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APPENDICES

APPENDIX A

MEDIA

The media were prepared by sterilization in the autoclave at 121 °C for 15 minutes.

1. Potato dextrose agar (PDA)

Potato, peeled and diced	200	g
Glucose	20.0	g
Agar	15.0	g
Distilled water	1000	ml

Boil 200 g of peels, dried potato for 1 hr in 1000 ml. of distilled water. Filter, and make up the filtrate to one liter. Add the glucose and agar and dissolve by streaming and sterilize by autoclaving at 121 °C for 15 minutes.

2. Cellulolysis basal medium (per liter) (Poiting, 1999)

$C_4H_{12}N_2O_6$	5.0	g
Yeast extracts	0.1	g
KH_2PO_4	1.0	g
$MgSO_4 \cdot 7H_2O$	2.0	g
$Ca_2Cl_2 \cdot 2H_2O$	0.001	g

pH 5.5

3. Mendel's medium (per liter) (Mandels and Weber, 1969)

Urea	0.3	g
(NH ₄) ₂ SO ₄	1.4	g
KH ₂ PO ₄	2.0	g
CaCl ₂ ·2H ₂ O	0.4	g
MgSO ₄ ·4H ₂ O	0.3	g
Peptone	1.0	g
FeSO ₄ ·7H ₂ O	5.0	mg
MnSO ₄ ·4H ₂ O	1.6	mg
ZnSO ₄ ·7H ₂ O	1.4	mg
CoCl ₂ ·6H ₂ O	2.0	mg
Tween 80	2.0	ml
Carbon source	1.0	g

pH 5..5

APPENDIX B**Preparation for non-denaturing polyacrylamide gel electrophoresis
(Native-PAGE)****1. Stock solutions****2 M Tris-HCl (pH 8.8)**

Tris (hydroxymethyl)-aminomethane 24.2 g
Adjusted pH to 8.8 with 1 M HCl and adjusted volume to 100 ml with
distilled water

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethyl)-aminomethane 12.1 g
Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with
distilled water.

1% Bromophenol blue (w/v)

Bromophenol blue 100 mg
Brought to 10 ml with distilled water and stirred until dissolved.
Filtration will remove aggregated dye.

2. Working solution**Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)**

Acrylamide 29.2 g
N,N,-methylene-bis-acrylamide 0.8 g
Adjust volume to 100 ml with distilled water

Solution B (1.5 M Tris-HCl pH 8.8)

2 M Tris-HCl (pH 8.8) 75 ml
Distilled water 25 ml

Solution C (0.5 M Tris-HCl pH 6.8)

1 M Tris-HCl (pH 6.8)	50 ml
Distilled water	50 ml

10% Ammonium persulfate

Ammonium persulfate	0.5 g
Distilled water	50 ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3 g
Glycine	14.4 g
Dissolved in distilled water to 1 litre without pH adjustment (final pH should be 8.3)	

5x sample buffer**(312.5 mM Tris-HCl pH 6.8, 50% glycerol, 1% bromophenol blue)**

1 M Tris-HCl (pH 6.8)	0.6 ml
Glycerol	5 ml
1% Bromophenol blue	0.5 ml
Distilled water	1.4 ml

3. Native-PAGE**7.5% Separating gel**

Solution A	2.5 ml
Solution B	2.5 ml
Distilled water	5 ml
10% Ammonium persulfate	50 μ l
TEMED	5 μ l

5.0% Stacking gel

Solution A	0.67 ml
Solution B	1 ml
Distilled water	2.3 ml
10% Ammonium persulfate	30 μ l
TEMED	5 μ l

APPENDIX C

Preparation for denaturing polyacrylamide gel electrophoresis

1. Stock solutions

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane	24.2 g
Adjusted pH to 8.8 with 1 M HCl and adjusted volume to 100 ml with distilled water	

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethyl)-aminomethane	12.1 g
Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water.	

10% SDS (w/v)

Sodium dodecyl sulfate (SDS)	10 g
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50% Glycerol (w/v)

100% Glycerol	50 ml
Added 50 ml of distilled water	

1% Bromophenol blue (w/v)

Bromophenol blue	100 mg
Brought to 10 ml with distilled water and stirred until dissolved.	
Filtration will remove aggregated dye.	

2. Working solution

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide	29.2 g
N,N,-methylene-bis-acrylamide	0.8 g
Adjust volume to 100 ml with distilled water	

Solution B (1.5 M Tris-HCl pH 8.8, 0.4% SDS)

2 M Tris-HCl (pH 8.8)	75 ml
10% SDS	4 ml
Distilled water	21 ml

Solution C (0.5 M Tris-HCl pH 6.8, 0.4% SDS)

1 M Tris-HCl (pH 6.8)	50 ml
10% SDS	4 ml
Distilled water	46 ml

10% Ammonium persulfate

Ammonium persulfate	0.5 g
Distilled water	5 ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine, 0.1% SDS)

Tris (hydroxymethyl)-aminomethane	3 g
Glycine	14.4 g
SDS	1 g

Dissolved in distilled water to 1 litre without pH adjustment
(final pH should be 8.3)

5x sample buffer

**(60 mM Tris-HCl pH 6.8, 25% glycerol, 2% SDS, 0.1% bromophenol blue,
14.4 mM 2-mercaptoethanol)**

1 M Tris-HCl (pH 6.8)	0.6 ml
Glycerol	5 ml
10% SDS	2 ml
1% Bromophenol blue	1 ml
2-mercaptoethanol	0.5 ml
Distilled water	0.9 ml

3. SDS-PAGE**12.5% Separating gel**

Solution A	4.2 ml
Solution B	2.5 ml
Distilled water	3.3 ml
10% Ammonium persulfate	50 μ l
TEMED	5 μ l

5.0% Stacking gel

Solution A	0.67 ml
Solution B	1 ml
Distilled water	2.3 ml
10% Ammonium persulfate	30 μ l
TEMED	5 μ l

APPENDIX D

Preparation for isoelectric focusing gel electrophoresis

Monomer-ampholyte solution

30% acrylamide solution	2 ml
Ampholyte pH 3-10	240 μ l
Distilled water	9.7 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50 μ l
TEMED	5 μ l

Fixative solution, 100 ml

Trichloroacetic acid	10 g
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Immerse gels in this solution for 30 minutes.

Staining solution, 100 ml

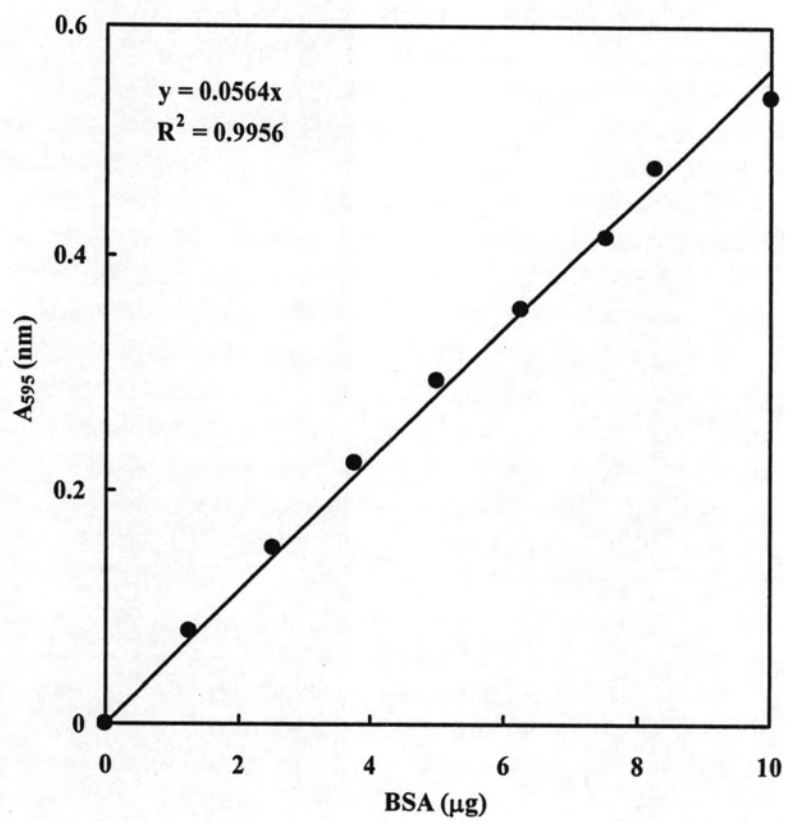
Ethanol	27 ml
Acetic acid	10 ml
Coomassie brilliant blue R-250	0.04 g
CuSO_4	0.5 g
Distilled water	63 ml

Dissolve the CuSO_4 in water before adding the alcohol. Either dissolve the dye in alcohol or add it to the solution at the end.

Immerse the gel in the stain for approximately 0.5-1 hours. After that immerse the gel in destain solution to remove the last traces of stain and CuSO_4 .

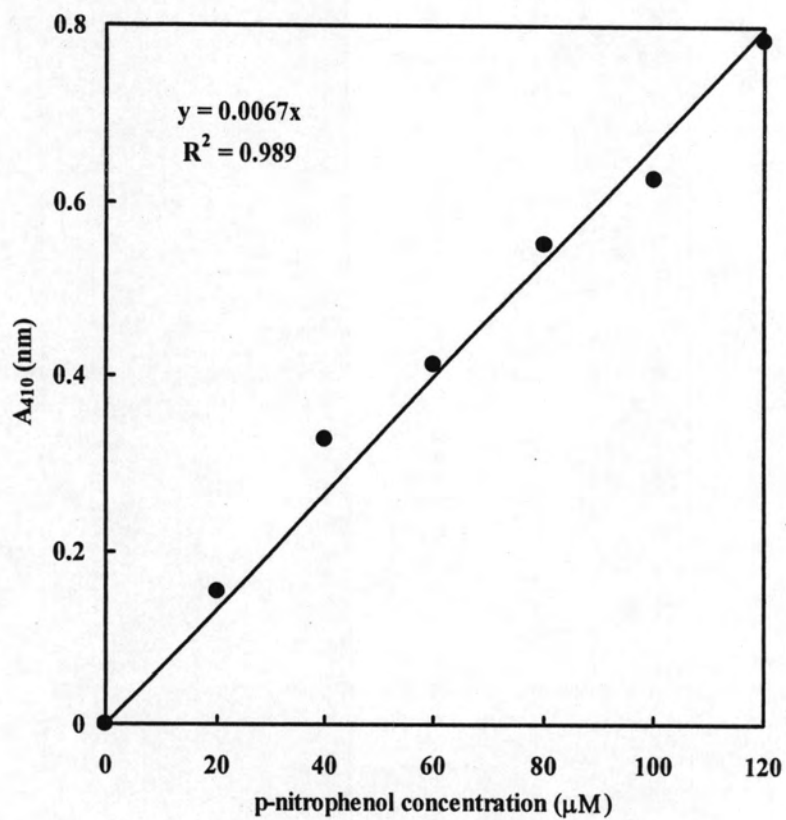
APPENDIX E

Calibration curve for protein determination Bradford method



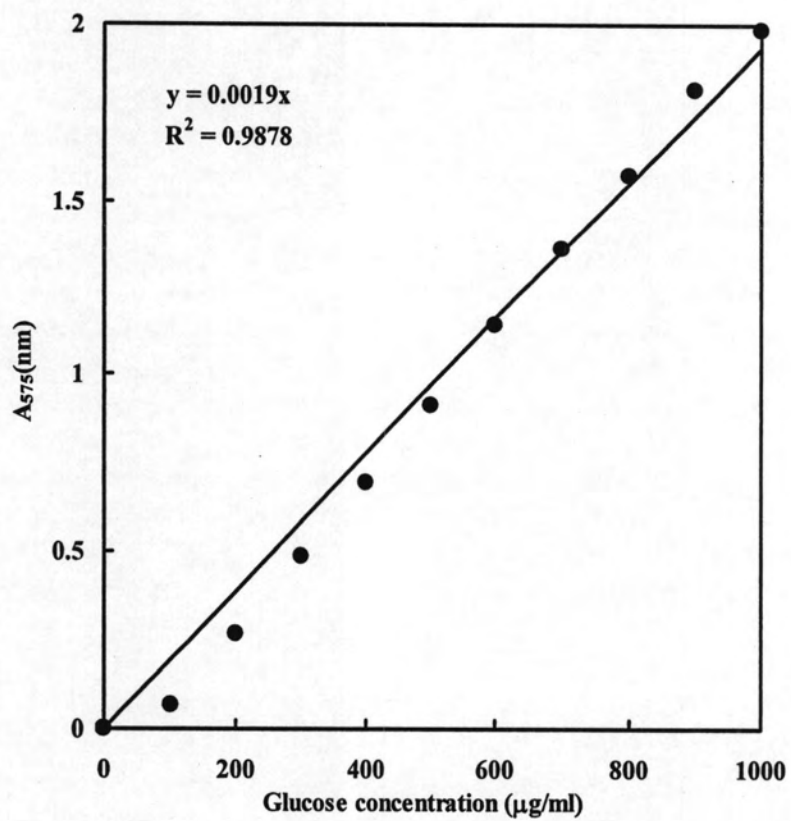
APPENDIX F

Calibration curve for various concentration of p-nitrophenol



APPENDIX G

Calibration curve for various concentration of glucose by
DNS method



APPENDIX H

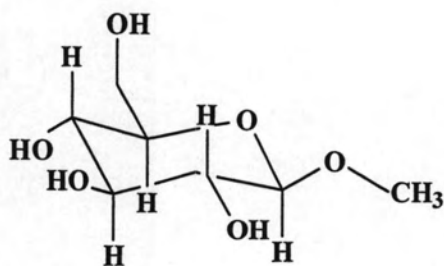
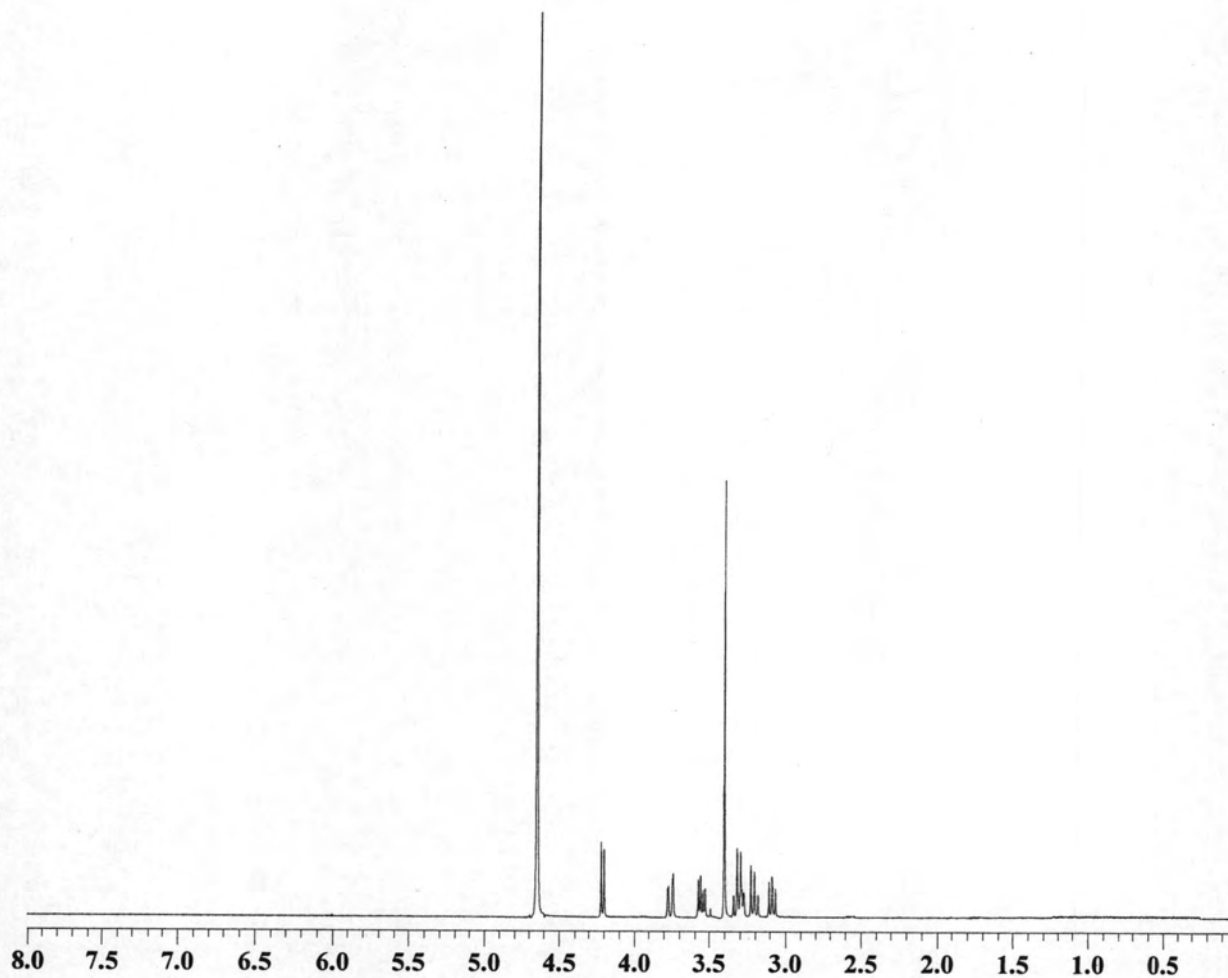


Figure H.1 $^1\text{H-NMR}$ spectrum of Methyl- β -D-glucoside

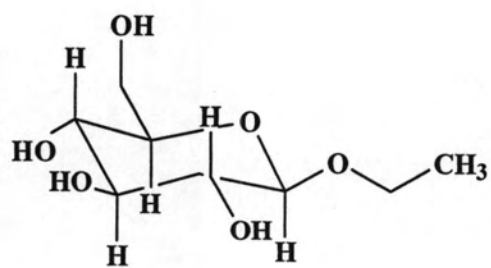
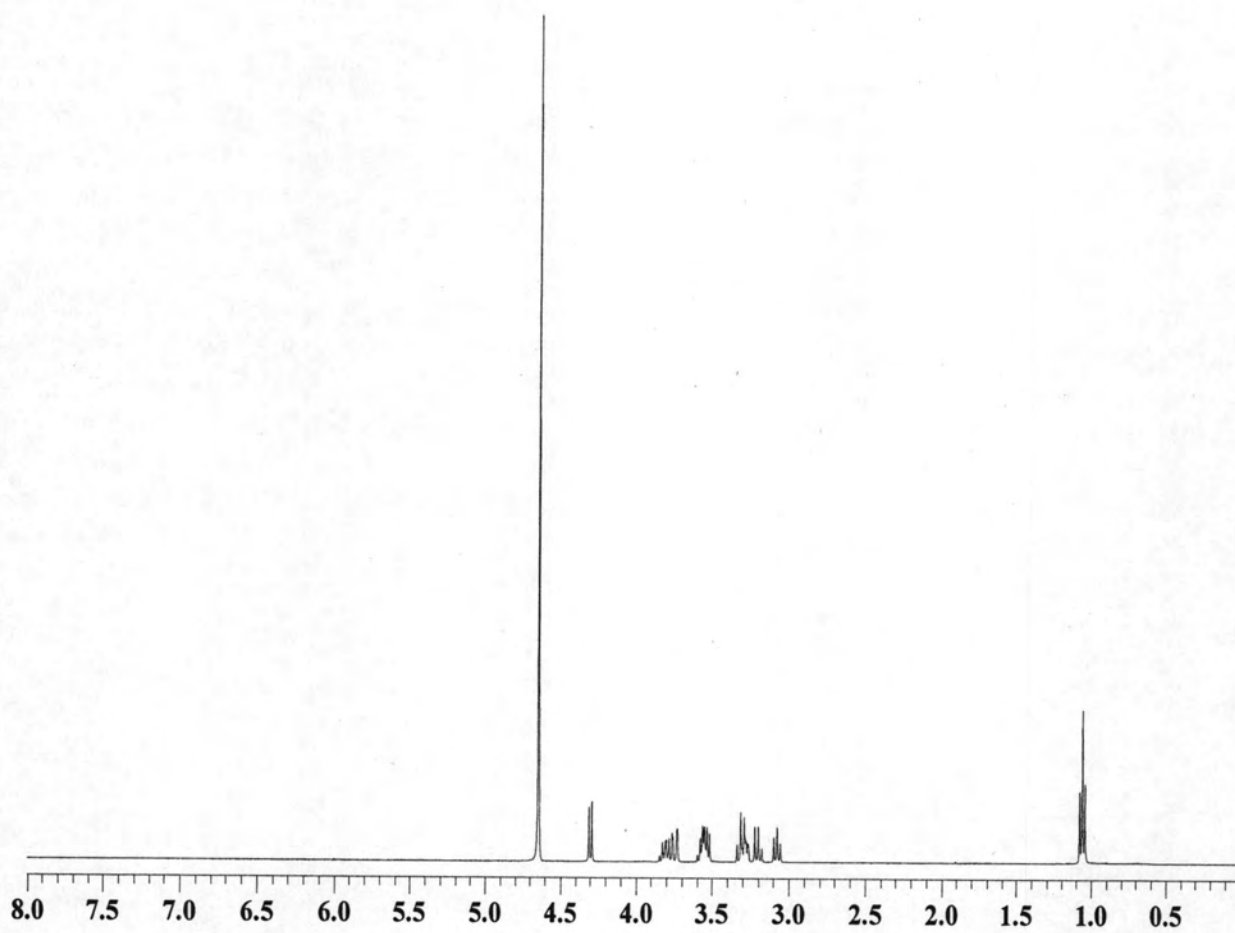


Figure H.2 $^1\text{H-NMR}$ spectrum of Ethyl- β -D-glucoside

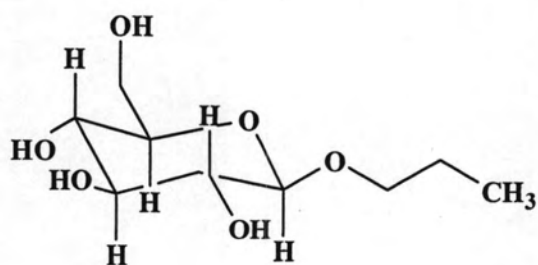
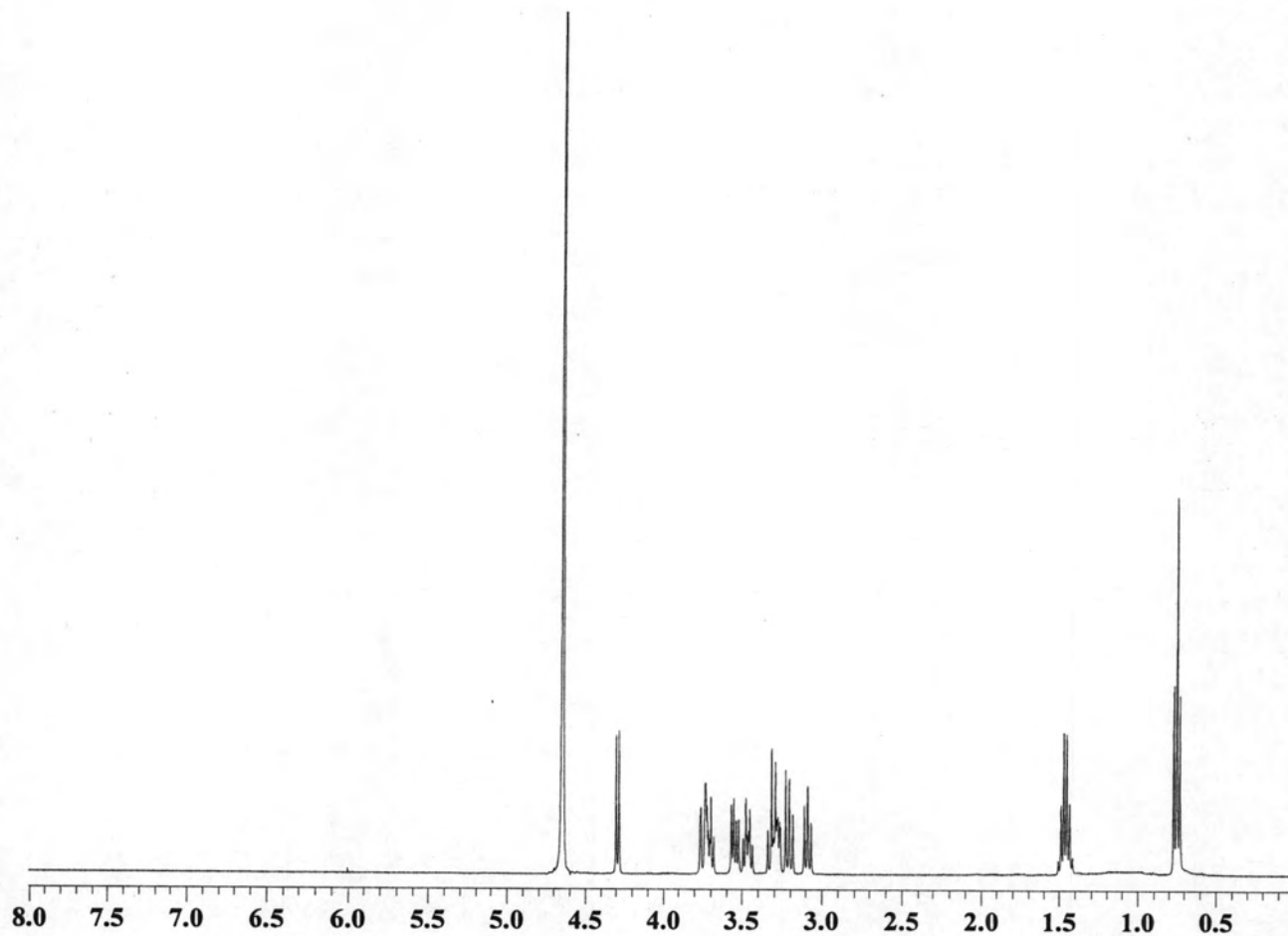


Figure H.3 $^1\text{H-NMR}$ spectrum of *n*-Propyl- β -D-glucoside

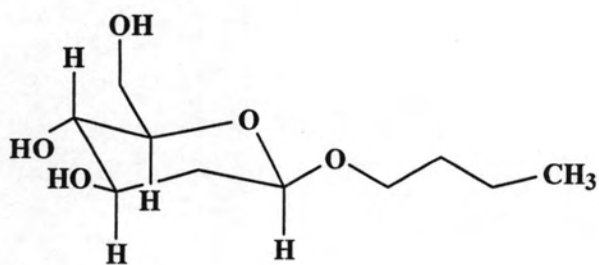
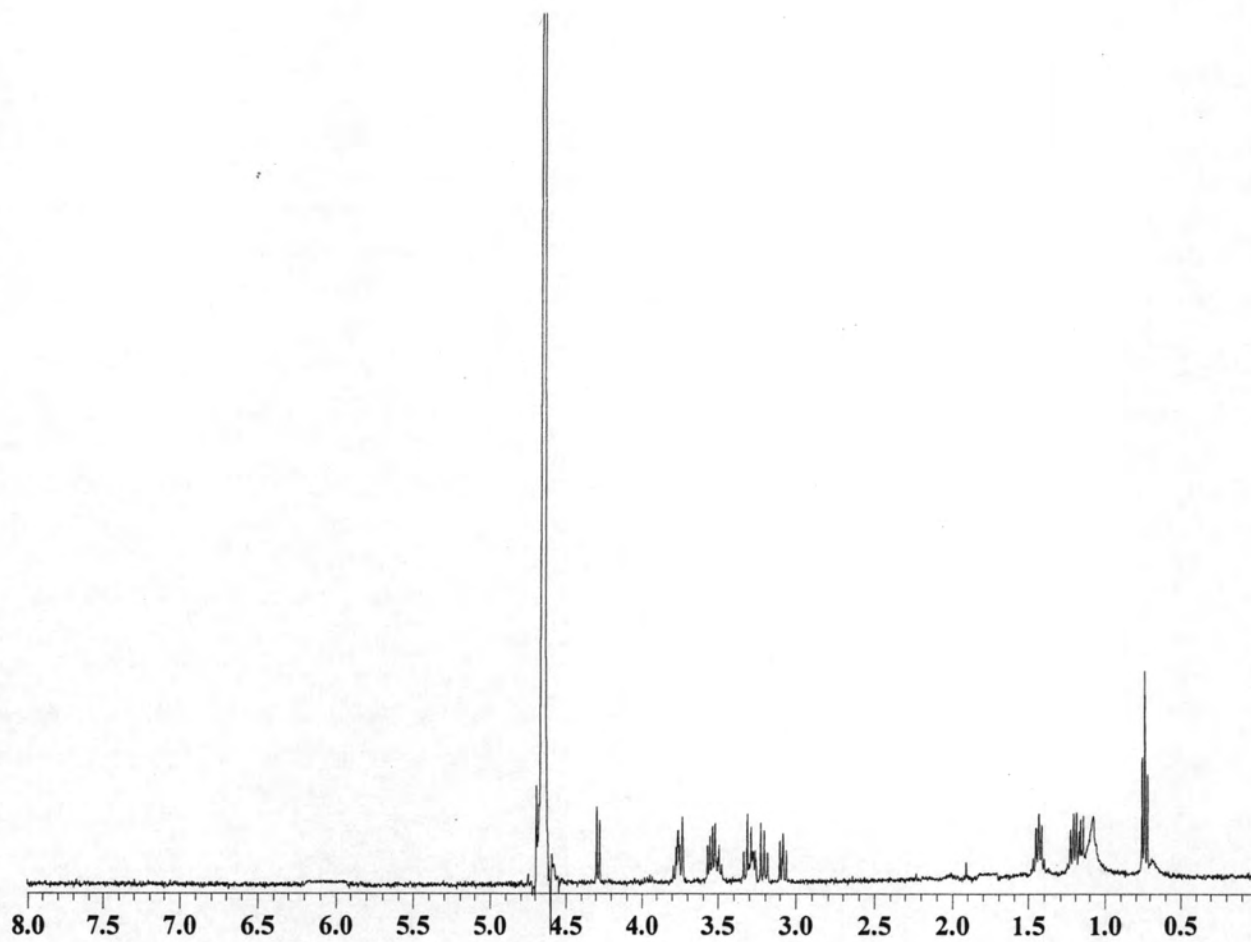


Figure H.4 $^1\text{H-NMR}$ spectrum of *n*-Butyl- β -D-glucoside

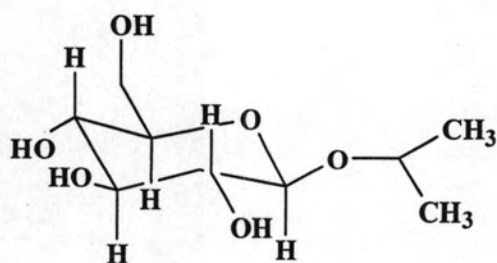
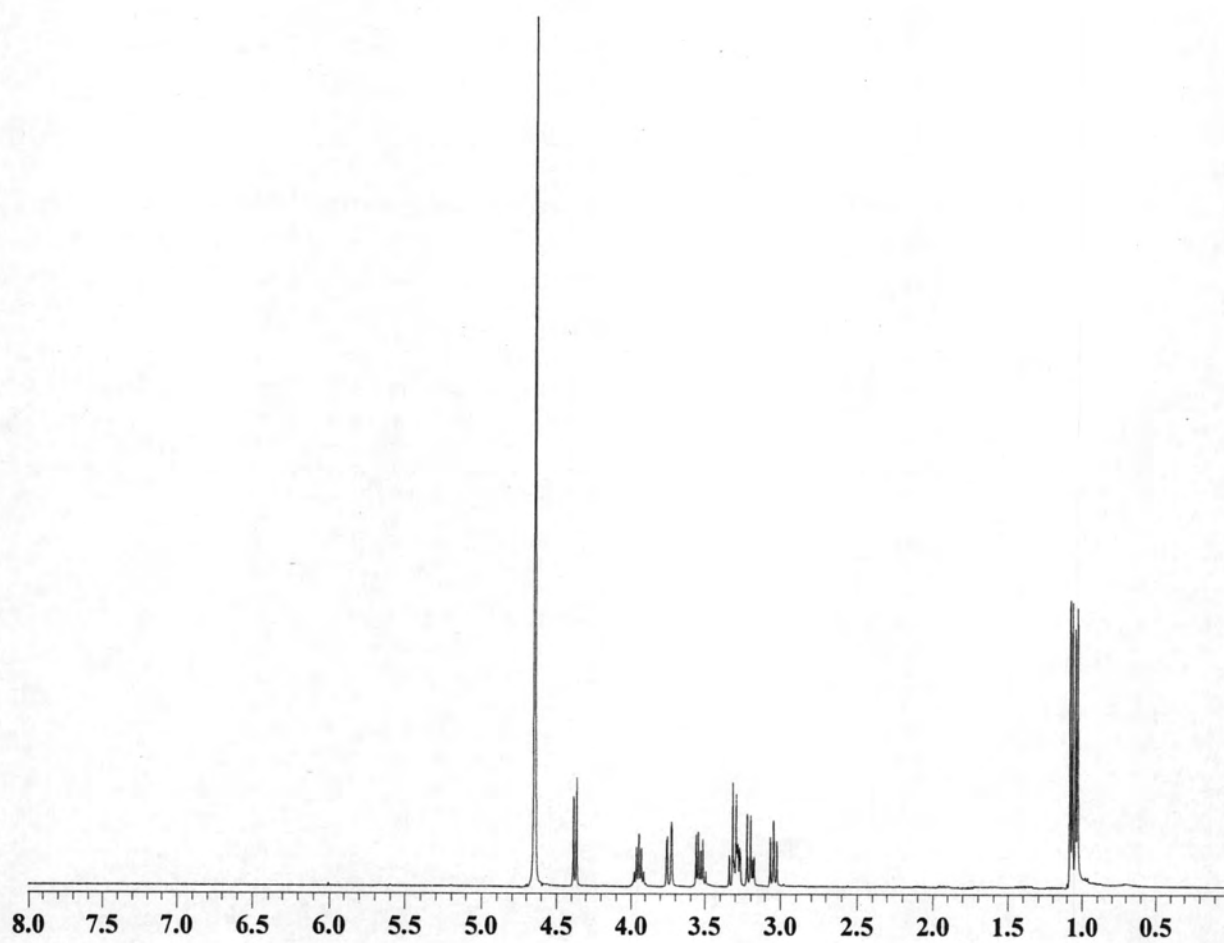


Figure H.5 $^1\text{H-NMR}$ spectrum of 2-Propyl- β -D-glucoside

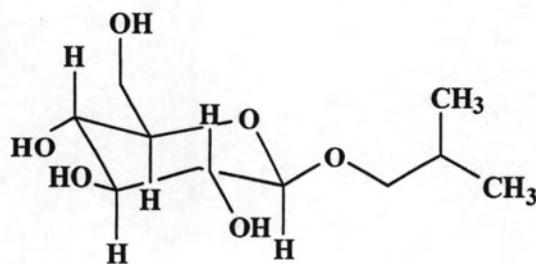
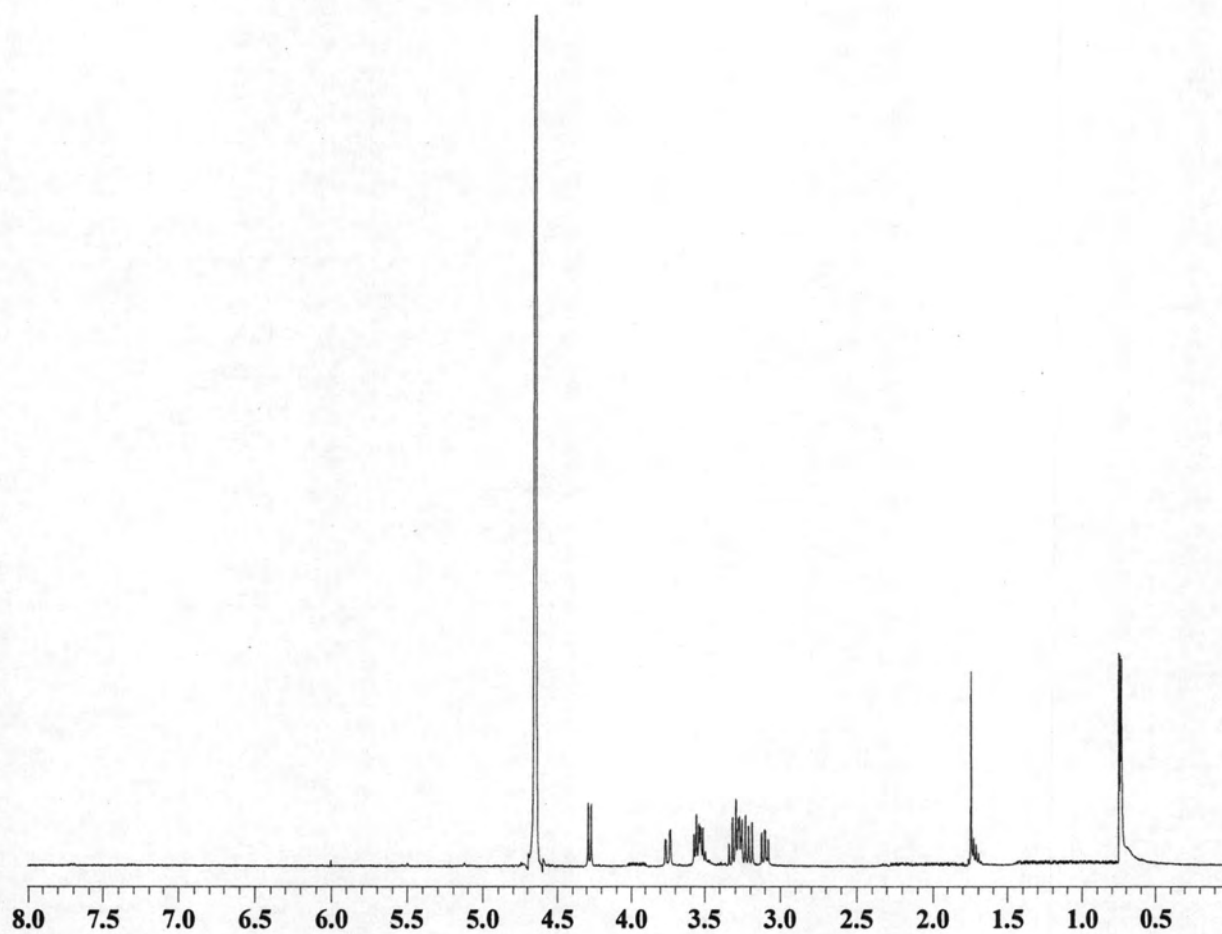


Figure H.6 $^1\text{H-NMR}$ spectrum of 2-Methyl-1-Propyl- β -D-glucoside

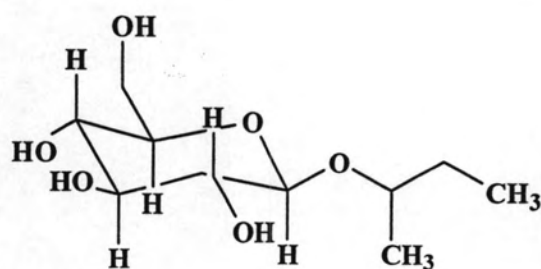
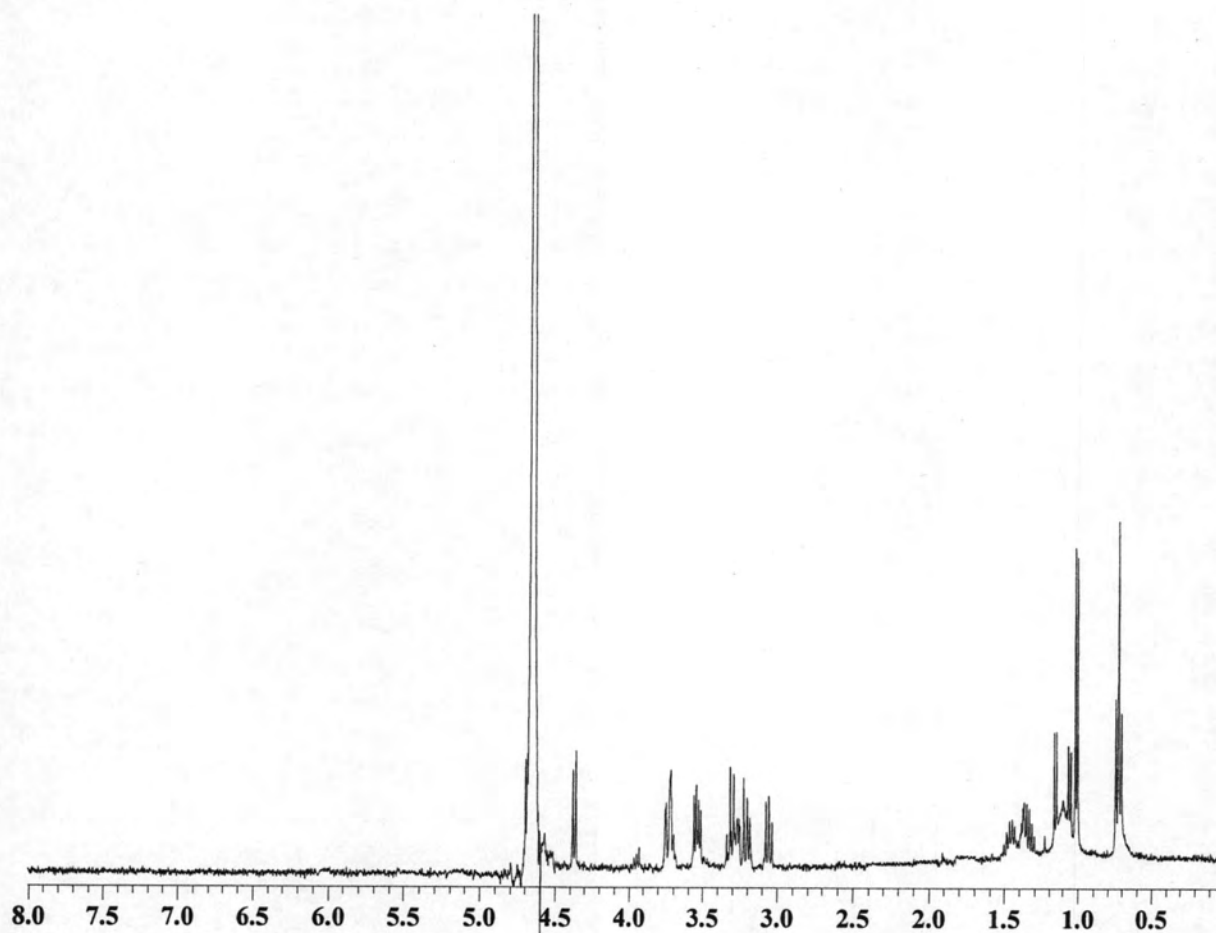


Figure H.7 $^1\text{H-NMR}$ spectrum of 2-Butyl- β -D-glucoside

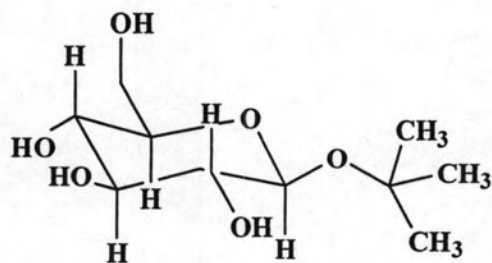
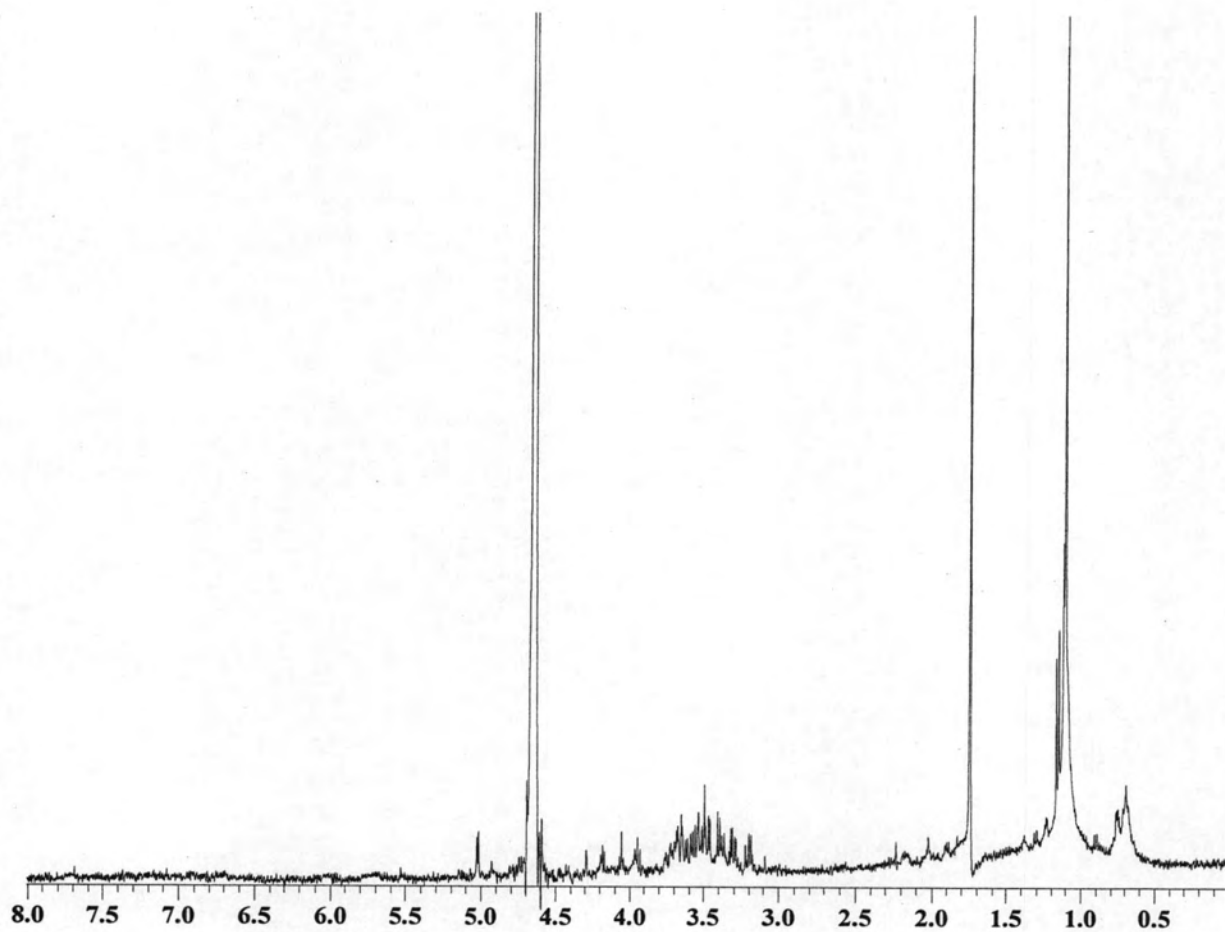


Figure H.8 $^1\text{H-NMR}$ spectrum of 2-Methyl-2-Propyl- β -D-glucoside

APPENDIX I



Figure I.1 *D. eschscholzii* on Mango Twig obtained from Dr. Jitra Piapukiew (a); Stromata on twig (b, c); cross section on the stromata having concentric zone.

APPENDIX J

Abbreviation for amino acid residues (Voet *et al.*, 1995)

Amino acid	3 Letter-Abbreviation	1 Letter-Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

BIOGRAPHY

Mr. Aphichart Karnchanatat was born on August 24, 1976 in Bangkok, Thailand. He graduated with a Bachelor Degree of Science in the Chemistry Department from the Faculty of Science, Ramkhumhang University in 1999, and Master Degree of Science in the Biochemistry Department from the Faculty of Science, Chulalongkorn University in 2001. He had been studying for a Doctor's Degree of Science in Biotechnology, the Faculty of Science, Chulalongkorn University since 2003. The Royal Golden Jubilee Program of the Thailand Research Fund financially supported his Ph.D. Program. He has publication:

1. Karnchanatat, A., Petsom, A., Sangvanich, P., Piapukiew, J., Whalley, A.J.S., Reynolds, C.D., and Sihanonth, P. 2007. Purification and biochemical characterization of extracellular β -glucosidase from the wood-decaying fungus *Daldinia eschscholzii* (Ehrenb.:Fr.) Rehm. FEMS Microbiology Letter 270: 162-170.