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The Degradation of Cholic Acid by *Pseudomonas* sp. N.C.I.B. 10590

By Michael E. TENNESON,*† J. David BATY,‡ Rodney F. BILTON* and Arthur N. MASON*
**Department of Chemistry and Biochemistry, Liverpool Polytechnic, Liverpool L3 3AF, U.K., and*
‡Department of Biochemical Medicine, University of Dundee, Dundee, DD2 1UD, Scotland, U.K.

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The microbial degradation of cholic acid by *Pseudomonas* sp. N.C.I.B. 10590 was studied, and two major products were isolated and identified as 7α,12β-*dihydroxyandrosta*-1,4-*diene*-3,17-*dione* and 7α,12α-*dihydroxy*-3-*oxopregna*-1,4-*diene*-20-*carboxylic acid*. Four minor products were isolated and evidence is given for the following structures: 7α,12α-*dihydroxyandrosta*-1,4-*diene*-3,17-*dione*, 12β-*hydroxyandrosta*-1,4,6-*triene*-3,17-*dione*, 7α,12β,17β-*trihydroxyandrosta*-1,4-*dien*-3-*one* and 7α,12α-dihydroxy-3-oxopregn-4-ene-20-carboxylic acid. The significance of the production of the steroid products is discussed, along with the possible enzymic mechanisms responsible for their production.

Reports on the microbial degradation of cholic acid have been extensive. However, most of the reports involve only minor transformations. Evidence has been shown of hydroxy steroid dehydrogenation of the 3α-, 7α- and 12α-hydroxy groups, oxido-reduction of the 3α- and 12α-hydroxy groups, dehydroxylation of the 3α-, 7α- and 12α-hydroxy groups and nuclear dehydrogenation between C-1 and C-2, C-4 and C-5, and C-6 and C-7 (Hayakawa, 1973; Midtvedt, 1974).

The microbial side-chain cleavage of cholic acid has been shown, most of the products being C_{22} steroids. *Aspergillus niger* (Furuta, 1959), *Corynebacterium equi*, *Streptomyces gelaticus* (Hayakawa, 1973) and *Mycobacterium mucosum* (Severina *et al.*, 1968, 1969) have all been shown to degrade cholic acid, giving rise to a series of 4-en-3-one and 4,6-dien-3-one C_{22} steroids. Some evidence has also been presented for the isolation of a 4-en-3-one C_{20} steroid from a culture of *Aspergillus niger* (Furuta, 1959) and a 4,6-dien-3-one C_{23} steroid from a culture of *Mycobacterium mucosum* (Severina *et al.*, 1969). Cholic acid has been found to be degraded under anaerobic conditions by a strain of *Escherichia coli* (Tenneson *et al.*, 1977) and a series of *Bacteroides* species (Owen *et al.*, 1977) to both C_{22} and C_{19} products.

Microbial degradation of the steroid nucleus of cholic acid has been observed and a series of hexahydroindane metabolites were isolated (Hayakawa, 1973).

In the present paper we provide evidence for the structure and configuration of the two main side-chain-cleavage products (2 and 6) isolated during the aerobic degradation of cholic acid by *Pseudomonas* sp. N.C.I.B. 10590. Four minor compounds were isolated and their structures are discussed.

† To whom requests for reprints should be sent, at present address: International Development Laboratory, E. R. Squibb and Sons Ltd., Reeds Lane, Moreton, Merseyside L46 1QW, U.K.

Experimental

Materials

Cholic acid, 5α-cholestane and androsta-1,4-diene-3,17-dione were obtained from Koch–Light Laboratories (Colnbrook, Bucks., U.K.). General reagents were of AnalaR grade and obtained from BDH Chemicals (Poole, Dorset, U.K.), and all solvents were redistilled before use.

Methods

Melting points, elemental analyses, i.r., u.v., p.m.r. and mass spectra, analysis by g.l.c. and t.l.c., and oxidation, acetylation and reduction on t.l.c. were performed as described before (Tenneson *et al.*, 1979).

The aerobic metabolism of cholic acid by *Pseudomonas* sp. N.C.I.B. 10590 was carried out in a buffered mineral-salts medium (Tenneson *et al.*, 1979). A 1-litre shake-flask culture was incubated at 28°C on an L.H. Engineering orbital incubator. The course of the transformation was followed by the direct determination of the 1,4-dien-3-one steroids present in the culture. The filtration of samples through a 0.45μm Millipore filter enabled A_{252} determination of the steroidal mixture. When the A_{252} value reached a maximum, after 14h, the culture was terminated by direct extraction of the steroid metabolites into ethyl acetate (3×300ml). After the extract had been dried over $MgSO_4$, the solvent was removed under reduced pressure at 50°C to yield 318mg of a tarry residue. The residue was then taken up in warm dichloromethane (5ml) and

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separated by preparative t.l.c. into a series of fractions from which steroids 2, 3, 4, 5, 6 and 7 were crystallized.

7α,12β - Dihydroxyandrosta - 1,4 - diene - 3,17 - dione (compound 2, Fig. 1)

Recrystallization of compound 2 from methanol/dichloromethane yielded white prisms (16mg), m.p. 245–246°C (Found: C, 72.00; H, 7.40. $C_{19}H_{24}O_4$ requires: C, 72.15; H, 7.59%). $\nu_{max.}$ 3550 (hydroxy), 3345 (hydroxy), 1726 (ketone), 1654 (3-ketone), 1608 and 1598 cm^{-1} ($C_{(1)}$–$C_{(2)}$ and $C_{(4)}$–$C_{(5)}$ double bonds); $\lambda_{max.}$ 244nm (ε 14860); δ 1.06, 1.31 (6H, s, 18-CH_3 and 19-CH_3), 2.44–2.68 (2H, m, 16-CH_2), 3.80 (1H, 4-line, m, $J = 5$, 10Hz, 12α-H), 4.24 (1H, s, 7β-H), 6.22 (1H, s, slight splitting, 4-H), 6.32 (1H, d, further splitting, $J = 10$Hz, 2-H) and 7.10 (1H, d, $J = 10$Hz, 1-H); M^+ 316 ($C_{19}H_{24}O_4$ requires M^+ 316), *m/e* 122 (1,4-dien-3-one), *m/e* 150 (7-hydroxy-1,4-dien-3-one), *m/e* 280 (M^+-2H_2O) and *m/e* 298 (M^+-H_2O). G.l.c. R_F 4.4; t.l.c. R_F 0.58, after oxidation R_F 0.91, after acetylation R_F 1.00 and after reduction R_F 0.34.

7α,12α - Dihydroxyandrosta - 1,4 - diene - 3,17 - dione (compound 3, Fig. 1)

Recrystallization of compound 3 from methanol/dichloromethane yielded white needles (2mg), m.p. 236–238°C. $\nu_{max.}$ 3385 (hydroxy), 1725 (ketone), 1655 (3-ketone), 1612 and 1600 cm^{-1} ($C_{(1)}$–$C_{(2)}$ and $C_{(4)}$–$C_{(5)}$ double bonds); $\lambda_{max.}$ 244nm (ε 14900); δ 1.05, 1.30 (6H, s, 18-CH_3 and 19-CH_3), 2.45–2.65 (2H, m, 16-CH_2), 4.10 (1H, t, $J = 3$Hz, 12β-H), 4.22 (1H, s. 7β-H), 6.21 (1H, s, slight splitting, 4-H), 6.31 (1H, d, further splitting, $J = 10$Hz, 2-H) and 7.09 (1H, d, $J = 10$Hz, 1-H); M^+ 316 ($C_{19}H_{24}O_4$ requires M^+ 316), *m/e* 122 (1,4-dien-3-one), *m/e* 150 (7-hydroxy-1,4-dien-3-one), *m/e* 280 (M^+-2H_2O) and *m/e* 298 (M^+-H_2O). G.l.c. R_F 4.5; t.l.c. R_F 0.44, after oxidation R_F 0.91, after acetylation R_F 0.99 and after reduction R_F 0.20.

12β-Hydroxyandrosta-1,4,6-triene-3,17-dione (compound 4, Fig. 1)

Recrystallization of compound 4 from methanol/dichloromethane yielded white needles (1mg), m.p. 230–232°C. $\nu_{max.}$ 3510 (hydroxy), 1737 (17-ketone), 1655 (3-ketone), 1619, 1599 and 1578 cm^{-1} ($C_{(1)}$–$C_{(2)}$, $C_{(4)}$–$C_{(5)}$ and $C_{(6)}$–$C_{(7)}$ double bonds); $\lambda_{max.}$ 224, 256 and 300nm (ε 12300, 12080 and 13220); M^+ 298 ($C_{19}H_{22}O_3$ requires M^+ 298), *m/e* 133 (1,4,6-trien-3-one) and *m/e* 280 (M^+-H_2O). G.l.c. R_F 2.6; t.l.c. R_F 0.86, after oxidation R_F 0.88, after acetylation R_F 0.98 and after reduction R_F 0.54.

7α,12β,17β - Trihydroxyandrosta - 1,4 - dien - 3 - one (compound 5, Fig. 1)

Recrystallization of compound 5 from methanol/dichloromethane yielded white needles (1mg), m.p. 201–203°C. $\nu_{max.}$ 3550 (hydroxy), 3340 (hydroxy), 1656 (3-ketone), 1615 and 1600 cm^{-1} ($C_{(1)}$–$C_{(2)}$ and $C_{(4)}$–$C_{(5)}$ double bonds); $\lambda_{max.}$ 244nm (ε 14320); M^+ 318 ($C_{19}H_{26}O_4$ requires M^+ 318), *m/e* 122 (1,4-dien-3-one), *m/e* 150 (7-hydroxy-1,4-dien-3-one), *m/e* 264 (M^+-3H_2O), *m/e* 282 (M^+-2H_2O), *m/e* 300 (M^+-H_2O). G.l.c. R_F 5.3; t.l.c. R_F 0.34, after oxidation R_F 0.91, after acetylation R_F 1.02 and after reduction R_F 0.34.

Methyl 7α,12α-dihydroxy-3-oxopregna-1,4-diene-20-carboxylate (methyl ester of compound 6, Fig. 1)

Recrystallization of the *methyl ester of compound 6* from methanol/dichloromethane yielded white prisms (9mg), m.p. 265–267°C (Found: C, 71.20; H, 8.32. $C_{23}H_{32}O_5$ requires: C, 71.13; H, 8.25%). $\nu_{max.}$ 3415 (hydroxy), 1710 (carboxy), 1652 (3-ketone), 1616 and 1600 cm^{-1} ($C_{(1)}$–$C_{(2)}$ and $C_{(4)}$–$C_{(5)}$ double bonds); $\lambda_{max.}$ 244nm (ε 14360); δ 0.81, 1.25 (6H, s, 18-CH_3 and 19-CH_3), 1.27 (3H, d, $J = 6$Hz, 21-CH_3), 3.71 (3H, s, 22-OCH_3), 3.96 (1H, t, $J = 3$Hz, 12β-H), 4.10 (1H, s, 7β-H), 6.20 (1H, s, slight splitting, 4-H), 6.30 (1H, d, further splitting, $J = 10$Hz, 2-H) and 7.06 (1H, d, $J = 10$Hz, 1-H); M^+ 388 ($C_{23}H_{32}O_5$ requires M^+ 388), *m/e* 121 (1,4-dien-3-one), *m/e* 150 (7-hydroxy-1,4-dien-3-one), *m/e* 265 (M^+ – side chain + $2H_2O$), *m/e* 352 (M^+-2H_2O) and *m/e* 370 (M^+-H_2O). G.l.c. R_F 9.1; t.l.c. R_F 0.60, after oxidation R_F 1.00, after acetylation R_F 1.10 and after reduction R_F 0.60.

Methyl 7α,12α-dihydroxy-3-oxopregn-4-ene-20-carboxylate (methyl ester of compound 7, Fig. 1)

Recrystallization of the methyl ester of compound 7 from methanol/dichloromethane yielded white prisms (5mg), m.p. 258–260°C. $\nu_{max.}$ 3410 (hydroxy), 1705 (carboxy), 1652 (3-ketone), 1615 ($C_{(4)}$–$C_{(5)}$ double bond); $\lambda_{max.}$ 241nm (ε 15200); δ 0.80, 1.24 (6H, s, 18-CH_3 and 19-CH_3), 1.26 (3H, d, $J = 6$Hz, 21-CH_3), 3.70 (3H, s, 22-OCH_3), 3.95 (1H, t, $J = 3$Hz, 12β-H), 4.11 (1H, s, 7β-H) and 6.17 (1H, s, 4-H); M^+ 390 ($C_{23}H_{34}O_5$ requires M^+ 390), *m/e* 124 (4-en-3-one), *m/e* 152 (7-hydroxy-4-en-3-one), *m/e* 267 (M^+ – side chain + $2H_2O$), *m/e* 354 (M^+-2H_2O) and *m/e* 372 (M^+-H_2O). G.l.c. R_F 8.0; t.l.c. R_F 0.64, after oxidation R_F 1.05, after acetylation R_F 1.15 and after reduction R_F 0.64.

Phenolic compounds

A crude mixture of compounds remained after crystallization of the steroidal metabolites: $\lambda_{max.}$ 218 and 275nm (methanol), 220 and 298nm (NaOH/methanol). G.l.c. showed two main metabolites, R_F 0.2 and 0.8, and t.l.c. two main metabolites, R_F 0.79 and 0.70; other metabolites had t.l.c. R_F 1.10, 0.86, 0.42 and 0.07.

Results

Pseudomonas sp. N.C.I.B. 10590 grew rapidly on sodium cholate, in a mineral salts medium. The concentration of 1,4-dien-3-one steroids in the medium showed a maximum after 1 h. The metabolites isolated after 14h transformation of cholic acid (1) are shown in Fig. 1.

The major neutral compound (2) shows a molecular ion at *m/e* 316 and intense ions at *m/e* 121 and 122 in the mass spectrum, suggesting a steroidal 1,4-dien-3-one A-ring structure (Budzikiewicz, 1972). Confirmation of this structure is provided by the i.r. spectrum (1654, 1608 and 1598 cm^{-1}, $\alpha\beta$-unsaturated ketone), by the u.v. spectrum ($\lambda_{max.}$ 244nm, di-β-substituted $\alpha\beta$-unsaturated ketone in a six-membered ring, double bond exocyclic) (Dorfman, 1953) and by the p.m.r. spectrum (three vinylic protons in the range δ 6.22–7.10p.p.m.). Compound 2 is easily oxidized, acetylated and reduced, suggesting the presence of both a hydroxy and a ketone group. This is confirmed by the i.r. spectrum, which contains peaks at 3550 and 3345 cm^{-1} characteristic of hydroxy groups and a peak at 1726 cm^{-1} characteristic of a ketone group. An intense ion at *m/e* 150 in the mass spectrum of compound 2 suggests the presence of a steroidal 7-hydroxy-1,4-dien-3-one structure. The p.m.r. spectrum of compound 2 shows a broad single peak centred at δ 4.24p.p.m. The 7β-proton bisects the dihedral angle between the two protons at C-6; the proton at C-8 presumably causes the expected triplet to be unresolved (Bridgeman *et al.*, 1970). One of the hydroxy groups of compound 2 is therefore assigned the 7α-configuration. This is in agreement with the assignment of the 7α-configuration to the hydroxy group of 7α-hydroxyandrosta-1,4-diene-3,17-dione (Tenneson *et al.*, 1979). The p.m.r. spectrum of compound 2 also shows a four-line multiplet centred

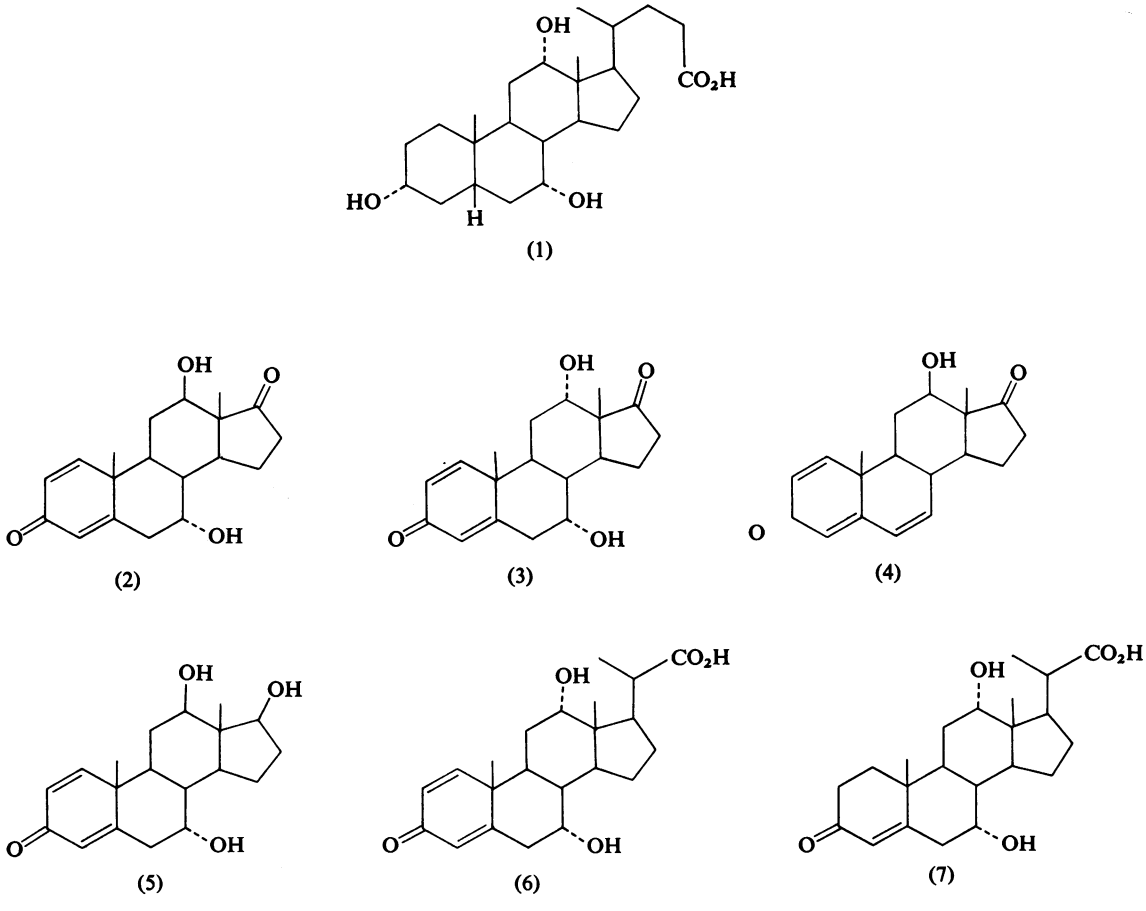

Fig. 1. *Metabolites isolated from the degradation of cholic acid by Pseudomonas sp. N.C.I.B.* 10590

at δ 3.80p.p.m. The 12α-proton causes spin–spin coupling with the two protons at C-11; the dihedral angles are about 60° and 180°, giving two different coupling constants of 5Hz and 10Hz. The ketone group at C-17 causes a de-shielding effect, lowering the expected chemical shift (Bridgeman *et al.*, 1970). On this basis the other hydroxy group of compound 2 is assigned the 12β-configuration. This is in agreement with the assignment of the 12β-configuration to the hydroxy group of 12β-hydroxyandrosta-1,4-diene-3,17-dione (Barnes *et al.*, 1976). Further support for the assignment of the configuration to each hydroxy group is provided by the observation (Zietz & Spiteller, 1974) that loss of the elements of water from the molecular ion in the mass spectrometer occurs more readily with axial hydroxy groups than with equatorial hydroxy groups. With compound 2 an intense ion at *m/e* 298 and a low-intensity ion at *m/e* 280 were observed, corresponding to the presence of an axial 7α-hydroxy group and an equatorial 12β-hydroxy group respectively. Compound 2 is therefore assigned the structure *7α,12β-dihydroxyandrosta-1,4-diene-3,17-dione.*

One of the minor neutral compounds (3) shows a molecular ion at *m/e* 316 and intense ions at *m/e* 121 and 122 in the mass spectrum, suggesting a steroidal 1,4-dien-3-one A-ring structure. Confirmation of this structure is provided by the i.r., u.v. and p.m.r. spectra. Compound 3 is easily oxidized, acetylated and reduced, suggesting the presence of both a hydroxy and a ketone group, and this is confirmed by the i.r. spectrum. One of the hydroxy groups is assigned the 7α-configuration from the mass spectrum, an intense ion at *m/e* 150, and the p.m.r. spectrum, a broad single peak centred at δ 4.22p.p.m. The p.m.r. spectrum of compound 3 also shows a triplet at δ 4.10p.p.m. The 12β-proton bisects the dihedral angle between the two protons at C-11, and only one coupling constant (J=3Hz) can be discerned. The C-17 ketone group again causes a lowering of the expected chemical shift (Bridgeman *et al.*, 1970). On this basis the other hydroxy group of compound 3 is assigned the 12α-configuration. This is in agreement with the assignment of the 12α-configuration to the hydroxy group of 12α-hydroxyandrosta-1,4-diene-3,17-dione (Barnes *et al.*, 1976). Intense ions at *m/e* 298 and 280 in the mass spectrum of compound 3 confirm the presence of the axial 7α- and 12α-hydroxy groups. Compound 3 is therefore assigned the structure *7α,12α-dihydroxyandrosta-1,4-diene-3,17-dione.*

Another minor neutral compound (4) shows a molecular ion at *m/e* 298 and an intense ion at *m/e* 133, suggesting a steroidal 1,4,6-trien-3-one A-ring structure (Budzikiewicz, 1972). Confirmation of this structure is provided by the i.r. spectrum (1655, 1619, 1599 and 1578cm^{-1}, $\alpha\beta,\gamma\delta$-unsaturated ketone) and by the u.v. spectrum ($\lambda_{max.}$ 224, 256 and 300nm, di-β-substituted $\alpha\beta$-unsaturated ketone in a six-membered ring, double bond exocyclic, extended by a double bond at $C_{(6)}$–$C_{(7)}$) (Dorfman, 1953). Compound 4 is easily oxidized, acetylated and reduced, suggesting the presence of both a hydroxy and a ketone group. This is confirmed by the i.r. spectrum, which contains a peak at 3510cm^{-1} characteristic of a hydroxy group and a peak at 1737cm^{-1} characteristic of a ketone group in a five-membered ring. The oxidation product of compound 4 is slightly more polar than the oxidation products of compounds 2 and 3. This shows that the oxygen substitution pattern is probably the same in all three compounds, the difference in polarity being caused by the different B-ring structures. Insufficient pure compound 4 is available to obtain p.m.r. spectra, so the stereochemistry of the hydroxy group is inferred from the mass-spectral analysis. Compound 4 shows a low-intensity ion at *m/e* 280 in the mass spectrum, indicating the presence of an equatorial 12β-hydroxy group. Compound 4 is therefore tentatively assigned the structure *12β-hydroxyandrosta-1,4,6-triene-3,17-dione.*

The other minor neutral compound (5) shows a molecular ion at *m/e* 318 and an intense ion at *m/e* 122, suggesting a steroidal 1,4-dien-3-one A-ring structure. Confirmation of this structure is provided by the i.r. and u.v. spectra. Compound 5 is easily oxidized and acetylated, but resists reduction, suggesting the presence of a hydroxy group. The i.r. spectrum confirms the presence of at least two hydroxy groups. An intense ion at *m/e* 150 in the mass spectrum of compound 5 suggests the presence of a steroidal 7-hydroxy-1,4-dien-3-one structure. The oxidation product of compound 5 shows the same polarity on t.l.c. as the oxidation product of both compounds 2 and 3. This shows that the oxygen substitution pattern is the same in all three compounds. The polarity of compound 5 on t.l.c. agrees with the reduction product of compound 2. This suggests that compound 5 contains three hydroxy groups, two of which are probably 7α and 12β in configuration. The mass spectrum of compound 5 confirms the presence of three hydroxy groups, showing an intense ion at *m/e* 300 and two low-intensity ions at *m/e* 282 and 264. The hydroxy groups are probably 7α (axial), 12β (equatorial) and 17β (equatorial) in configuration. Compound 5 is therefore tentatively assigned the structure *7α,12β,17β-trihydroxyandrosta-1,4-dien-3-one.*

The major acidic compound (6) was isolated as a crystalline solid. The methyl ester of compound 6 shows a molecular ion at *m/e* 388 and an intense ion at *m/e* 121, suggesting a steroidal 1,4-dien-3-one A-ring structure. Confirmation of this structure is provided by the i.r., u.v. and p.m.r. spectra. Compound 6 is easily oxidized and acetylated, but resists reduction, suggesting the presence of a hydroxy group. This is confirmed by the i.r. spectrum.

An intense ion at *m/e* 150 in the mass spectrum of the methyl ester of compound 6 suggests the presence of a steroidal 7-hydroxy-1,4-dien-3-one structure. The p.m.r. spectrum of the methyl ester of compound 6 shows a broad single peak centred at δ 4.10p.p.m. and a triplet centred at δ 3.96p.p.m., and on this basis the hydroxy groups of compound 6 are assigned the 7α- and 12α-configurations. Confirmation for this assignment is provided by the presence of two intense ions at *m/e* 370 and 352 in the mass spectrum. An intense ion at *m/e* 265 in the mass spectrum of the methyl ester of compound 6 corresponds to loss of the side chain from C-17. Compound 6 is therefore assigned the structure *7α,12α-dihydroxy-3-oxopregna-1,4-diene-20-carboxylic acid.*

A minor acidic compound (7) was isolated, the methyl ester of which shows a molecular ion at *m/e* 390 and an intense ion at *m/e* 124 in the mass spectrum, suggesting a steroidal 4-en-3-one A-ring structure (Budzikiewicz, 1972). Confirmation of this structure is provided by the i.r. spectrum (1652 and 1615cm^{-1}, $\alpha\beta$-unsaturated ketone), by the u.v. spectrum ($\lambda_{max.}$ 241 nm, di-β-substituted $\alpha\beta$-unsaturated ketone in a six-membered ring, double bond exocyclic) (Dorfman, 1953) and by the p.m.r. spectrum (one vinylic proton at 6.17δ). Compound 7 is easily oxidized and acetylated, but resists reduction, suggesting the presence of a hydroxy group. This is confirmed by the i.r. spectrum. An intense ion at *m/e* 152 in the mass spectrum of the methyl ester of compound 7 suggests the presence of a steroidal 7-hydroxy-4-en-3-one structure. As with compound 6, the hydroxy groups are assigned the 7α- and 12α-configuration from the p.m.r. spectrum. Intense ions at *m/e* 372, 354 and 267 in the mass spectrum confirm the loss of a 7α- and 12α-hydroxy group and the side chain from C-17. Compound 7 is therefore 7α,12α-dihydroxy-3-oxopregn-4-ene-20-carboxylic acid, previously isolated by Severina *et al.* (1969).

The yield of the steroidal metabolites isolated is listed in Table 1.

Table 1. *Yield of metabolites with respect to starting material after 14h incubation*
Experimental details are given in the text.

Metabolite	Yield (%)
7α,12β-Dihydroxyandrosta-1,4-diene-3,17-dione (2)	20
7α,12α-Dihydroxyandrosta-1,4-diene-3,17-dione (3)	2
12β-Hydroxyandrosta-1,4,6-triene-3,17-dione (4)	1
7α,12β,17β-Trihydroxyandrosta-1,4-diene-3-one (5)	2
7α,12α-Dihydroxy-3-oxopregna-1,4-diene-20-carboxylic acid (6)	11
7α,12α-Dihydroxy-3-oxopregn-4-ene-20-carboxylic acid (7)	5

A crude mixture was left after the removal of the steroidal metabolites. This mixture contained two main metabolites, both of which agreed on t.l.c. and g.l.c. with the non-steroidal phenolic compounds isolated from the degradation of chenodeoxycholic acid by *Pseudomonas* sp. N.C.I.B. 10590 (Tenneson *et al.*, 1979).

Discussion

The isolation and identification of steroidal metabolites 2, 3, 4 and 5 during the degradation of cholic acid by *Pseudomonas* sp. N.C.I.B. 10590 is the first recorded instance of the microbial side-chain cleavage of cholic acid under aerobic conditions yielding androstanes. Partial side-chain-cleavage products have been isolated previously, and these include compound 7 (Severina *et al.*, 1969). However, the present findings include the first recorded instance of the isolation of compound 6 from the aerobic degradation of cholic acid.

The microbial degradation of bile acids is potentially of importance in the commercial production of physiologically active steroids (Appleweig, 1974). Cholic acid is the most commonly occurring bile acid and is freely available in bovine bile. *Pseudomonas* sp. N.C.I.B. 10590 has been shown to contain the enzymes necessary for the production of androstanes from cholic acid. Therefore physiologically active steroids could be produced from cholic acid by microbial degradation and subsequent chemical modification.

All side-chain-cleavage products isolated from the degradation of cholic acid by *Pseudomonas* sp. N.C.I.B. 10590 are either 4-en-3-one or 1,4-dien-3-one steroids. Such transformations have been shown to be necessary before side-chain cleavage can occur (Hayakawa, 1973). This is illustrated in the degradation of lithocholic acid (Tenneson *et al.*, 1978*a*) and chenodeoxycholic acid (Tenneson *et al.*, 1979) by *Pseudomonas* sp. N.C.I.B. 10590. Side-chain cleavage proceeds probably by β-oxidation from a C_{24} bile acid through a C_{22} metabolite to a C_{19} androstane. Such cleavage has been reported in the degradation of taurocholic acid and glycocholic acid by *Pseudomonas* sp. N.C.I.B. 10590 (Tenneson *et al.*, 1978*b*). The hydroxy group at C-12 is epimerized from the α-position in the C_{22} steroids (compounds 6 and 7) to the β-position in the major C_{19} steroid (compound 2). This epimerization, which is also shown in the degradation of deoxycholic acid by *Pseudomonas* sp. N.C.I.B. 10590 (Barnes *et al.*, 1976), is probably necessary before side-chain cleavage of the C_{22} steroids can occur. This would explain why the degradation of cholic acid by other micro-organisms proceeds only as far as a C_{22} steroid, the 12α-hydroxy group being inhibitory to any further side-chain cleavage.

Cholic acid is degraded to non-steroidal products (Hayakawa, 1973) by *Corynebacterium simplex*. The non-steroidal metabolites isolated from the degradation of cholic acid, chenodeoxycholic acid (Tenneson *et al.*, 1979), taurocholic acid and glycocholic acid (Tenneson *et al.*, 1978*b*) by *Pseudomonas* sp. N.C.I.B. 10590 are phenolic in nature. The mechanism of formation of these metabolites is probably similar to the pathway shown by the microbial degradation of androsta-1,4-diene-3,17-dione (Sih & Whitlock, 1968).

Compounds 2 and 6 are produced under anaerobic conditions by an *Escherichia coli* strain isolated from a faecal sample of a colon-cancer patient (Tenneson *et al.*, 1977). Since cholic acid is the major bile acid in man, a study on the carcinogenicity of compounds 2 and 6 would be of value. If these compounds are carcinogenic, this would add support to the hypothesis that the degradation of bile acid by bacteria is implicated in the aetiology of colon cancer (Hill, 1975).

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References

Appleweig, N. (1974) *Chem. Week* 10 July, 31–36

Barnes, P. J., Baty, J. D., Bilton, R. F. & Mason, A. N. (1976) *Tetrahedron* **32**, 89–93

Bridgeman, J. E., Cherry, P. C., Clegg, A. S., Evans, J. M., Jones, E. R. H., Kasal, A., Meakins, G. D., Morisawa, Y., Richards, E. E. & Woodgate, P. D. (1970) *J. Chem. Soc. C* 250–257

Budzikiewicz, H. (1972) in *Biochemical Applications in Mass Spectrometry* (Waller, G. R., ed.), pp. 251–289, Wiley–Interscience, New York

Dorfman, L. (1953) *Chem. Rev.* **53**, 47–144

Furuta, T. (1959) *Hiroshima J. Med. Sci.* **8**, 311–322

Hayakawa, S. (1973) *Adv. Lipid Res.* **11**, 143–192

Hill, M. J. (1975) *Cancer* **36**, 2387–2400

Midtvedt, T. (1974) *Am. J. Clin. Nutr.* **27**, 1341–1347

Owen, R. W., Bilton, R. F. & Tenneson, M. E. (1977) *Biochem. Soc. Trans.* **5**, 1711–1713

Severina, L. O., Torgov, I. V., Skrjabin, G. K., Zaretskii, V. I., Wulfson, N. S. & Papernaja, I. B. (1968) *Tetrahedron* **24**, 2145–2153

Severina, L. O., Torgov, I. V., Skrjabin, G. K., Zaretskii, V. I., Wulfson, N. S. & Papernaja, I. B. (1969) *Tetrahedron* **25**, 485–491

Sih, C. J. & Whitlock, H. W. (1968) *Annu. Rev. Biochem.* **37**, 661–694

Tenneson, M. E., Owen, R. W. & Mason, A. N. (1977) *Biochem. Soc. Trans.* **5**, 1758–1760

Tenneson, M. E., Bilton, R. F. & Mason, A. N. (1978*a*) *FEBS Lett.* **91**, 140–143

Tenneson, M. E., Bilton, R. F. & Mason, A. N. (1978*b*) *Biochem. Soc. Trans.* **6**, 975–977

Tenneson, M. E., Baty, J. D., Bilton, R. F. & Mason, A. N. (1979) *J. Steroid Biochem.* **10**, 311–316

Zietz, E. & Spiteller, G. (1974) *Tetrahedron* **30**, 586–596