The metabolism of lithocholic acid-3α-sulphate by *Pseudomonas* sp. NCIB 10590 under anaerobic conditions

ROBERT W. OWEN* and RODNEY F. BILTON

Department of Chemistry and Biochemistry, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, U.K.

A proportion of LA which is produced by 7α-dehydroxylation of chenodeoxycholic acid by colonic bacteria (Hill & Drasar, 1968), after absorption, reaches the liver via the hepatic portal vein. When LA reaches the liver it is rendered non-hepatoxic by sulphation to form LASO (Palmer, 1967). The majority of LASO is excreted in urine but a proportion undergoes enterophaeic circulation and thus becomes susceptible to microbial attack in the large intestine. Kelsey et al. (1978) have shown faecal bacteria can produce LA, ILA, 3KLA, 5β-cholanoic acid and Δ3-cholanic acid from LASO *in vitro*. Similar results were obtained by Borriello & Owen (1982) who also demonstrated that of the human faecal bacteria the genus *Clostridium* is the major group of organisms involved in the desulphation process.

Because unsaturated bile acids have been incriminated in the aetiology of colon cancer the metabolism of LASO was studied using *Ps* sp. 10590, which is a potent bile acid-degrading organism (Owen & Bilton, 1983a,b; Owen et al., 1984).

The anaerobic biotransformation of LASO by *Ps* sp. 10590 was conducted in a buffered mineral salts medium comprising (g/l): LASO, 1.0; K2HPO4, 0.7; KH2PO4, 0.3; KNO3, 1.0; MgSO4.7H2O, 0.1; FeSO4.7H2O, 0.0025; ZnSO4.7H2O, 0.0025; and MnSO4.4H2O; 0.0025; final pH 7.2.

Cells obtained from a 1 litre aerobic culture of *Ps* sp. 10590 by centrifugation were used to inoculate 1 litre of the culture medium. The culture was incubated anaerobically for 3 weeks at 28°C under 90%H2/10% CO2 (with palladium catalyst to remove residual oxygen). At the end of the fermentation the culture was extracted twice with an equal volume of dichloromethane. After drying over anhydrous MgSO4 the solvent was removed *in vacuo* at 50°C to yield 810mg of residue. The residue was separated into six major products by preparative t.l.c. yielding LA (160mg), ILA (120mg), 3KLA (100mg), chol-4-ene-3-one-24-oic acid (30mg), 3-oxopregna-1,4-diene-3-one-20-carboxylic acid (130mg) and androsta-1,4-diene-3,17-dione (50mg).

The products were identified by reference to t.l.c., g.l.c. mobilities, u.v., i.r., n.m.r. and mass spectra of authentic standards and to compounds previously isolated and identified from the degradation of hyodeoxycholic acid by *Ps* sp. 10590 (Owen & Bilton, 1983a).

The results show (Fig. 1) that *Ps* sp. 10590 is capable of extensive metabolism of LASO under anaerobic conditions. The production of LA (2), ILA (3) and 3KLA (4) is similar to the metabolism of LASO by mixed faecal bacteria. However, the unsaturated metabolites (5), (6) and (7) differ substantially from Δ3-cholanic acid described by Kelsey et al. (1978) and Borriello & Owen (1982). The production of Δ3-cholanic acid by mixed faecal bacteria probably occurs by either dehydroxylation of LA or ILA or by transesterification of the sulphate ester group of LASO. In contrast the production of the unsaturated compounds (5), (6) and (7) in this study probably arose by dehydrogenation of LA and ILA to give 3KLA which in turn is oxidized to chol-4-ene-3-one-24-oic acid (5), which, after introduction of a double bond at C1–C2, undergoes partial side-chain cleavage yielding 3-oxopregna-1,4-diene-3-one-20-carboxylic acid (6) which in turn is converted to androsta-1,4-diene-3,17-dione (7). The probable biotransformation pathway of LASO by *Ps* sp. 10590 is depicted in Fig. 1.

These results vindicate those of Kelsey et al. (1978) and Borriello & Owen (1982) which have shown that although sulphation of LA is probably a detoxifying process in humans, the metabolism of LASO by bacteria may represent a pro-carcinogenic process in that it results in the formation of unsaturated bile acids which may be promoters of colon carcinogenesis.

R. W. O. was in receipt of a Liverpool Education Research Assistantship during this study. We should like to thank Dr. M. H. Thompson, PHLS, CAMR, Bacterial Metabolism Research Laboratory, for the mass spectra conducted on equipment funded by the Cancer Research Campaign.


---

*Abbreviations used: LA, lithocholic acid; LASO, lithocholic acid-3α-sulphate; ILA, iso-lithocholic acid; 3KLA, 5β-cholane-3α-oxo-24-oic acid; Ps sp. 10590, *Pseudomonas* sp. NCIB 10590.*