-dehydrogingerdione)

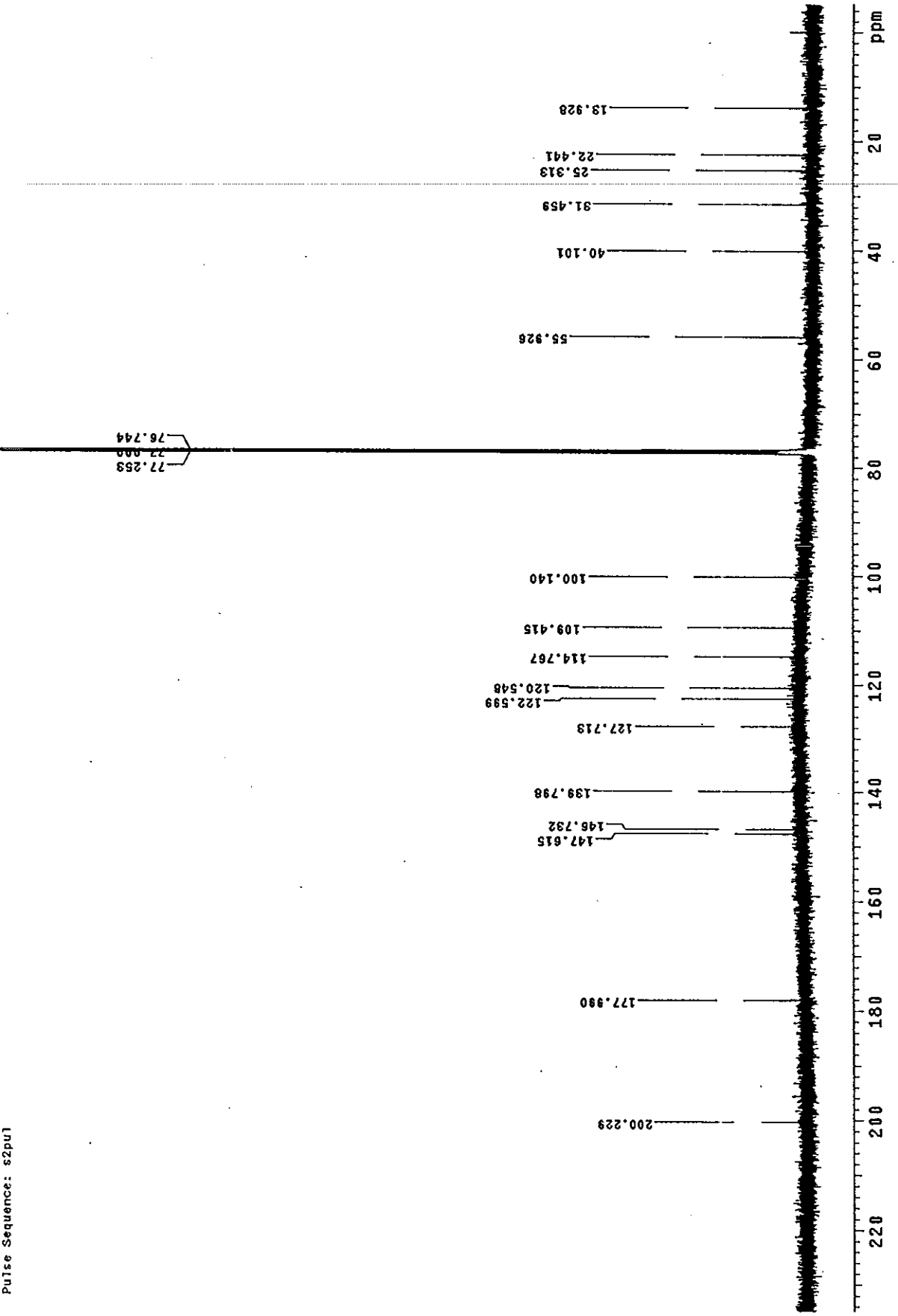

Figure 48 ^{13}C-NMR spectrum of ZOM1 (6-dehydrogingerdione)

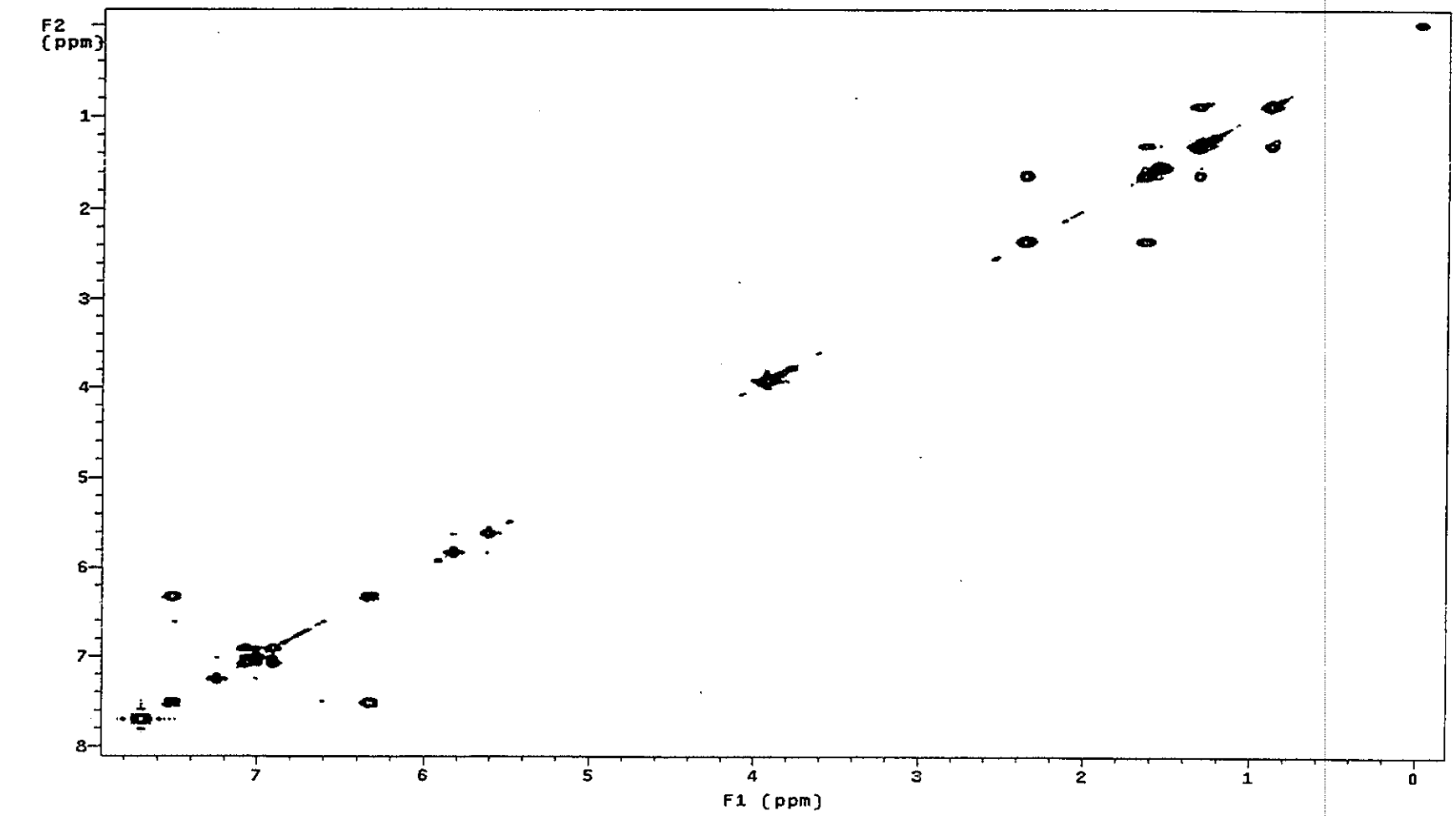

Figure 49 ^{1}H-^{1}H COSY spectrum of ZOM1 (6-dehydrogingerdione)

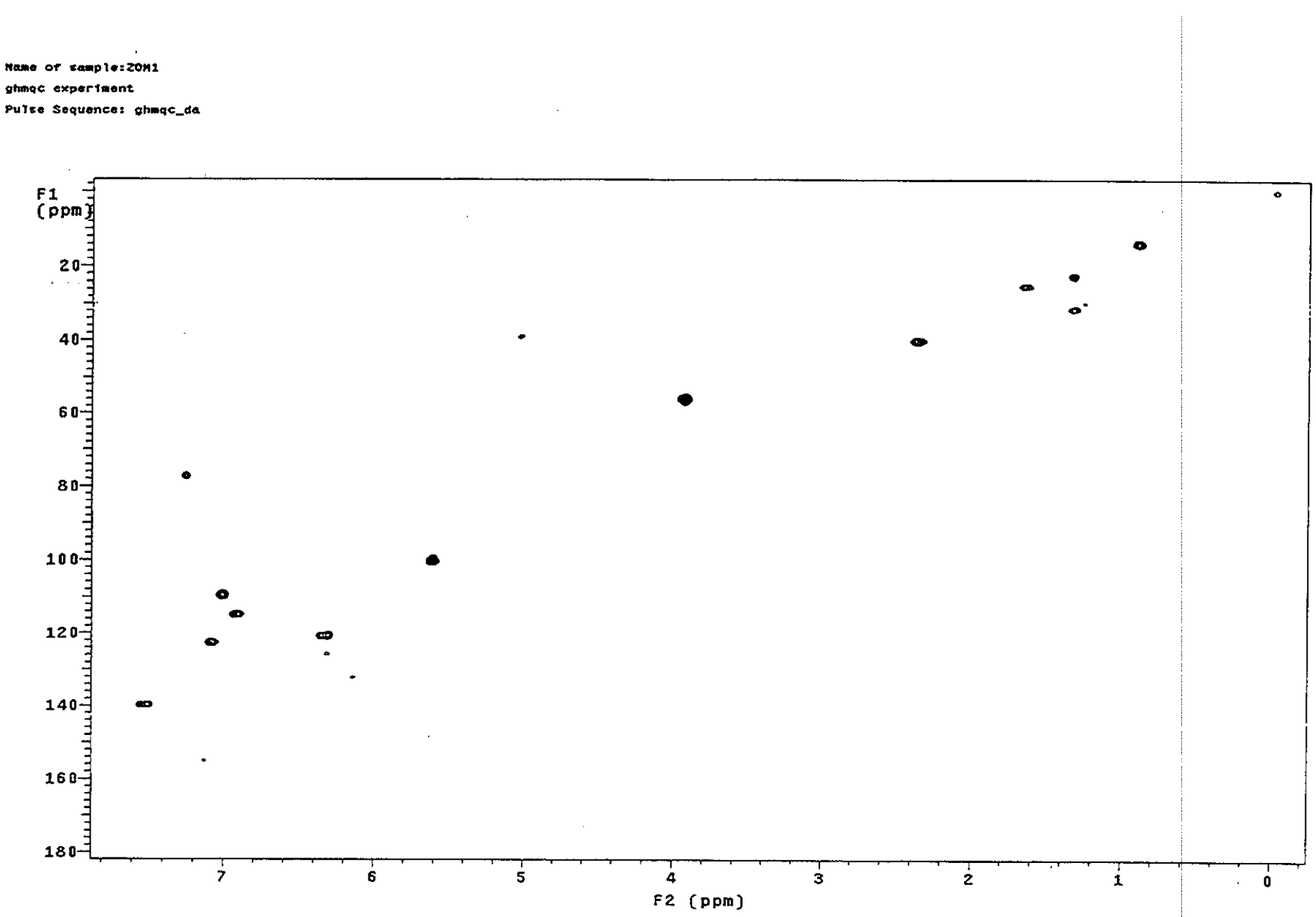

Figure 50 HMQC spectrum of ZOM1 (6-dehydrogingerdione)

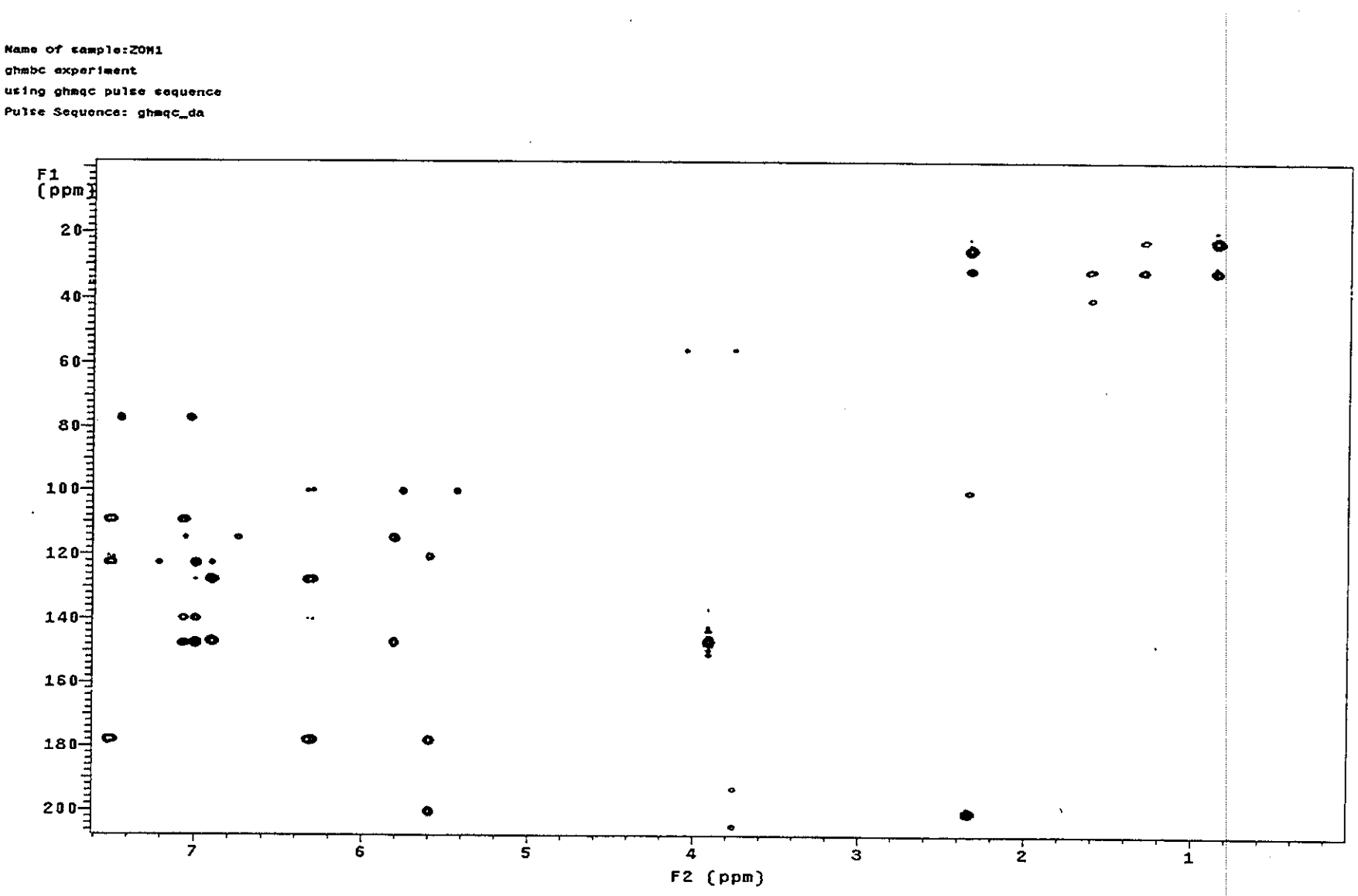

Figure 51 HMBC spectrum of ZOM1 (6-dehydrogingerdione)

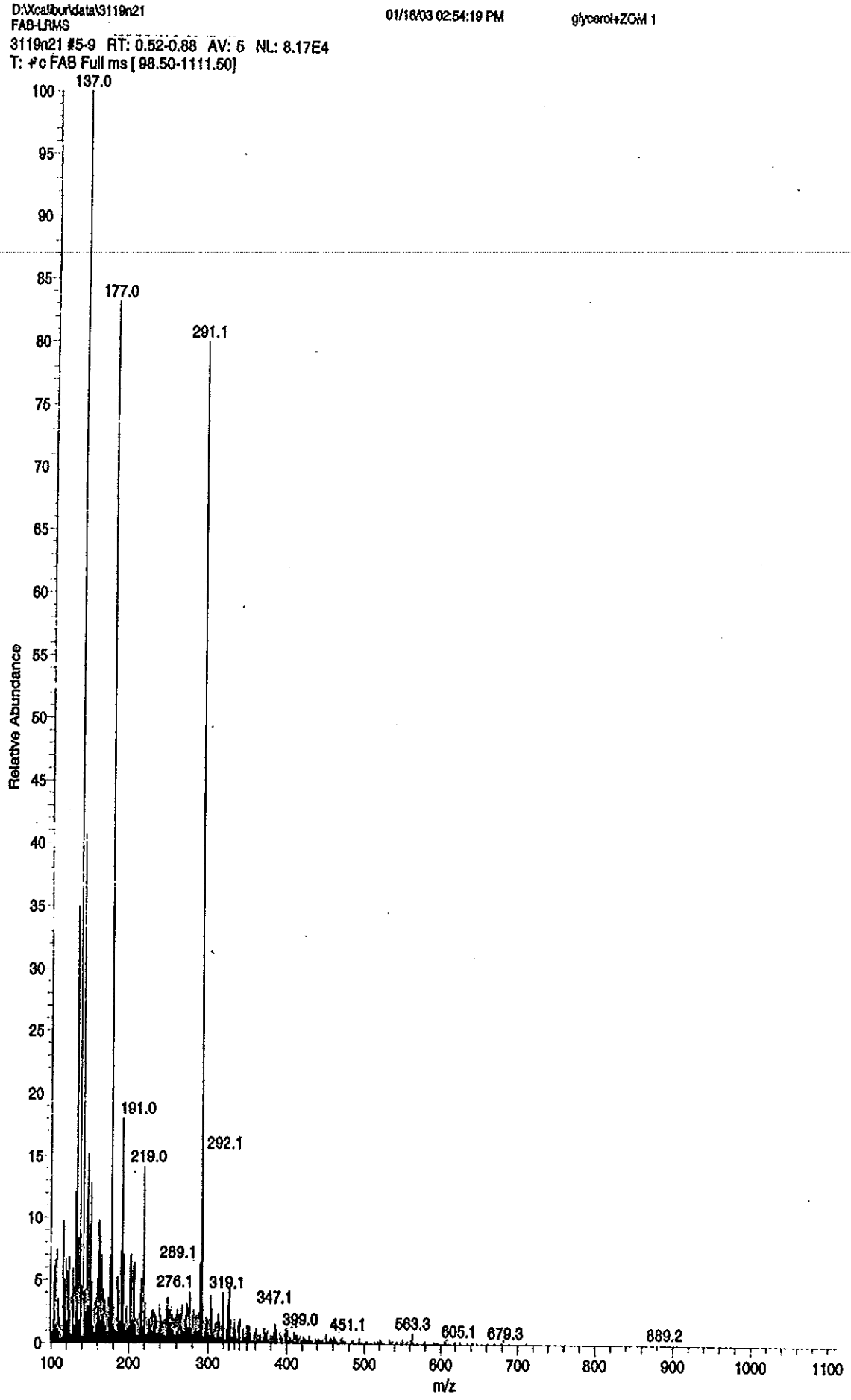

Figure 52 Mass spectrum (FAB) of ZOM1 (6-dehydrogingerdione)

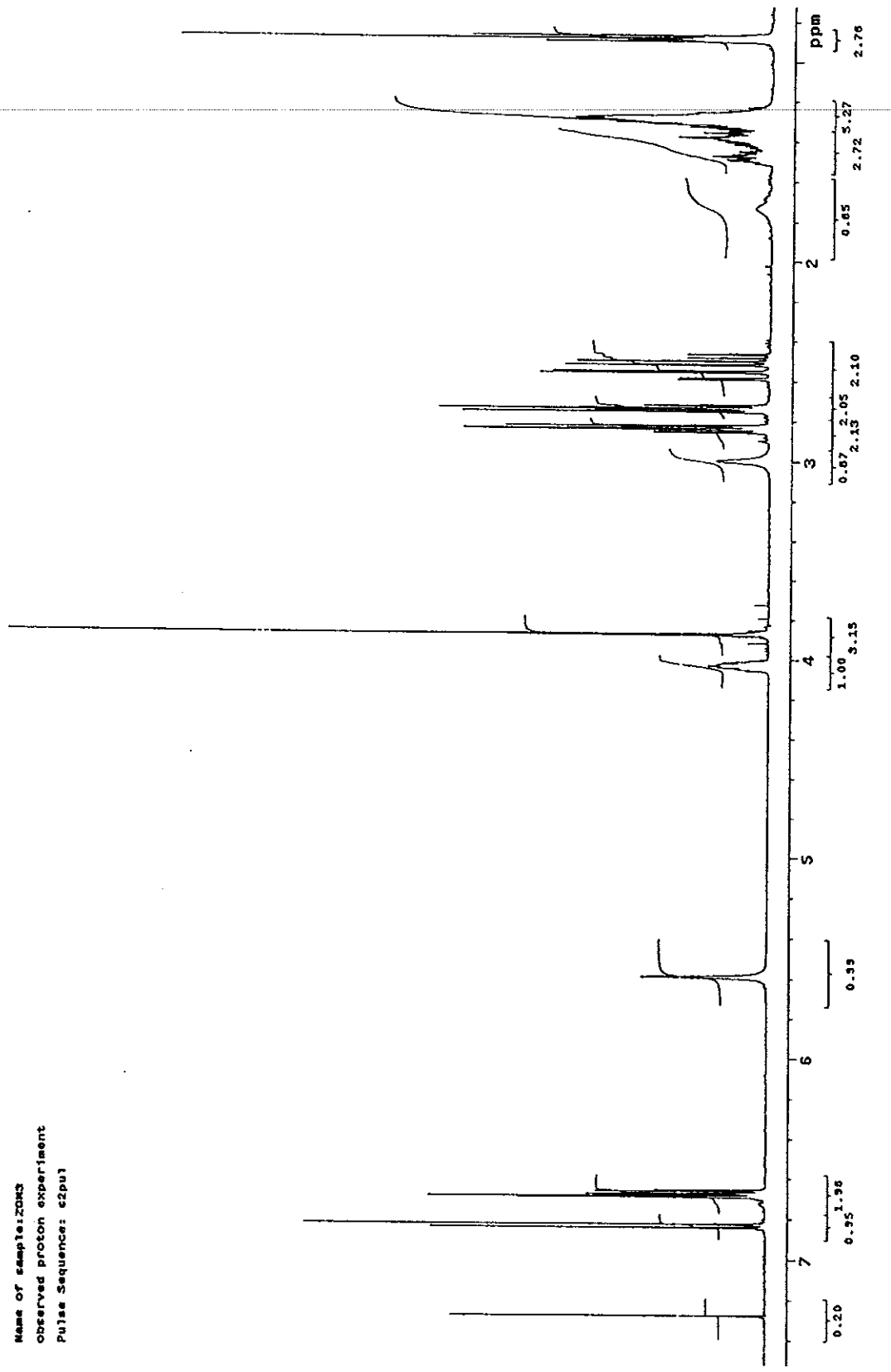

Figure 53 ^{1}H-NMR spectrum of ZOM3 (6-gingerol)

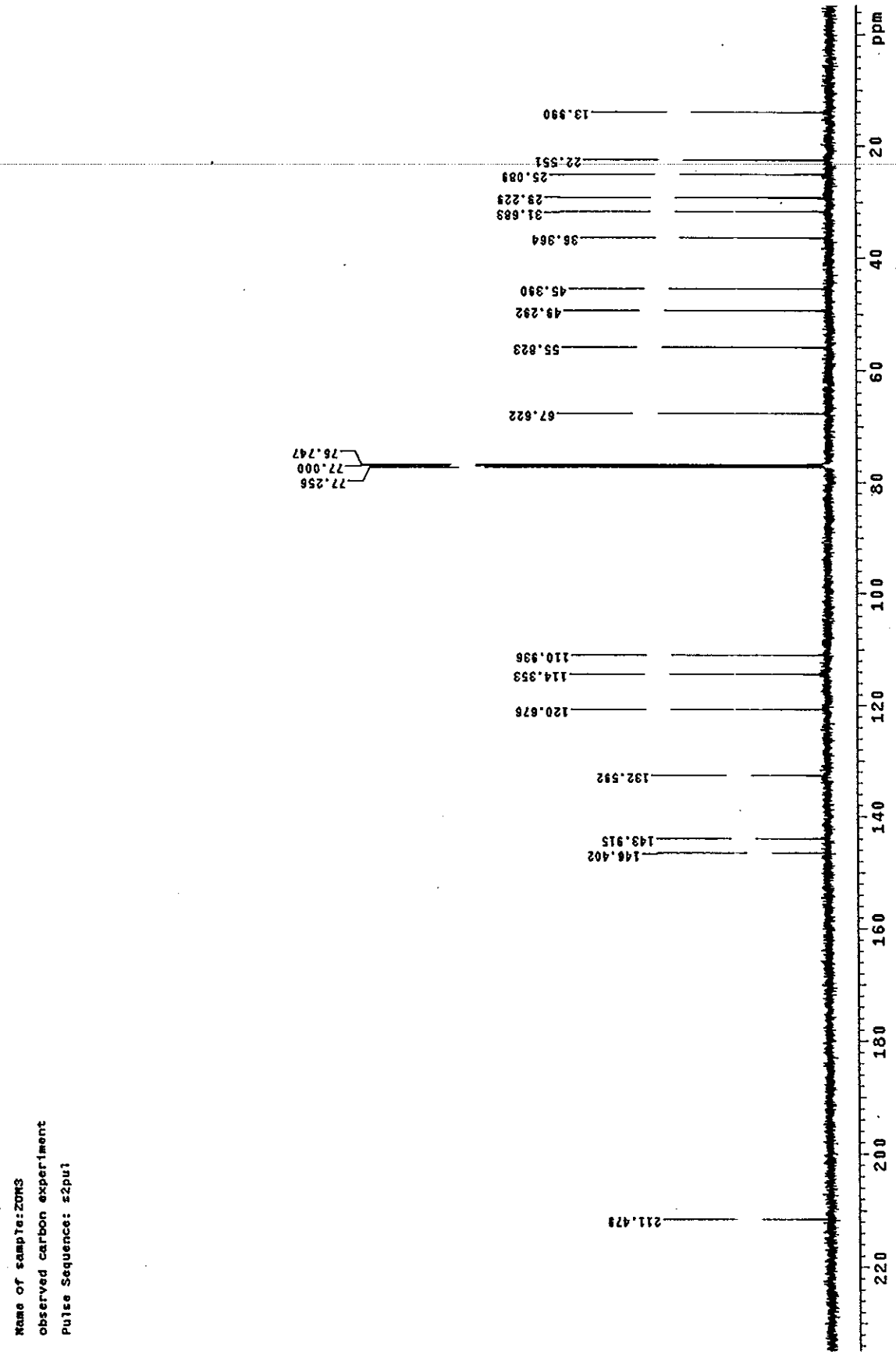

Figure 54 ^{13}C-NMR spectrum of ZOM3 (6-gingerol)

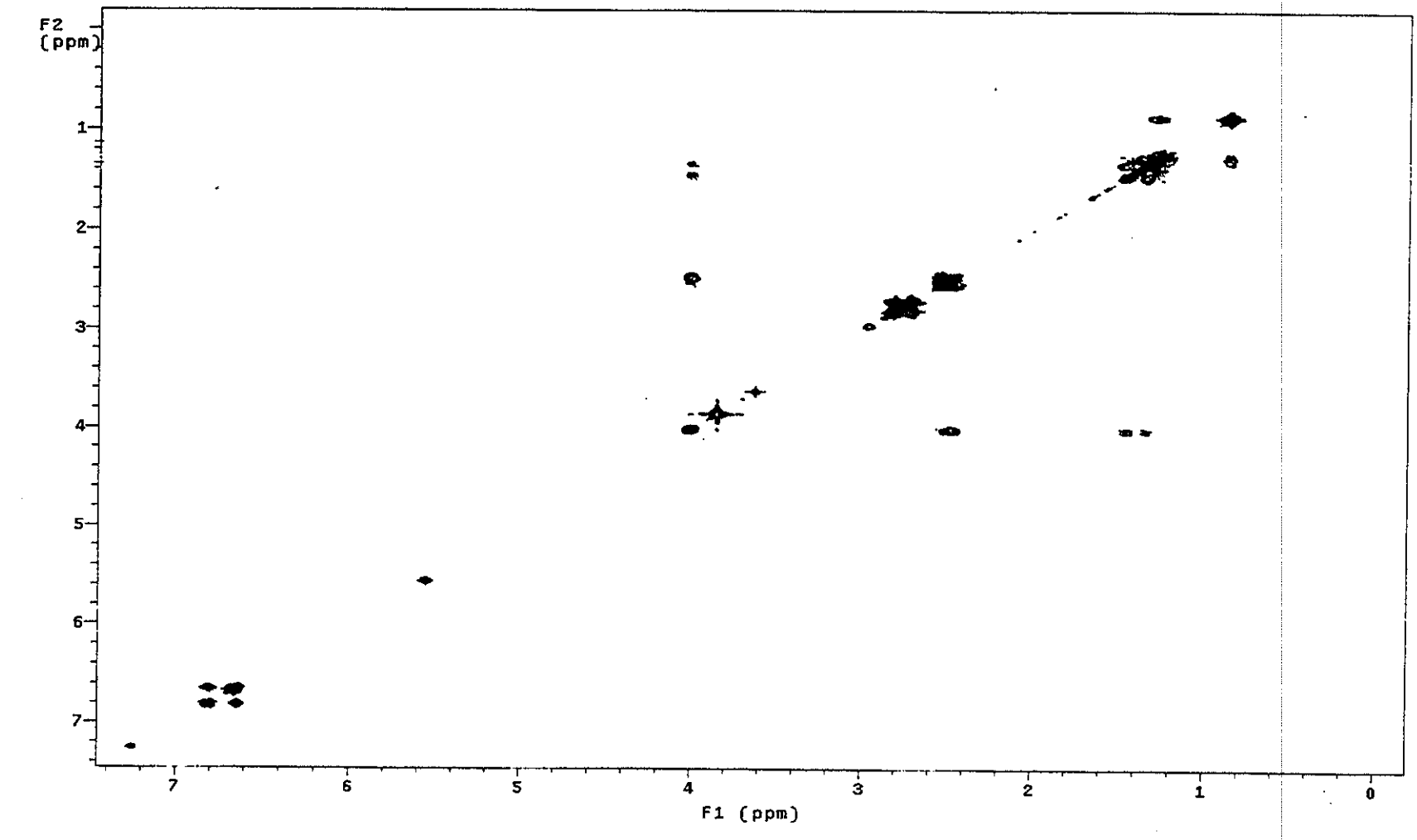

Figure 55 ^{1}H-^{1}H COSY spectrum of ZOM3 (6-gingerol)

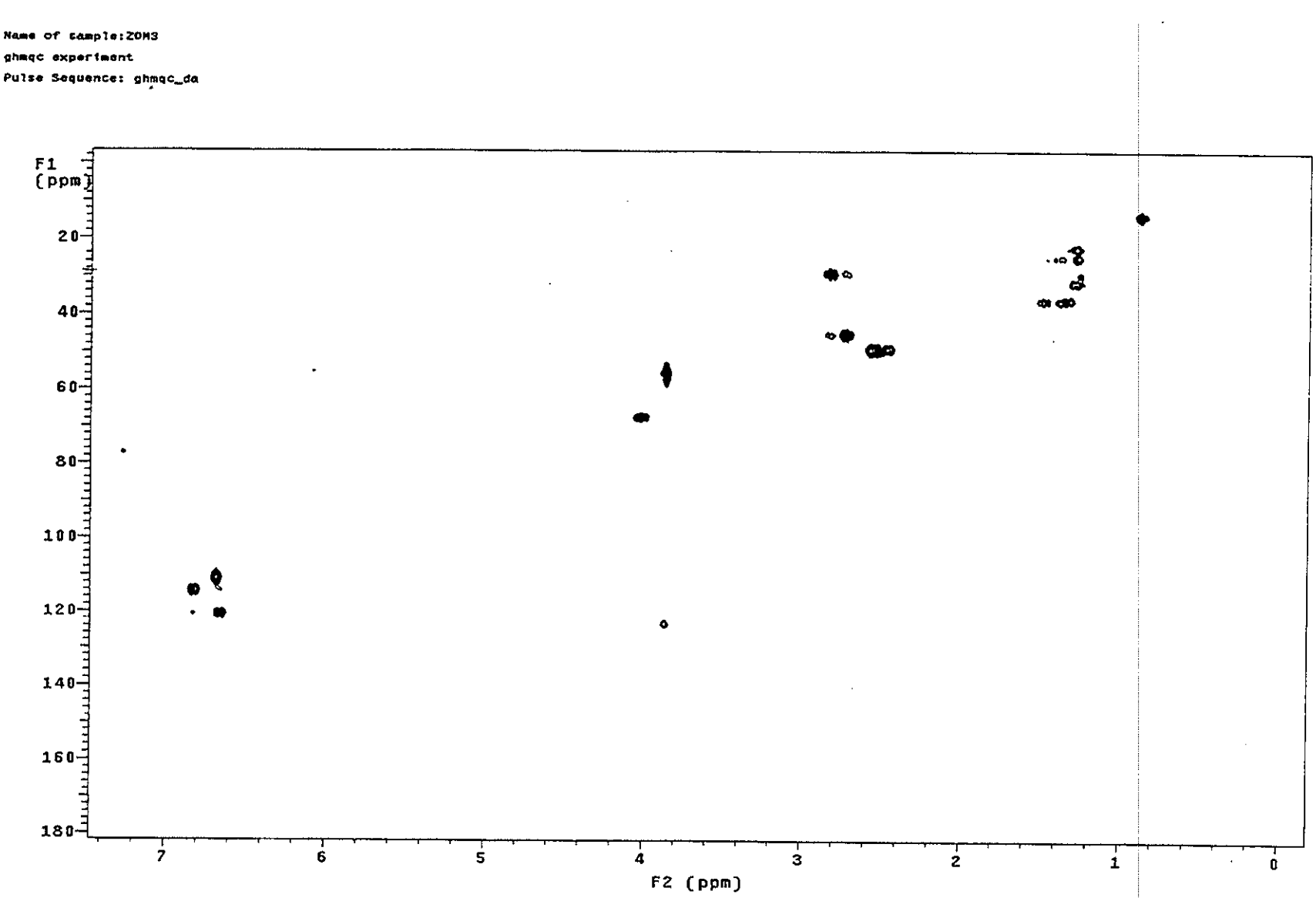

Figure 56 HMQC spectrum of ZOM3 (6-gingerol)

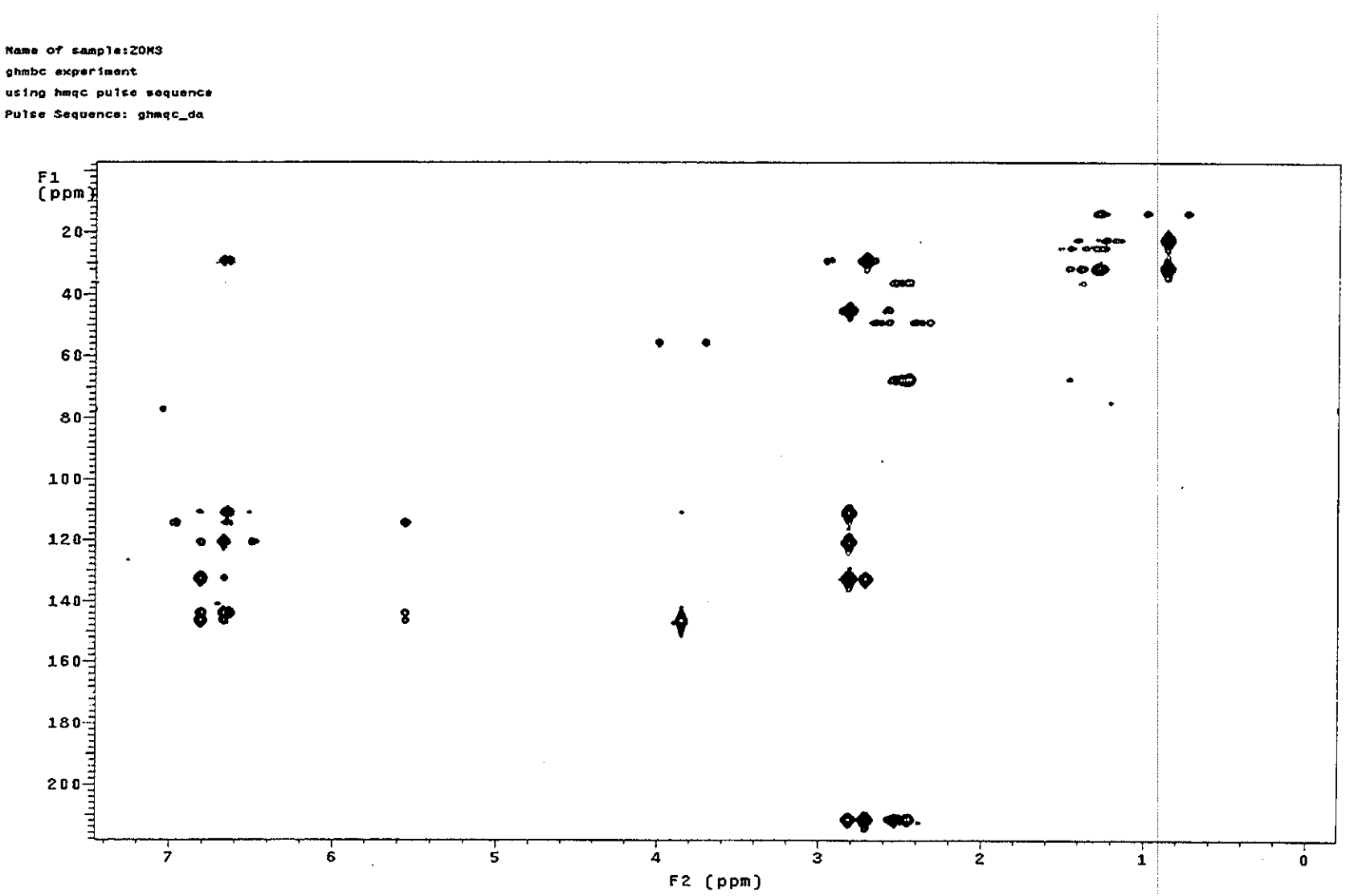

Figure 57 HMBC spectrum of ZOM3 (6-gingerol)

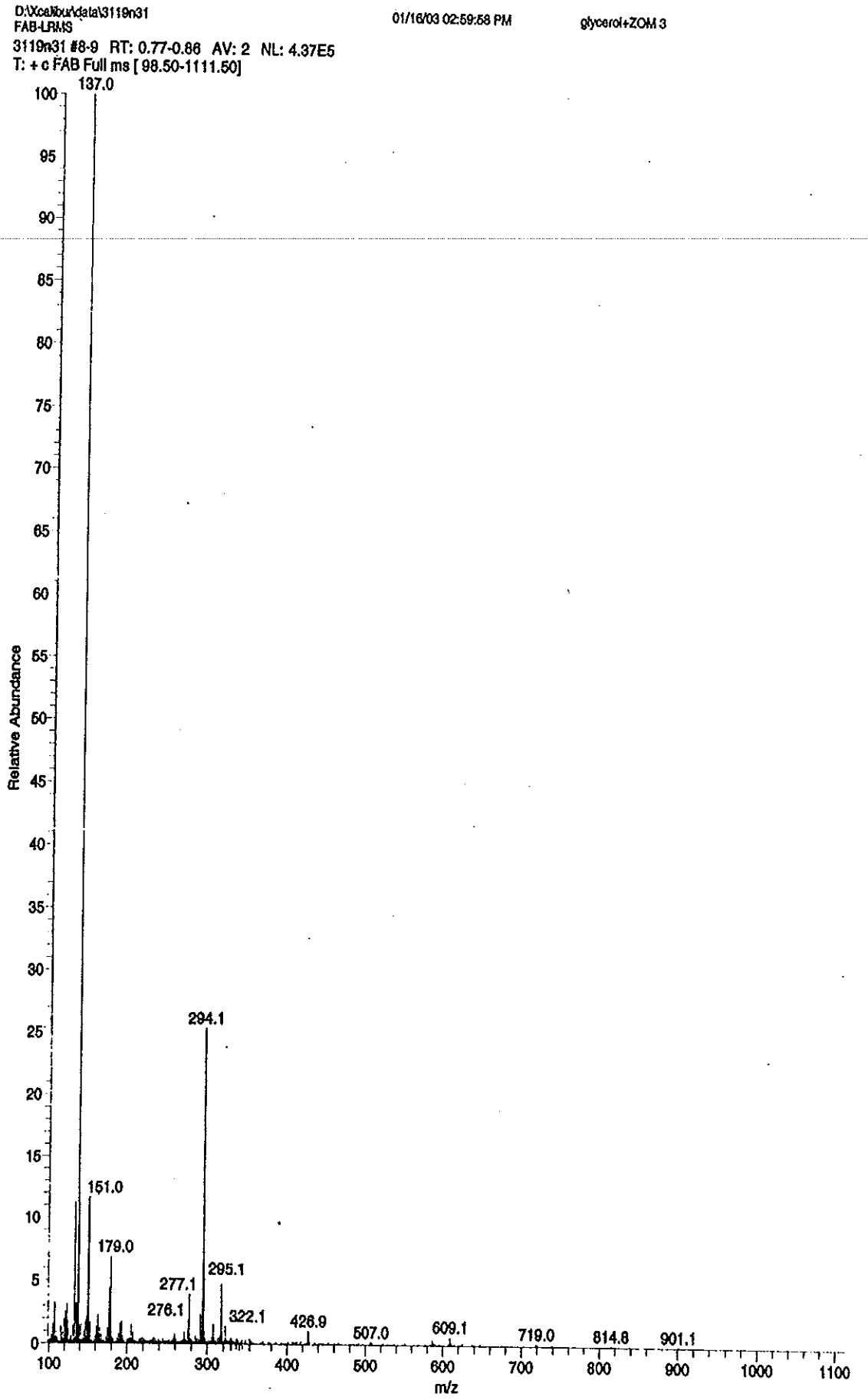

Figure 58 Mass spectrum (FAB) of ZOM3 (6-gingerol)

VITAE

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1. Shell Centennial Education Fund (The Shell Company of Thailand Limited) for students in Master's degree and Ph.D. programmes
2. Graduate School, Prince of Songkla University
3. Graduate Studies Related Research Fund (GSRRF) for the year 2002, Prince of Songkla University

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1. Poster presentation entitled Cytotoxic Activity Against Human Colon Tumour Cells of Turmeric (*Curcuma longa*) at The Fourth Regional IMT-GT UNINET Conference, Penang, Malaysia, 15-17 October 2002
2. Poster presentation entitled Chemical Constituents and Cytotoxic Activity Against Human Breast Tumour Cells of Volatile Oils from Zingiberaceous Rhizomes used as Spices at 28th Congress on Science and Technology of Thailand, Bangkok, Thailand, 24-26 October 2002
3. Oral presentation entitled Cytotoxic Activity Against Human Colon Tumour Cells of Ginger (*Zingiber officinale*) at The First PSU Symposium on Graduate Research, Prince of Songkla University, Songkhla, Thailand, 12 March 2003