CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (ENGLISH)	iv
ABSTRACT (THAI)	vi
LIST OF TABLES	xi
LIST OF ILLUSTRATIONS	xii
LIST OF ABBREVIATIONS	xvii
PART I: Isolation of Glycosides by Electrolytic Decolourization	
CHAPTER I : Introduction	
1. Definition and History	1
2. Mechanisms and Advantages/disadvantages	. 3
3. Applications	7
4. Purpose of Research	9
5. References	10
CHAPTER II: Model studies of aplication of electrocoagulation	13
1. Electrocoagulation unit	13
2. Model studies	14
2.1 Preliminary Investigation of Electrolytic Decolourization	14
2.2 Electrocoagulation of dyes	16
2.2.1 Synthetic dyes	16
2.2.2 Natural dyes	21
2.3 Electrocoagulation of phenolic compounds	24
2.4 Electrocoagulation of pure glycosides	26
3. Model studies of electrocoagulation of chlorophyll	29
3.1 Electrocoagulation of standard chlorophyllin	29
3.2 Electrocoagulation of chlorophyll from Stevia	31

	3.3 Electrocoagulation of standard chlorophyllin and stevia extract	32
	in difference ratio of water-ethanol solutions	
4.	Conclusion	35
6.	Experimental	36
7.	References	36
CHAI	PTER III: Isolation of Glycosides and Other Natural Compounds from	38
	Plants using Electrolytic Decolourization	
1.	Glycyrrhizin from Glycyrrhiza radix (Licorice)	38
	1.1 Introduction	38
	1.2 Results and discussion	43
	1.3 Experimental	44
	1.4 References	47
2.	D-Pinitol from Cassia siamea Lamk	50
	2.1 Introduction	50
	2.2 Results and discussion	56
	2.3 Experimental	60
	2.4 References	61
3.	Asiaticoside from Centella asiatica	64
	3.1 Introduction	64
	3.2 Results and discussion	67
	3.3 Experimental	74
	3.4 References	75
4.	Mukurozioside from Sapindus rarak	77
	4.1 Introduction	77
	4.2 Results and discussion	78
	4.3 Experimental	89
	4.4 References	90
5.	Electrocoagulation study on various plants	91
	5.1 Introduction	91

	5.2 Results and discussion	91
	5.3 Experimental	93
6.	Conclusion	93
PART	III: Synthesis of Pentenomycin	94
1.	Introduction	95
. 2.	Objective	112
3.	Proposed synthetic approaches to pentenomycin	112
4.	Results and Discussion	112
	4.1 Synthesis of cyclopentenones prior to pentenomycins	112
	4.2 Epoxidation of Cyclopentenone leading to pentenomycins	122
	4.3 Hydrolysis of spiro epoxide leading to pentenomycins and	133
_	epipentenomycins	
5.	Experimental	140
6.	References	152
APPE	NDIX	155
VITA		165

LIST OF TABLES

	Page
PART I: Isolation of Glycosides by Electrolytic Decolourization	
CHAPTER I : Introduction	
CHAPTER II: Model studies of aplication of electrocoagulation	
Table 1. The absorption of dye solutions after electrocoagulation.	16
Table 2. Electric current value during electrolysis of each extraction.	35
CHAPTER III: Isolation of Glycosides and Other Natural Compounds from	
Plants using Electrolytic Decolourization	
1. Glycyrrhizin from Glycyrrhiza radix	
2. Pinitol from Cassia siamea Lamk	
Table 1. The 100 MHz ¹³ C NMR (DMSO) spectral data of pinitol	57
3. Asiaticoside from Centella asiatica	
Table 1. ¹ H NMR and ¹³ C spectral data of asiaticoside, (in CD ₃ OD)	73
4. Mukurozioside from Sapindus rarak	
Table 1. ¹³ C-NMR chemical shifts of aglycone moieties of mukurozioside IIb	83
(CD ₃ OD, TMS as internal standard).	
Table 2. ¹³ C-NMR chemical shifts of sugar moieties of mukurozioside IIb	84
(CD ₃ OD, TMS as internal standard).	
5. Electrocoagulation study on various plants	
Table 1. TLC analysis before and after the electrocoagulation method.	92
PART II : Synthesis of Pentenomycin	
Table 1. Bacteriostatic Activity of 67 (in acetone)	130

LIST OF ILLUSTRATIONS

	Page
PART I: Isolation of Glycosides by Electrolytic Decolourization	
CHAPTER I: Isolation of Glycosides by Electrolytic Decolourization	
Figure 1a. Chromium Water, before/after electrocoagulation	3
Figure 1b. Steam Cleaner Wastewater, before/after electrocoagulation	3
Figure 2. Complexation of phenols with Al ³⁺	5
Figure 3. Reduction of quinones to phenols, followed by complexation with Al ³⁺	5
CHAPTER II: Model studies of aplication of electrocoagulation	
Figure 1. Bench-scale Electrocoagulation reactor with electrode and a small	13
suction pump (Solution sampling).	
Figure 2. Plots of absorbance and electrolysis time, 0.01% w/v of xylenol Blue,	18
424 nm	
Figure 3. Plots of absorbance and electrolysis time, 0.01% w/v of	18
eriochromeblack T, 503 nm	
Figure 4. Plots of absorbance and electrolysis time, 0.01% w/v of tropaeoline O,	19
486 nm	
Figure 5. Plots of absorbance and electrolysis time, 0.01% w/v of O-nitrophenol,	19
266-284 nm	
Figure 6. Plot of the residual weight percentage and electrolysis time for ★,	20
xylenol blue; \blacksquare , eriochromeblack T; \blacksquare , tropaeoline O and \triangle , O-nitrophenol.	
Figure 7. Plots of absorbance and electrolysis time, 0.01% w/v of crocin, 464 nm	22
Figure 8. Plots of absorbance and electrolysis time, 0.01% w/v of morin, 262 nm	22
Figure 9. Plots of absorbance and electrolysis time for Curcumin 0.1(a) and	23
0.2(b) % w/v in 5% potassium oleate aqueous solution.	
Figure 10. Plot of the residual weight percentage and electrolysis time for	25
each phenolic compound: ★, phenol; ♠, resorcinol; ♠, pyrocatechol;	

■, pyrogallol; ×, phloroglucinol; ●, n-propyl 3,4,5-trihydroxybenzoate;	
+, orcinol; O, hydroquinone; _, tannin.	
Figure 11. Plots of absorbance and electrolysis time, 0.01% w/v of saponin rein,	28
194 nm	
Figure 12. Plots of absorbance and electrolysis time, 0.01% w/v of stevioside,	28
196 nm	
Figure 13. Plots of absorbance and electrolysis time, 0.01% w/v of	29
glycyrrhizic acid, 256 nm	
Figure 14. Plots of absorbance and electrolysis time, 0.01% w/v of standard	31
chlorophylline, A: aluminium electrode, B: iron electrode.	
Figure 15. Plots of absorbance and electrolysis time of Stevia extract,	32
A: aluminium electrode, B: iron electrode.	
Figure 16. Plots of the absorbance value and electrolysis time for each	33
ethanol: water ratio of chlorophyllin solution; A: Aluminium electrode,	
B: Iron electrode; ◆, water; ★, 25% ethanol; ■, 50% ethanol;	
▲, 75% ethanol.	
Figure 17. Plots of the absorbance value and electrolysis time for each	34
ethanol: water ratio of Stevia extract; A: Aluminium electrode,	
B: Iron electrode; ♦, water; ★, 25% ethanol; ■, 50% ethanol;	
▲, 75% ethanol .	
CHAPTER III: Isolation of Glycosides and Other Natural Compounds from	
Plants using Electrolytic Decolourization	
1. Glycyrrhizin from Glycyrrhiza radix (Licorice)	
Figure 1. Glycyrrhiza radix (Licorice)	38
Figure 2. Structures of compounds 1-6: Glycyrrhizin (1), 3-O-[β-D-	42
glucuronopyranosyl-(1 \Rightarrow 2)- β -D-glucuronopyranosyl]liquiritic acid (2),	
3- <i>O</i> - $[\alpha$ -D-glucopyranosyl- $(1\rightarrow 4)$ - β -D-glucuronopyranosyl- $(1\rightarrow 2)$ - β -D-	
glucuronopyranosyl]glycyrrhetinic acid (3), 3- <i>O</i> -[α-D-glucopyranosyl-(1-)-	(4

$-\beta$ -D-glucuronopyranosyl- $(1\rightarrow 2)$ - β -D-glucuronopyranosyl]-liquiritic acid	(4),
3-O-[α -D-glucopyranosyl-(1→4)- α -D-glucopyranosyl-(1→4)- β -D-glucuro	no
pyranosyl- $(1\rightarrow 2)$ - β -D-glucuronopyranosyl]glycyrrhetinic acid (5), 3-O-	
[α -D-glucopyranosyl-($1\rightarrow 4$)- α -D-glucopyranosyl-($1\rightarrow 4$)- β -D-	,
lucuronopyranosyl- $(1\rightarrow 2)$ - β -D-glucuronopyranosyl] liquiritic acid (6).	
Figure 3. Crude glycyrrhizin analysis chromatogram, data from GL Science	43
HPLC company,	
Figure 4. HPLC of crude licorice extract without electrolytic decolourization.	44
Figure 5. a. Standard glycyrrhizin b. Isolated glycyrrhizin after electrolytic	45
decolourization (small peak is impurity) (c). Isolated glycyrrhizin after	
electrolytic decolourization and resin ion-exchange (small peak is impurit	y).
HPLC conditions; detection wavelength 248 nm; column Inertsil (C18)OI	OS-3,
4.6ID × 150 mm; eluting solvent CH ₃ CN: H ₂ O(65:35); flow-rate 0.5 ml	/min ;
retention time for glycyrrhizin 2.6 min.	
Figure 6. a. Mass spectrum of extracted product, b. Mass spectrum of standard	46
sample.	
2. D-Pinitol from Cassia siamea Lamk	
Figure 1. Cassia siamea Lamk.	50
Figure 2. Structures of compounds (1)-(5), luteolin (1), cassia chromone (5-aceton	yl 52
-7-hydroxy-2-methylchromone) (2), 5-acetonyl-7-hydroxy-2-hydroxymeth	ıyl-
chromone (3), 4- (trans)-acetyl-3,6,8-trihydroxy-3-methyldihydronaphtha	lenone
(4), and 4-(cis)-acetyl-3,6,8- trihydroxy- 3-methyldihydronaphthalenone (5).
Figure 3. Barakol	53
Figure 4. Structures of compounds (7) and (8)	53
Figure 5. D-Pinitol, (D-chiro-Inositol, 3-O-methyl)	54
Figure 6. Structure of an inositol	55
Figure 7. The 400 MHz HMQC spectrum of pinitol (DMSO)	56
Figure 8. The 400 MHz ¹ H-NMR spectrum of pinitol (DMSO)	58
Figure 9. The 100 MHz ¹³ C-NMR spectrum of pinitol (DMSO)	59

Figure 10. The 100 MHz DEPT spectrum of pinitol (DMSO)	59
Figure 11. The 400 MHz COSY spectrum of pinitol (DMSO)	60
3. Asiaticoside from Centella asiatica	
Figure 1. Centella Asiatica	64
Figure 2. The sequence and linkage position of monosaccharide and triterpenoid	67
established by HMBC and TOCSY experiments.	
Figure 3. 500 MHz HMBC NMR spectrum for the sequence and linkage position of	68
monosaccharide and triterpenoid (methanol-d4) of asiaticoside	
Figure 4. 500 MHz TOCSY NMR spectrum for the sequence and linkage position of	69
monosaccharide and triterpenoid (methanol-d4) of asiaticoside	
Figure 5. 500 MHz ¹ H COSY NMR spectrum (methanol-d ₄) of asiaticoside.	70
Figure 6. 500 MHz ¹ H-NMR spectrum (methanol-d ₄) of asiaticoside	71
Figure 7. 75 MHz ¹³ C-NMR spectrum (methanol-d ₄) of asiaticoside	72
4. Mukurozioside from Sapindus rarak DC	
Figure 1. Sapindus rarak DC	77
Figure 2. Structure of mukurozioside lib	78
Figure 3. 300 MHz ¹ H-NMR (CDCl ₃) of mukurozioside	79
Figure 4. 300 MHz ¹ H COSY NMR (CDCl ₃) of mukurozioside	80
Figure 5. 75 MHz ¹³ C-NMR (CDCl ₃) of mukurozioside	81
Figure 6. 75 MHz DEPT NMR (CDCl ₃) of mukurozioside	82
Figure 7. Structure of monodesmoside	82
Figure 8. 300 MHz ¹ H-NMR (CD ₃ OD) of the unknown compound	85
Figure 9. 300 MHz COSY NMR spectrum (CD ₃ OD) of the unknown compound	86
Figure 10. 75 MHz ¹³ C-NMR spectrum (CD ₃ OD) of the unknown compound	87
Figure 11. 75 MHz DEPT NMR spectrum (CD ₃ OD) of the unknown compound	87
Figure 12. 300 MHz HSQC NMR spectrum (CD ₃ OD) of the unknown compound	88
Figure 13. 300 MHz HMBC NMR spectrum (CD ₃ OD) of the unknown compound	88
PART II: Synthesis of Pentenomycin	
Figure 1. X-ray structure of compound 64a	120

Figure 2. X-ray structure of compound 64b	121
Figure 3. NOESY spectra of 67	127
Figure 4. NOESY spectra of 70	128
Figure 5a. The 300 MHz ¹ H-NMR (CDCl ₃) of compound 69	131
Figure 5b. X-ray structure of 69	132
Figure 6. The 300 MHz ¹ H-NMR (D ₂ O) of the mixture of epipentenomycin I (major)135
and pentenomycin I (minor) from the hydrolysis reaction of the mixture	
of 67 and 68.	
Figure 7. The 300 MHz ¹ H-NMR (D ₂ O) of epipentenomycin I from the hydrolysis	136
reaction of pure 67.	

LIST OF ABBREVIATIONS

Anal. Analysis

aq Aqueous

ArH Aromatic proton (in ¹H NMR)

atm Atmosphere(s), atmospheric

Bp. Boiling point (°C)

calc Calculate

cat Catalyst

COSY Correlation Spectroscopy

cm Centimeter(s)

cm⁻¹ Reciprocal centimeter (wave munber)

dec., decomp.

Decompose(s)

DEPT Distortionless Enhancement by Polarization Transfer

DIBAH Diisobutyl aluminium hydride

DME 1,2-dimethoxyethane
DMF Dimethylformamide

DCM Dichloromethane

DMSO Dimethyl sulfoxide

CCC Classical Column Liquid Chromatography

Electrophile(s)

e.g. (Exempli gratia) for example(s)

eq Equivalent(s)

et al (et alii) and others

g Gram Glu Glucose

h Hour

HMPA Hexamethylphosphoramide

HMQC Heteronuclear Multiple Quantum Coherence

xvii**i**

HPLC High Performance Liquid Chromatography
HSQC Heteronuclear Singel Quantum Coherence

Hz Hertz

IR Infrared spectroscopy

LDA Lithium diisopropylamide

M Molar

MCPBA Metachloroperbenzoic acid

Mp. Melting point (°C)

MHz Megahertz
min Minute
ml Mililiter

NMR Nuclear Magnetic Resonance

2D NMR Two Dimentional Nuclear Magnetic Resonance
NOESY Nuclear Overhauser Enhancement Spectroscopy

PLC Preparative Layer Chromatography

ppm Part per million

Rha Rhamnose

TLC Thin Layer Chromatography
TMEDA Tetramethylethylenediamine

TOCSY Total Correlation Spectroscopy

V Volt(s)
Vol Volume

μ Micron (micrometer)

 $[\alpha]_D^{20}$ Specific rotation (°) Weight by volume

δC ¹³C chemical shift (ppm)

δΗ ¹H chemical shift (ppm)

Coupling constant (Hz)

s Singlet

dd Double doublet

br d Broad double of doublet

ddd Double double of doublet

dq Double quartet

t Triplet

dt Double triplet

q Quartet

m Multiplet UV Ultraviolet

 λ Wavelenght

Ref. Reference(s)

Δ Heat, reflux, warm