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APPENDICES

APPENDIX A

1. Media

The media were sterilized by autoclaving at 121°C, 15 lb/in^2 for 15 minutes.

1.1 Malt czapek agar (MCzA)

Czapek stock solution A	50.0	ml
Czapek stock solution B	50.0	ml
Malt extracts	40	g
Sucrose	30	g
Agar	15.0	g
Distilled water	1000	ml

1.2 Malt Czapek Broth (MCz) pH 5.0

Czapek solution A	100	ml
Czapek solution B	100	ml
Malt Extract	80	g
Sucrose	60	g
Zinc solution	2	ml
Copper solution	2	ml
Distilled water to	2000	ml

Czapek stock solution A

$NaNO_3$	4.0	g
KCl	1.0	g
$MgSO_4.7H_2O$	10.0	g
$FeSO_4.7H_2O$	0.02	g
Distilled water	1000	ml

Keep in a refrigerator.

Czapek stock solution B

K_2HPO_4	20.0	g
Distilled water	1000	ml

Keep in a refrigerator.

Zinc solution

$ZnSO_4.7H_2O$	0.5	g
Distilled water to	50	ml

Copper solution

$CuSO_4.5H_2O$	0.25	g
Distilled water to	50	ml

1.3 Czapek Yeast Autolysate Agar (YCz)

Czapek solution agar	49	g
Yeast extracts	4.9	g
Agar	15	g
Distilled water	1000	ml

1.4 Malt extract agar (MEA)

Malt extracts	20.0	g
Glucose	20.0	g
Peptone	1.0	g
Agar	15.0	g
Distilled water	1000	ml

1.5 Sabouraud's dextrose agar (SDA)

Dextrose	40.0	g
Peptone	10.0	g
Agar	15.0	g
Distilled water	1000	ml

1.6 Potato dextrose agar (PDA)

Potatoes, peeled and diced	200.0	g
Dextrose	20.0	g
Agar	15.0	g
Distilled water	1000	ml

Boil 200 g of peels, diced potatoes for 1 hr in 1000 ml of distilled water. Filter, and adjust the filtrate to 1000 ml. Add the dextrose and agar and dissolve by steaming and sterilize by autoclaving at 121°C for 15 min.

1.7 Yeast extract sucrose agar (YES)

Yeast extracts	20.0	g
Sucrose	150.0	g
Agar	20.0	g
Distilled water	1000	ml

1.8 Corn meal agar

Corn meal	30	g
Agar	15	g
Distilled water up to	1000	ml

1.9 Water agar

Agar	15	g
Distilled water up to	1000	ml

2. Reagent and buffer for DNA amplification by PCR

2.1 Lysis buffer

Tris-HCl (pH 7.2)	50	mM
EDTA	50	mM
SDS	3 %	
2-mercaptoethanol	1 %	

2.2 Chloroform : TE-saturated phenol 1 : 1 (v/v)

2.3 TE for resuspending pellet

Tris-HCl	10	mM
EDTA	0.1	mM

2.4 Gel loading buffer

Bromophenol blue	0.25 %
Sucrose in water	40 % (w/v)

2.5 5x Tris-Borate-EDTA (TBE)

Tris base	54	g
Boric acid	27.5	g
0.5 M EDTA pH 8.0	20	ml

The working solution was 1x TBE, diluted with four volume of distilled water.

2.6 10 x buffer

Tris HCl pH 9.0	100	ml
KCl	500	ml
Triton X-100	1 %	

2.7 2 mM dNTP (dATP, dCTP, dGTP, dTTP mix)

dATP	100	mM
dCTP	100	mM
dGTP	100	mM
dTTP	100	mM

Mixed equal volume of each dNTP to get 25 mM dNTP, then dilute to 2 mM dNTP with sterile double distilled wate

APPENDIX B

Table B1 The anti-*C. albicans* activity of endophytic fungal active isolates.

Isolate Code	Anti-*C. albicans* activity		Synergist with ketoconazole			Synergist with nystatin		
	Culture	Clear zone dia. (mm)	Culture	Clear zone dia. (mm)		Culture	Clear zone dia. (mm)	
				Inwards**	Outwards***		Inwards**	Outwards***
HANT 2	MCz	10.8	MCz	11.9*	–	MCz	11.6*	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	9.7	PDA	–	–	PDA	9.8*	–
	SDA	10.1	SDA	9.9*	–	SDA	10.2*	–
	YCz	11.8	YCz	10.6*	–	YCz	10.9*	–
	YES	11.0	YES	11.4*	–	YES	12.1*	–
HANT 4	MCz	10.6	MCz	11.3*	–	MCz	10.7*	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	11.3	SDA	12.1*	–	SDA	11.3*	–
	YCz	7.4	YCz	7.7*	–	YCz	–	–
	YES	11.9	YES	11.8*	–	YES	11.9*	–
HANT 5	MCz	7.2	MCz	–	–	MCz	8.1*	–
	MEA	10.8	MEA	10.9*	–	MEA	10.6*	–
	PDA	8.1	PDA	11.0*	–	PDA	10.8*	–
	SDA	11.2	SDA	11.6*	–	SDA	11.3*	–
	YCz	10.5	YCz	10.3*	–	YCz	10.8*	–
	YES	10.6	YES	10.4*	–	YES	10.1*	–

* diameter ** clear zone is on the same side as the drug disc. *** clear zone is on the opposite side of the drug disc.

Table B2 The anti-*C. albicans* activity of endophytic fungal active isolates (continue).

Isolate Code	Anti-*C. albicans* activity		Synergist with ketoconazole			Synergist with nystatin		
	Culture	Clear zone dia. (mm)	Culture	Clear zone dia. (mm)		Culture	Clear zone dia. (mm)	
				Inwards**	Outwards***		Inwards**	Outwards***
HANT 6	MCz	–	MCz	–	–	MCz	–	–
	MEA	9.1	MEA	10.2*	–	MEA	8.8*	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	9.8	YES	9.5*	–	YES	–	–
HANT 9	MCz	–	MCz	–	–	MCz	–	–
	MEA	8.6	MEA	8.7*	–	MEA	9.7*	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	7.7	SDA	10.0*	–	SDA	9.9*	–
	YCz	–	YCz	–	–	YCz	10.1*	–
	YES	7.8	YES	10.0*	–	YES	–	–
HANT 14	MCz	–	MCz	7.2*	–	MCz	–	–
	MEA	8.6	MEA	8.5*	–	MEA	–	–
	PDA	8.7	PDA	–	–	PDA	–	–
	SDA	–	SDA	9.3*	–	SDA	10.0*	–
	YCz	8.4	YCz	–	–	YCz	–	–
	YES	–	YES	–	–	YES	11.7*	–

* diameter ** clear zone is on the same side as the drug disc. *** clear zone is on the opposite side of the drug disc.

Table B3 The anti-*C. albicans* activity of endophytic fungal active isolates (continue).

Isolate Code	Anti-*C. albicans* activity		Synergist with ketoconazole			Synergist with nystatin		
	Culture	Clear zone dia. (mm)	Culture	Clear zone dia. (mm)		Culture	Clear zone dia. (mm)	
				Inwards**	Outwards***		Inwards**	Outwards***
HANT 17	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	4.5	–	MEA	–	–
	PDA	1.1	PDA	3.2	–	PDA	–	–
	SDA	–	SDA	3.1	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	–	YES	–	–	YES	–	–
HANT 26	MCz	7.8	MCz	9.3*	–	MCz	9.3*	–
	MEA	–	MEA	9.5*	–	MEA	–	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	9.3*	–	SDA	–	–
	YCz	–	YCz	9.5*	–	YCz	10.5*	–
	YES	–	YES	10.0*	–	YES	9.2*	–
HANT 27	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	9.3	PDA	1.1	0.8	PDA	1.5	1.1
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	–	YES	–	–	YES	–	–

* diameter ** clear zone is on the same side as the drug disc. *** clear zone is on the opposite side of the drug disc.

Table B4 The anti-*C. albicans* activity of endophytic fungal active isolates (continue).

Isolate Code	Anti-*C. albicans* activity		Synergist with ketoconazole			Synergist with nystatin		
	Culture	Clear zone dia. (mm)	Culture	Clear zone dia. (mm)		Culture	Clear zone dia. (mm)	
				Inwards**	Outwards***		Inwards**	Outwards***
HANT 36	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	10.5*	–	SDA	8.5*	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	–	YES	11.6*	–	YES	8.9*	–
HANT 37	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	4.3	–	YCz	–	–
	YES	–	YES	–	–	YES	–	–
HANT 41	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	10.0	PDA	–	–	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	9.9	YES	–	–	YES	–	–

* diameter ** clear zone is on the same side as the drug disc. *** clear zone is on the opposite side of the drug disc.

Table B5 The anti-*C. albicans* activity of endophytic fungal active isolates (continue).

Isolate Code	Anti-*C. albicans* activity		Synergist with ketoconazole			Synergist with nystatin		
	Culture	Clear zone dia. (mm)	Culture	Clear zone dia. (mm)		Culture	Clear zone dia. (mm)	
				Inwards**	Outwards***		Inwards**	Outwards***
HANT 44	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	8.8	YCz	9.6*	–	YCz	8.1*	–
	YES	–	YES	–	–	YES	–	–
HANT 52	MCz	–	MCz	10.1*	–	MCz	–	–
	MEA	–	MEA	9.3*	–	MEA	–	–
	PDA	–	PDA	8.1*	–	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	–	YES	–	–	YES	8.2*	–
HANT 55	MCz	9.5	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	9.5*	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	10.5	YES	10.4*	–	YES	8.8*	–

* diameter ** clear zone is on the same side as the drug disc. *** clear zone is on the opposite side of the drug disc.

Table B6 The anti-*C. albicans* activity of endophytic fungal active isolates (continue).

Isolate Code	Anti-*C. albicans* activity		Synergist with ketoconazole			Synergist with nystatin		
	Culture	Clear zone dia. (mm)	Culture	Clear zone dia. (mm)		Culture	Clear zone dia. (mm)	
				Inwards**	Outwards***		Inwards**	Outwards***
HANT 57	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	9.5	YES	9.7*	–	YES	9.5*	–
HANT 58	MCz	–	MCz	7.3	–	MCz	–	–
	MEA	–	MEA	7.9	–	MEA	–	–
	PDA	–	PDA	9.1	–	PDA	–	–
	SDA	–	SDA	8.6	–	SDA	–	–
	YCz	–	YCz	9.3	–	YCz	–	–
	YES	–	YES	–	–	YES	–	–
HANT 59	MCz	–	MCz	6.5	–	MCz	–	–
	MEA	–	MEA	6.8	–	MEA	–	–
	PDA	–	PDA	6.3	–	PDA	–	–
	SDA	–	SDA	7.0	–	SDA	–	–
	YCz	–	YCz	7.0	–	YCz	–	–
	YES	–	YES	–	–	YES	–	–

* diameter ** clear zone is on the same side as the drug disc. *** clear zone is on the opposite side of the drug disc.

Table B7 The anti-*C. albicans* activity of endophytic fungal active isolates (continue).

Isolate Code	Anti-*C. albicans* activity		Synergist with ketoconazole			Synergist with nystatin		
	Culture	Clear zone dia. (mm)	Culture	Clear zone dia. (mm)		Culture	Clear zone dia. (mm)	
				Inwards**	Outwards***		Inwards**	Outwards***
HANT 60	MCz	–	MCz	–	–	MCz	–	–
	MEA	–	MEA	–	–	MEA	–	–
	PDA	–	PDA	1.0	0.5	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	1.6	0	YCz	–	–
	YES	–	YES	1.8	1.6	YES	–	–
HANT 64	MCz	–	MCz	–	–	MCz	–	–
	MEA	8.7	MEA	2.4	2.3	MEA	1.0	1.0
	PDA	–	PDA	–	–	PDA	–	–
	SDA	–	SDA	–	–	SDA	–	–
	YCz	–	YCz	–	–	YCz	–	–
	YES	12.0	YES	4.3	2.2	YES	2.0	2.6

* diameter
** clear zone is on the same side as the drug disc.
*** clear zone is on the opposite side of the drug disc.

APPENDIX C

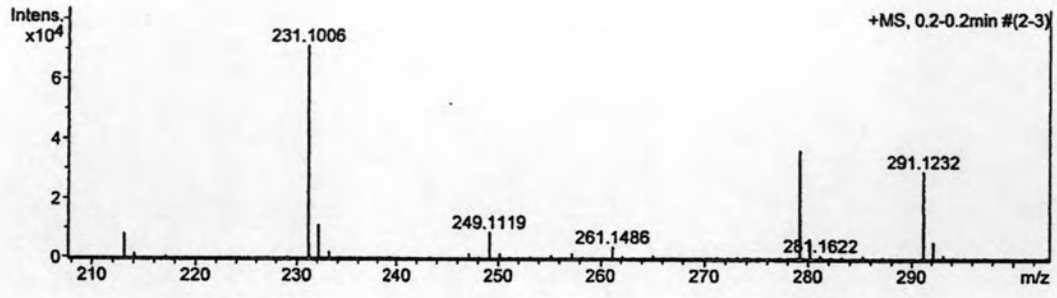

Figure C1 ESI-TOFMS mass spectrum of compound **1**.

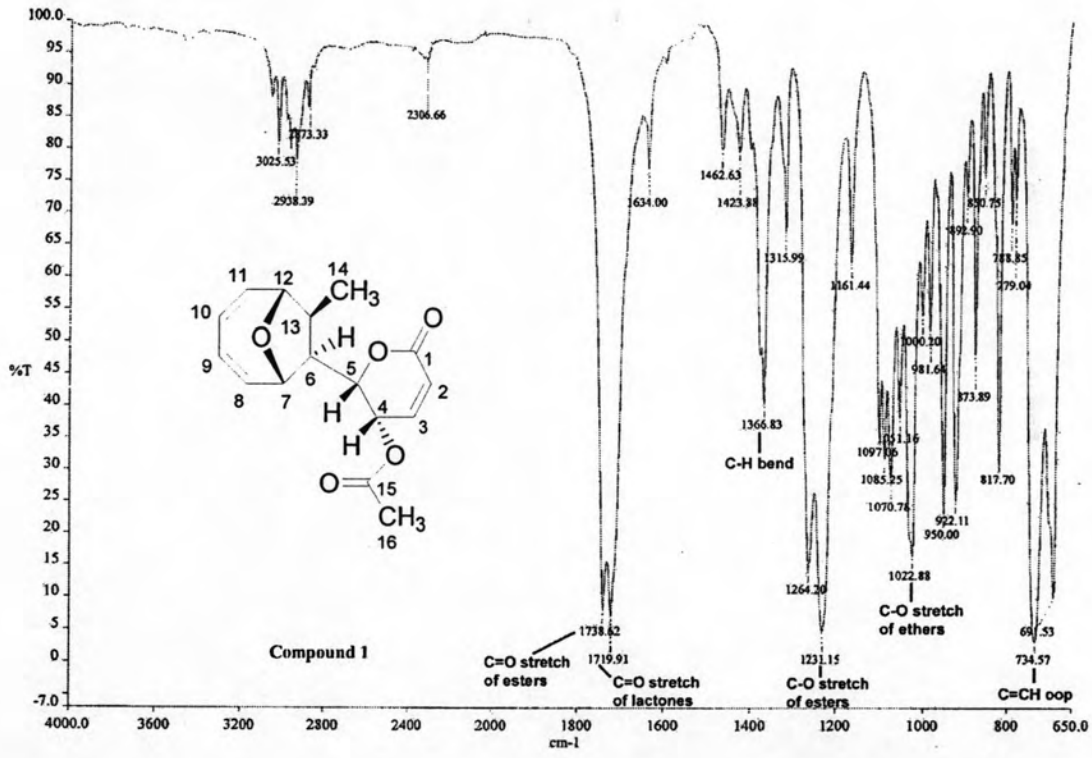

Figure C2 IR spectrum of compound **1**.

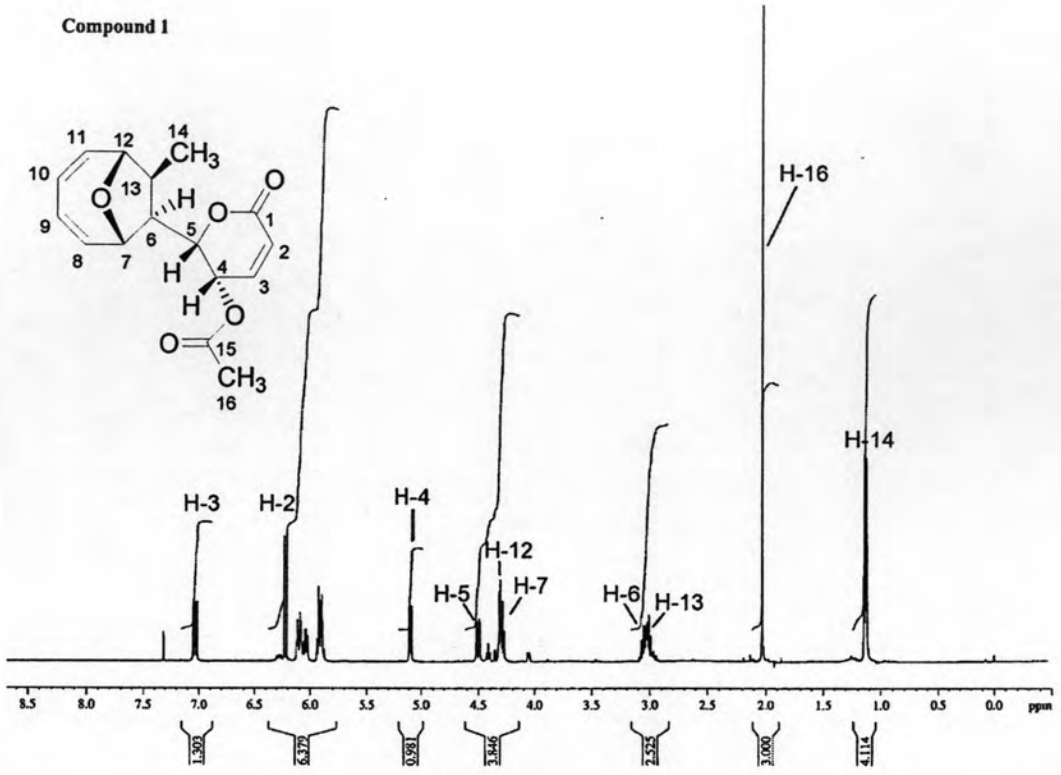

Figure C3 ^{1}H-NMR spectrum of compound **1**.

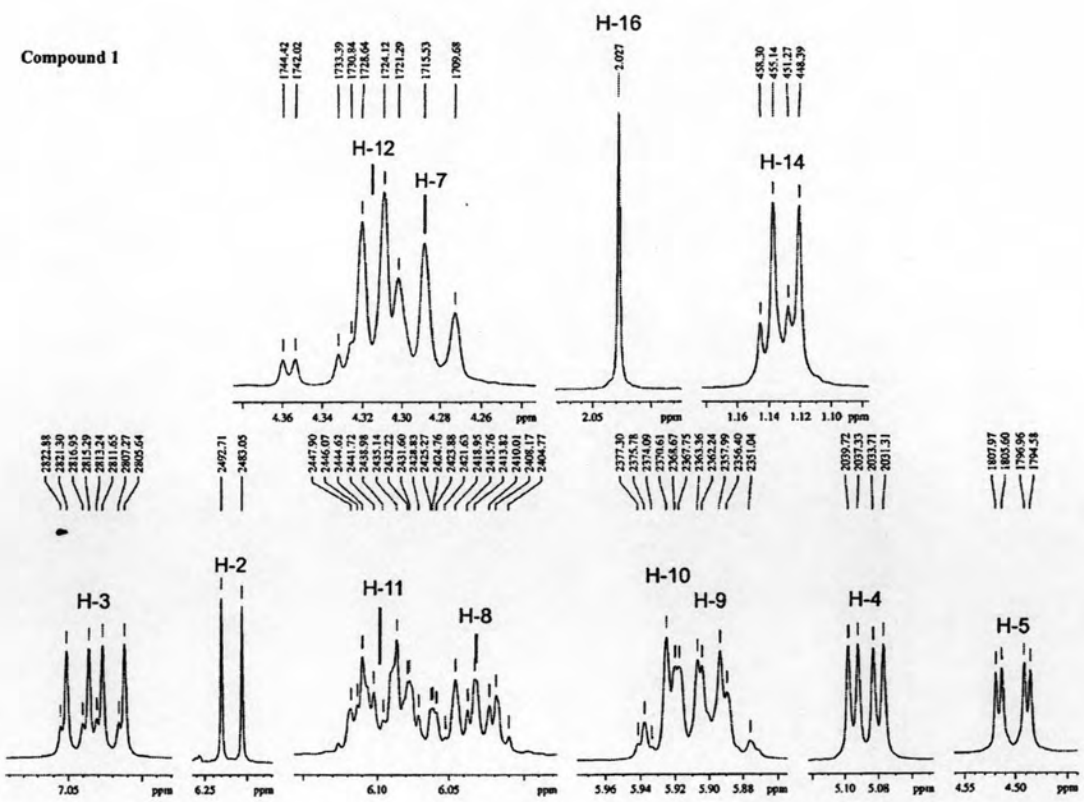

Figure C4 Expansion of Figure C2.

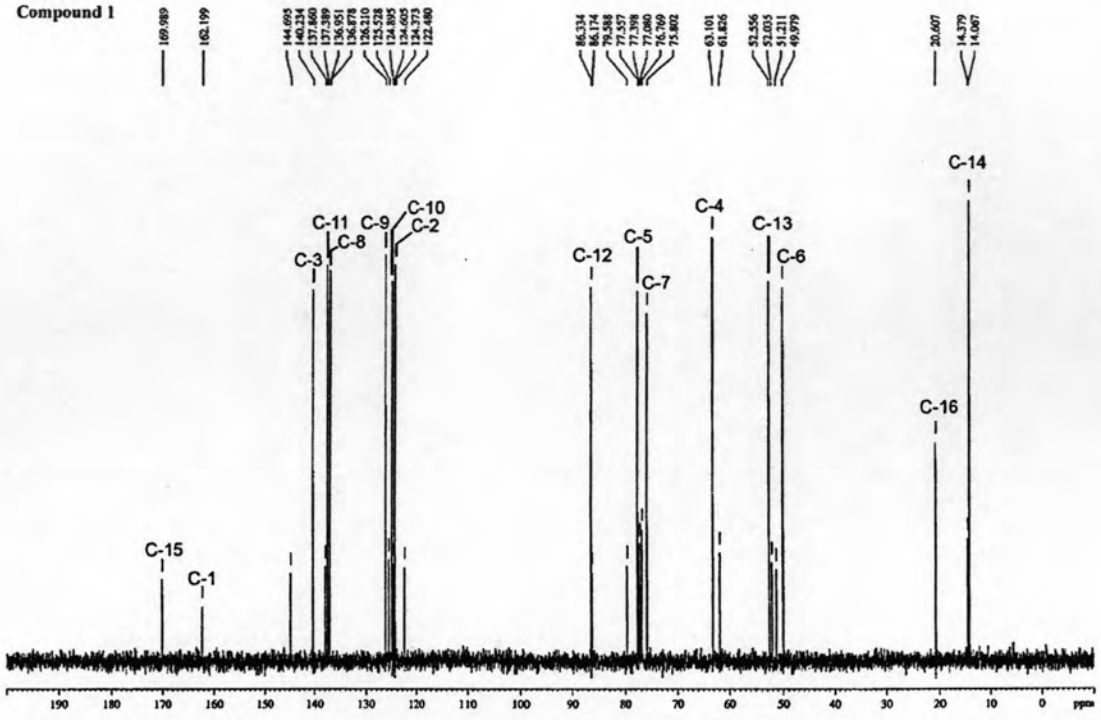

Figure C5 ^{13}C-NMR spectrum of compound **1**.

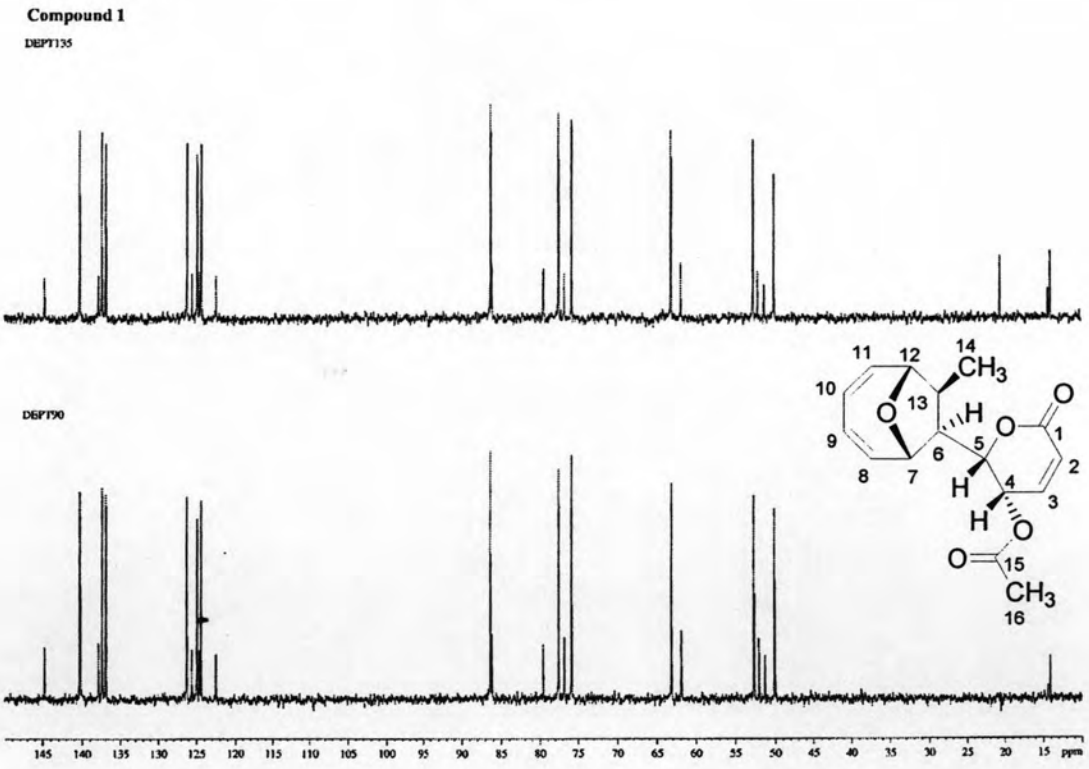

Figure C6 DEPT spectrum of compound **1**.

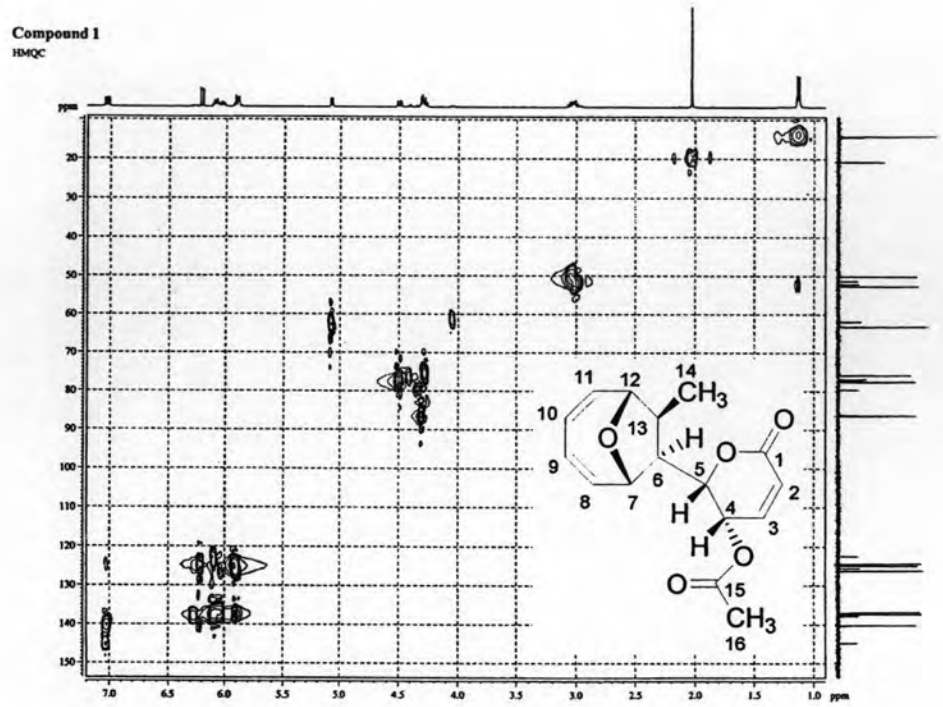

Figure C7 HMQC spectrum of compound **1**.

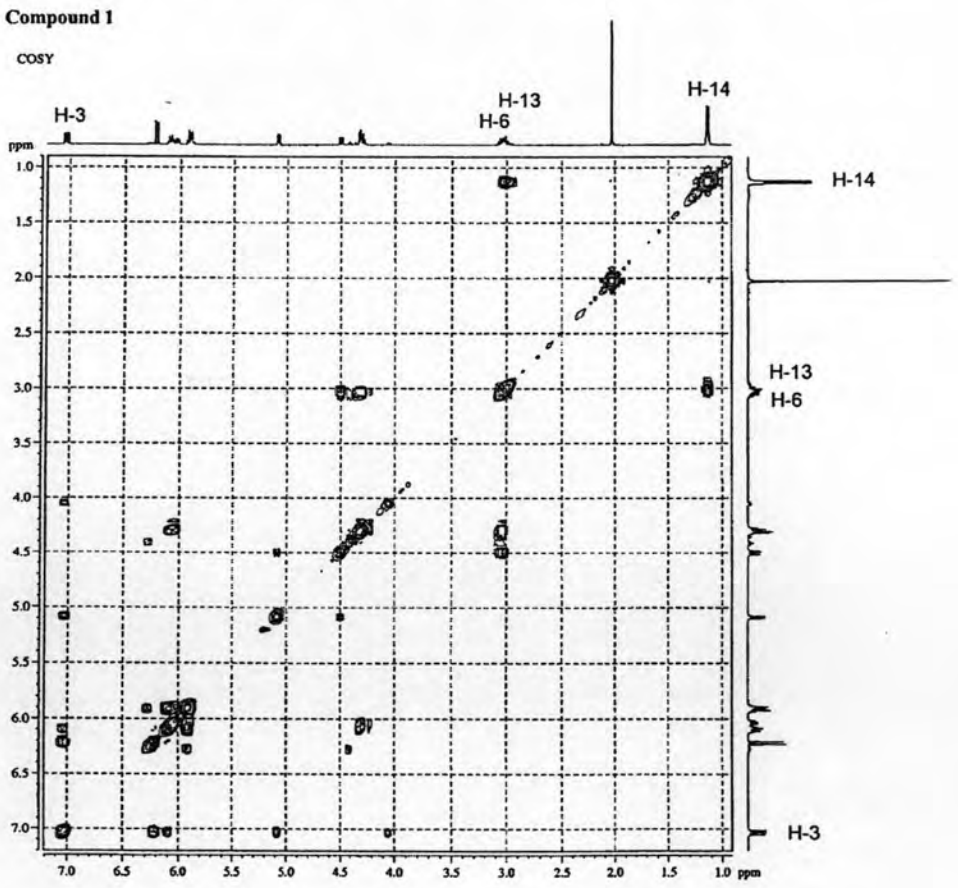

Figure C8 COSY spectrum of compound **1**.

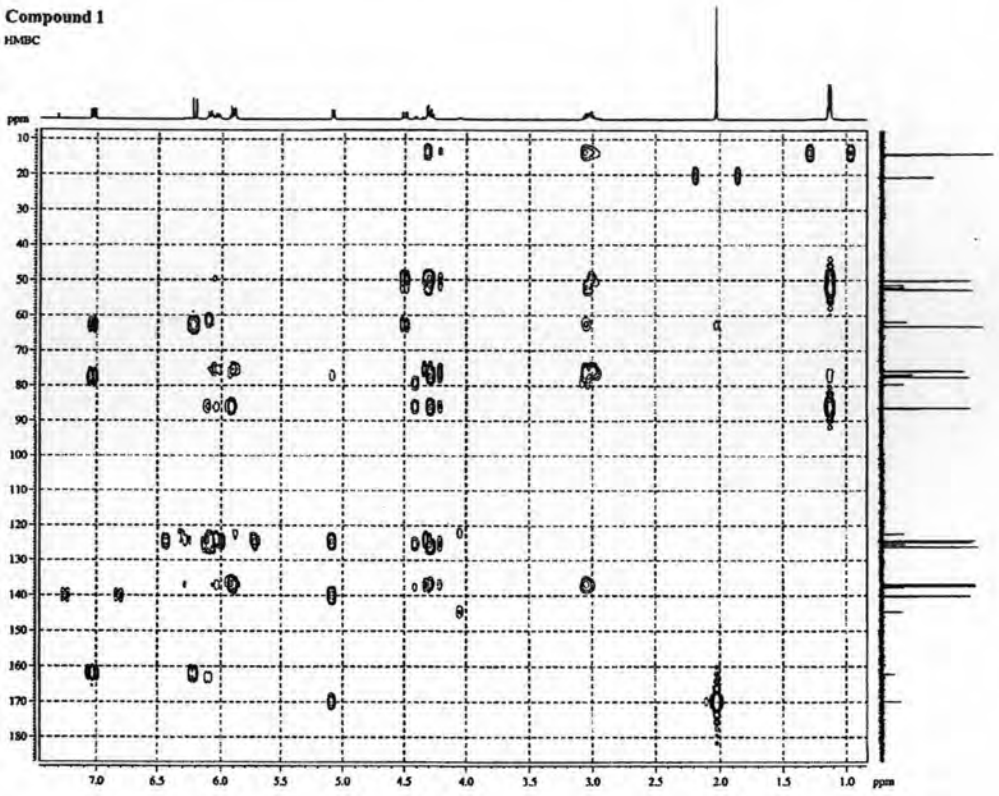

Figure C9 HMBC spectrum of compound **1**.

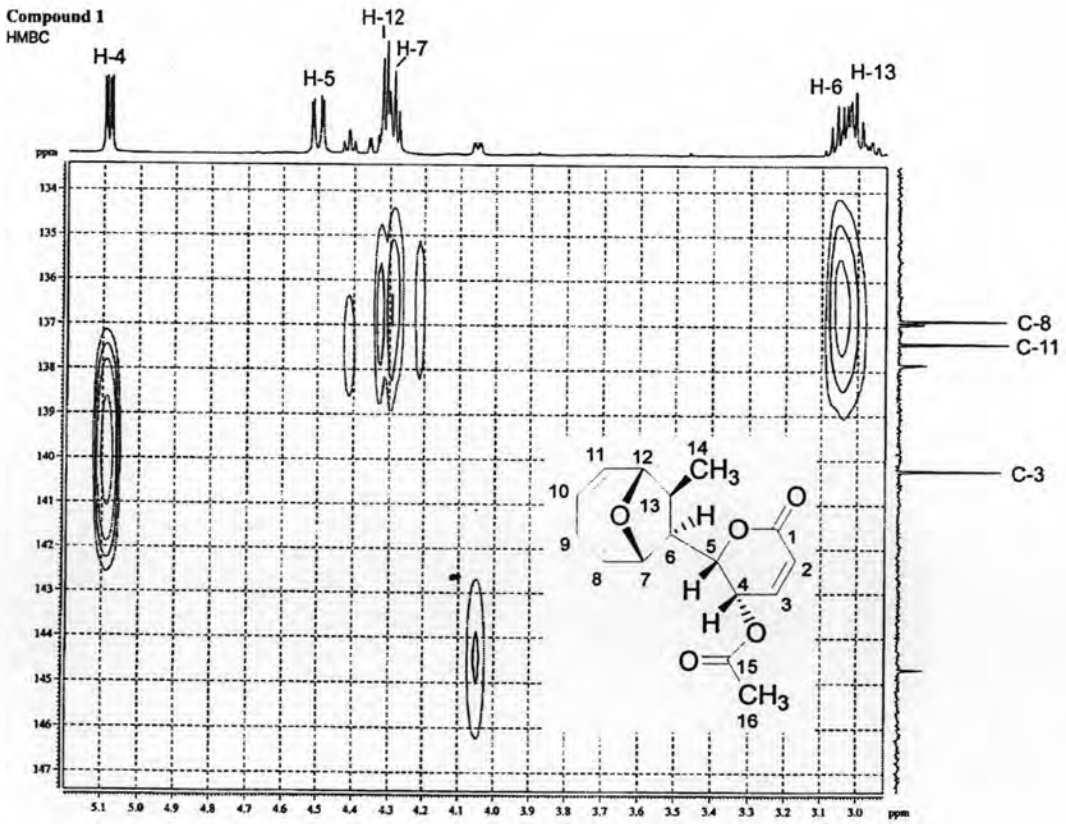

Figure C10 Expansion of Figure C8.

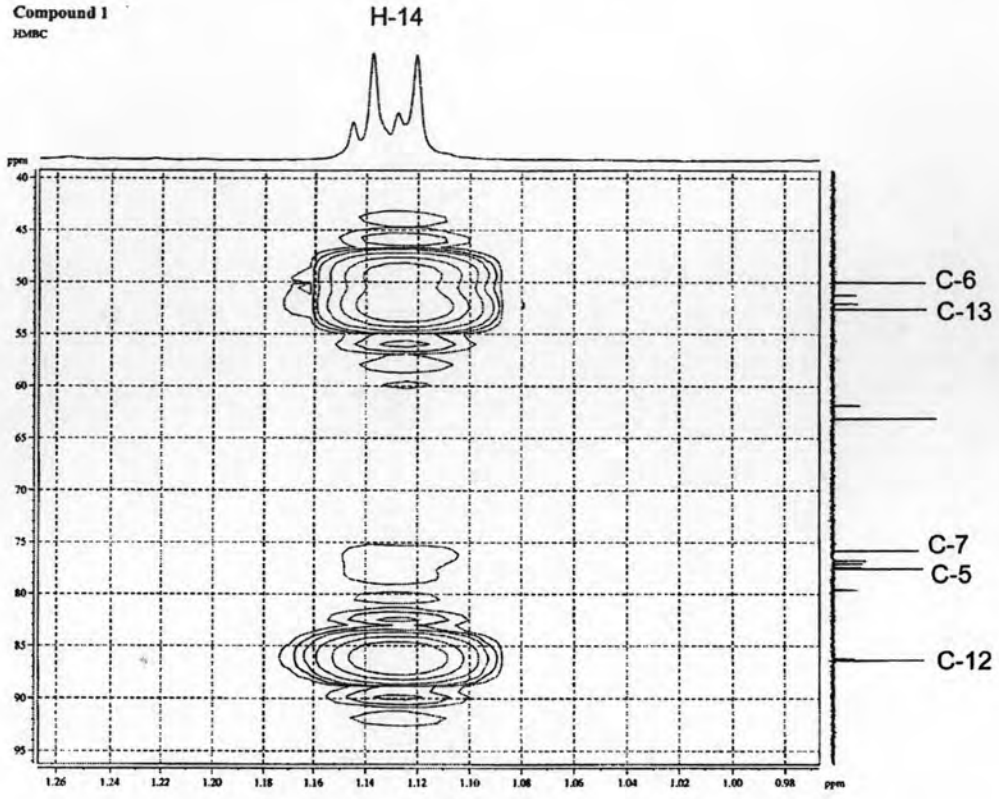

Figure C11 Expansion of Figure C8 (continue).

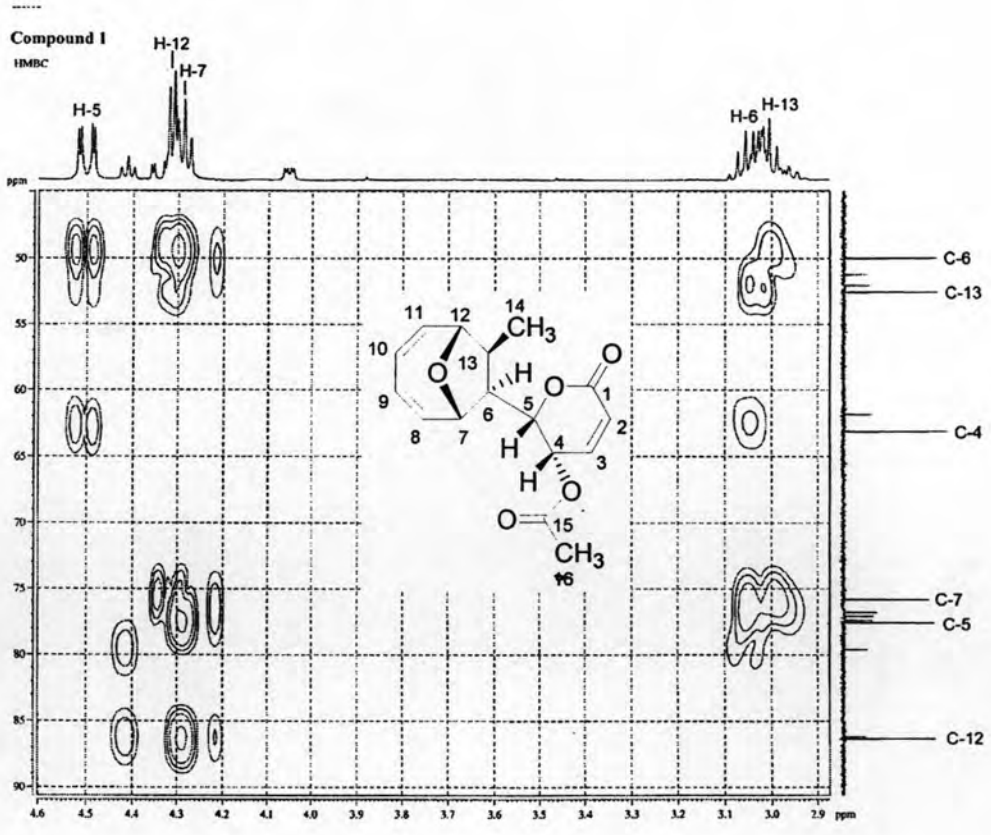

Figure C12 Expansion of Figure C8 (continue).

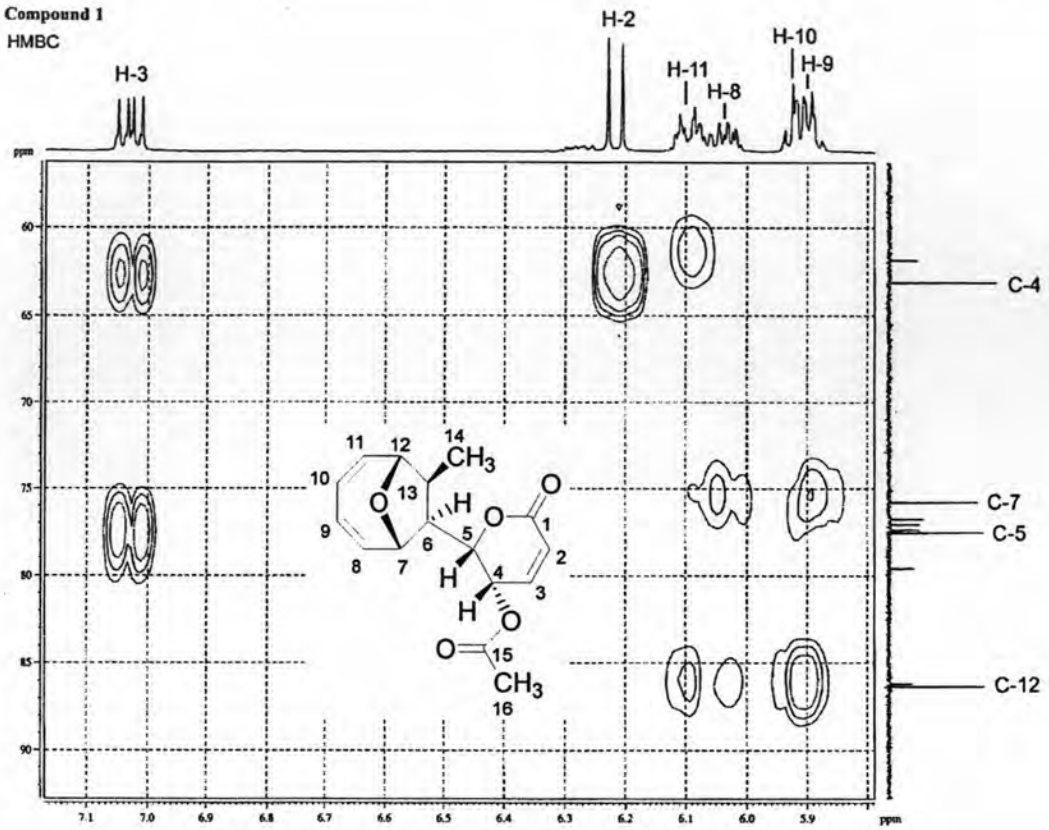

Figure C13 Expansion of Figure C8 (continue).

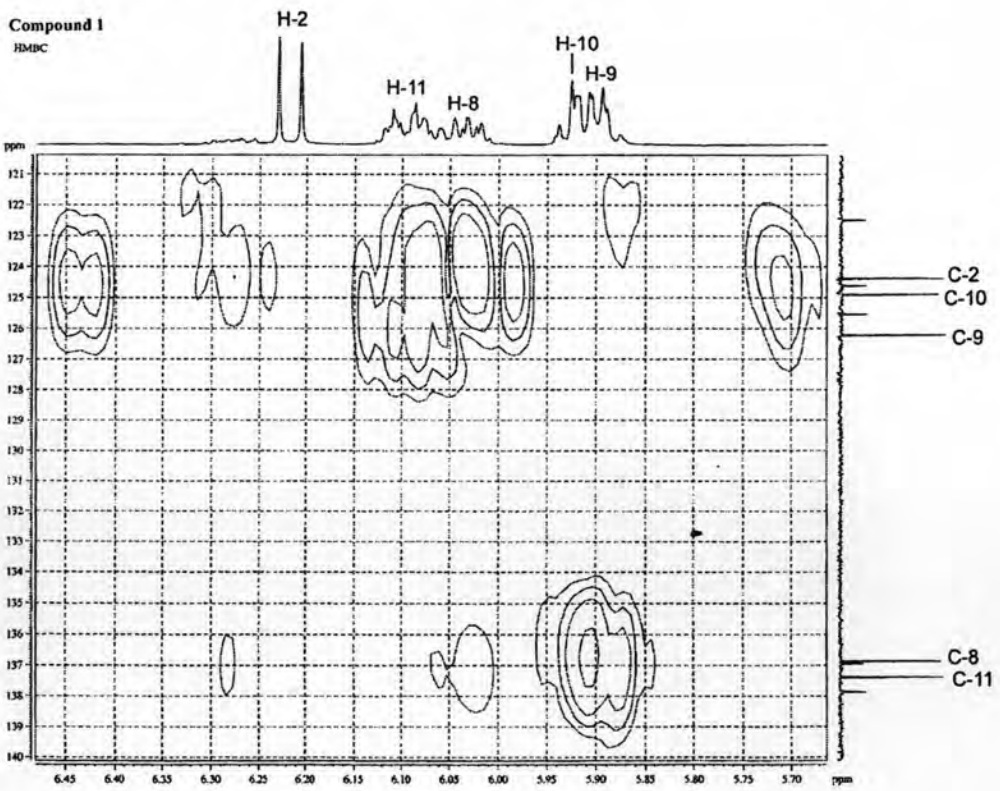

Figure C14 Expansion of Figure C8 (continue).

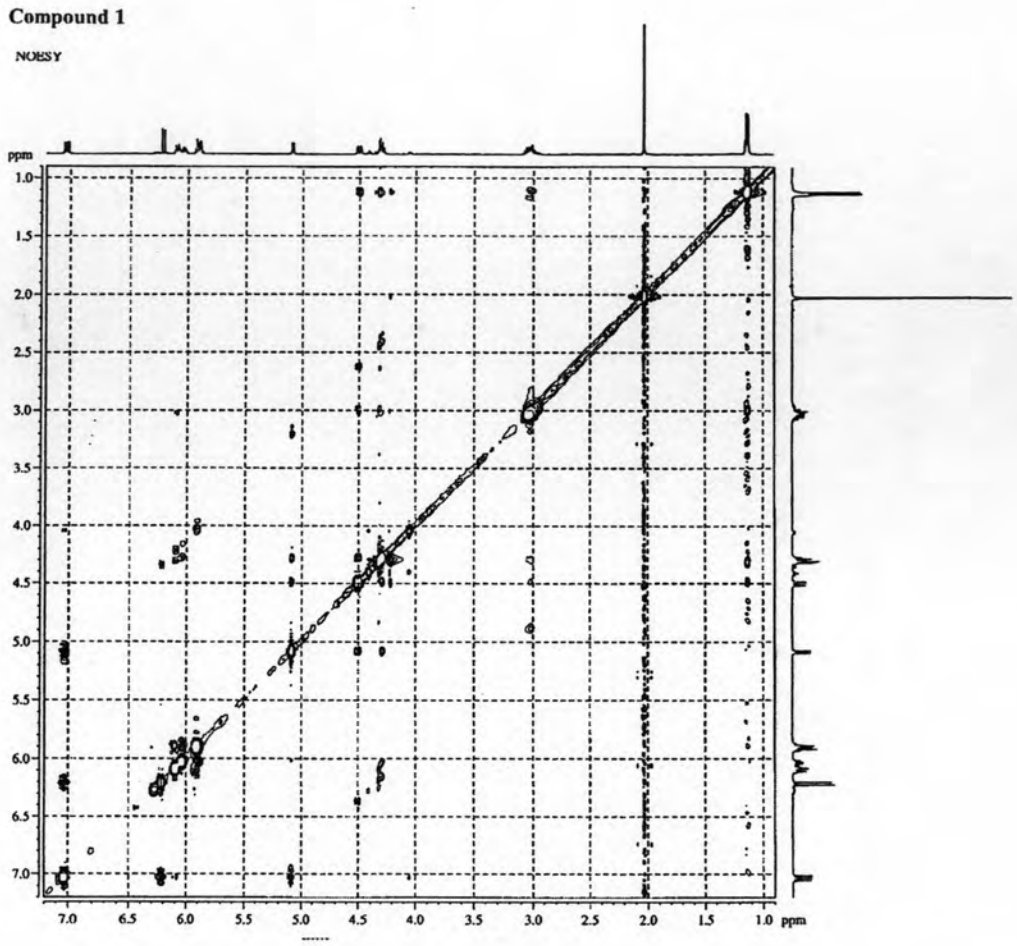

Figure C15 NOESY spectrum of compound **1**.

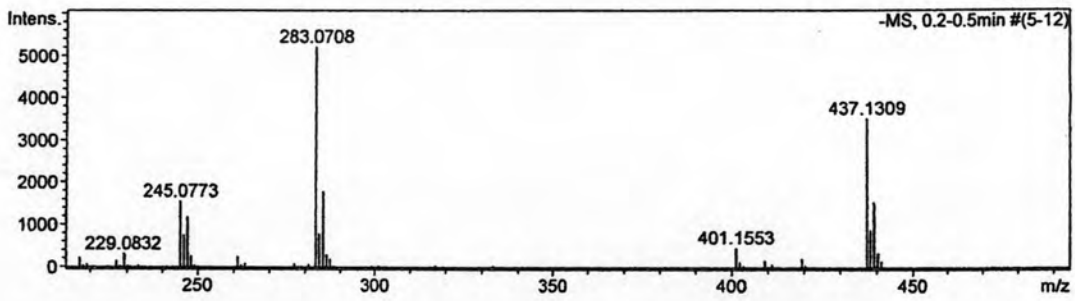

Figure C16 ESI-TOFMS mass spectrum of compound **2**.

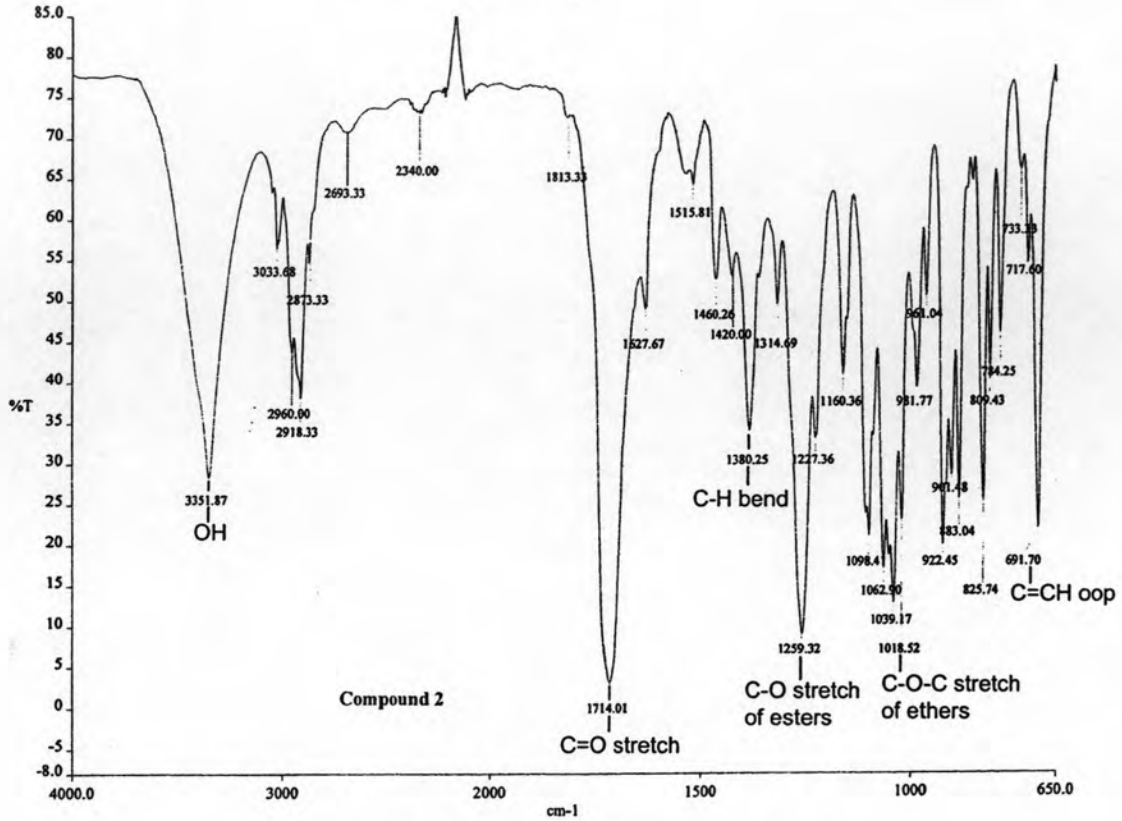

Figure C17 IR spectrum of compound **2**.

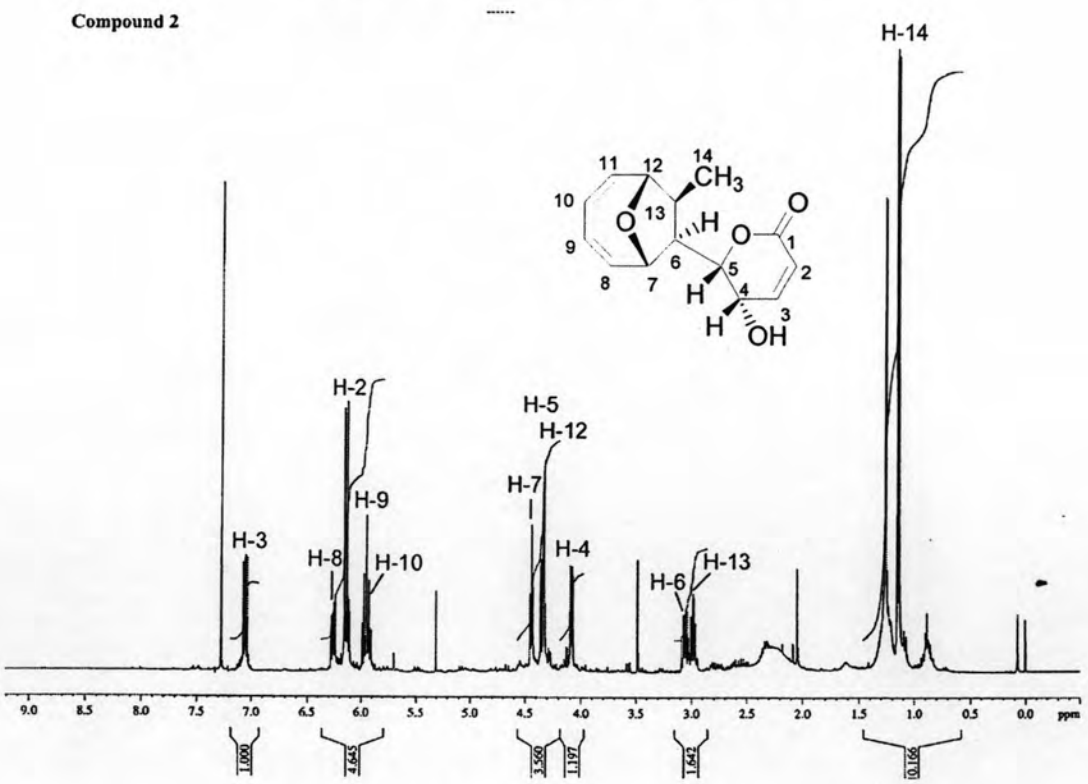

Figure C18 ^{1}H-NMR spectrum of compound **2**.

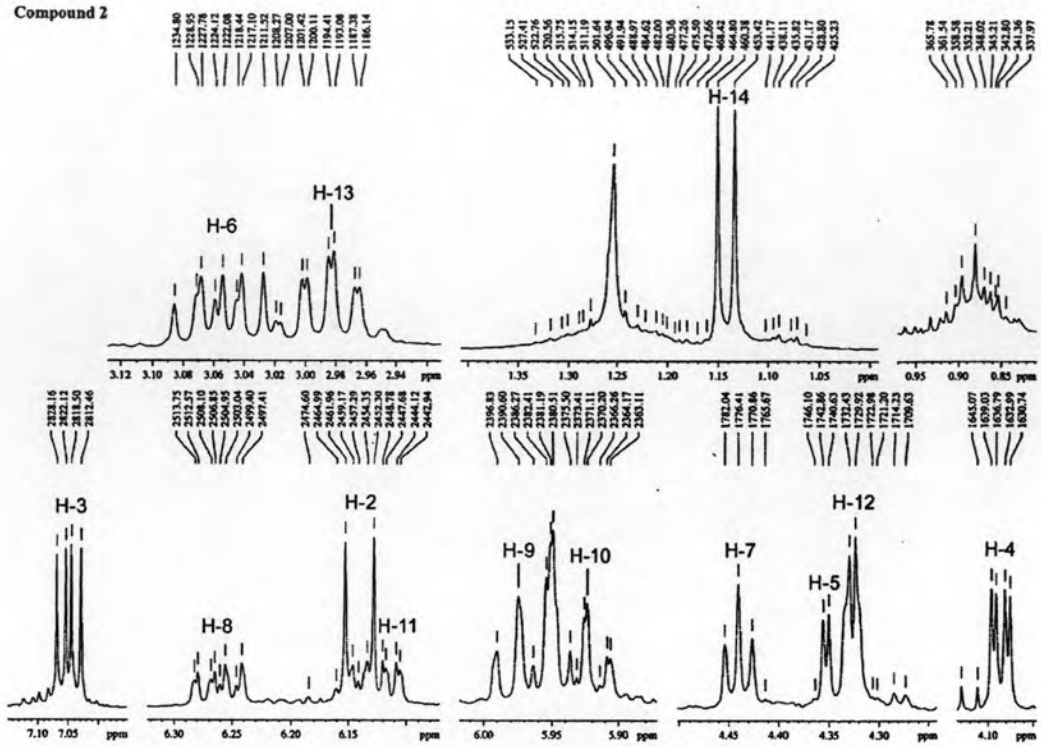

Figure C19 Expansion of Figure C16.

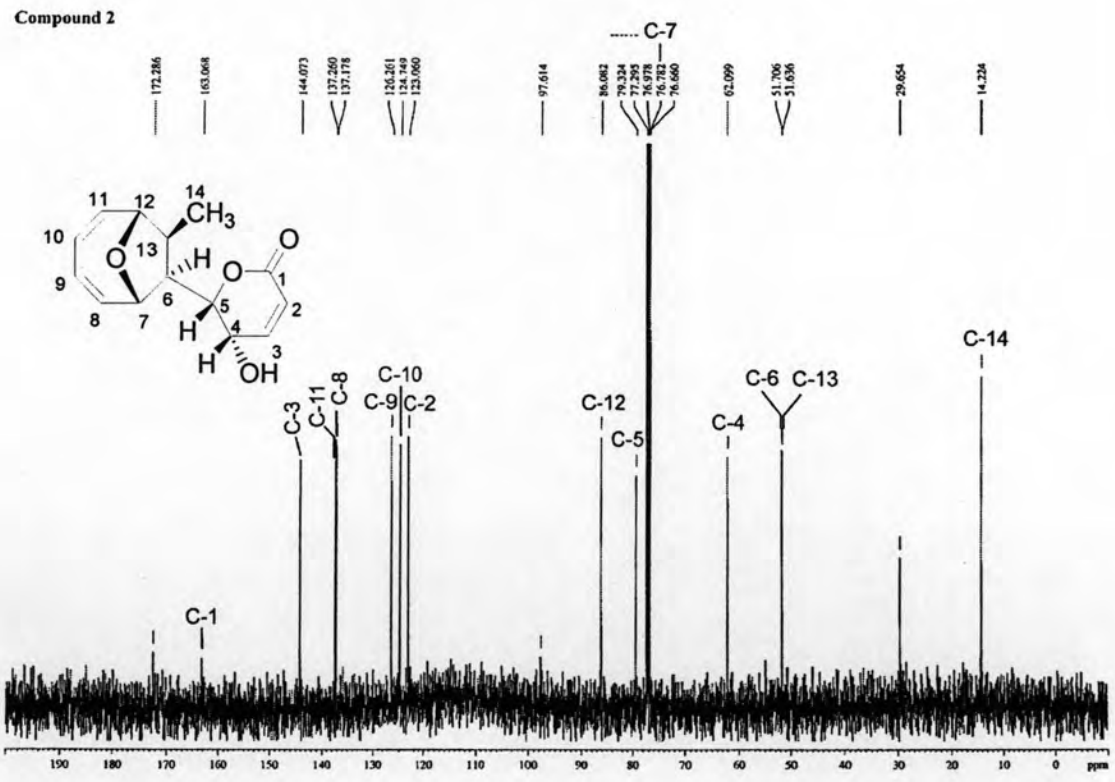

Figure C20 ^{13}C-NMR spectrum of compound **2**.

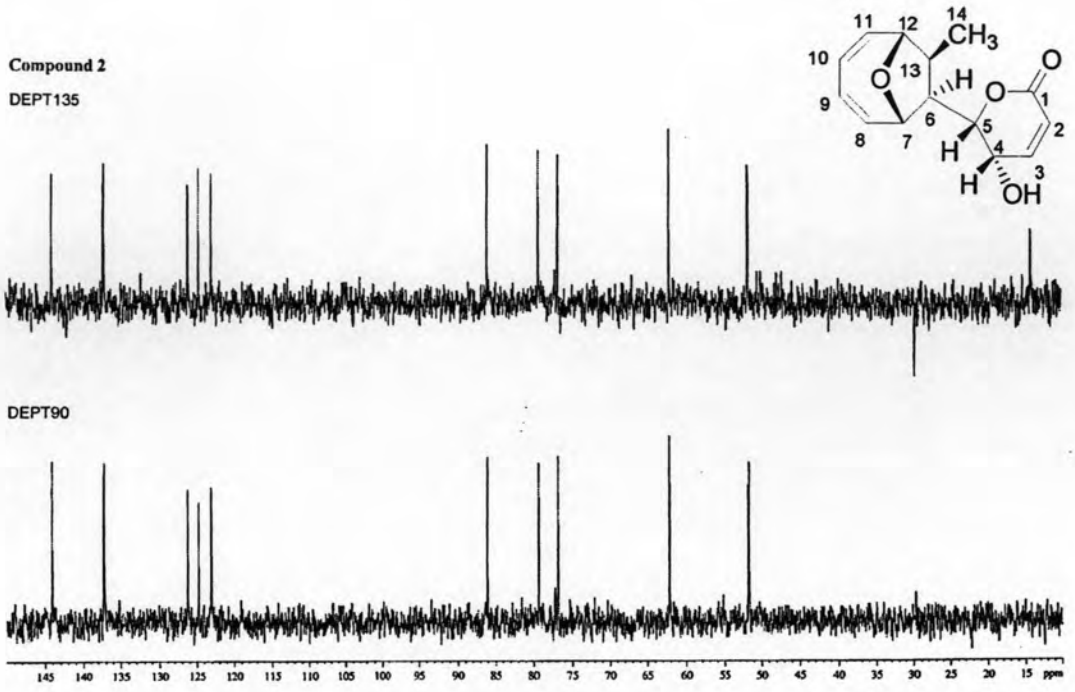

Figure C21 DEPT spectrum of compound **2**.

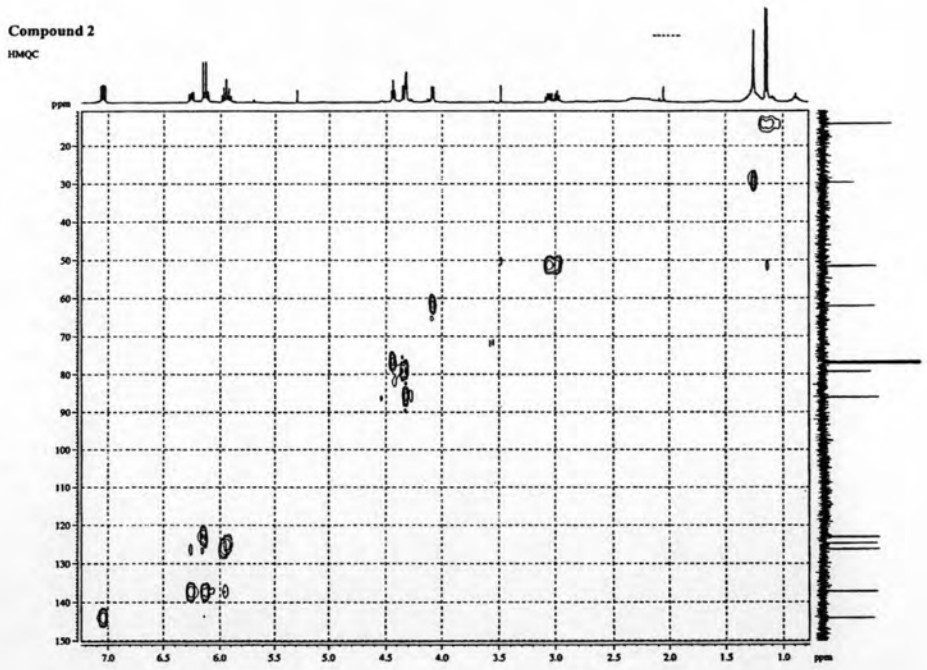

Figure C22 HMQC spectrum of compound **2**.

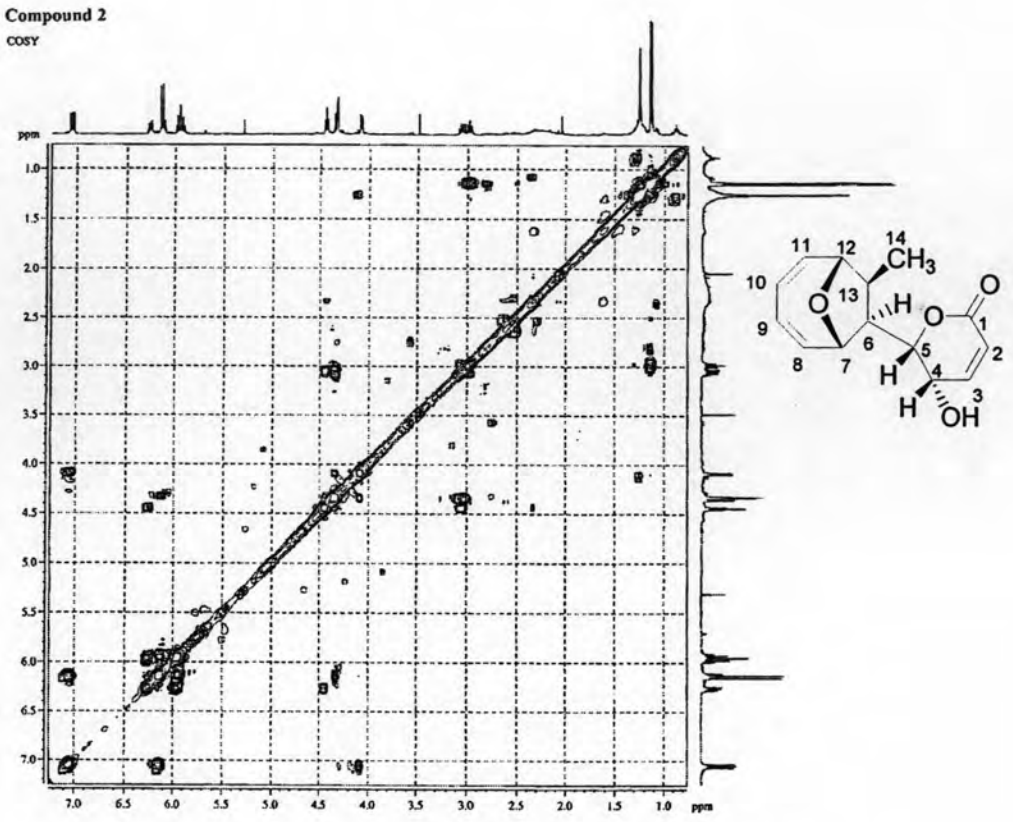

Figure C23 COSY spectrum of compound **2**.

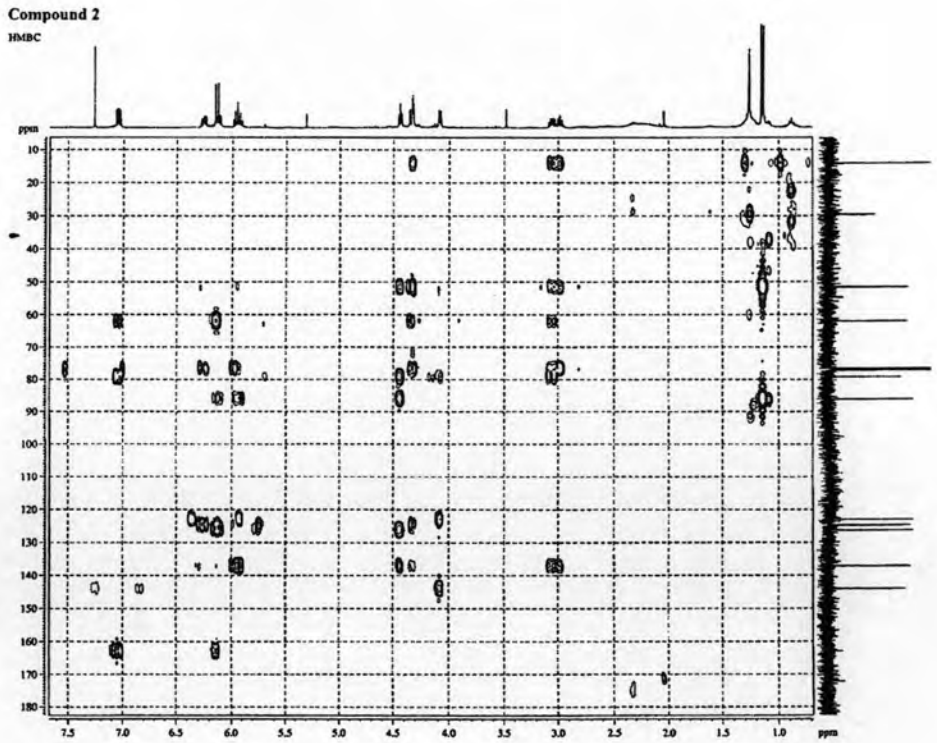

Figure C24 HMBC spectrums of compound **2**.

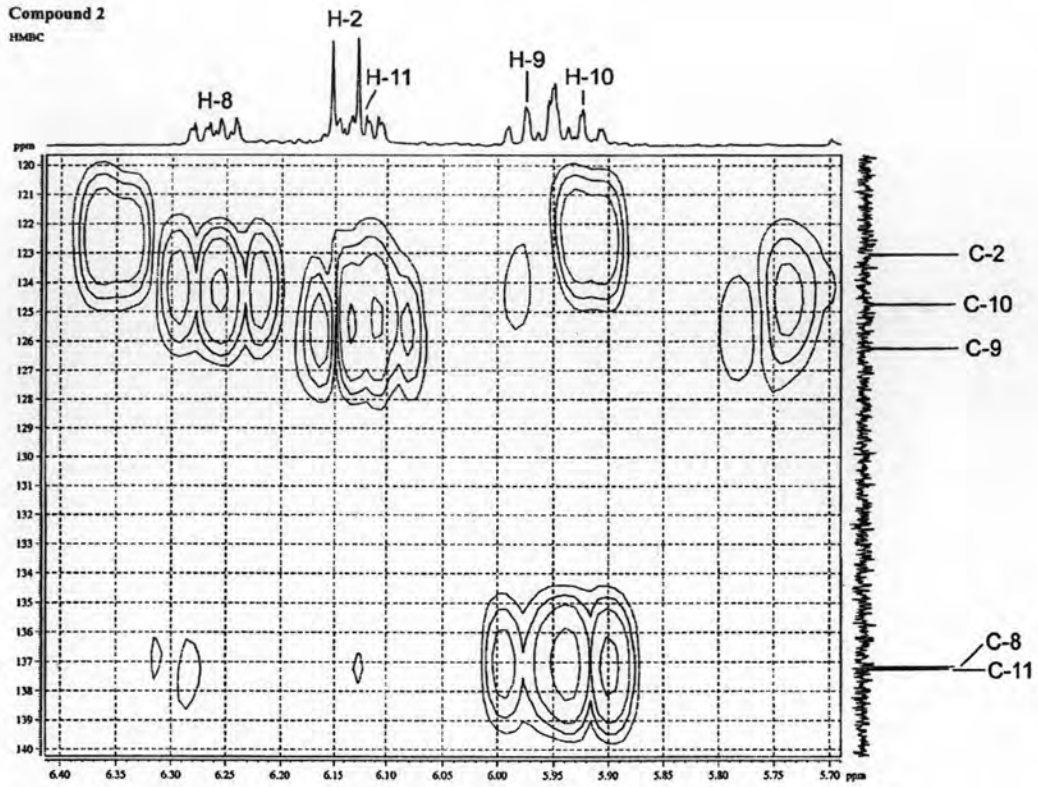

Figure C25 Expansion of Figure C22.

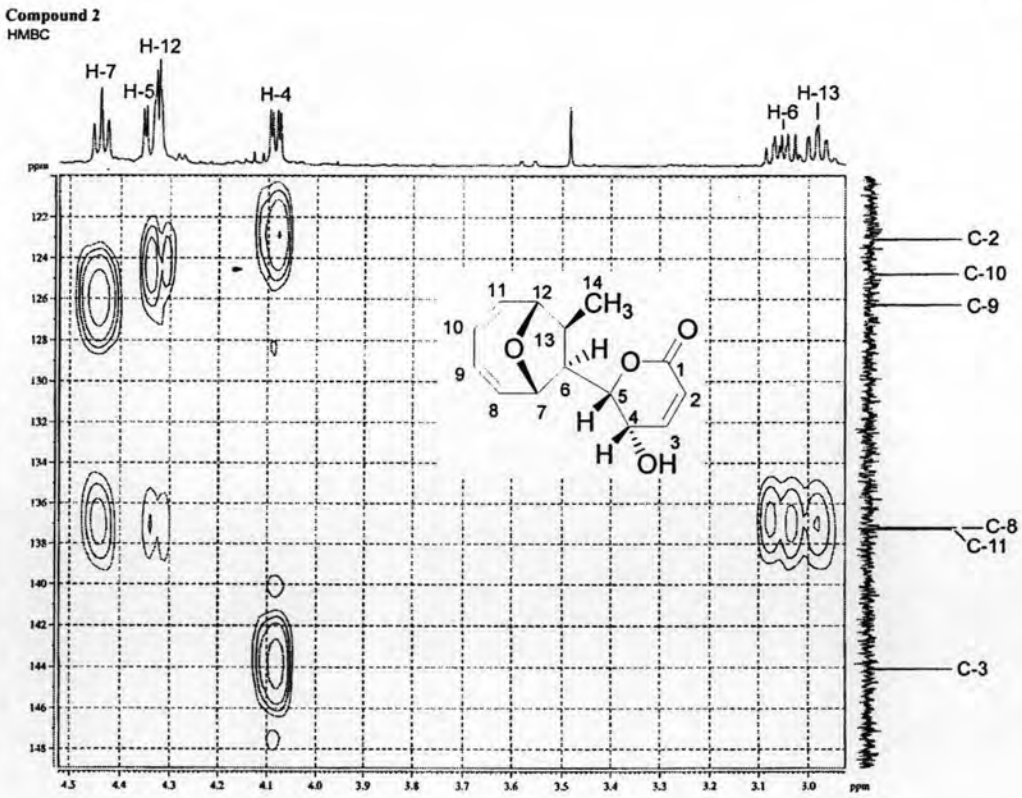

Figure C26 Expansion of Figure C22 (continue).

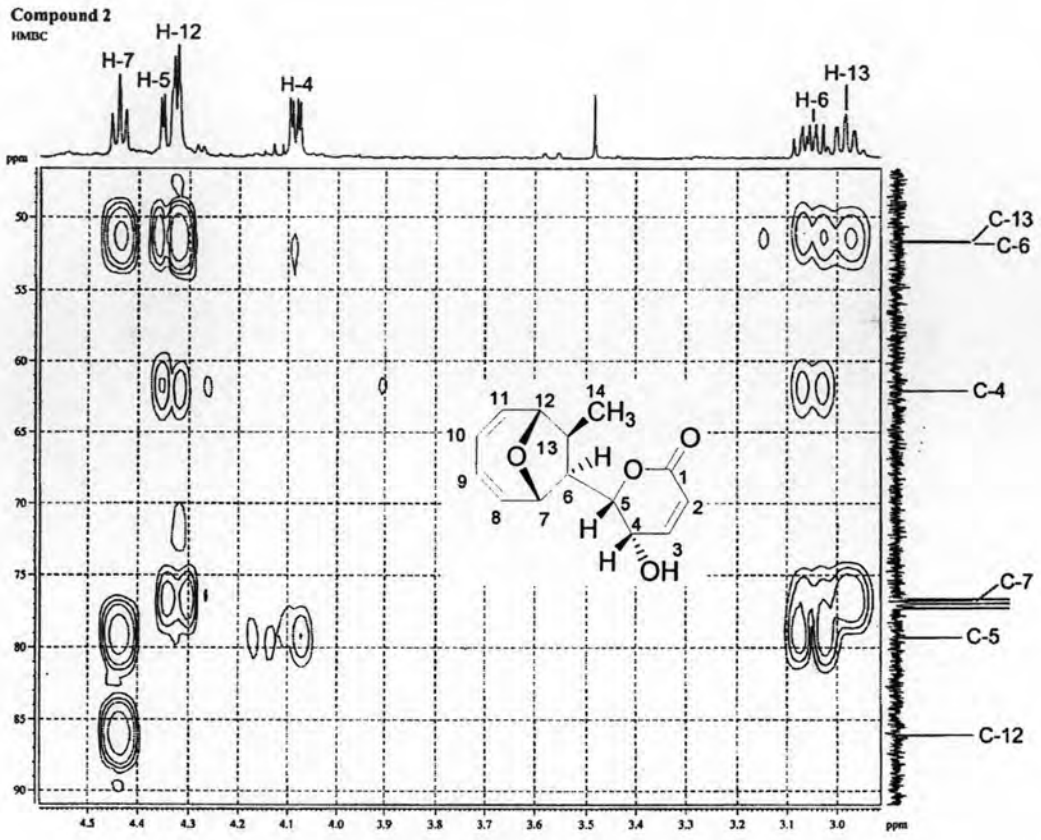

Figure C27 Expansion of Figure C22 (continue).

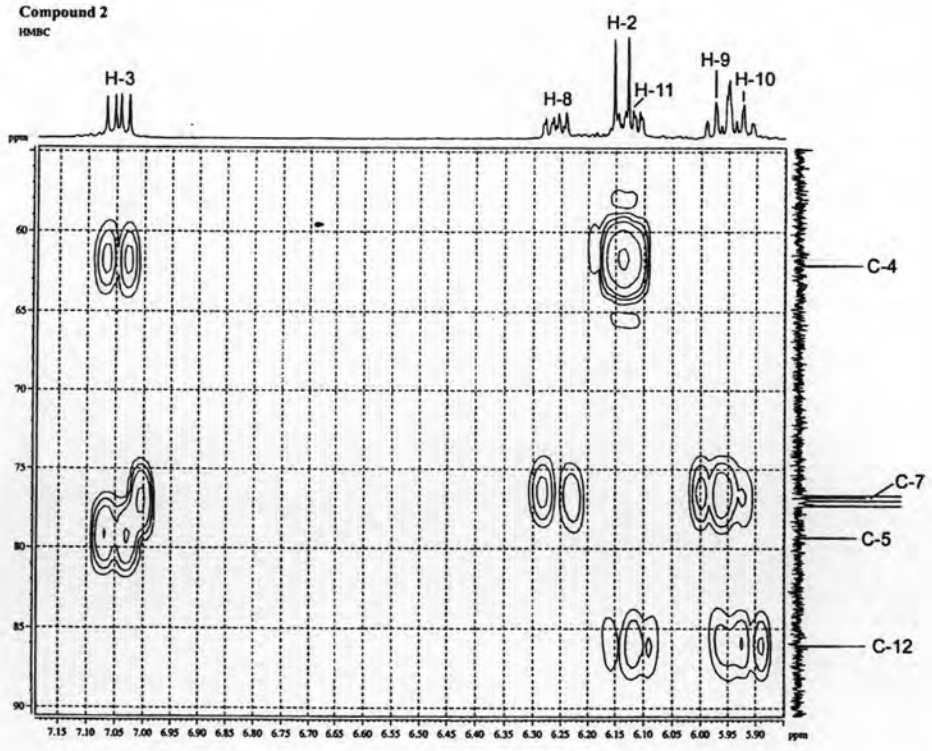

Figure C28 Expansion of Figure C22 (continue).

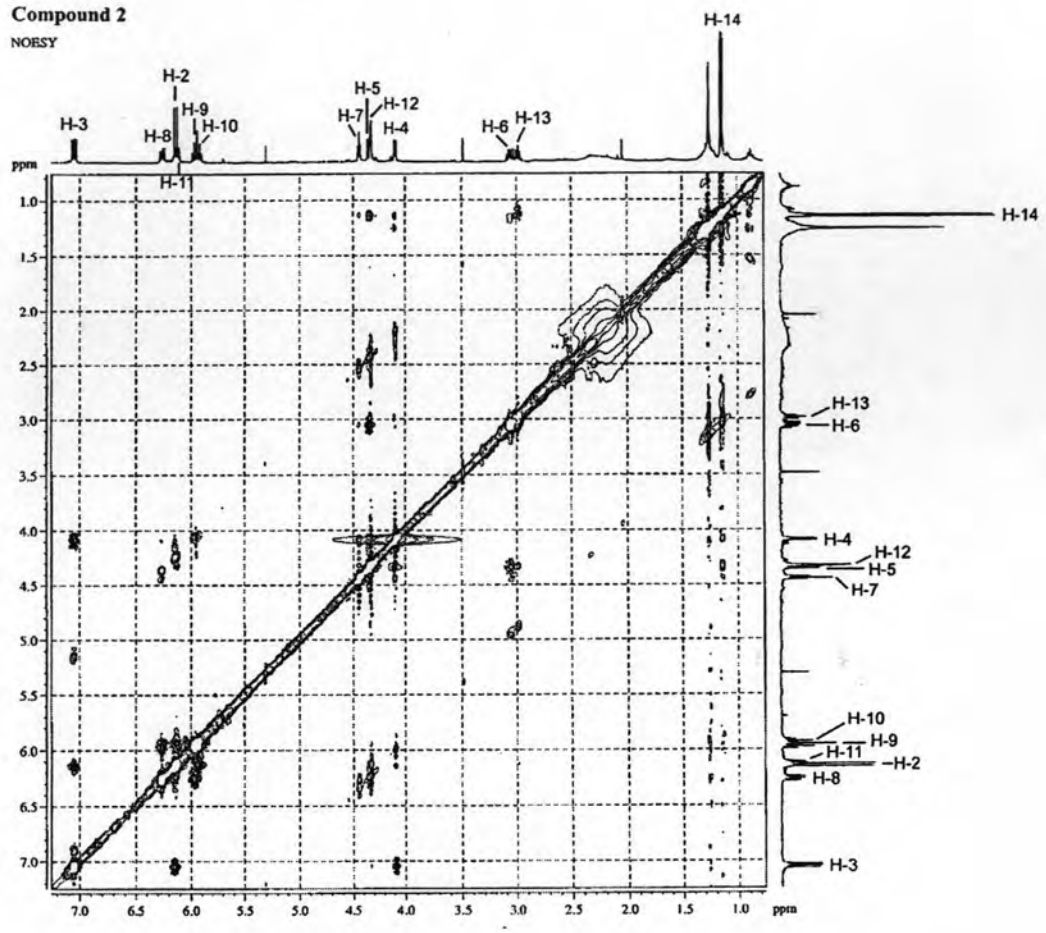

Figure C29 NOESY spectrum of compound **2**.

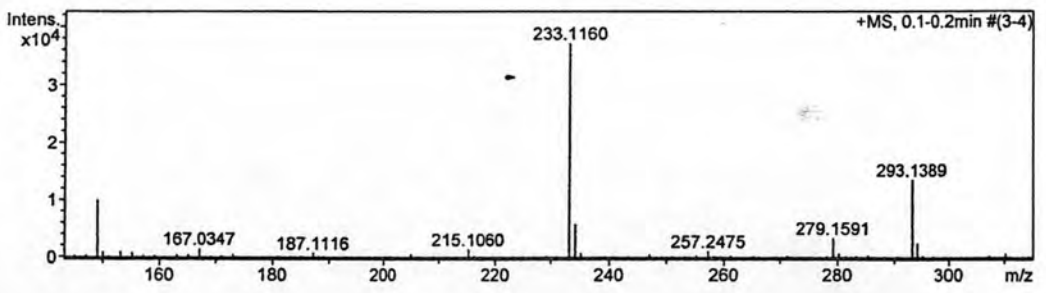

Figure C30 ESI-TOFMS mass spectrum of compound **3**.

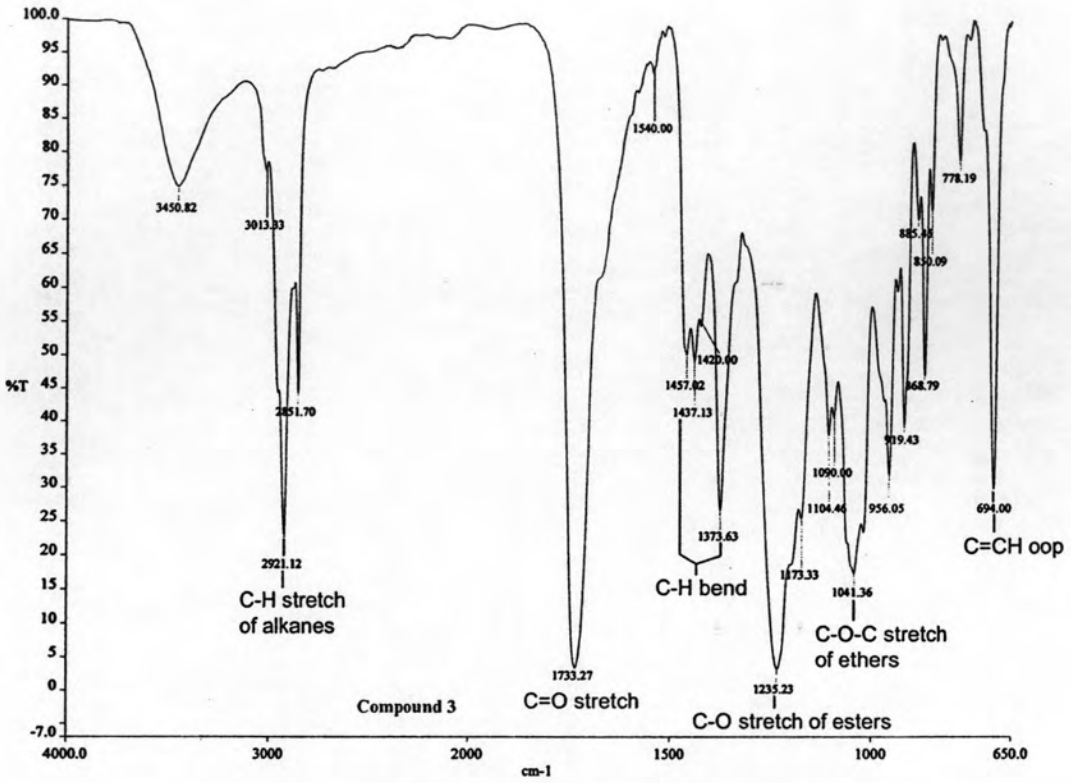

Figure C31 IR spectrum of compound **3**.

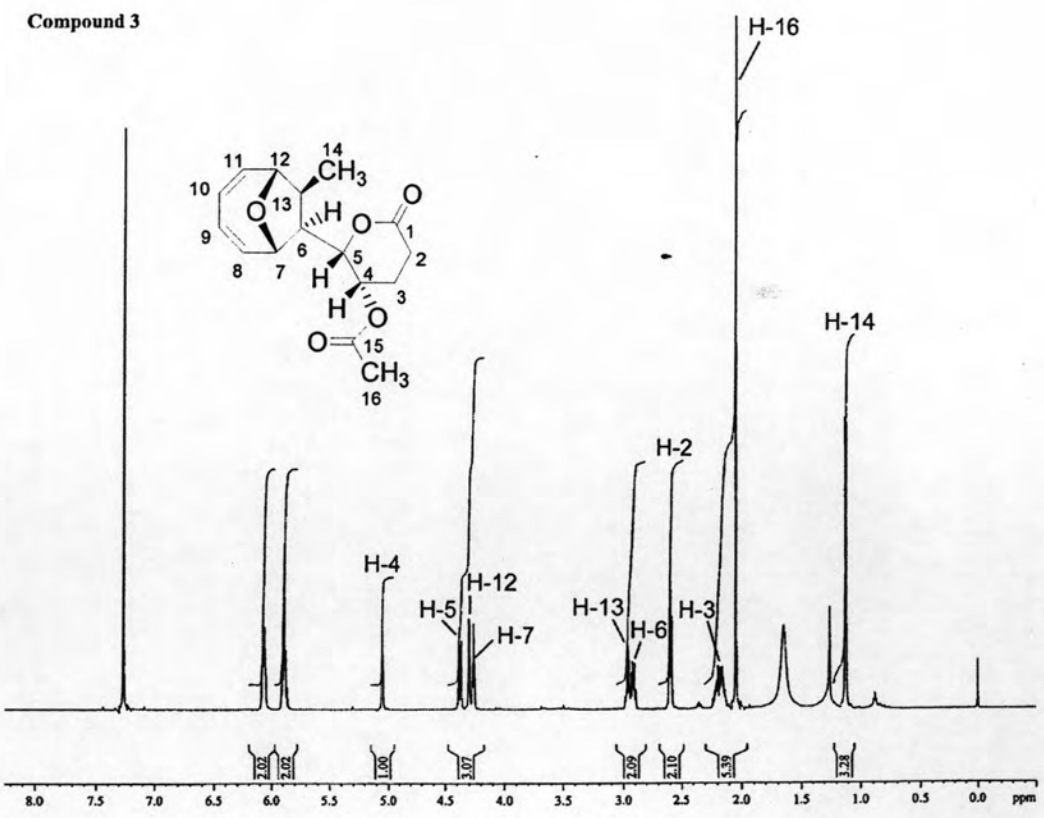

Figure C32 ^{1}H-NMR spectrum of compound **3**.

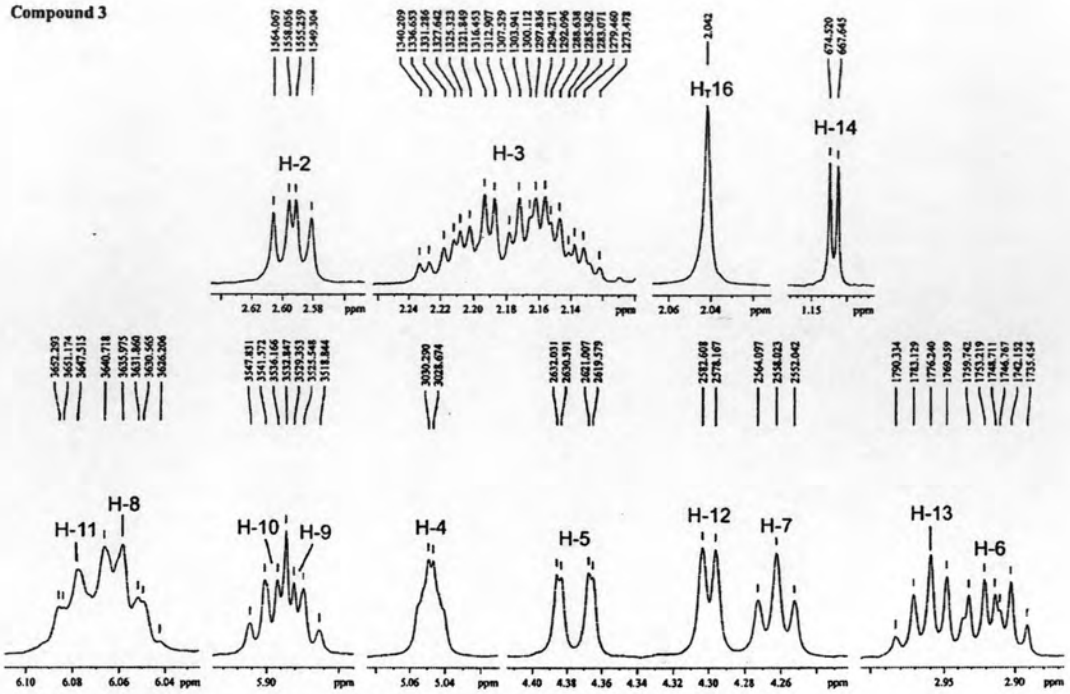

Figure C33 Expansion of Figure C29.

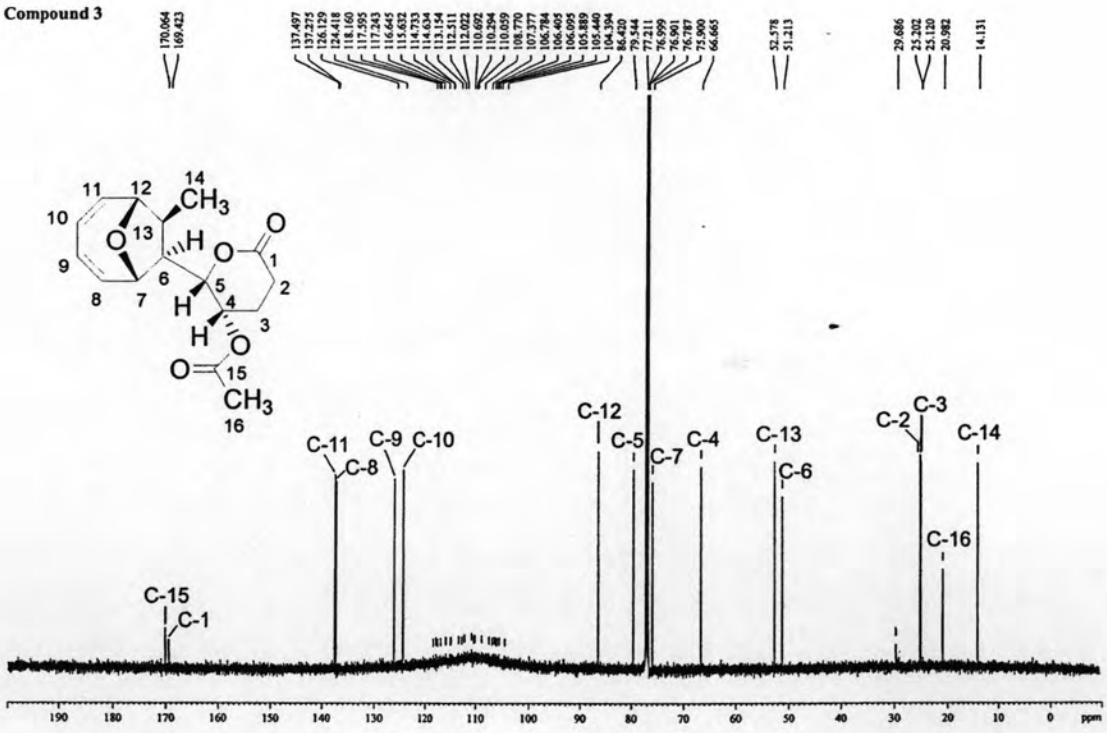

Figure C34 ^{13}C-NMR spectrum of compound **3**.

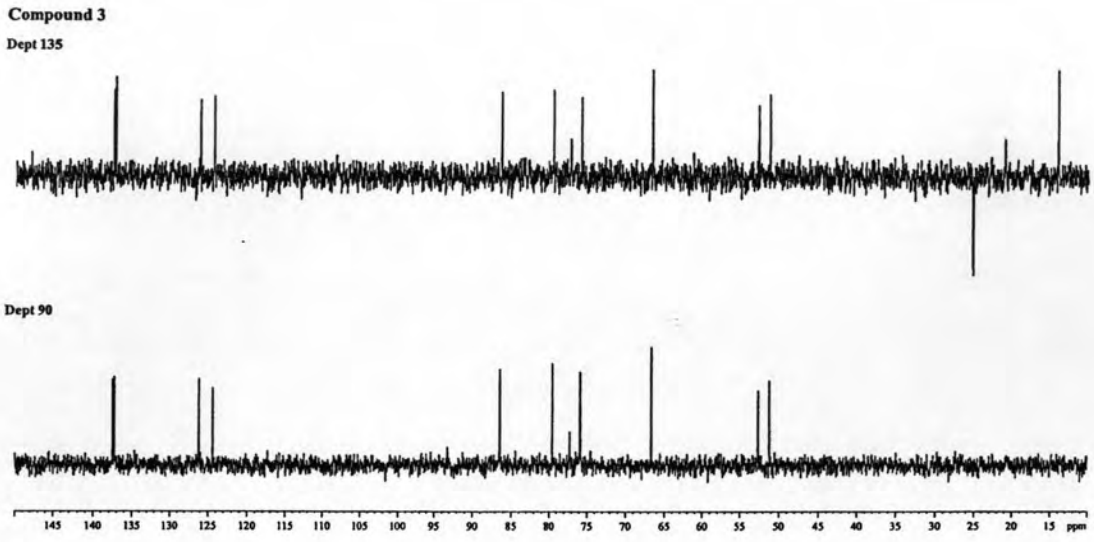

Figure C35 DEPT spectrum of compound **3**.

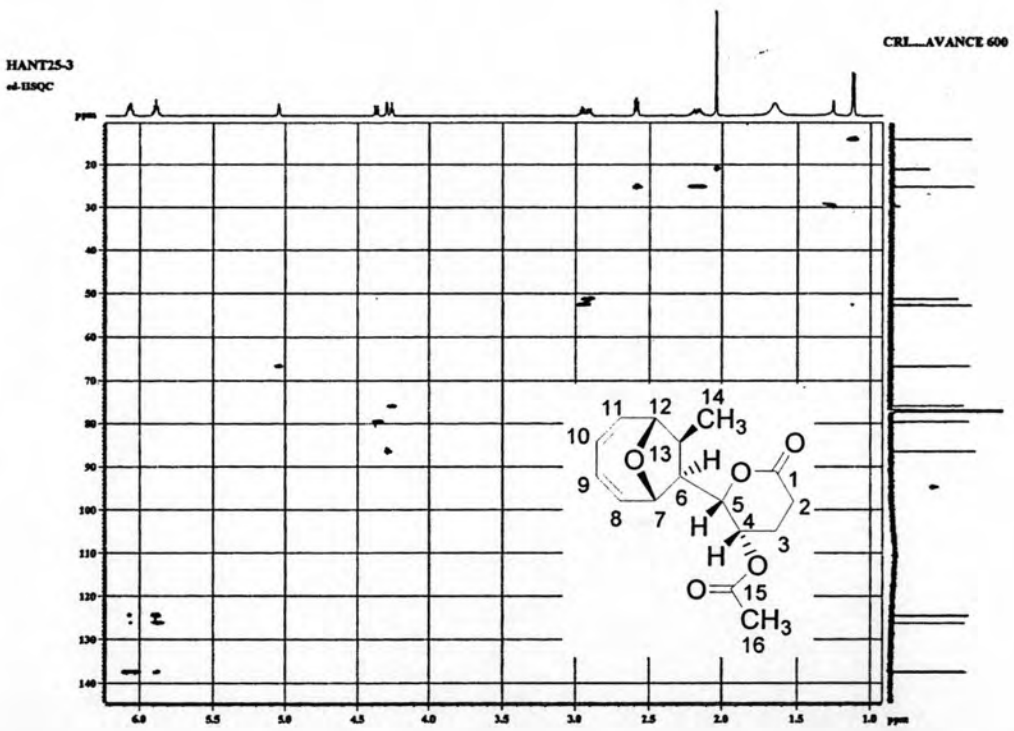

Figure C36 HSQC spectrum of compound **3**.

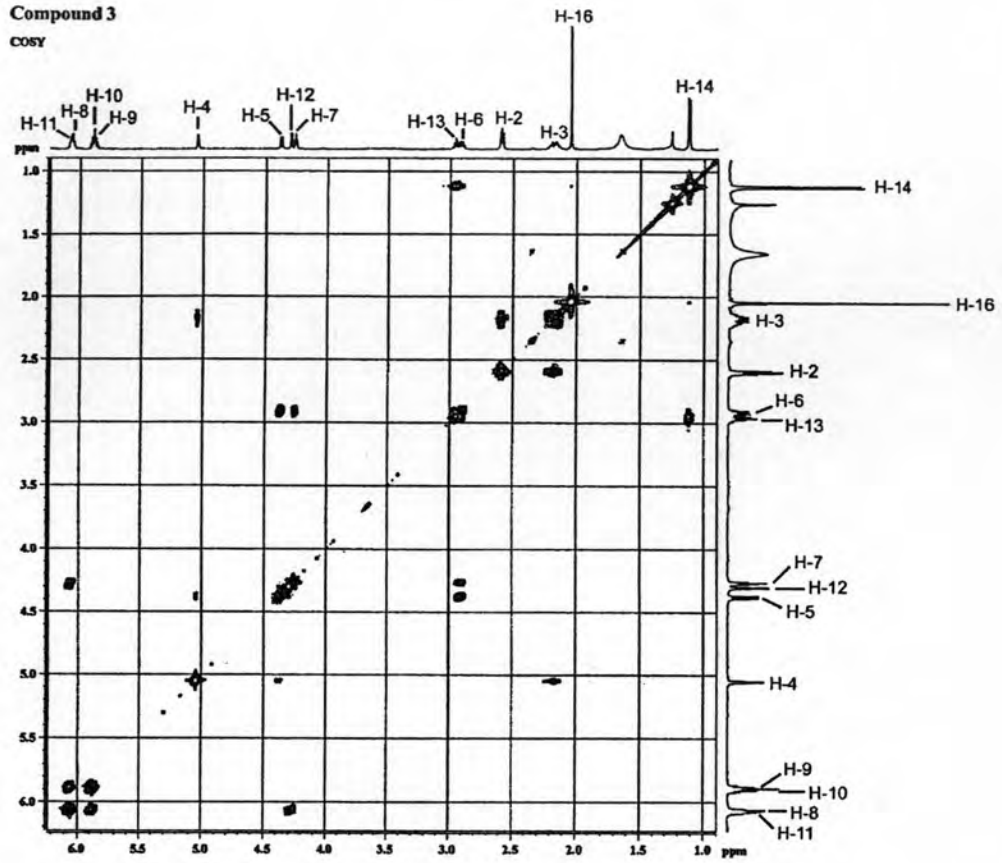

Figure C37 COSY spectrum of compound **3**.

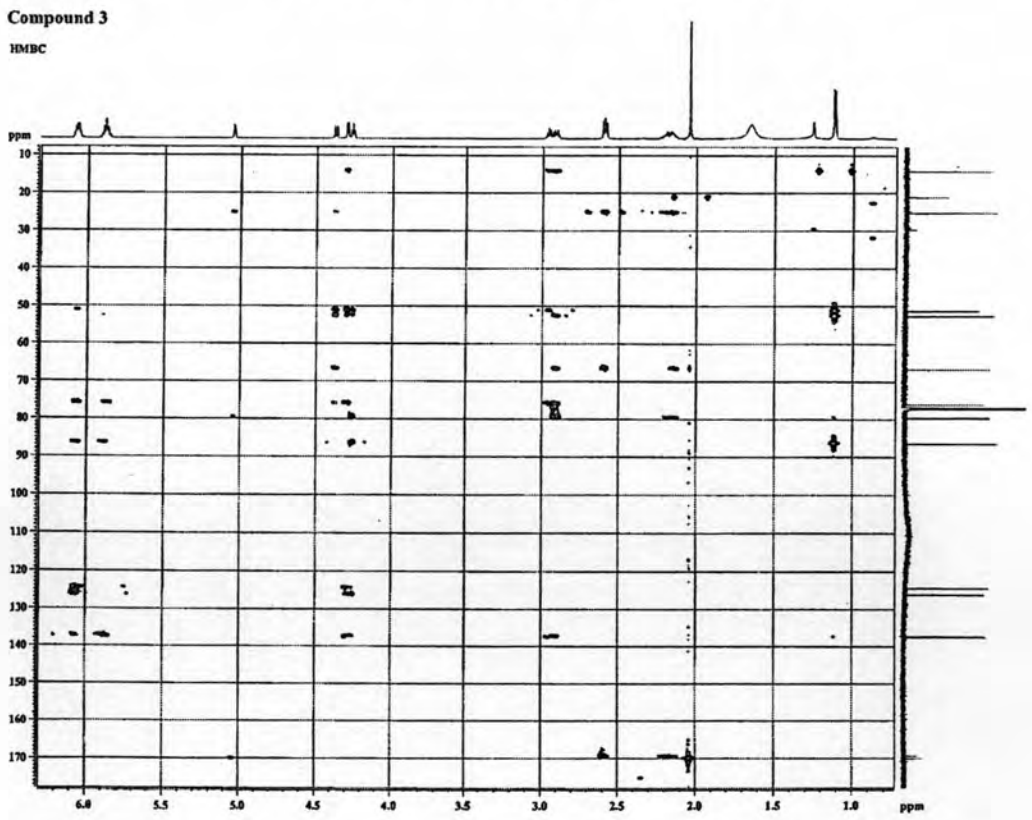

Figure C38 HMBC spectrum of compound **3**.

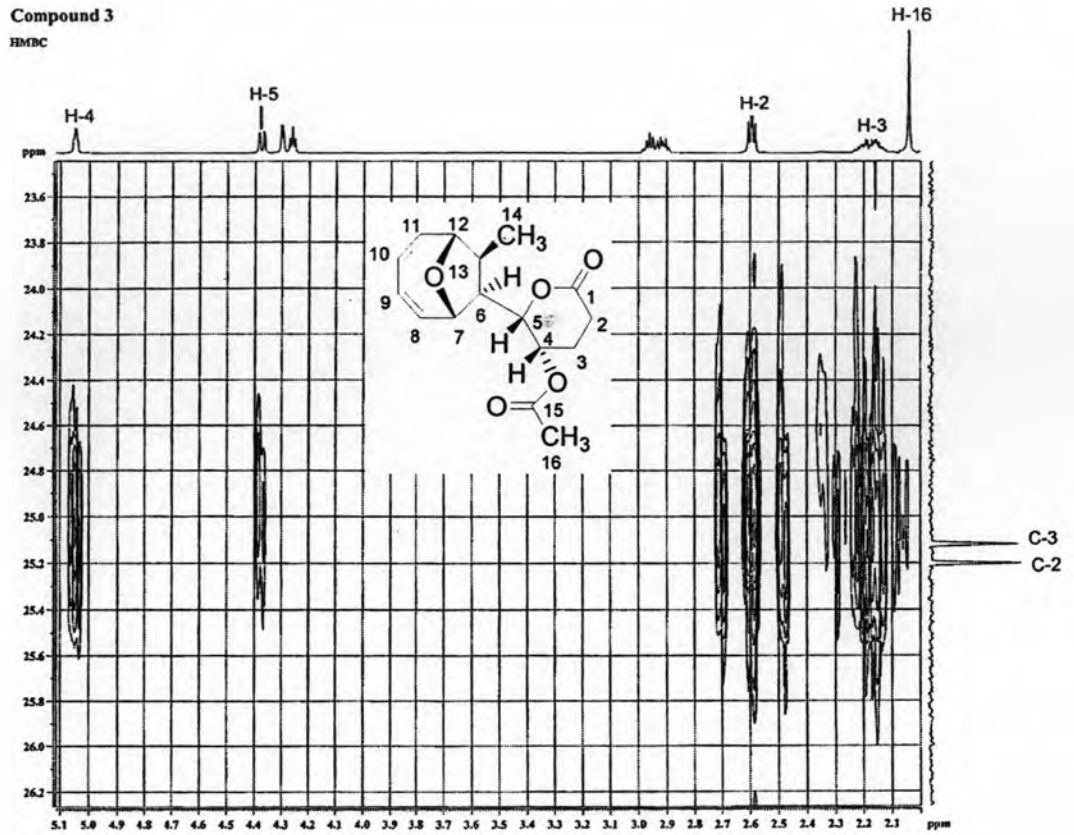

Figure C39 Expansion of Figure C35.

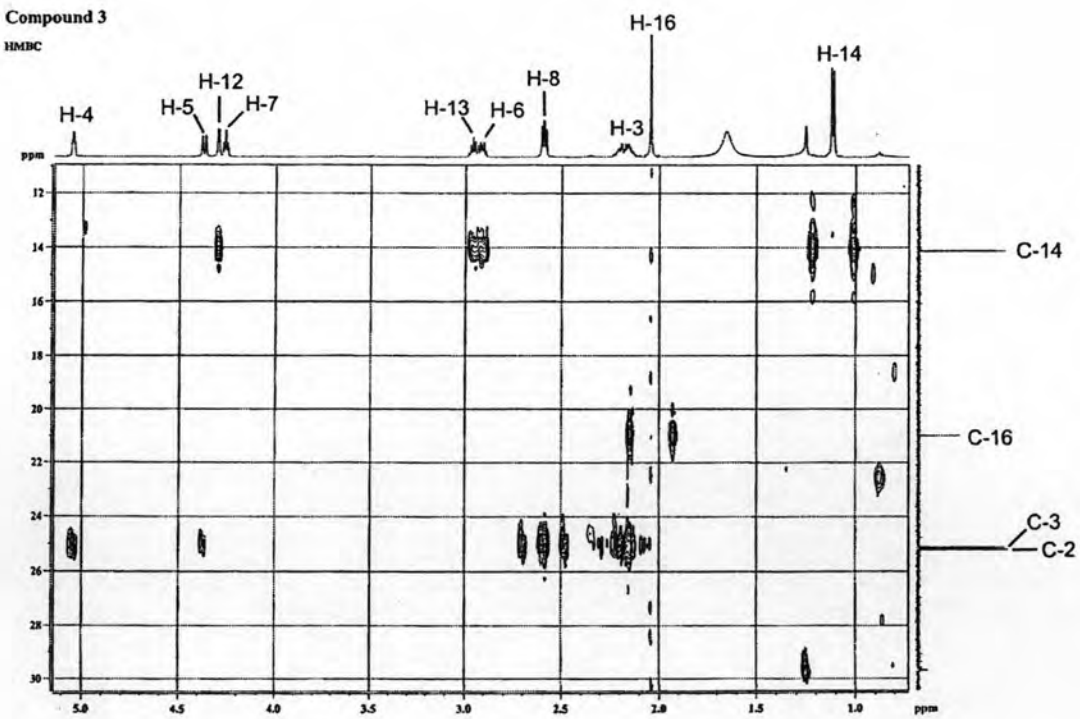

Figure C40 Expansion of Figure C35 (continue).

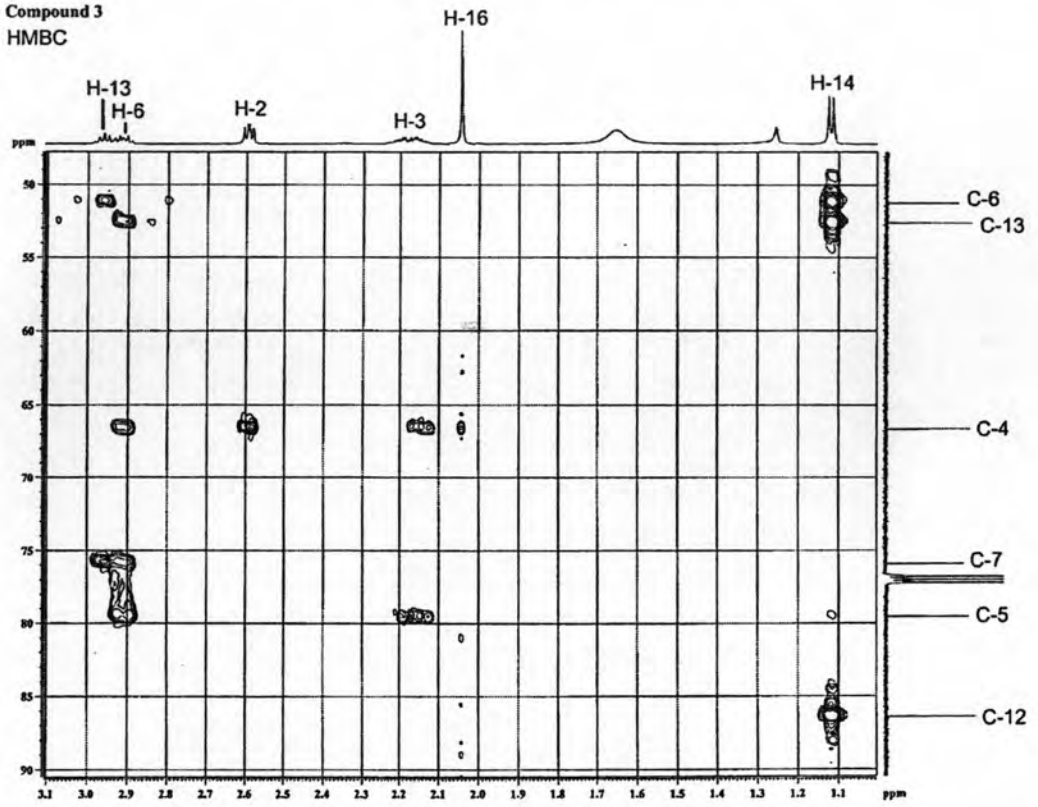

Figure C41 Expansion of Figure C35 (continue).

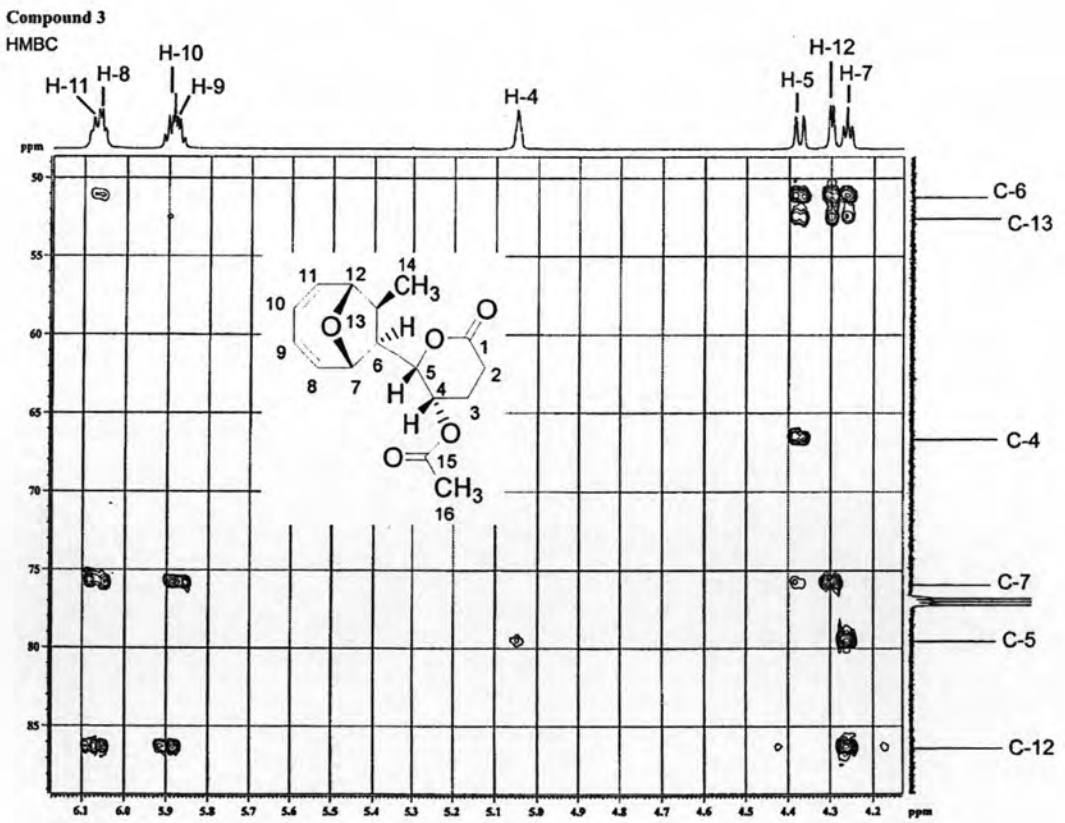

Figure C42 Expansion of Figure C35 (continue).

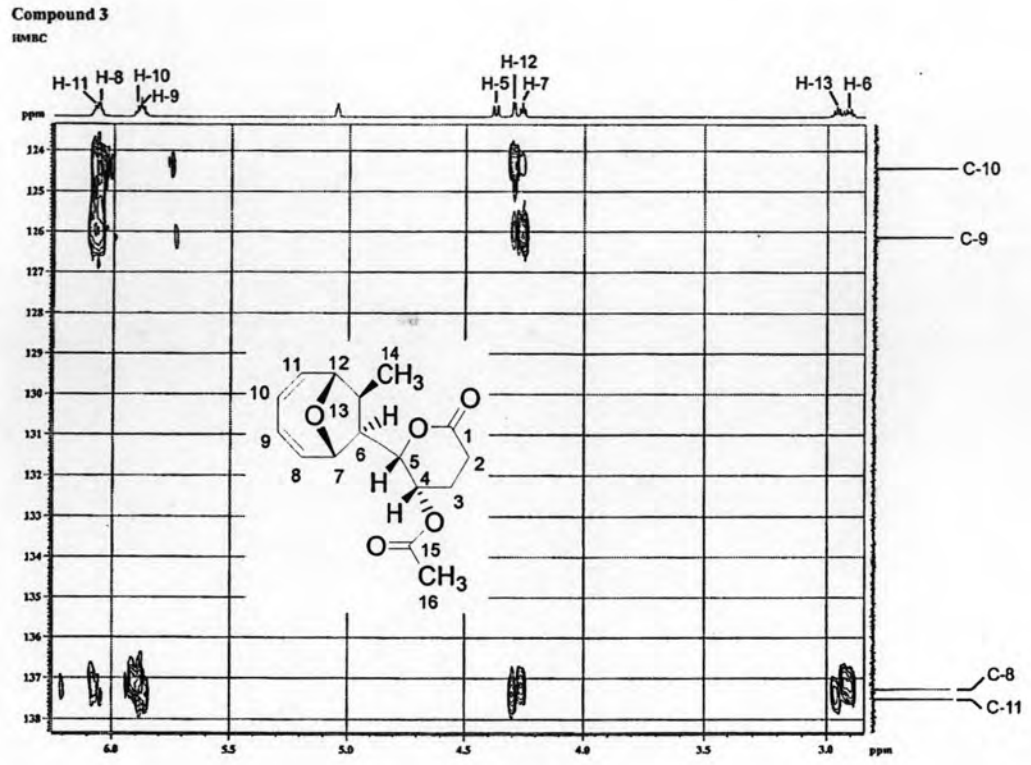

Figure C43 Expansion of Figure C35 (continue).

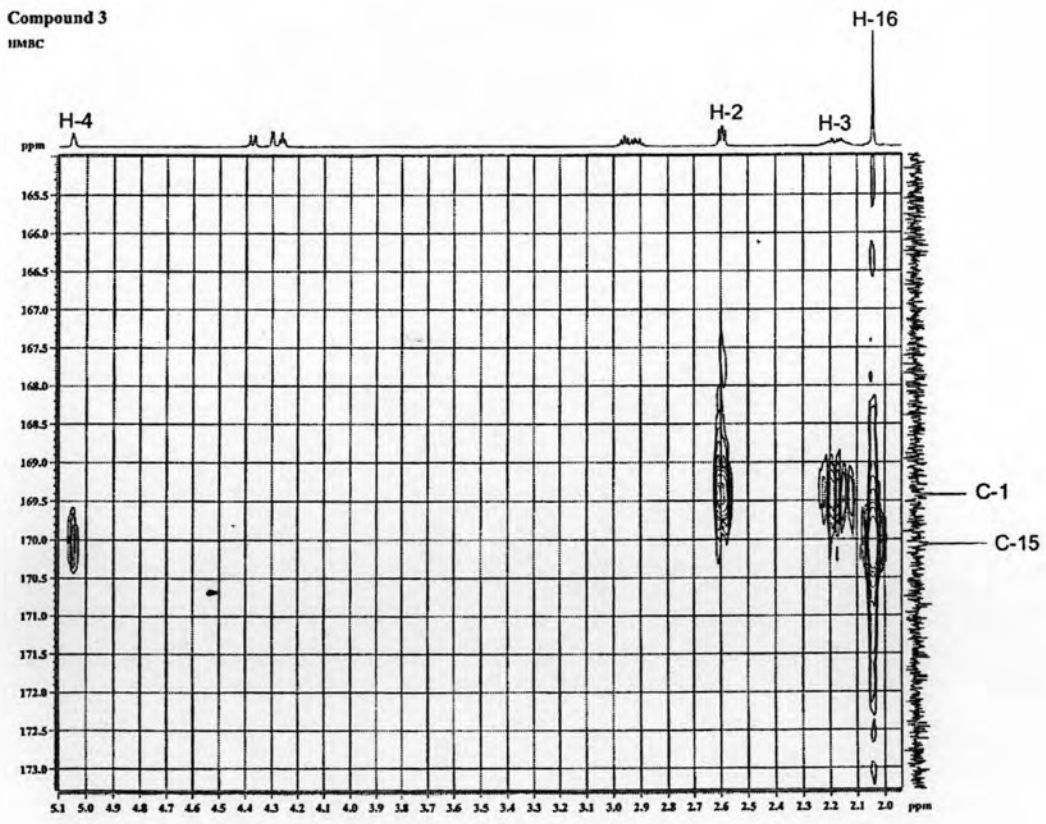

Figure C44 Expansion of Figure C35 (continue).

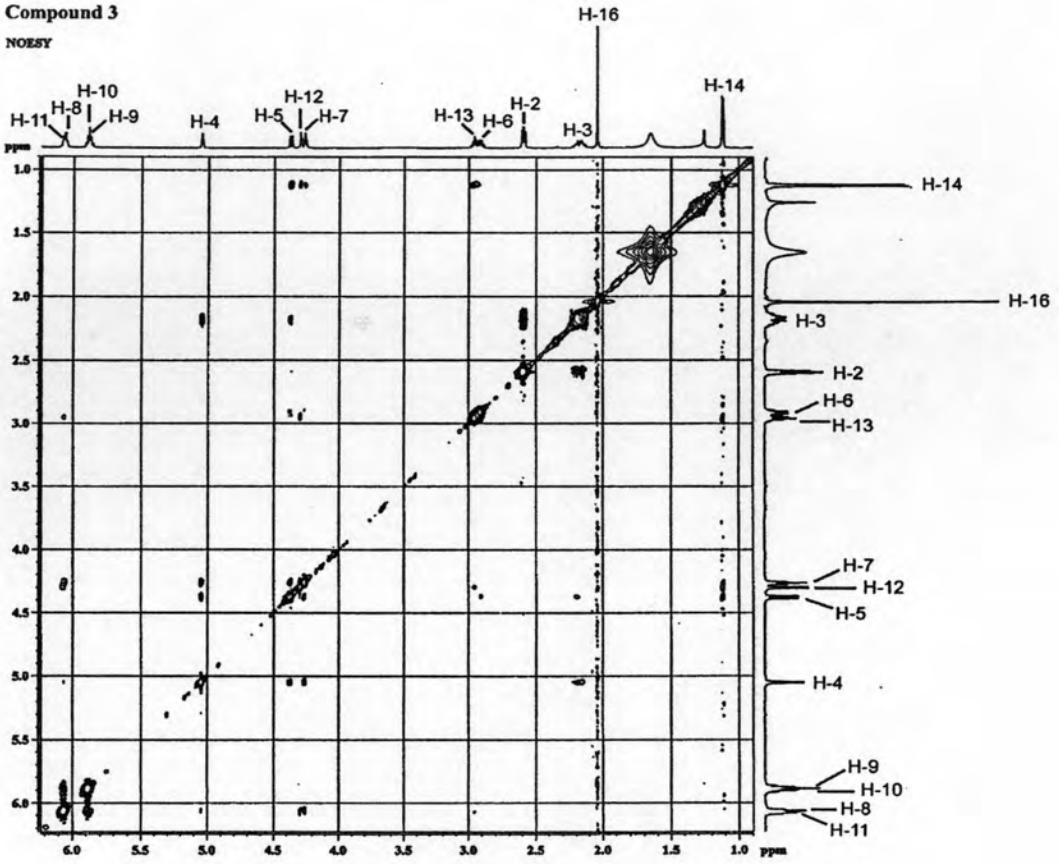

Figure C45 NOESY spectrum of compound **3**.

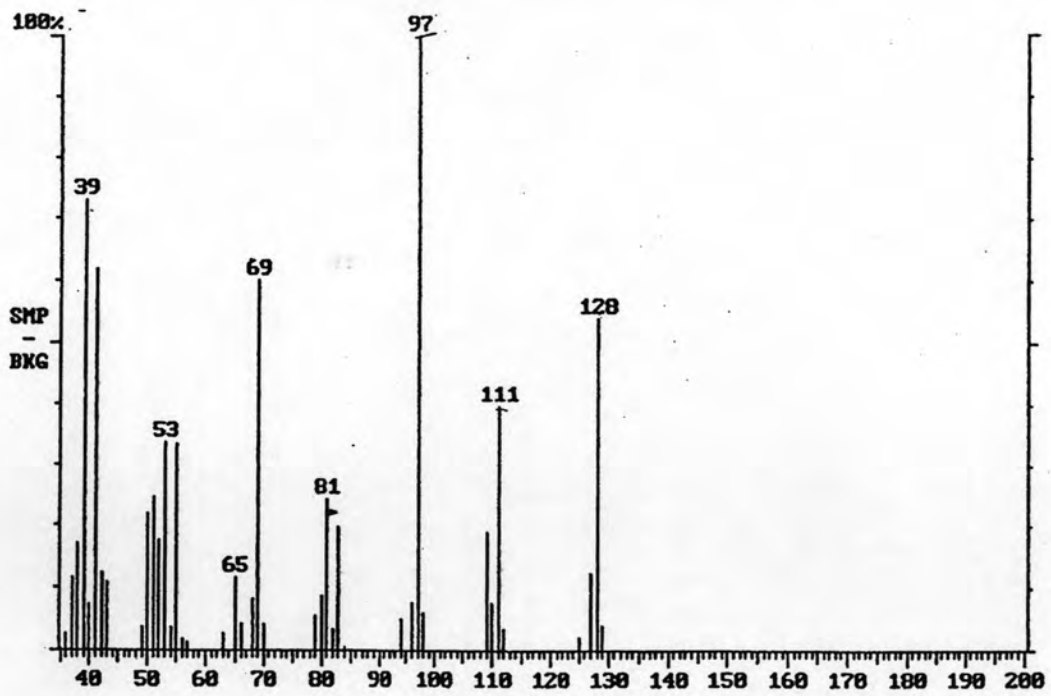

Figure C46 GC-MS spectrum of compound **4**.

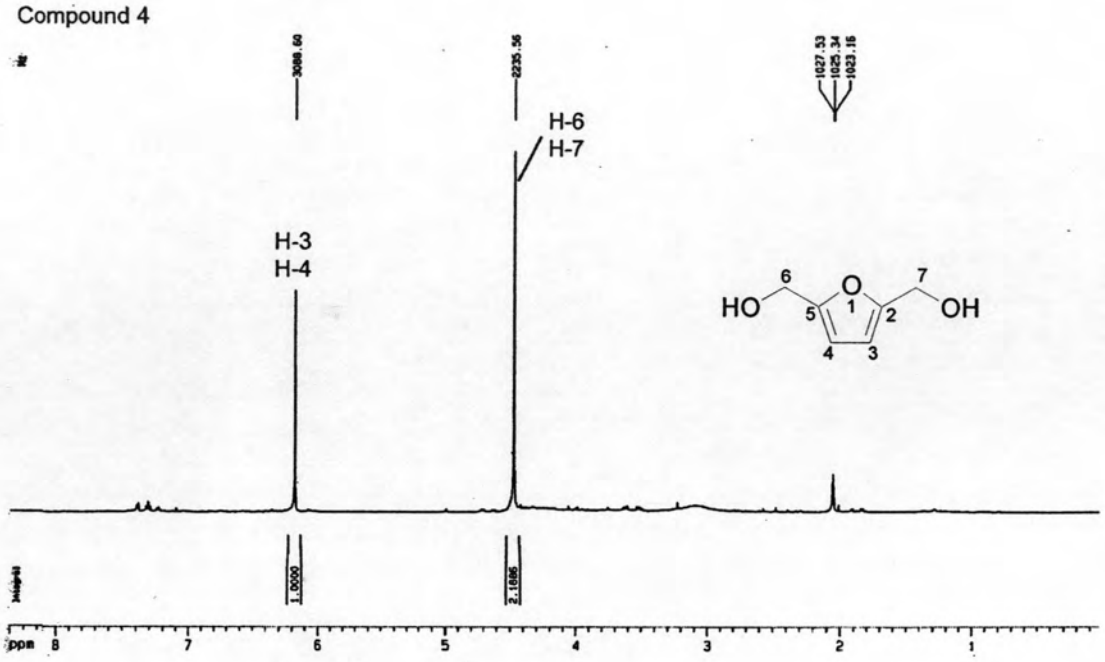

Figure C47 ^{1}H-NMR spectrum of compound **4**.

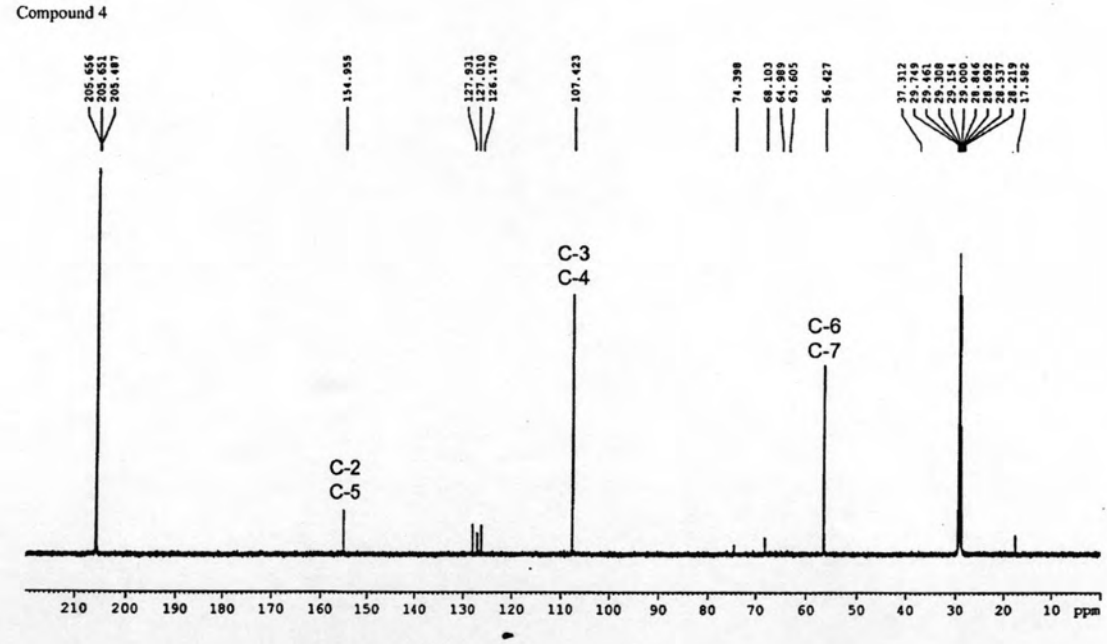

Figure C48 ^{13}C-NMR spectrum of compound **4**.

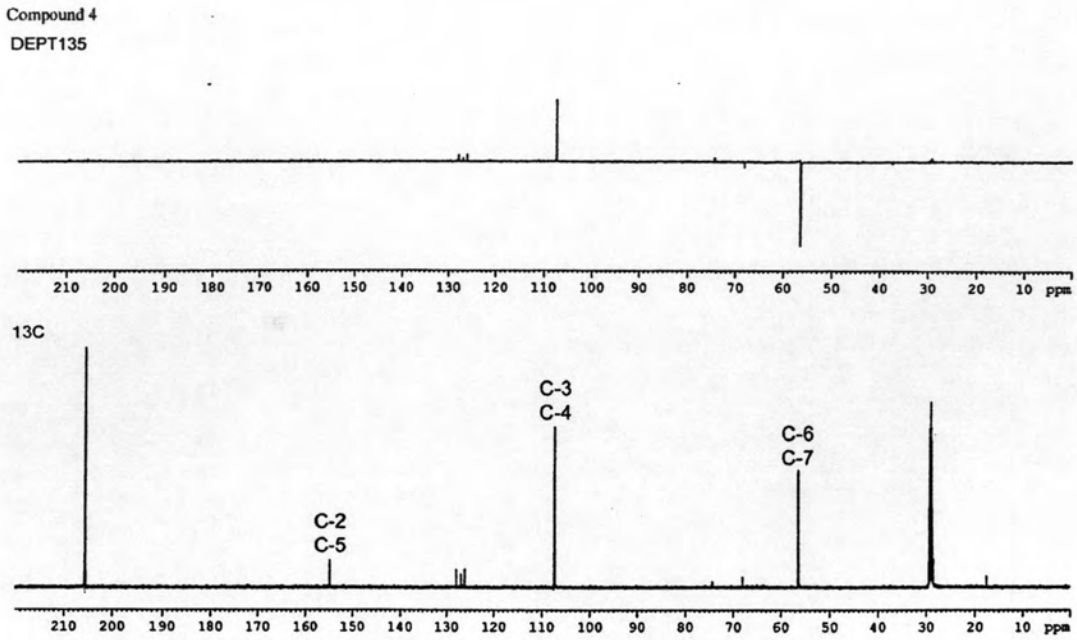

Figure C49 DEPT spectrum of compound **4**.

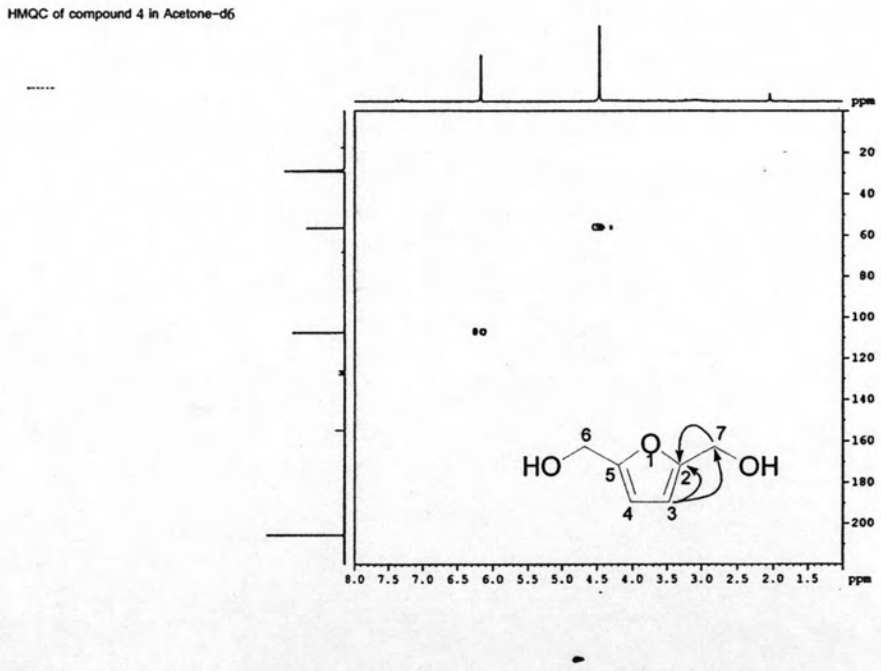

Figure C50 HMQC spectrum of compound **4**.

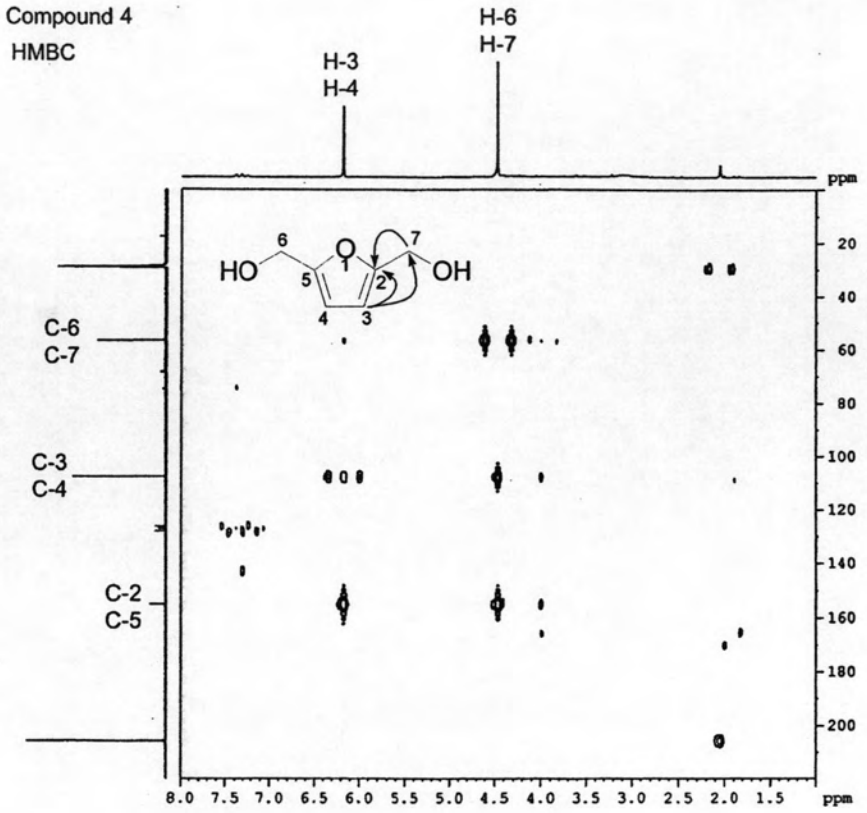

Figure C51 HMBC spectrum of compound **4**.

BIOGRAPHY

Mr. Surasak Prachya was born on August 11, 1976 in Nakhonpathom province, Thailand. He graduated with a Bachelor Degree of Science in Biotechnology Department from the Faculty of Industrial Technology, Silpakorn University, Thailand in 2001. He had been studying for a Master Degree of Science in Biotechnology, Faculty of Science, Chulalongkorn University, Thailand since 2003.