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HYDROLYSIS OF BILE ACID CONJUGATES
AND DEHYDROXYLATION OF CHOLIC ACID
BY CLOSTRIDIUM BIFERMENTANS

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy in Biotechnology
at Massey University

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1980

AC111-03

ABSTRACT

The transformation of bile acids by *Clostridium bifermentans* was studied with a view to developing a process whereby the bile acid conjugates of New Zealand mutton and beef gall may be converted to deoxycholic acid.

Statistically designed experiments were employed to maximise 7 α -dehydroxylation of cholic acid to deoxycholic acid and to minimise the 7 α -dehydrogenation of cholic acid to 7-ketodeoxycholic acid. Both transformations showed optima near pH 7. High deoxycholate yields were associated with conditions less favourable to strong growth and with relatively high electrode potentials. 7-ketodeoxycholic acid production was not as sensitive to environmental factors as was 7 α -dehydroxylation and could not be eliminated merely by manipulating fermentation variables.

Studies on the 7 α -dehydrogenation of cholic acid with washed resting-stage cells of *Cl. bifermentans* indicated several means of manipulating 7-ketodeoxycholate yields which were then tested using batch fermentation. In the presence of Zn⁺⁺ions, 7-ketodeoxycholate yields were reduced but dehydroxylation was completely inhibited. In the presence of EDTA, 7 α -dehydrogenation was almost quantitative but deoxycholate yields were again nil. Both transformations were enhanced during aerobic incubation. The highest deoxycholate yield observed during the work (50 molar %) was obtained by sweeping the fermenter headspace with air.

Growing cells of *Cl. bifermentans* effected the near-quantitative hydrolysis of glycodeoxycholate, taurodeoxycholate and taurocholate within 48 h whilst glycocholate was 90% deconjugated. At substrate concentrations greater than 0.1% w/v however, taurine conjugates were less well hydrolysed.

ACKNOWLEDGEMENTS

I wish to acknowledge the following:

- Dr I.S. Maddox and Dr R. Chong, for their supervision and assistance.
- The Department of Scientific and Industrial Research, for financial assistance.
- Dr R.P. Garland and New Zealand Pharmaceuticals Ltd., for advice, the provision of bile acids and the use of analytical equipment.
- Professor Annamarie Ferrari of Milan University, for a culture of *Clostridium bifermentans* SD 10.
- Mr J.T. Alger and Mr D.W. Couling, for their assistance in building and maintaining equipment.
- Mr L.E. O'Brien and Dr I.F. Boag, for advice on the statistical design of experiments.
- My mother, for typing, and my father, for critical advice.
- My wife, Deborah, for support and for draft typing and proof-reading.

TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT	i
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
TABLE OF FIGURES	vi
TABLE OF TABLES	ix
BILE ACID NOMENCLATURE	xi
ABBREVIATIONS	xii
<u>CHAPTER 1</u> PRELUDE	1
<u>CHAPTER 2</u> INTRODUCTION AND LITERATURE REVIEW	3
2.1 Dehydroxylation	4
2.2 Dehydrogenation	9
2.2.1 7 α -Hydroxycholanoyl Dehydrogenases	10
2.2.2 3 α -Hydroxycholanoyl Dehydrogenases	12
2.2.3 12 α -Hydroxycholanoyl Dehydrogenases	13
2.3 Cholanoylglycine and Cholanoyltaurine Hydrolases	15
2.4 Occurrence of Multiple Transformation Abilities in Single Organisms	18
2.5 Choice of Organism	21
2.6 <i>Clostridium bifermentans</i>	22
2.7 Inhibition of Bacterial Growth by Bile Acids	23
<u>CHAPTER 3</u> METHODS	25
3.1 Melting Points	25
3.2 Materials	25
3.2.1 Media	25
3.2.2 Chromatography Materials	25
3.2.3 Bile Acids	25
3.2.3.1 7-Ketodeoxycholic Acid	26
3.2.3.2 3 α , 7 α -dihydroxy-5 β -chol-11- en-24-oic-acid	26
3.2.3.3 Conjugates	26
3.2.4 Solvents	28
3.2.5 Gases and Other Chemicals	28
3.3 Organisms	29
3.4 Sterilisation of Media	29
3.5 Cleaning of Glassware	30
3.6 Analytical Methods	30
3.6.1 pH Measurement	30
3.6.2 Dry Weight Determination	30
3.6.3 Cell Counts	30
3.6.4 Thin-layer Chromatography	30
3.6.5 High Performance Liquid Chromatography	31
3.6.5.1 Mobile Phase	31
3.6.5.2 Resolution	31
3.6.5.3 Operation	33
3.6.6 Infra-red Spectrophotometry	33

3.7	Culture Conditions	33
3.7.1	Small Scale Experiments	33
3.7.2	Fermentation	33
3.7.2.1	Equipment	33
3.7.2.2	Instrumentation	38
3.7.2.3	Sterilisation	41
3.7.2.4	Inoculum Preparation	41
3.7.2.5	Operation	42
3.7.2.6	Sampling Technique	42
3.8	Washed Cell Methodology	43
3.8.1	Cell Production	43
3.8.2	Equipment	43
3.8.3	Harvesting and Washing	43
3.8.4	Incubation	43
3.9	Extraction and H.P.L.C. Sample Preparation	47
3.9.1	Solvent Extraction	47
3.9.2	Freeze Dry Extraction	47
3.10	Product Characterisation	48
3.10.1	Action of Strain ATCC 9714 on Cholic Acid	48
3.10.2	Action of Strain SD 10 on Conjugates	49
3.10.3	Action of Strain ATCC 9714 on Conjugates	51
3.11	Calculations	52
3.11.1	H.P.L.C. Data Analysis	51
3.11.2	Calculation of Molar Compositions	52
3.11.3	Mass Balance Calculations	52
3.12	Discussion of Methods	53
<u>CHAPTER 4</u>	EXPERIMENT 1: EFFECT OF pH AND ATMOSPHERIC COMPOSITION ON CHOLIC ACID TRANSFORMATION	56
4.1	Introduction	56
4.2	Experiment Design	56
4.3	Fermentation Conditions	57
4.4	Results	58
4.4.1	Statistical Analysis	58
4.5	Discussion of Experiment 1	76
4.6	Conclusions	83
<u>CHAPTER 5</u>	EXPERIMENT 2: IDENTIFICATION OF FERMENTATION VARIABLES IMPORTANT TO DEHYDROXYLATION AND DEHYDROGENATION OF CHOLIC ACID	85
5.1	Introduction	85
5.2	Experiment Design	85
5.3	Fermentation Conditions	90
5.4	Results	90
5.5	Discussion of Experiment 2	112
5.6	Conclusions	117
<u>CHAPTER 6</u>	CHOLIC ACID TRANSFORMATION USING WASHED RESTING CELLS OF Cl. BIFERMENTANS ATCC 9714	119
6.1	Introduction	119
6.2	Fermentation Conditions and Cell Preparation	120
6.3	Results and Discussion	120
6.3.1	Time Scale and Reproducibility of the Reaction	121
6.3.2	Effect of Aerobic Incubation	121
6.3.3	Effect of Atmosphere, pH, Incubation Time and Temperature	124
6.3.4	Effects of Common Enzyme Inhibitors and Other Miscellaneous Chemicals	127
6.4	Conclusions	131

<u>CHAPTER 7</u>	EFFECT OF AEROBIC INCUBATION AND MISCELLANEOUS COMPOUNDS ON CHOLIC ACID TRANSFORMATION DURING BATCH FERMENTATION	133
7.1	Introduction	133
7.2	Fermentation Conditions	133
7.3	Results and Discussion	134
	7.3.1 Effect of Very Late Substrate Addition	134
	7.3.2 Effect of Zn^{++} ion	137
	7.3.3 Effect of High Thioglycollate Levels	137
	7.3.4 Effect of EDTA on Cholic Acid Transformation	139
	7.3.5 Effect of Air on Cholic Acid Transformation	144
7.4	Conclusions	151
<u>CHAPTER 8</u>	HYDROLYSIS OF BILE ACID CONJUGATES BY CLOSTRIDIUM BIFERMENTANS	152
8.1	Introduction	152
8.2	Fermentation Conditions	152
8.3	Results	154
8.4	Discussion	155
8.5	Conclusions	167
<u>CHAPTER 9</u>	GENERAL DISCUSSION	169
<u>CHAPTER 10</u>	GENERAL CONCLUSIONS	178
<u>REFERENCES</u>		180
<u>APPENDICES</u>		
Appendix 1	Infra-red Spectra	189
Appendix 2	Parsimonious Models and Regression Statistics for Experiment 1	195
Appendix 3	Parsimonious Models and Regression Statistics for Experiment 2	201
Appendix 4	Electrode Potentials of Cell Production Fermentation	209
Appendix 5	Raw Data for Deconjugation Experiments Conducted on the Small Scale	210

TABLE OF FIGURES

	<u>Page</u>
3.1 The Assembled Fermenter	34
3.2 The Assembled Fermenter - Key to Figure 3.1	35
3.3 The 2-litre Fermenter Vessel Head	36
3.4 Plan of the 2-litre Fermenter Vessel Head	37
3.5 The Gas-Mixer/Flow-Regulator	39
3.6 Schematic of Gas-Mixer/Flow-Regulator	40
3.7 Cell Washing Equipment	44
3.8 Washed Cell Incubation Rack	45
3.9 Cell Washing Equipment - Diagram	46
3.10 Differential Refractometer Linearity Characteristic	54
4.1 Course of Growth and Transformation for Exp. 1, Run 1	59
4.2 Run 2	60
4.3 Run 3	61
4.4 Run 4	62
4.5 Run 5	63
4.6 Run 6	64
4.7 Run 7	65
4.8 Run 8	66
4.9 Run 9	67
4.10 Run 10	68
4.11 Run 11	69
4.12 Residual Plot Number 2 for the Parsimonious Model for Deoxycholate Yield	72
4.13 Predicted Deoxycholate Yield (via log-transform model)	73
4.14 Predicted 7-Ketodeoxycholate Yield	74
4.15 Predicted Cell Yield (as L.M.C., log of maximum observed number of cells per millilitre of culture)	75
4.16 Course of Growth and Transformation for Experiment 1, Run 1 Repeated	78
5.1 Course of Growth and Transformation for Exp. 2, Run 1	91
5.2 Run 2	92
5.3 Run 3	93
5.4 Run 4	94
5.5 Run 5	95
5.6 Run 6	96

	<u>Page</u>
5.7 Course of Growth and Transformation for Exp. 2, Run 7	97
5.8 Run 8	98
5.9 Run 9	99
5.10 Run 10	100
5.11 Run 11	101
5.12 Run 12	102
5.13 Run 13	103
5.14 Run 14	104
5.15 Run 15	105
5.16 Run 16	106
5.17 Half-Normal Plot for Deoxycholate Yield from Exp. 2	111
5.18 Residual Plot Number 2 for the Parsimonious Model for 7-Ketodeoxycholate Yield from Experiment 2	111
6.1 Time Scale of Dehydrogenation by Whole Resting Cells	122
6.2 Effect of Aerobic Incubation on Dehydrogenation by Whole Resting Cells	123
6.3 Effect of pH and Atmosphere on Dehydrogenation	125
6.4 Effect of pH, Atmosphere and Incubation Time on Dehydrogenation by Whole Resting Cells	125
7.1 Course of Growth and Transformation for Fermentation with 18-hour Substrate Addition	135
7.2 Course of Growth and Transformation for Fermentation with 6-hour Addition of Zn^{++} Ion to 5mM	138
7.3 Course of Growth and Transformation for Fermentation with 6-hour Addition of Sodium Thioglycollate to 0.15% w/v	140
7.4 Course of Growth and Transformation for Fermentation with 6-hour Addition of EDTA. (a)	142
7.5 Course of Growth and Transformation for Fermentation with 6-hour Addition of EDTA. (b)	143
7.6 Course of Growth and Transformation for Fermentation with Air-Sweeping. (a)	146
7.7 Course of Growth and Transformation for Fermentation with Air-Sweeping. (b)	147
7.8 Course of Growth and Transformation for Fermentation with Air-Sweeping. (c)	148
8.1 Effect of Substrate Concentration on Deconjugation Yields	156
8.2 Effect of Substrate Concentration on Total Extent of Deconjugation	157
8.3 Course of Growth of Strain SD 10 and Transformation of Glycocholic Acid in Batch Fermentation	158
8.4 Course of Growth of Strain SD 10 and Transformation of Glycodeoxycholic Acid in Batch Fermentation	159

Page

8.5	Course of Growth of Strain SD 10 and Transformation of Sodium Taurocholate in Batch Fermentation	160
8.6	Course of Growth of Strain SD 10 and Transformation of Sodium Taurodeoxycholate in Batch Fermentation	161
8.7	Course of Growth of Strain SD 10 and Transformation of Glycocholic Acid and Sodium Taurocholate in Batch Fermentation with 6-hour Substrate Addition	162
8.8	Course of Growth of Strain SD 10 and Transformation of Glycocholic Acid and Sodium Taurocholate in Batch Fermentation with Air-Sweeping from 7.5 to 19 hours	163
A2.1	Residual Plot Number 1 for the Parsimonious Model for 7-Ketodeoxycholate Yield	200
A2.2	Residual Plot Number 3 for the Parsimonious Model for L.M.C. (i.e. log of maximum observed cell count)	200
A4.1	Electrode Potential Curves and Dry Weights of Cell Suspensions for Washed Cell Production Runs	209

TABLE OF TABLES

	<u>Page</u>
2.1 7 α -Dehydroxylation Yields Previous Published in the Literature	7
3.1 Melting Points of Synthetic Conjugates	27
3.2a Retention Volumes of Bile Acids	32
3.2b Retention Volumes of Bile Acids	32
3.3 Melting Points of Deconjugation Products	51
4.1 Design and Run Order, Experiment 1	57
4.2 The Effect of pH and Gas on Cell Growth and Cholic Acid Transformation	58
4.3 Full Regression Models for Experiment 1	70
4.4 Matrix of Correlation Coefficients for the Pairs of Response Variables in Experiment 1	76
5.1 Design Matrix and Variables for Experiment 2	89
5.2a Raw Transformation and Growth Data for Experiment 2	107
5.2b Raw Electrode Potential Data for Experiment 2	108
5.3 Coefficients from the Full Regression Models of Experiment 2	109
5.4 Matrix of Correlation Coefficients for the Pairs of Response Variables in Experiment 2	110
6.1 Effects of Miscellaneous Compounds on Cholate Transformation by Whole Cells	129
8.1 Yield of Deconjugation after 48 h	154
Parsimonious Models and Regression Statistics for Experiment 1:	
A2.1 For 7-Ketodeoxycholate Yield	195
A2.2 For Cholic Acid Remaining	196
A2.3 For Deoxycholic Acid Yield	197
A2.4 For the Natural Logarithm of Deoxycholic Acid Yield	198
A2.5 For the Base Ten Logarithm of the Maximum Number of Cells Observed. (L.M.C.)	199
Parsimonious Models and Regression Statistics for Experiment 2:	
A3.1 For 7-Ketodeoxycholate Yield	201
A3.2 For the Natural Logarithm of 7-Ketodeoxycholate Yield	202
A3.3 For the Cholic Acid Remaining at 48 Hours	203
A3.4 For Deoxycholate Yield	204
A3.5 For the Base 10 Logarithm of Maximum Observed Cell Counts (L.M.C.)	205
A3.6 For the Maximum Rate of Electrode Potential Decline $\left(\frac{\Delta E_c}{\Delta t}\right)_{\max}$	206

Parsimonious Models and Regression Statistics for
Experiment 2 (continued):

A3.7	For Minimum Electrode Potential	207
A3.8	For Initial Electrode Potential (E_{c_i})	208
Raw Data for Deconjugation Experiments Conducted on the Small Scale:		
A5.1	Extent of Transformation of Conjugate over 48 h	210
A5.2	Effect of Substrate Concentration on Transformation of Conjugates After 48 h	211

BILE ACID NOMENCLATURE

Throughout this work, bile acids will be referred to by the trivial names employed in "The Bile Acids" (Matschiner, 1971). These are listed below, together with the appropriate I.U.P.A.C. systematic chemical names (I.U.P.A.C. - I.U.B., 1969).

cholic acid	-	3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oic acid
deoxycholic acid	-	3 α ,12 α -dihydroxy-5 β -cholan-24-oic acid
chenodeoxycholic acid	-	3 α ,7 α -dihydroxy-5 β -cholan-24-oic acid
ursodeoxycholic acid	-	3 α ,7 β -dihydroxy-5 β -cholan-24-oic acid
lithocholic acid	-	3 α -hydroxy-5 β -cholan-24-oic acid
7-ketodeoxycholic acid		
	-	3 α ,12 α -dihydroxy-7-oxo-5 β -cholan-24-oic acid
7-ketolithocholic acid		
	-	3 α -hydroxy-7-oxo-5 β -cholan-24-oic acid
glycocholic acid	-	3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oylglycine
glycodeoxycholic acid	-	3 α ,12 α -dihydroxy-5 β -cholan-24-oylglycine
taurocholic acid	-	3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oyltaurine
taurodeoxycholic acid	-	3 α ,12 α -dihydroxy-5 β -cholan-24-oyltaurine

Acids will be referred to by the suffixes "-ate" and "-oic acid" interchangeably, for example 7-ketodeoxycholic acid and 7-ketodeoxycholate.

ABBREVIATIONS

Abbreviations of bile acid names (from Eneroth and Sjövall, 1971):

C	cholic acid
D	deoxycholic acid
7KD	7-ketodeoxycholic acid
GC	glycocholic acid
GD	glycodeoxycholic acid
TC	taurocholic acid
TD	taurodeoxycholic acid

Abbreviations of units:

g	gramme
h	hour
l	litre
m	metre
M	moles per litre
min	minutes
Pa	Pascal (Newton per square metre)
rev	revolution
tonne	tonne (1,000 Kg)
s	second
V	volt

Other abbreviations:

A	coded value of pH in Experiment 1 ($A = \text{pH} - 7.0$)
ATCC	American Type Culture Collection
B	coded value of gas in Experiment 1 ($B = 1$ for H_2 , $B = 0$ for N_2 , $B = -1$ for $\text{N}_2\text{-CO}_2$, 9:1)
DMSO	dimethylsulphoxide
Ec	electrode potential relative to the saturated calomel electrode

Ec_{min}	minimum Ec observed during a fermentation
Ec_i	Ec prevailing at inoculation
$(\Delta Ec/\Delta t)_{max}$	maximum rate of decline of Ec during a fermentation
EDTA	ethylenediaminetetraacetic acid
E_h	electrode potential relative to the standard hydrogen electrode
F	ratio of mean sums of squares (i.e. F ratio)
H.P.L.C.	high performance liquid chromatography
K_m	the Michaelis constant
L.M.C.	base 10 logarithm of the maximum cell count observed during a fermentation
\ln	natural logarithm
\log	base 10 logarithm
$\log K_1$	first formation constant of chelate complex
MS_{lof}	mean sum of squares due to lack of fit
MS_{pe}	mean sum of squares due to pure error
N	number of independent variables in an experiment design
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NCIB	National Collection of Industrial Bacteria
pCMB	parachloromercuribenzoate
R_f	T.L.C. mobility relative to solvent front mobility
T.L.C.	thin-layer chromatography
Tris	tris(hydroxymethyl)methylamine
\hat{y}	estimated value of response variable being modelled