HYDROLYSIS OF BILE ACID CONJUGATES

AND DEHYDROXYLATION OF CHOLIC ACID

BY CLOSTRIDIUM BIFERMENTANS

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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 7α-dehydroxylation of cholic acid to deoxycholic acid and to minimise the 7α-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 7α-dehydroxylation and could not be eliminated merely by manipulating fermentation variables.

Studies on the 7α-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, 7α-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.

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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	- 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid
deoxycholic acid	- 3α,12α-dihydroxy-5β-cholan-24-oic acid
chenodeoxycholic acid	- 3α,7α-dihydroxy-5β-cholan-24-oic acid
ursodeoxycholic acid	- 3α,7β-dihydroxy-5β-cholan-24-oic acid
lithocholic acid	- 3α-hydroxy-5β-cholan-24-oic acid
7-ketodeoxycholic acid	- 3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid
7-ketolithocholic acid	- 3α-hydroxy-7-oxo-5β-cholan-24-oic acid
glycocholic acid	- 3α,7α,12α-trihydroxy-5β-cholan-24-oylglycine
glycodeoxycholic acid	- 3α,12α-dihydroxy-5β-cholan-24-oylglycine
taurocholic acid	- 3α,7α,12α-trihydroxy-5β-cholan-24-oyltaurine
taurodeoxycholic acid	- 3α,12α-dihydroxy-5β-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 = 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 N_2-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
Eh	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
Km	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 chromotography
Tris	tris(hydroxymethyl)methylamine
Y	estimated value of response variable being modelled