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APPENDICES

Appendix I Preparation of Alkaline Copper Solution

Following to Oyama V.I. Eagle H.(304), Alkaline
Copper

Solution is the mixture of 2 kinds of solution.

Solution A is composed of

Na_2CO_3	200	g
NaOH	40	g
NaK-Tartrate	2	g
distilled water q.s. to	10	litre

Solution B is composed of

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	5	g
distilled water q.s. to	1	litre

Alkaline Copper Solution or Lowry's solution C is
freshly prepared by mixing 50 parts of solution A' to 1 part of
solution B.

Appendix II Preparation of Hanks' Balance Salt Solution (HBSS)

(327,328)

It is the admixture of two solutions.

Solution A

NaCl	80.0 g
CaCl ₂ .2H ₂ O	1.9 g
KCl	4.0 g
MgSO ₄ .7H ₂ O	2.0 g

dissolve these reagents in slightly less than 500 ml
distilled water, then q.s. to 500.0 ml

Solution B

Na ₂ HPO ₄ .12H ₂ O	1.5 g
KH ₂ PO ₄	0.6 g
Glucose	10.1 g
0.2% Phenol red	100.0 ml
distilled water q.s. to	500.0 ml

Solution B is added slowly to solution A with stirring
This stock solution is sterilized by filtration, or diluted
1:10 (Ca = 5 mg%, P = 2.4 mg%) and autoclaved at 10 Lbs pres-
sure for 10 minutes. Autoclaved 1.4% (isotonic) NaHCO₃ is
added to the diluted solution at the ratio of 2.5 ml:100 ml
and the solution stored in the refrigerator for CO₂ equili-
brium to pH 7.6 before final tightening of the cap of bottle.

Appendix III Preparation of Phosphate Buffer Saline (PBS)(329)

For preparation of this solution, all compounds in the
following order are dissolved in distilled water.

NaCl	8.0	g
KH ₂ PO ₄	0.2	g
Na ₂ HPO ₄	0.15	g
KCl	0.2	g
distilled water q.s. to	1000.0	ml

The solution was sterilized by autoclaving at 121°C, 15 lbs. for 15 minutes and stored at room temperature.

Appendix IV Preparation of Trypan Blue Dye

This dye solution is prepared following to Phillips H. J. (305). The 0.4% aqueous solution of trypan blue has a pH of about 6.5 and is hypotonic to animal cells. Therefore, stock solutions of the dye are made up as an isotonic salt solution buffered at pH 7.2-7.3 .

To about 95 ml distilled water in a 250 ml beaker are added 0.4 g trypan, 0.81 g sodium chloride, 0.06 g potassium phosphate monobasic, and 0.05 g methyl-p-hydroxybenzoate as a preservative. The solution is heated to boiling to dissolve all materials, then cooled, and pH is adjusted to 7.2-7.3 with 1.0 N sodium hydroxide (about 8 drops). The final volume is adjusted with distilled water to 100 ml. This solution is stable indefinitely at room temperature.

Appendix V Preparation of 0.25% trypsin in HEPES-buffered
Saline

This solution was prepared as described by Shipman C. et al (330). It is separated into 2 steps.

Step 1 Preparation of HEPES-buffered saline (HBS)(331)

Dissolved the reagents given below in 1,000 ml distilled water then adjust pH to 7.4 at 22°C with NaOH

NaCl	8.00 g
KCl	0.40 g
Na ₂ HPO ₄	0.10 g
Dextrose	1.00 g
HEPES	2.38 g

HEPES = 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic
Acid

Step 2 Preparation of 0.25% trypsin in HBS

2.5 g of trypsin (1:250)* is added to 1000 ml of HBS and stirred overnight at 4°C. On the following day, the solution is warmed to room temperature. The pH is adjusted to 7.4 (corresponding to approximately 7.2 at 37°C). The solution is then sterilized by membrane filtration.

* :One part of trypsin will convert 250 parts of casein to protease, peptones and amino acid under the conditions of the N.F. assay for pancreatin.

Appendix VI Measurement of cell growth in cell culture

Materials:

1. Earle's salt solution (332) or any similar balanced salt solution for washing the cell layer in the culture flask.

2. Alkaline copper solution (see appendix I) to dissolve the cell.

3. Folin-ciocalteau reagent (333). The reagent should be standardized by determining that concentration which gives the maximum and most stable color. This standardization need be carried out only once for each lot. Crystalline bovine albumin may be used as the reference standard. One part of a solution at 50 mcg/ml is added to 5 parts of solution C. Six ml are then distributed into each of 6 cuvettes, and a parallel series is prepared using H₂O instead of the protein solution. Dilutions of the F-C reagent are prepared as indicated below:

reagent	5	5	5	5	5	5
H ₂ O	4	5	6	7	8	9
Dilution	5/9	5/10	5/11	5/12	5/13	5/14

0.5 ml of each of these dilutions is rapidly blown into 1 cuvette containing the protein solution, and 1 control cuvette containing H₂O. The color is read after 15, 30, 60, 90, 120, 180, and 240 minutes, in each case against the corresponding water control. That dilution of the F-C reagent which reaches maximum color within approximately 1/2 hour, and remains sta-

ble for 2 hours thereafter, is the one used in the test.

Details of procedure:

Step 1. Washing of cultures. The culture flasks are drained of medium by inversion, and the adherent cell layer washed twice with PBS (334). For the culture flasks with a surface area of approximately 25 sq.cm, and 40 ml capacity, a 5 ml PBS is used. After washing the flasks are left inverted over clean gauze to drain for approximately 15 minutes, when clean stoppers are inserted. At this stage, the washed and dry cells may be stored in original culture flasks for several days with no change in the protein analysis.

Step 2. Solution of cells in alkaline copper tartrate. Five ml of Lowry's reagent C are added to the flasks with an automatic syringe and allowed to remain in contact with the cell layer for 10 minutes, after which the flasks are shaken with mixer (Thermo-Mixed Model TM 105, Thermonics CO., Ltd. Japan) to complete the dissolution of the cells. At this stage also, the flasks may be stored for several days with no loss in protein.

Step 3. Color development. A biuret color is produced by the interaction of the alkaline copper sulfate tartrate solution C with the protein, and the intensity of this color may serve as a rough measure of the amount of cells, and thus of the amount of the solution which must be used in the final test in order to produce a color within the optimum measurable

range (optical density= <0.4). When the color is not detectable, 2 ml is taken for analysis; when the color is pale violet, 0.5 ml will suffice, while with a deep violet color, the solution should be diluted with 1 or 2 volumes of solution c, and 0.5 ml taken for analysis. The appropriate amount of the cell solution is pipetted into a cuvette, and solution C added to a total of 5 ml. One ml of H₂O is then added and finally, 0.5 ml of the properly diluted F-C reagent is jetted in by an automatic pipette. The rapid admixture of the color reagent with the solution is essential, and the slow addition of the reagent without such rapid admixture leads to erroneous results. The control consist of 3 cuvettes, each containing 1 ml of crystalline bovine albumin at 50 mcg/ml instead of the H₂O used in the test proper. A water blank is also run. The volume of solutions in step 2 and 3 was summarized in Table 7. Absorption is read after 30 min at 660 nm; but the color remains stable for 2 hours.

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Table VII The volume of solutions in protein determination

Reagent(ml)	Blank	Protein control			Day 0			Day 4		
		1	2	3	1	2	3	1	2	3
Cell solution	-	-	-	-	1	1	1	1	1	1
BSA std.(50 mcg/ml)	-	1	1	1	-	-	-	-	-	-
Solution C	5	5	5	5	4	4	4	4	4	4
Distilled water	1	-	-	-	1	1	1	1	1	1
Folin:H ₂ O = 5:9 "rapidly mixed"	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Size of cell inoculum on day 0 about 4×10^4 cells/ml

Absorption wavelength at 660nm, Slit=1

Read after 30 min, Determine within 2 hrs.

Calculation of results

Results of the test are most simply expressed in terms of the bovine albumin equivalent of the unknown cell culture. However, the results, referred to a bovine serum albumin standard, may be converted to dry weight-nitrogen or cell count by appropriate conversion factors.

APPENDIX VII Antineoplastic sesquiterpene and related lactones

This table shows sesquiterpene and related lactones from plants with activity in various tumor systems, reviewed by Hartwell, J. L. and Abbot, B. J. in 1969 (236).

Compound name	Tumor system*	Evaluation [#]		
		TWI(%)	ILS(%)	ED ₅₀
Santonin	SA	18		140
	CA	~25		
	LE		0	
	KB			
Gibberellin	SA	~25		
Isocolumbin	SA	0		
	CA	21		
	LE		7	
Gibberellic acid	LL	0		>100
	WM	0		
	LE		0	
	KB			

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation#		
		TWI(%)	ILS(%)	ED ₅₀
Tenulin	SA	17		
	CA	8		
	WM	0		
	LE		1	
Pyrethrosin	SA	6		
	CA	0		
	WA	28		
	LE		15	
	DL		0	
Lactone from <i>Arctium minus</i>	CA	0		
	LE	0		
Lactone from <i>Centaurea maculosa</i>	SA	0		
	CA	22		
	LE		0	
Pulvinic acid di- γ - lactone (pulvic anhydride)	SA	2		
	LE		20	

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation†		
		TWI(%)	ILS(%)	ED ₅₀
I- β -Desmotroposantonin	SA	13	0	>100
	LE			
	KB			
I- β -Desmotroposantonin acetate	SA	15	0	
	CA	10		
	LE			
I- α -Desmotroposantonin	SA	0	0	
	CA	2		
	LE			
β -Santonin	SA	25	0	>100
	CA	43		
	LE			
	KB			
Endesma-3,5,7(11)-trien- 6,13-olide, 3-hydroxy, acetate	SA	0	0	
	CA	0		
	LE			

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation†		
		TWI(%)	ILS(%)	ED ₅₀
Santonin oxime	SA	9		
	CA	25		
	LE		0	
Pinnatifidin	SA	10		
	LL	9		
	8P	13		
	LE		4	
	DA		0	
	KB			1.7
Ambrosin	SA	13		
	8P	7		
	LE		0	
	DA		0	
	KB			0.04

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation†		
		TWI(%)	ILS(%)	ED ₅₀
Helenalin	SA	0		0.22
	LL	33		
	8P	23		
	HI	0		
	WM	0		
	LE		4	
	DA		0	
	KB			
Ivalin	SA	20		0.72
	8P	0		
	LE		10	
	DA		0	
	KB			
Asperilin	8P	12		1
	LE		20	
	DA		4	
	KB			

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation#		
		TWI(%)	ILS(%)	ED ₅₀
Parthenin	SA	0		0.025
	LL	21		
	8P	26		
	WM	0		
	KB			
Neotenuclin, deacetyl-	8P	0		100
	LE		17	
	DA		0	
	KB			
Ivasperin	SA	30		1.6
	8P	36		
	LE		22	
	DA		0	
	H1	35		
	KB			

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation [†]		
		TWI(%)	ILS(%)	ED ₅₀
Coronopilin	SA	17		1.4
	8P	0		
	LE		6	
	DA		0	
	KB			
Pseudoivalin	SA	17		1.8
	LL	24		
	8P		2	
	DA		0	
	KB			
Pulehellin	SA	5		1.8
	8P	0		
	LE		18	
	DA		0	
	KB			
Microcephalin	8P	0		22
	LE		3	
	DA		13	
	KB			

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation [#]		
		TWI(%)	ILS(%)	ED ₅₀
Gaillardilin	8H1	0		2.2
	KB			
Isotenulin	8P	36		16
	LE		0	
	DA		0	
	KB			
Flexuosin B	8P	0		26
	KB			
Damsin	KB			0.58
Spathulin	KB			4.5
Alantolactone	SA	28		1.4
	LL	29		
	WM	0		
	LE		0	
	KB			

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation†		
		TWI(%)	ILS(%)	ED ₅₀
Pulchellin B	KB			7
Pulchellin C	KB			>100
Pulchellin E	KB			-1.0
Fastigilin B	KB			1.9
Fastigilin c	KB			0.34
Mikanolide	WM	55 ^a		
	LE		12	
	KB			<1
Elephantopin	SA	0		
	LL	0		
	8P	6		

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation [#]		
		TWI(%)	ILS(%)	ED ₅₀
Elephantopin	WM	78 ^a		0.32
	LE		28	
	PS		71	
	DA		8	
	KB			
Elephantin	WM	70		1.6
	KB			
Euparotin	KB			0.21
Euparotin acetate	WM	77 ^b		0.22
	PS		18	
	KB			
Psilostachyin A	WM	0		5.4
	KB			
Psilostachyin B	WM	11		
Psilostachyin C	WM	25		

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation*		
		TWI(%)	ILS(%)	ED ₅₀
Gaillardin	WM	9	0	0.8
	LE			
	KB			
Isogaillardin	KB			1.6
Vernolepin methanol adduct	WM	7	40	25
	PS			
	KB			
Vernolepin	WM	70		2.0
	KB			
Costunolide	WM	11	0	0.26
	LE			
	KB			
Tulipinolide	WM	20	2	0.52
	LE			
	KB			

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation [#]		
		TWI(%)	ILS(%)	ED ₅₀
Fomannosin	WM	35		
	LE		5	
Laserolide	WM	43		
Scabiolide	WM	0		
Pulchellin C epoxide	WM	49		
Gingko lactone	KB			>100
Plumericine	LE		4	
Isoplumericine	LE		6	
Dihydroparthenolide	KB			>100
Vernomenin	WM	56 ^d		
	KB			20

APPENDIX VII Antineoplastic sesquiterpene and related lactones
(continued.)

Compound name	Tumor system*	Evaluation [#]		
		TWI(%)	ILS(%)	ED ₅₀
Picrotoxin	SA	43		
	CA	0		
	LE		0	
	KB			40

^a Results erratic.

^b Results not reproducible.

^c Single injection procedure only.

^d Erratic toxicity.

*Tumor System. The host-tumor assays used for screening are coded as follows:

A3. Lieberman plasina cell No.1. Mouse

CA. Adenocarcinoma 755. Mouse

DA. Dunning leukemia (ascites). Rat

DL. Dunning leukemia(solid).Rat

EA. Ehrlich ascites.Mouse

FV. Friend virus leukemia. Mouse

- 5H1. HS1 human sarcoma. Rat
- 8H1. HS1 human sarcoma. Egg
- HE. Hepatomla 129. Mouse
- KB. Human epidermoid carcinoma of the nasopharynx. Cell culture
- 3L8. L-5178Y lymphatic leukemia. Mouse
- LE. Leukemia L-1210. Mouse(intraperitoneal)
- LL. Lewis Lung carcinoma. Mouse
- LZ. Leukemia L-1210. Mouse(subcutaneous). Delayed treatment
- MM. Melanotic melanoma. Hamster
- MS. Murphy Sturm lymphosarcoma. Rat
- OS. Osteogenic sarcoma He 10734. Mouse
- P1. Plasmacytoma No 1. Hamster
- P4. P-1534 Leukemia. Mouse
- 8P. P-1798 lymphosarcoma. Mouse
- PS. P-388 lymphocytic leukemia. Mouse
- SA. Sarcoma 180. Mouse
- WA. Walker carcinosarcoma 256. Rat (subcutaneous)
- WI. Walker carcinosarcoma 256 ascites. Rat
- WM. Walker carcinosarcoma 256. Rat (intramuscular)
91. S-91 Cloudman melanoma. Mouse


* Evaluation criteria of each tumor system are as follows:

TWI. Tumor weight inhibition. A reproducible TWI in drug treated animals vs untreated control animals => 58% is considered significant.

ILS. Increase in life span. A reproducible ILS => 25% for teated vs control animals is considered significant.

ED₅₀. Dose level in mcg/ml at 50% inhibition of growth of cells (*in vitro*) is noted vs untreated controls.

A value ≤ 1.0 is considered significant.

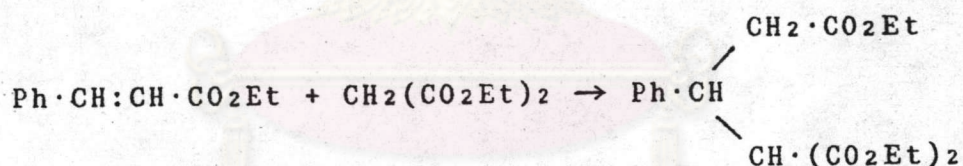


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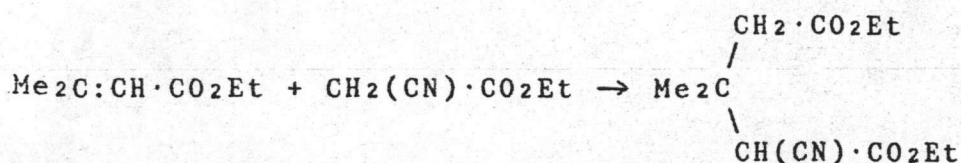
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- WM. Walker carcinosarcoma 256. Rat (intramuscular)
91. S-91 Cloudman melanoma. Mouse

APPENDIX IIX Micheal Reaction.

The Michael reaction, essentially the base-catalysed addition of a pseudo-acidic ketone, ester, nitrile, or nitro-compound, to the $\alpha\beta$ -double bond of a conjugated unsaturated ketone, ester, or nitrile, stands in the same relation to the above-mentioned additions of hydrogen cyanide to an $\alpha\beta$ -double bond, as does the aldol addition reaction to ordinary cyanohydrin formation. Michael's original example, discovered in 1887, was the addition of ethyl malonate to ethyl cinnamate, under the influence of sodium ethoxide in ethyl alcohol, to give an addition product, which could be hydrolysed, and decarboxylated, to β -phenylglutaric acid:



Another example, one which found distinguished employment in the course of the work of W.H. Perkin Jr. on the synthesis of deg2adat)gN prndubts gf camphor, is the addition of ethyl malonate or of ethyl cyanoacetate to ethyl $\beta\beta$ -dimethylacrylate, to form an ester, which can be hydrolysed, with loss of a carboxyl group, to $\beta\beta$ -dimethylglutaric acid:



The direction of these additions is always as illustrated, the anionic part of the pseudo-acidic addendum going to the β end of the $\alpha\beta$ -double bond.

There are three standard ways of carrying out a Michael reaction. Michael's original method employed one molecular proportion of sodium ethoxide. Then there is the so-called "catalytic" method, in which are uses a much smaller amount of sodium ethoxide. And then there is Knoevenagel's method, in which the catalyst is, not sodium ethoxide, but some secondary amine, such as piperidine. Reaction by the second and third of these methods is slower than by the first method.

The reversibility of the Michael reaction was first noticed by Vorlander, it was studied further by Powell, Perren, and the writer. Most Michael additions are exothermic, and so a larger yield of addition product results at lower temperatures, provided that one can wait the extra time that the reaction then requires. In his original experiments with ethyl cinnamated and ethyl malonate, Michael records a high yield of addition product obtained by reaction at room temperature, and a poor yield obtained by reaction at the

boiling point of the alcoholic solution. Equilibrium also depends on the experimental method, in a way that can be understood if one assumes, as seems probable, that the adding pseudo-acid is a stronger acid than the pseudoacidic adduct, or, in other words, that the former has the more stable anion. In a reaction conducted in the presence of one equivalent of sodium ethoxide, the equilibrium which determines the eventual composition of the bulk of the material is not between the three esters, in examples such as those formulated above, but between the unsaturated ester and the anions of the other two esters; and so, the stability difference just mentioned between the anions is thermodynamically inhibitory to addition. Suppose now that we change the method, employing only "catalytic" amounts of sodium ethoxide, or alternatively, using the Knoevenagel method; then the composition-determining equilibrium will really be between the three esters, and the thermodynamically inhibitory factor will have been removed; and therefore, provided that equilibrium is attained, we shall get a better yield of addition product.

As to the effect of structure on equilibrium, certain relations are fairly well established. Alkyl groups, and still more aryl groups, in either the α - or the β -position of the unsaturated ester are thermodynamically inhibitory towards addition. Presumably this is because they

hyperconjugate or conjugate with the double bond, thereby tending towards its preservation. A primary steric effect may contribute to the result. Preparative yields of Michael addenda with ethyl malonate or ethyl cyanoacetate may be obtained, when one conjugated phenyl group is, or two hyperconjugated methyl groups are, present in the unsaturated ester as illustrated for cinnamic and $\beta\beta$ -dimethylacrylic esters but not when two such phenyl groups, or three methyl groups, are present. Cyanoacetic esters give generally higher equilibrium yields of addition products than do malonic esters: why this is so, is not known definitely.

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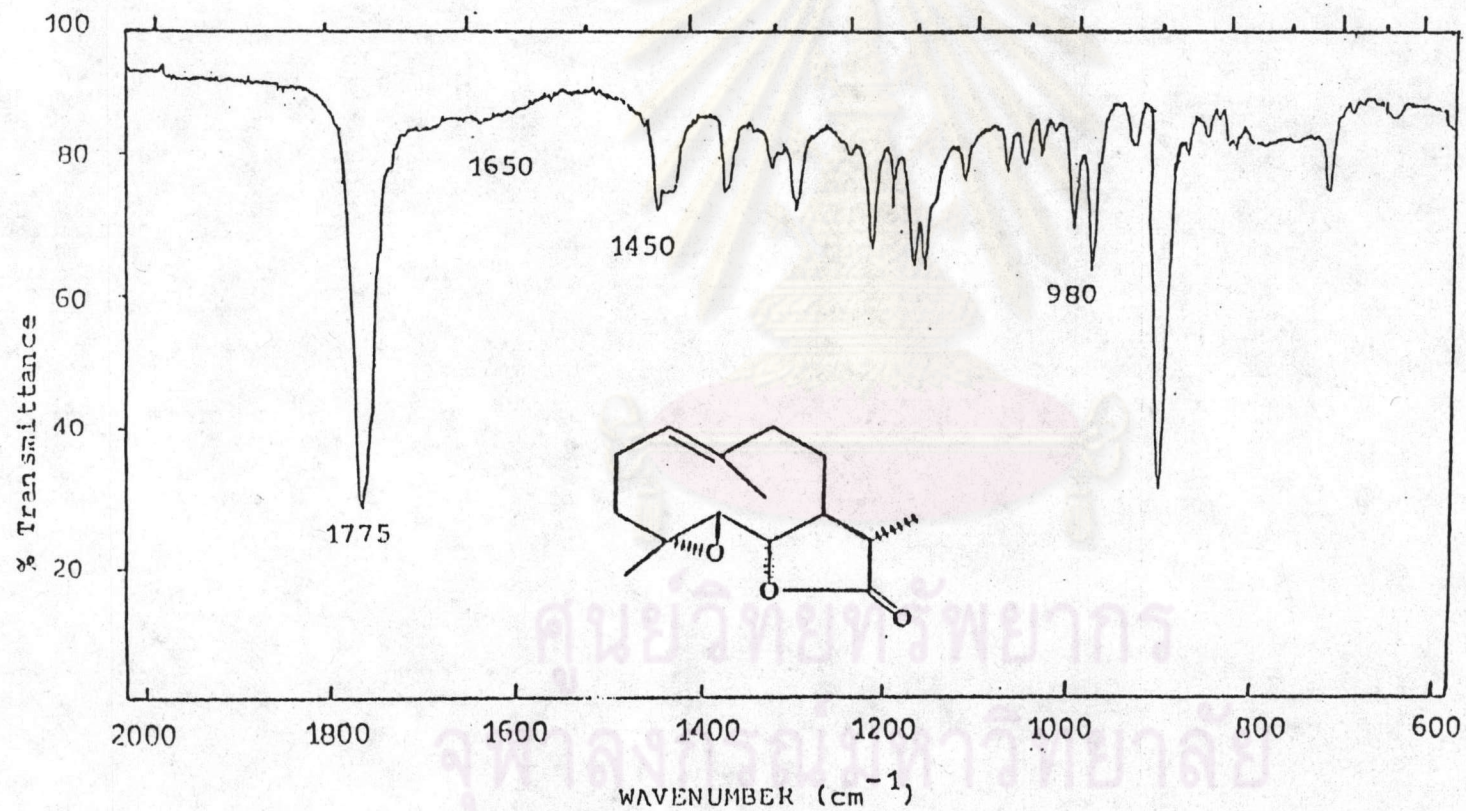


Figure 4 Infrared absorption of PB-1 in CCl₄

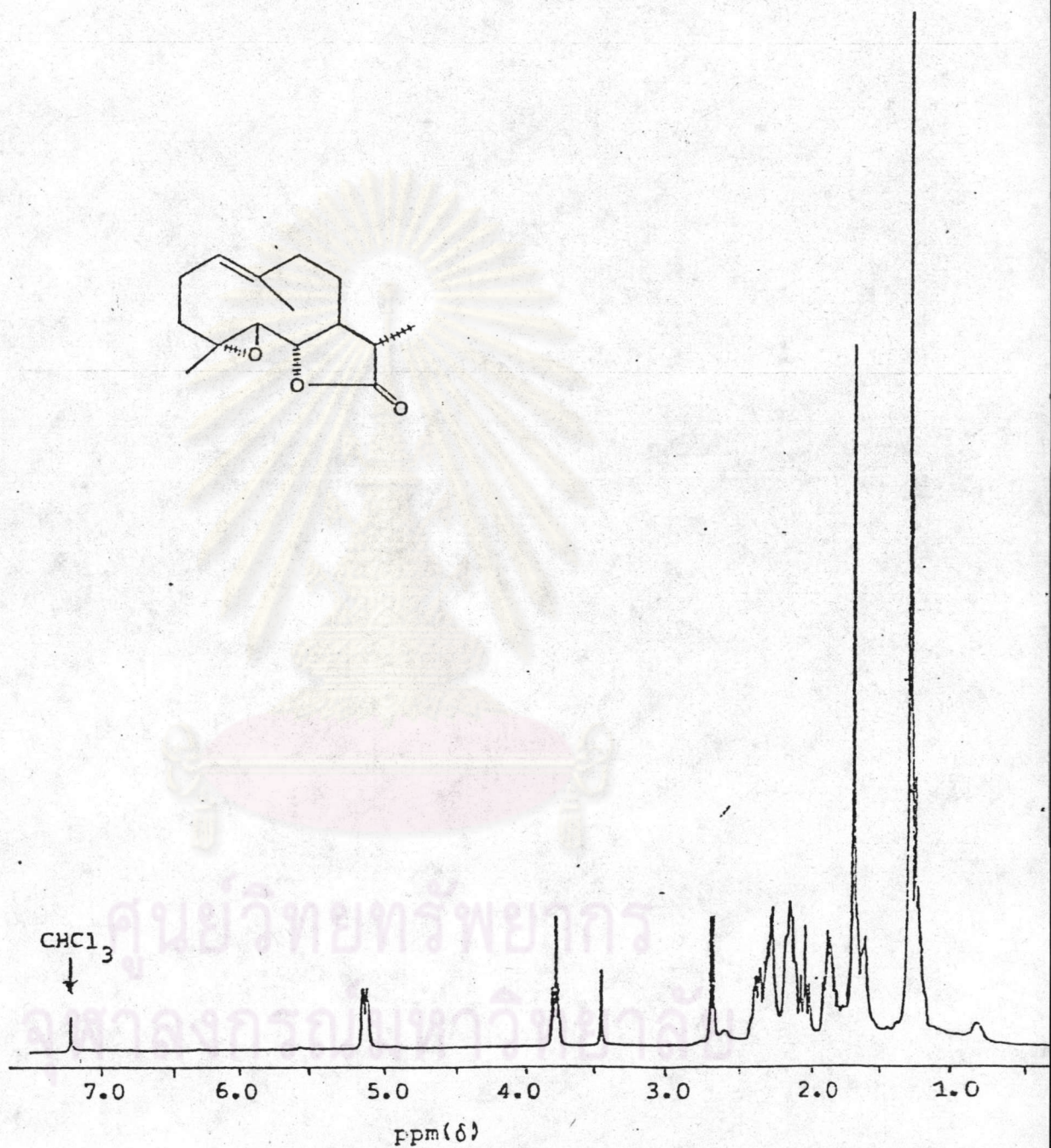


Figure 5 ^1H -nuclear magnetic resonance spectrum (400 MHz) of PB-1 in CDCl_3

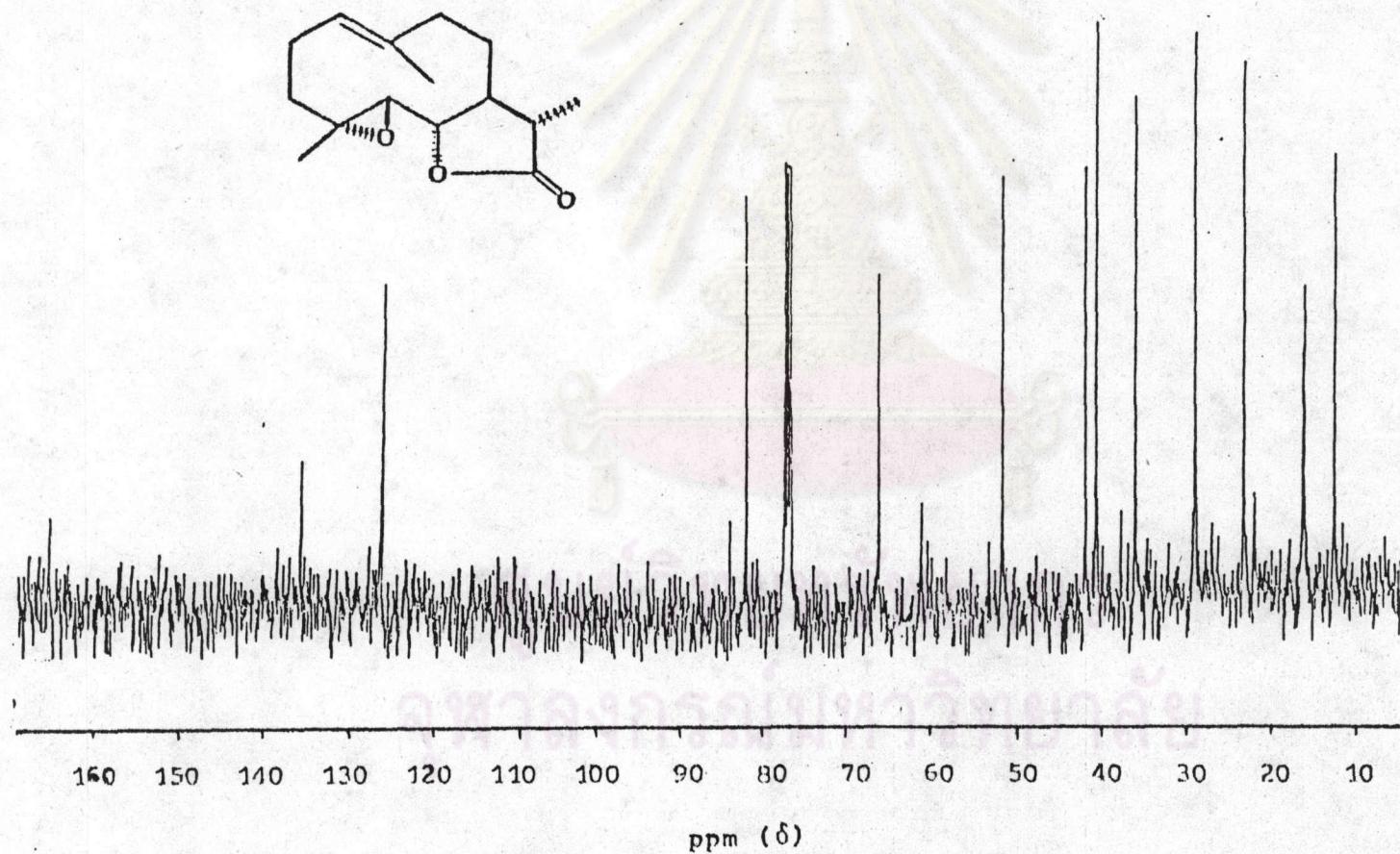


Figure 6 ^{13}C -nuclear magnetic resonance spectrum
(100 MHz) of PB-1 in CDCl_3

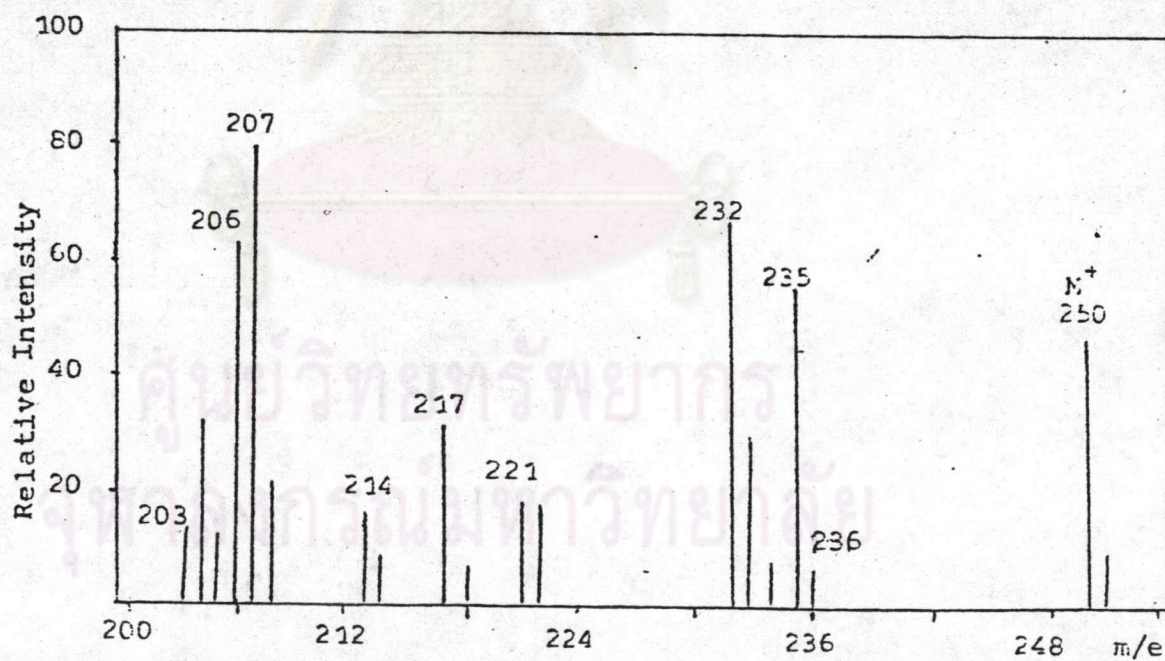
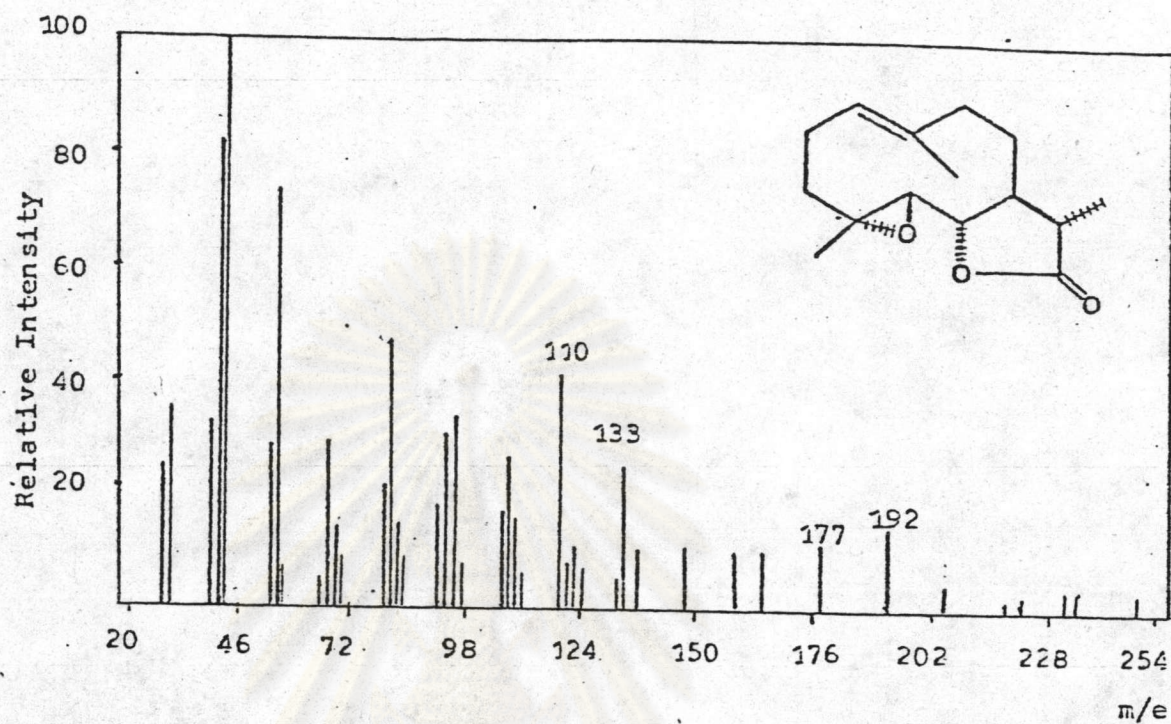


Figure 7 Electron impact mass spectrum of PB-1

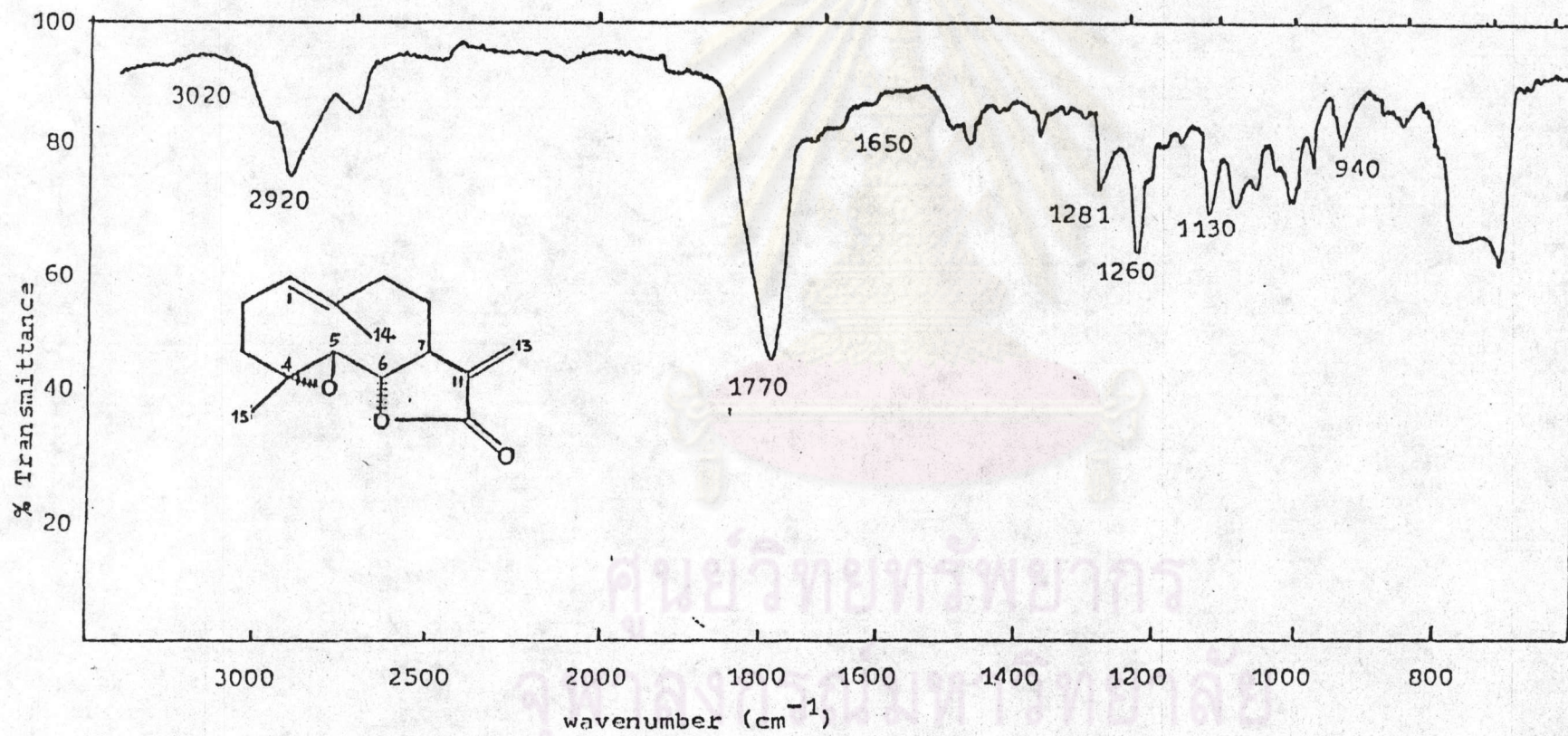


Figure 8 Infrared absorption of PB-2 in CCl₄

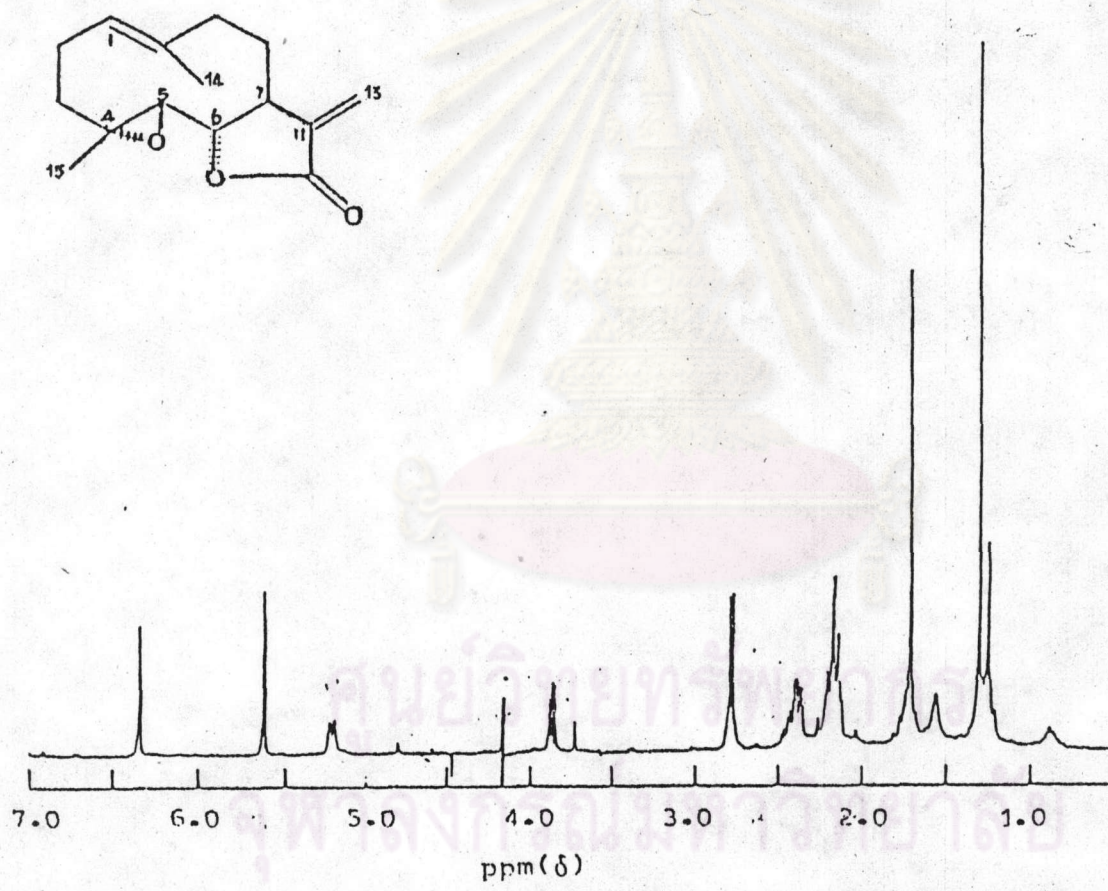


Figure 9 ¹H-nuclear magnetic resonance spectrum (400 MHz) of PB-2 in CDCl₃

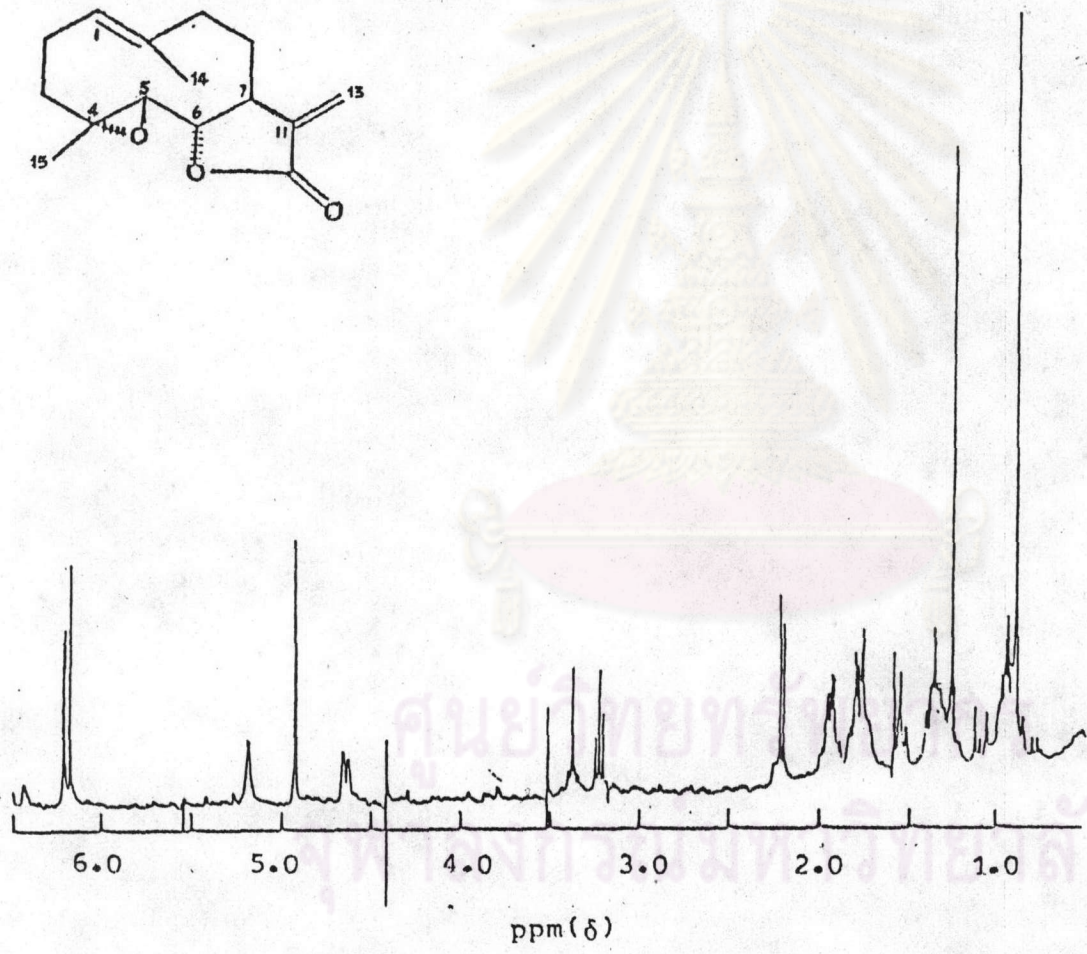


Figure 10 ^1H -nuclear magnetic resonance spectrum (400 MHz) of PB-2 in C_6D_6

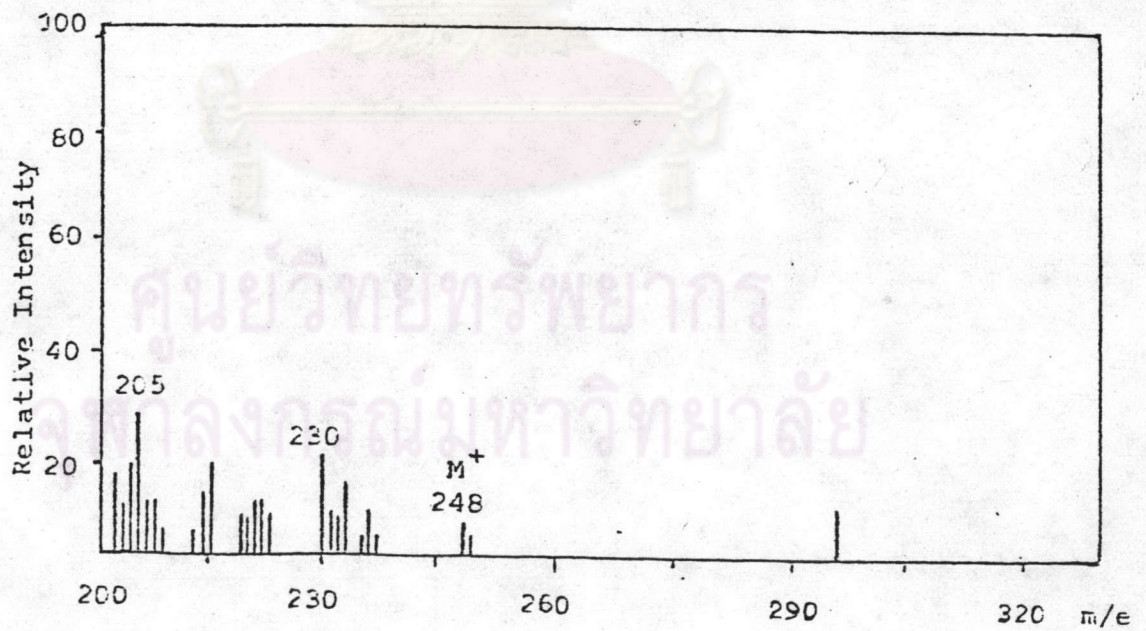
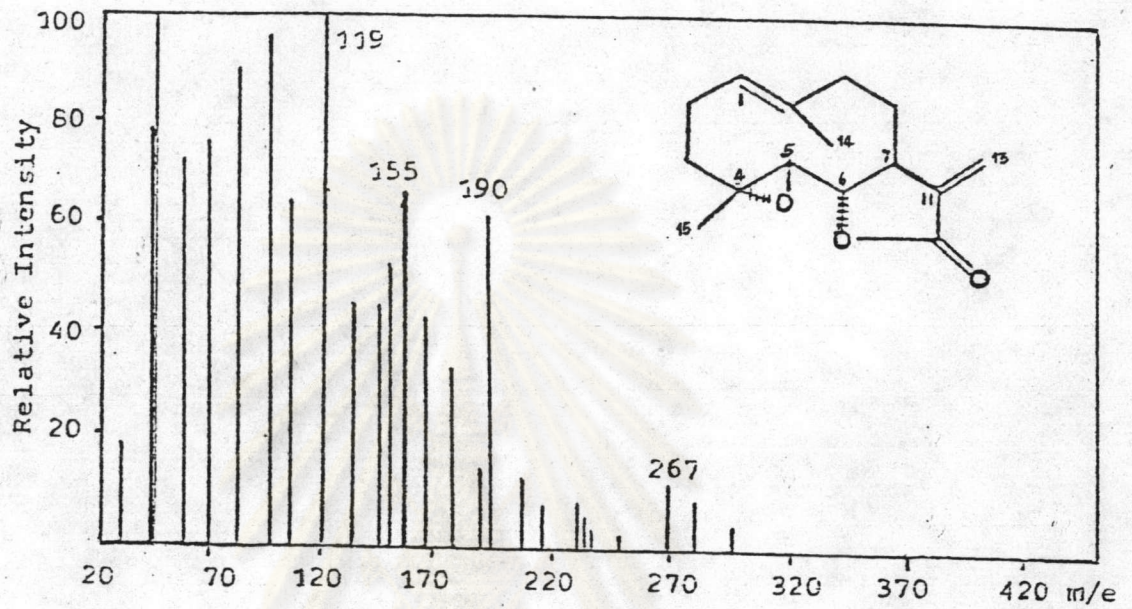


Figure 11 Electron impact mass spectrum of PB-2

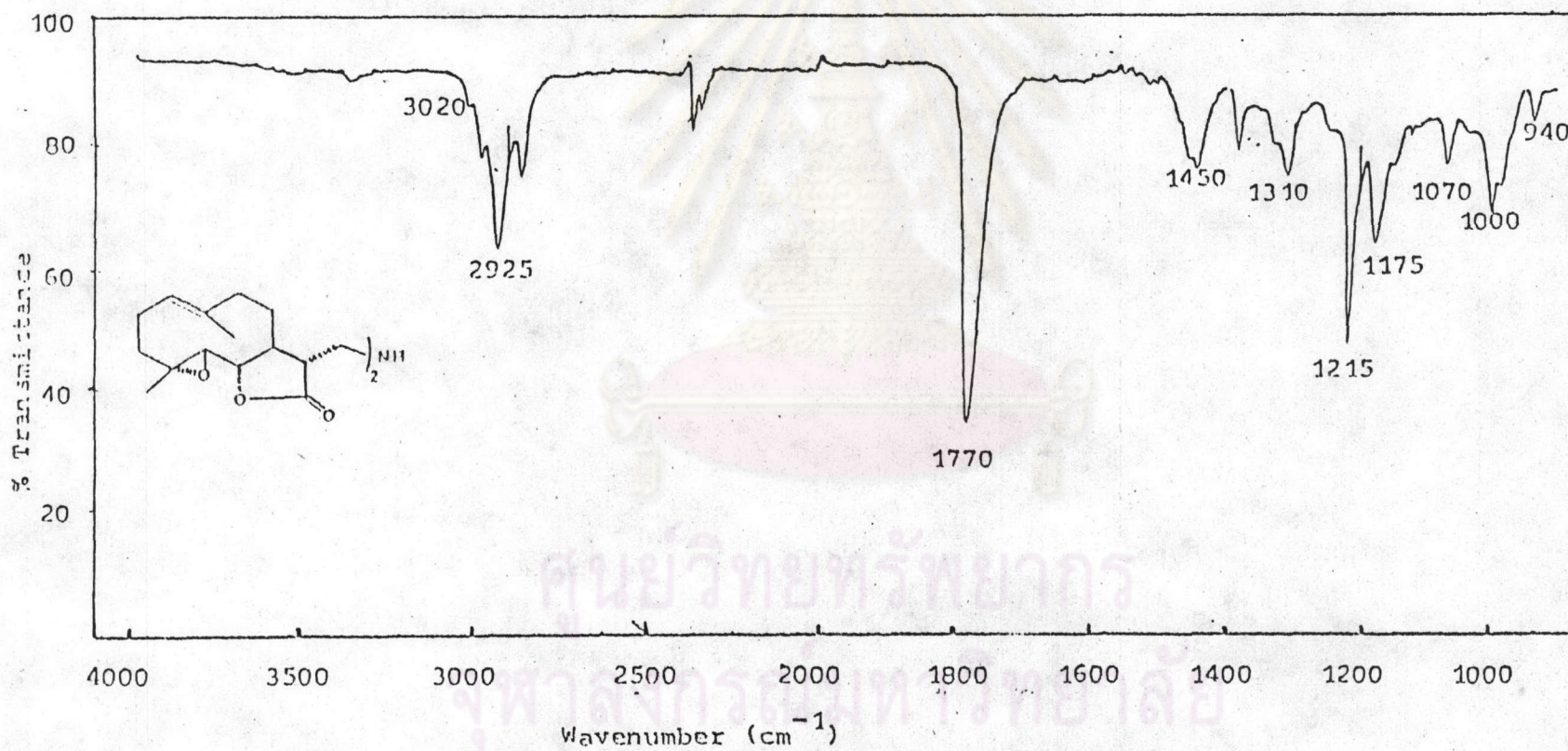


Figure 12 Infrared absorption of PB-3 in CCl₄

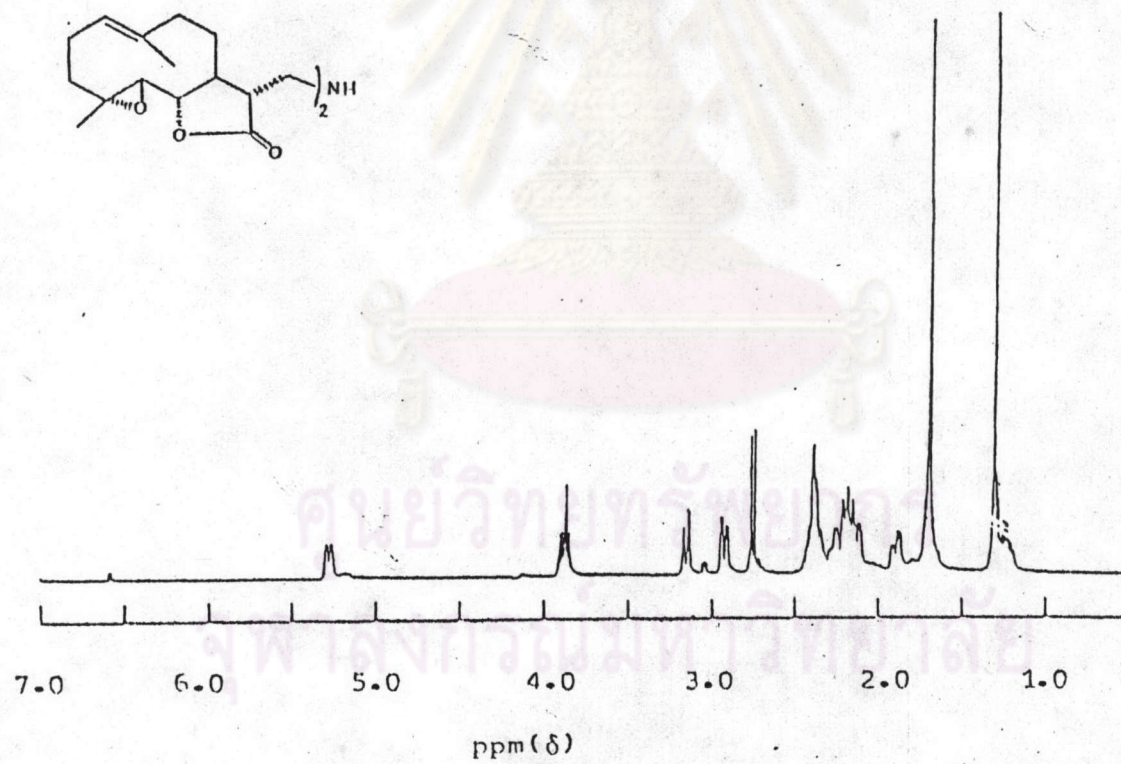


Figure 13 ¹H-nuclear magnetic resonance spectrum (400 MHz) of PB-3 in CDCl₃

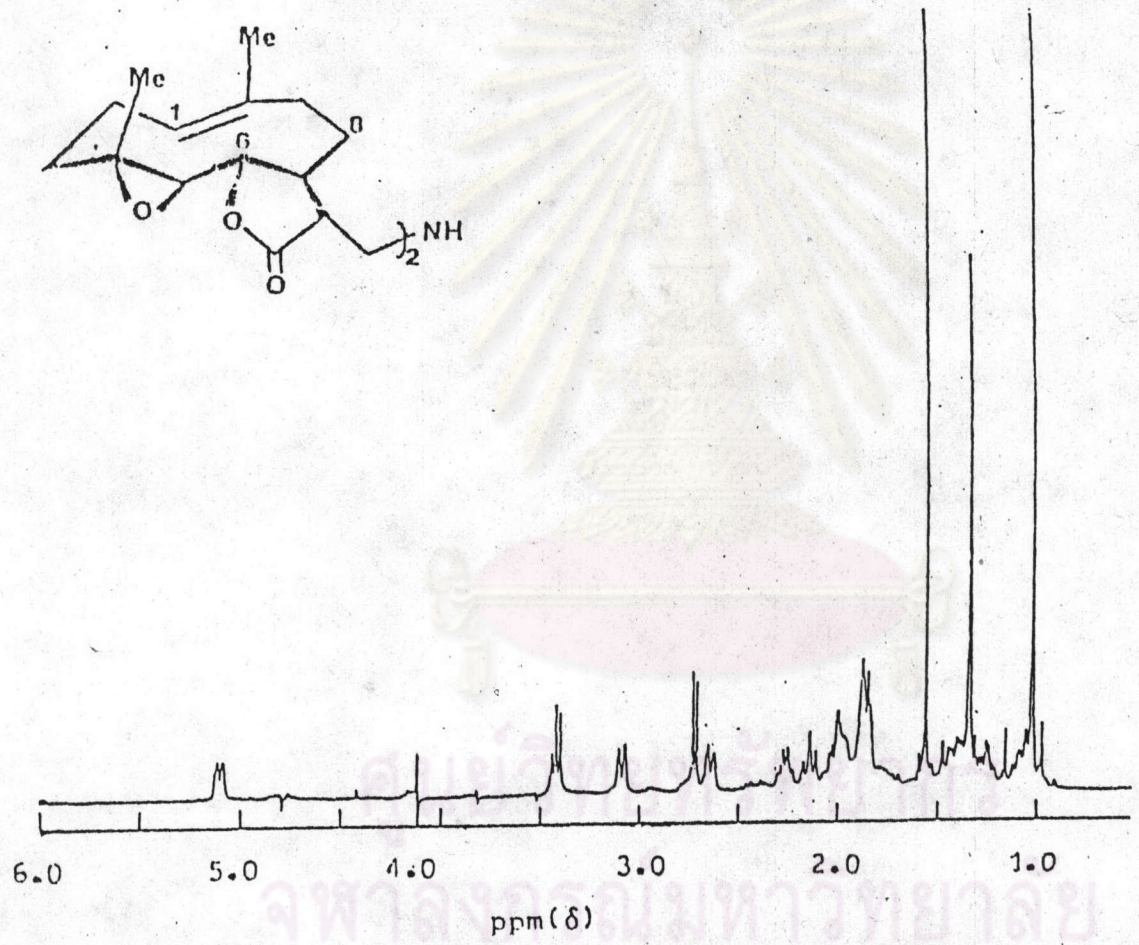


Figure 14 ¹H-nuclear magnetic resonance spectrum (400 MHz) of PB-3 in C₆D₆

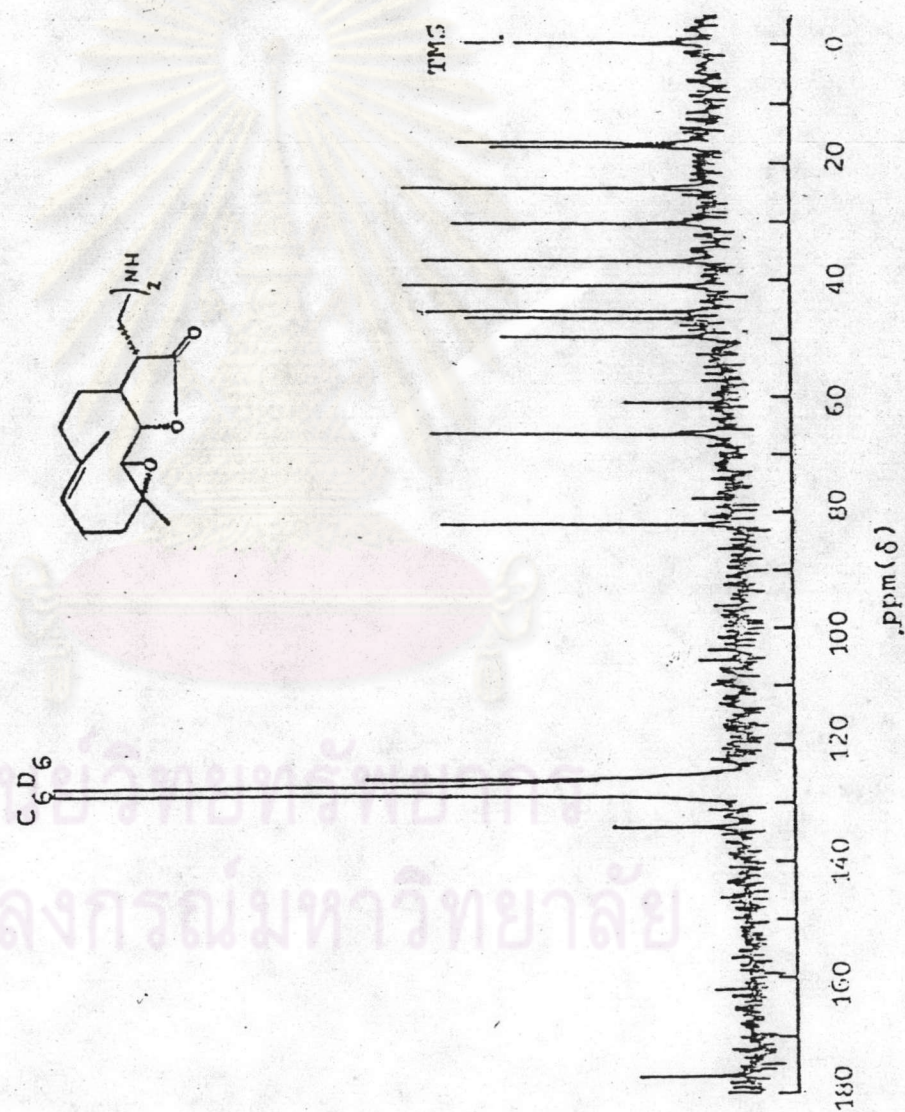


Figure 15 ^{13}C -nuclear magnetic resonance spectrum (100 MHz) of PB-3 in C_6D_6 (^1H -decoupled)

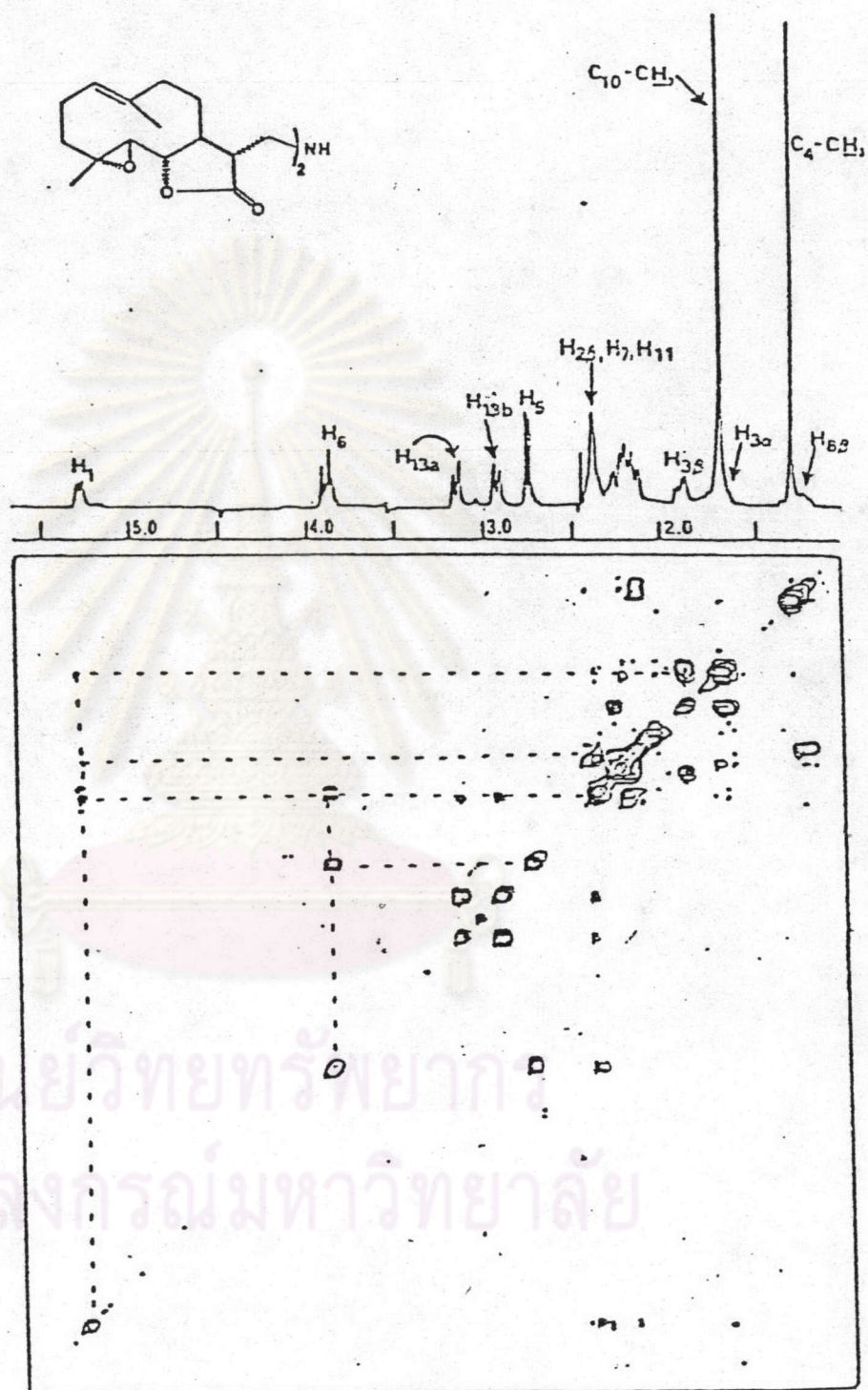


Figure 16 ^1H - ^1H COSY nuclear magnetic resonance spectrum (400 MHz) of PB-3 in CDCl_3

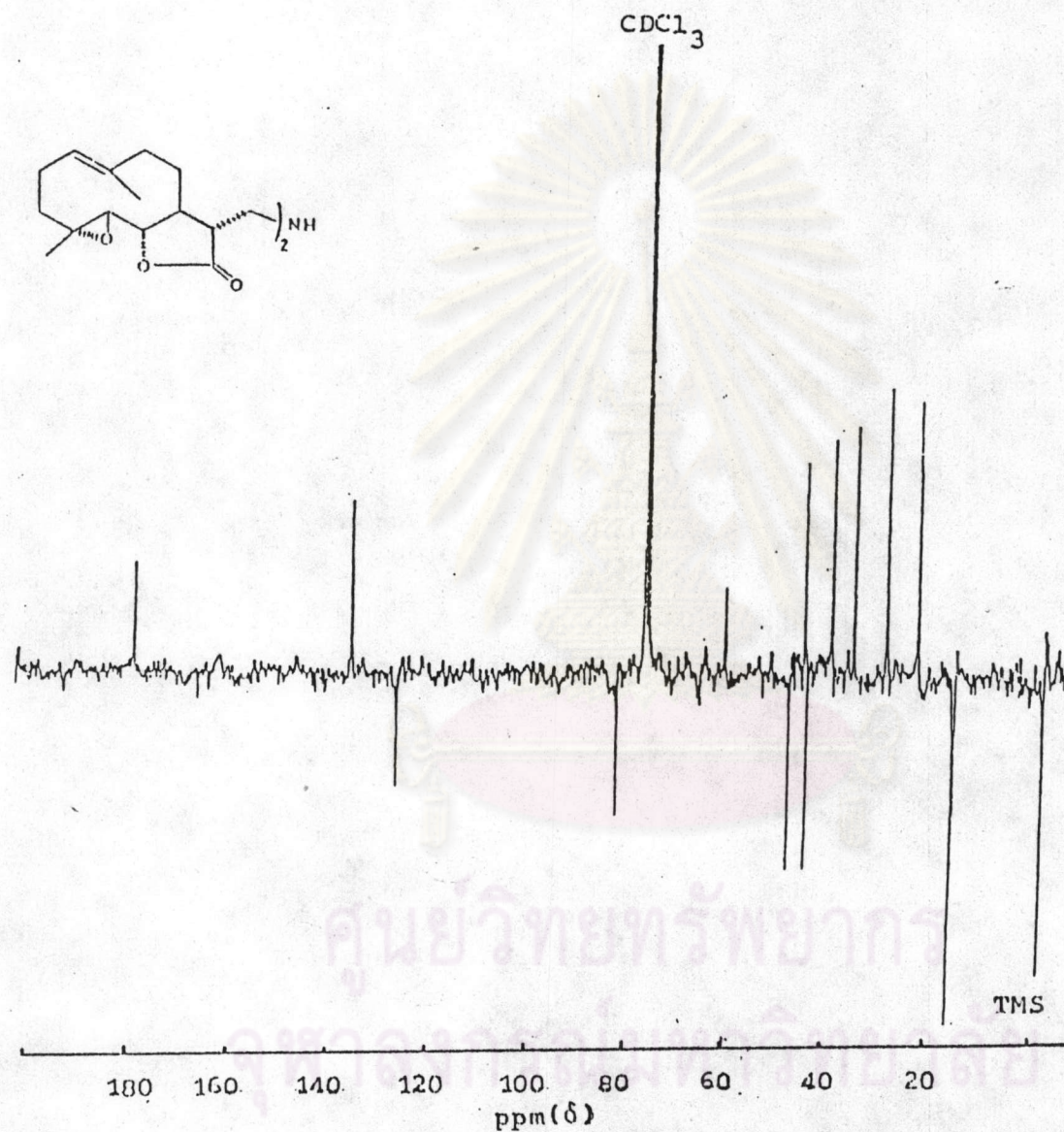


Figure 17 ^{13}C -nuclear magnetic resonance spectrum (100 MHz) of PB-3 in CDCl_3 . (Attached Proton Test)

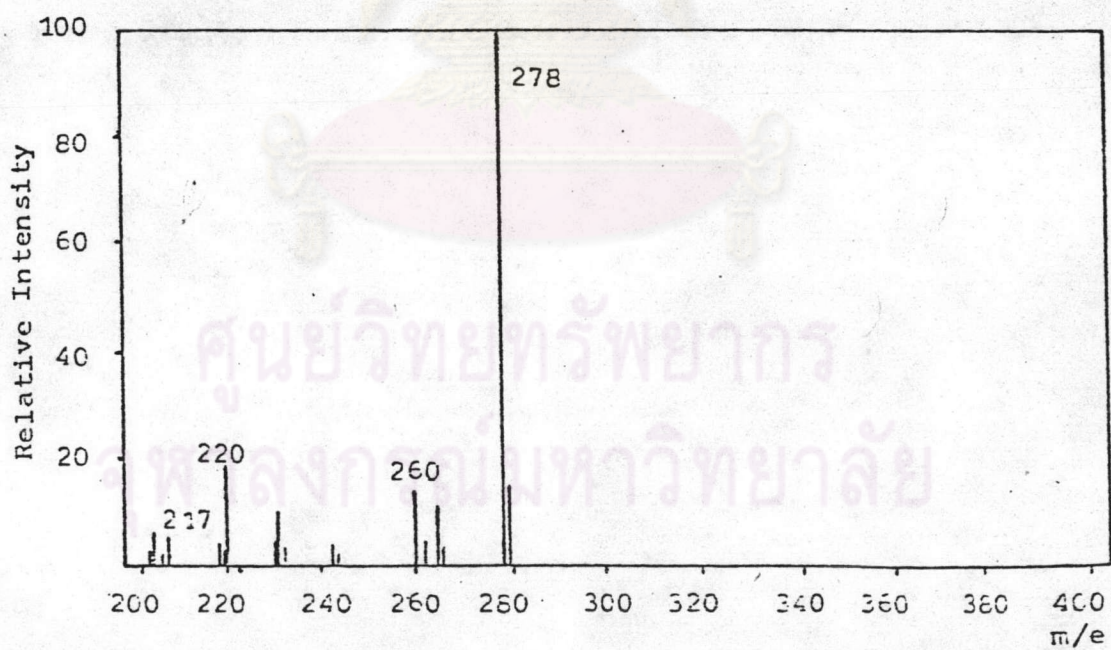
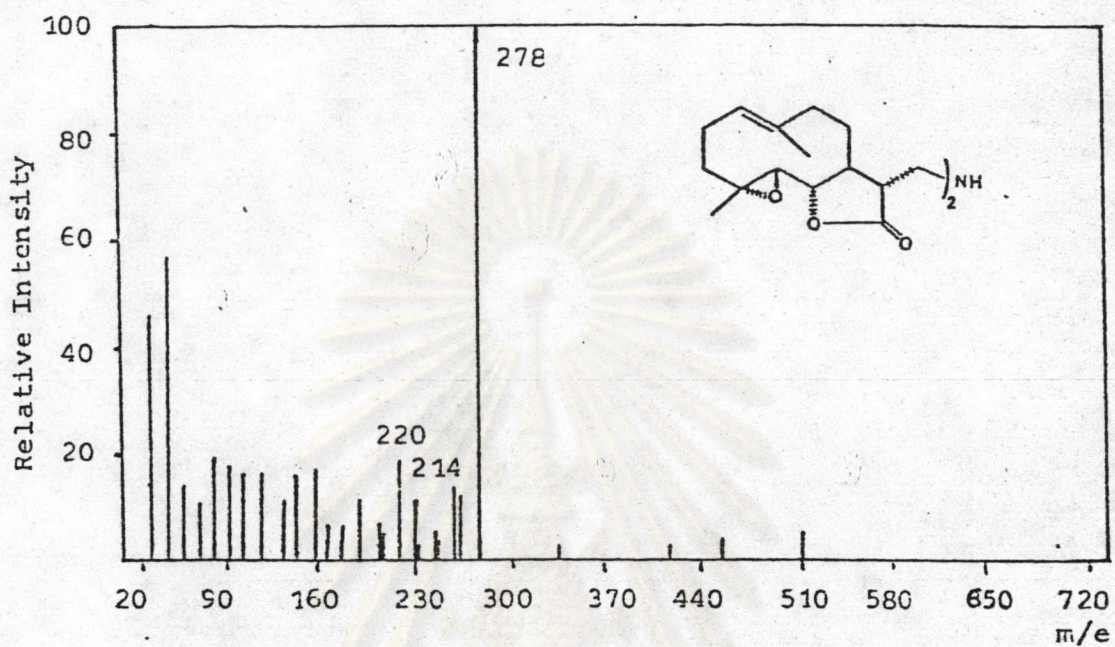


Figure 18 Electron impact mass spectrum of PB-3

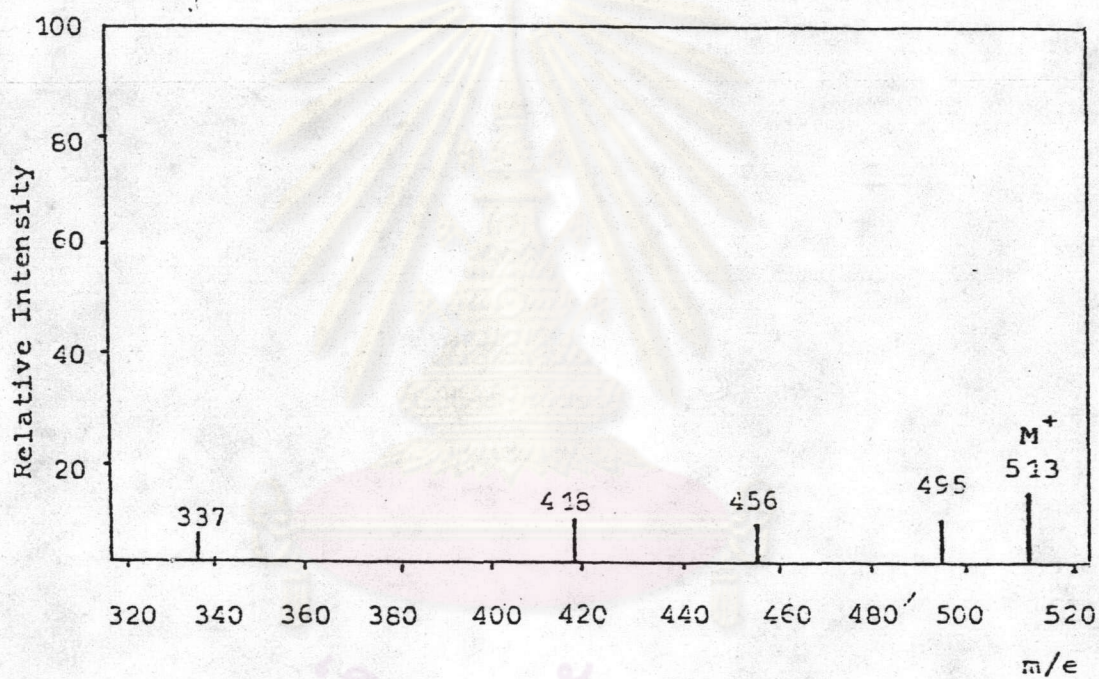


Figure 18 Electron impact mass spectrum of PB-3
(continued)

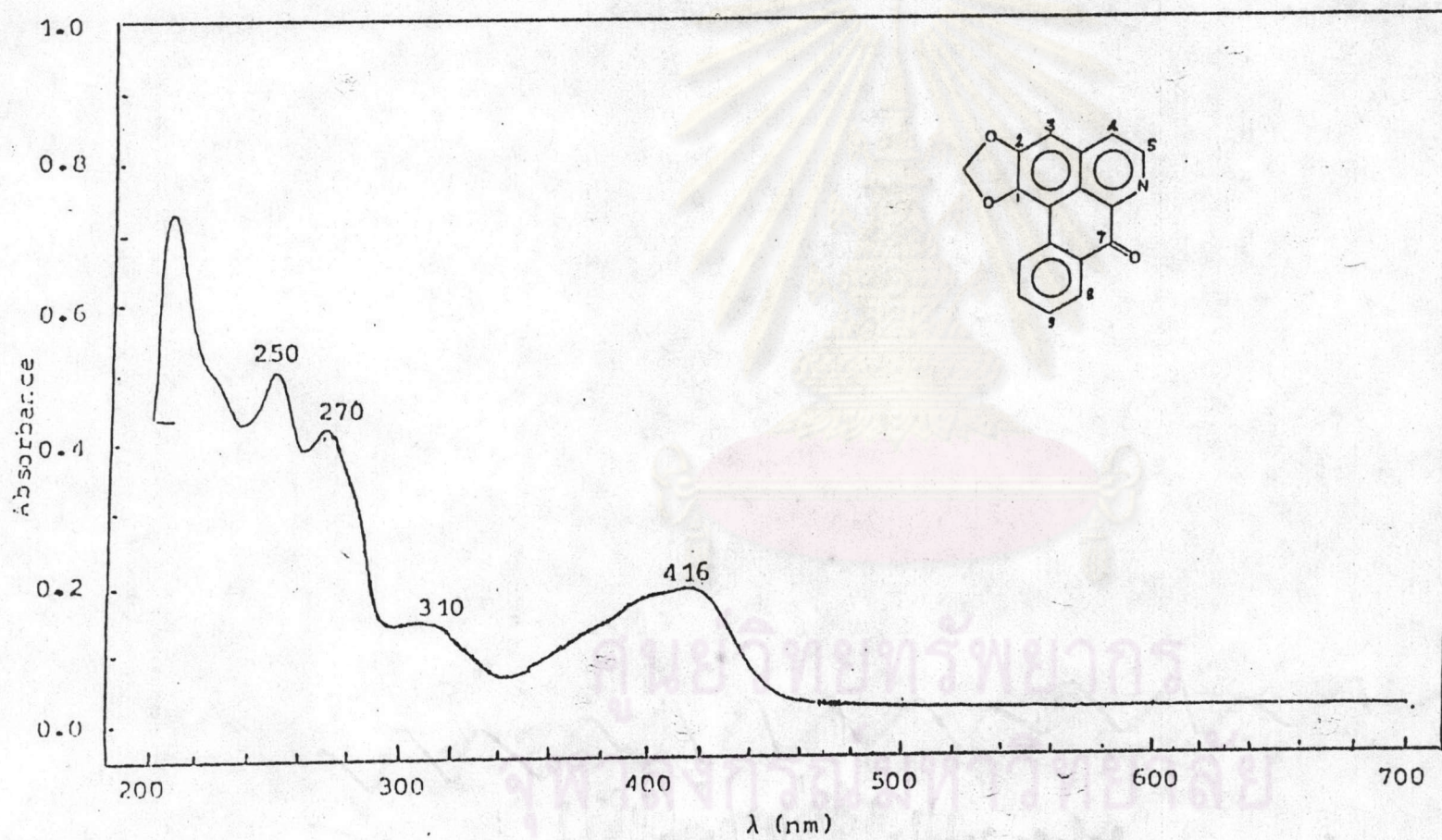


Figure 19 Ultraviolet absorption spectrum of PB-4 in EtOH

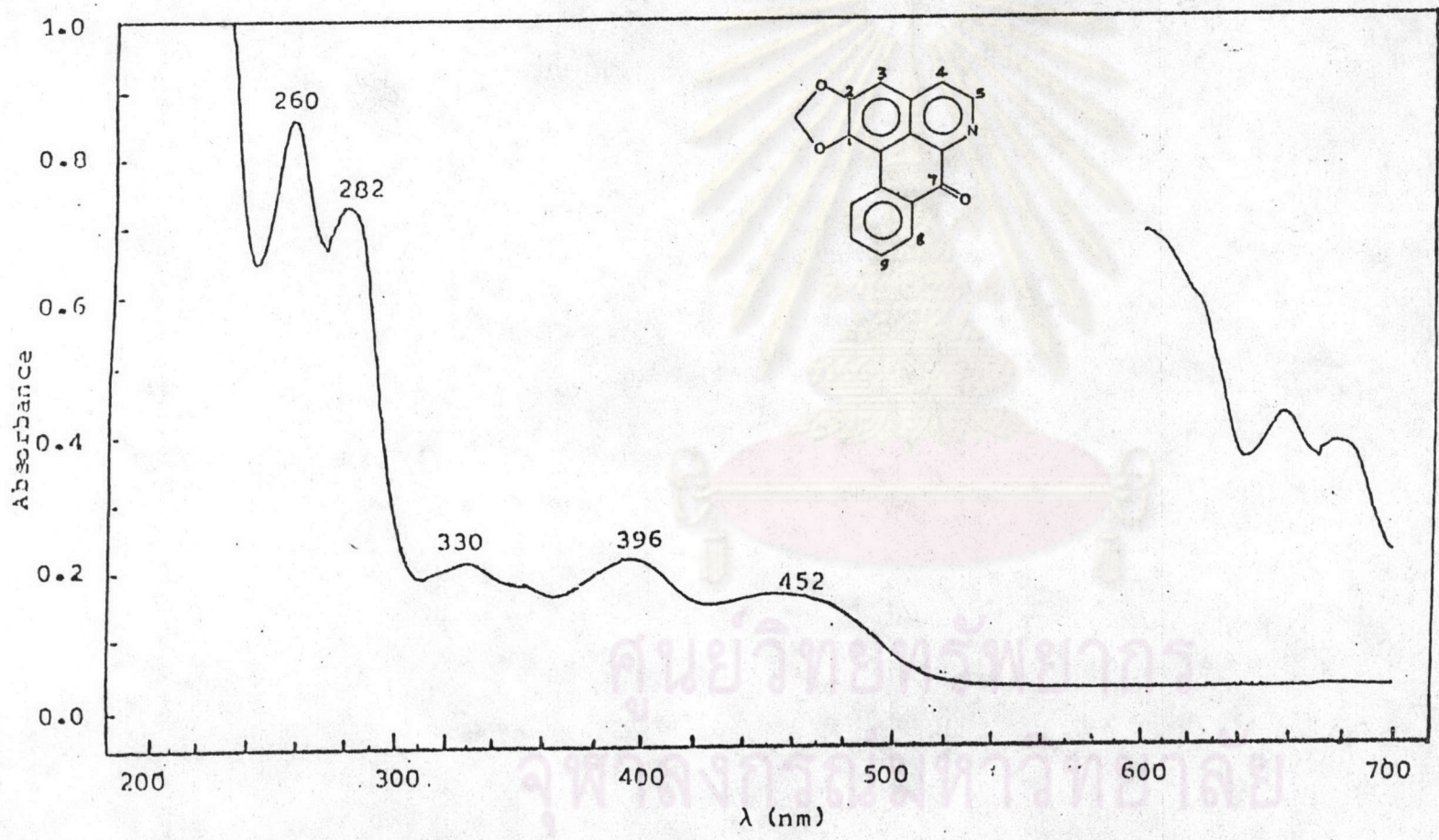


Figure 20 Ultraviolet absorption spectrum of PB-4 in 0.1 N HCl in EtOH

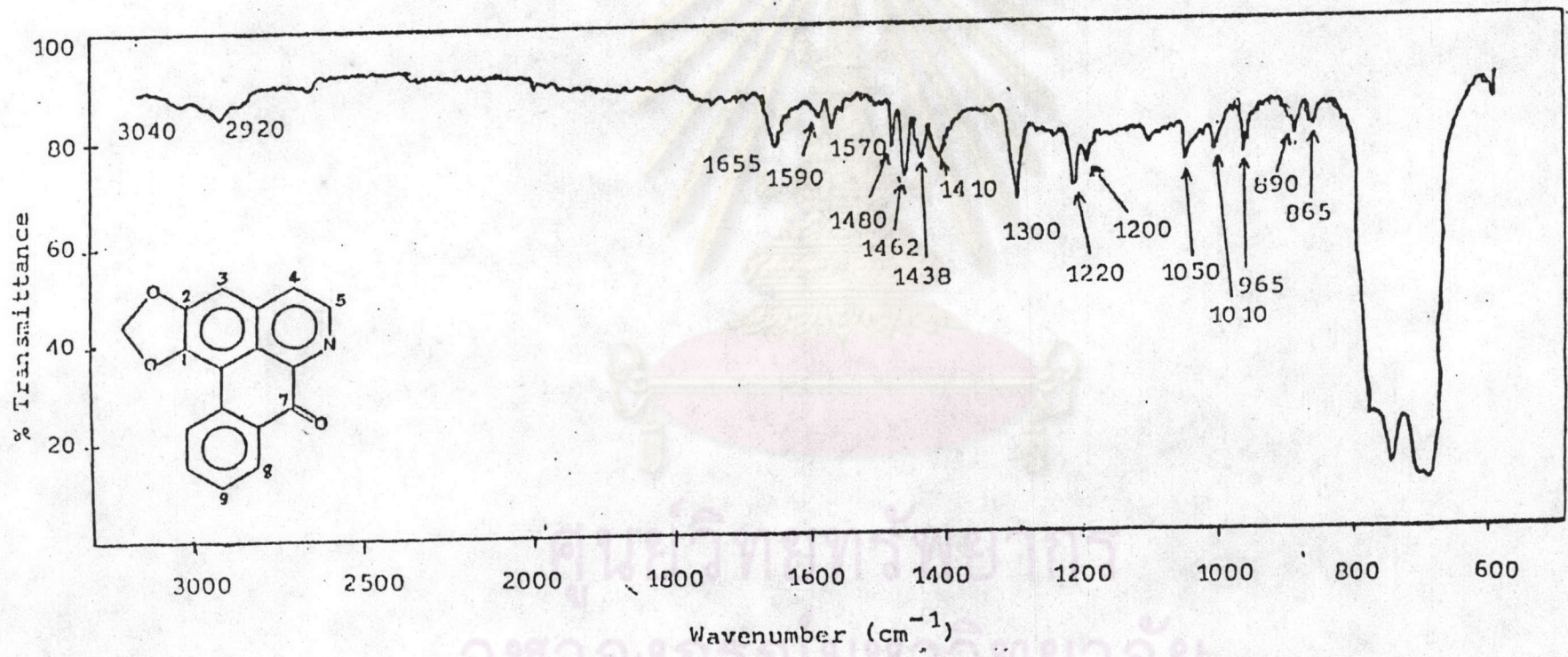


Figure 21 Infrared absorption of PB-4 in CH₂Cl₂

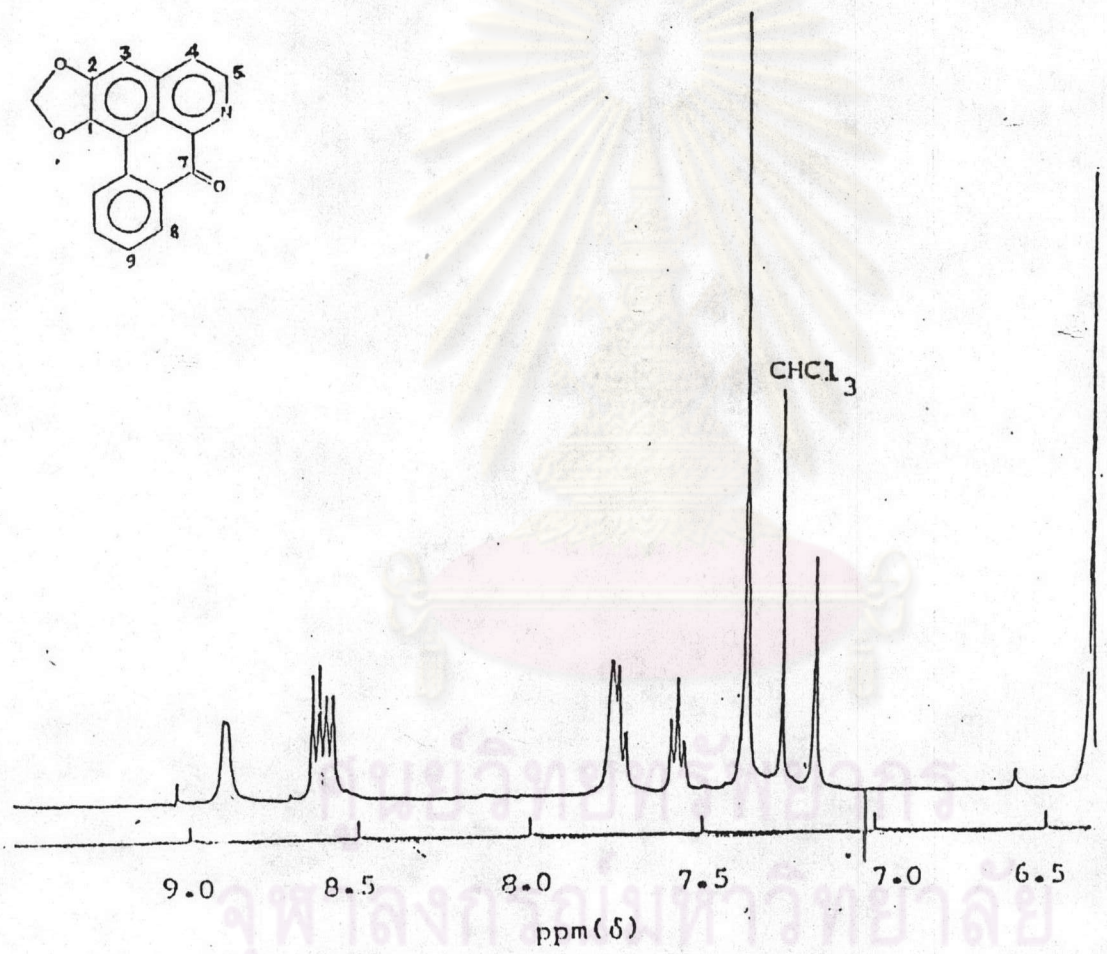


Figure 22 ¹H-nuclear magnetic resonance spectrum (400 MHz) of PB-4 in CDCl₃

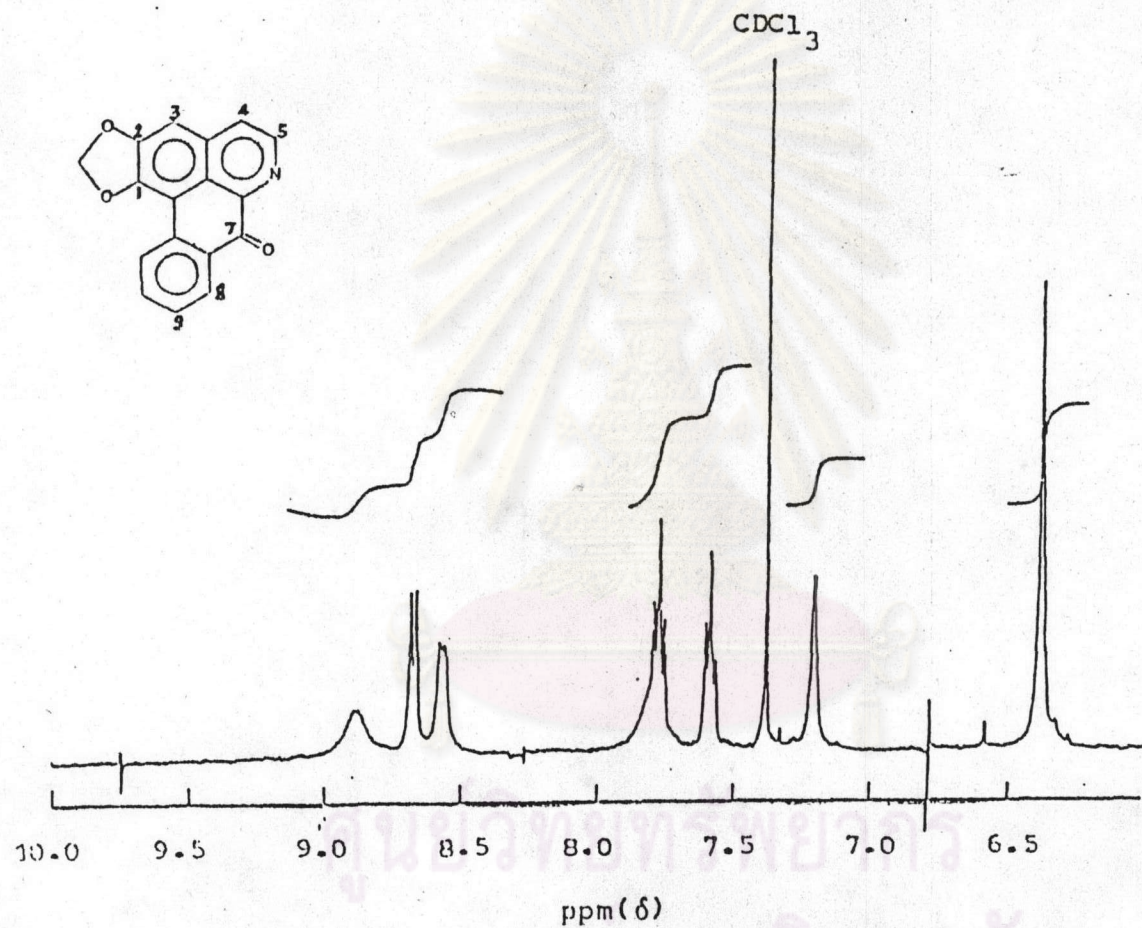


Figure 23 ^1H -nuclear magnetic resonance spectrum^x (400 MHz) of PB-4 in CDCl_3 + few drops of DMSO-D_6

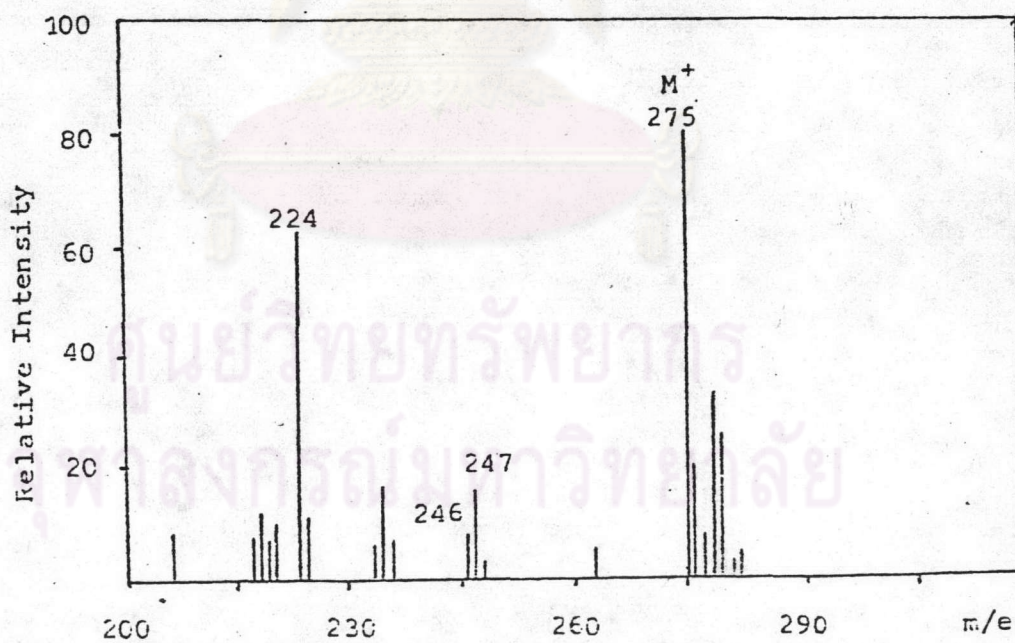
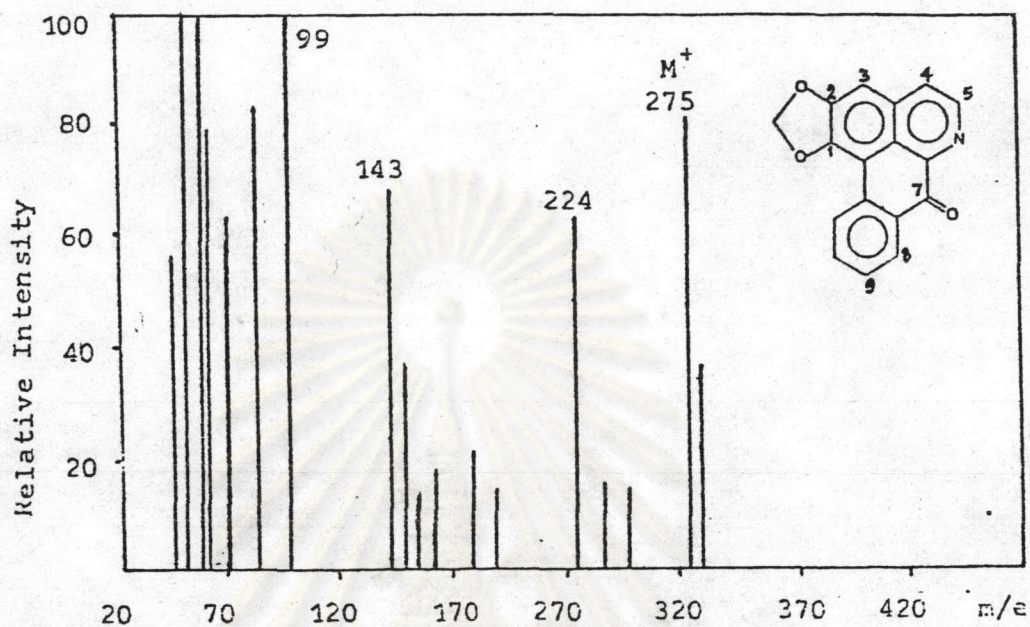


Figure 24 Electron impact mass spectrum of PB-4



VITA

Mr. Arthorn Rivepiboon was born on February 9, 1958 in Nakorn Pathom Province, Thailand. He received his Bachelor of Science in Pharmacy in 1981 from the Faculty of Pharmacy, Mahidol University, Bangkok. Since graduation, he received Certificates of Primary Health Care Development from Asian Training Center for Primary Health Development, Bangkok, in 1984 and of "Zielorientierte Projekplanung (ZOPP) : GTZ's Objectives Oriented Project Planing System" from German Agency for Technical Co-operation, Frankfurt, Federal Republic of German in 1985. He used to work as Medical Representative, Bristol-Myers Co., Ltd. during 1981-1982 and Chief of Pharmacy Section, Bua Yai Hospital, Nakorn Ratchasima, during 1982-1984. At present, he is working at Traditional Medicine and Herbs Promotion Unit, Office of the Primary Health Care, Ministry of Public Health, Bangkok, Thailand.

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