Tetrahedron Vol. 40, No. 2, pp. 421 to 426, 1984
Printed in Great Britain.

0040-4020/84 $3.00 + .00
Pergamon Press Ltd.

THE ANAEROBIC DEGRADATION OF DEOXYCHOLIC ACID BY *PSEUDOMONAS* SP. NCIB 10590

R. W. OWEN,† A. N. MASON and R. F. BILTON
Department of Chemistry and Biochemistry, Liverpool Polytechnic, Liverpool, England

(*Received in UK 17 February 1983*)

Abstract—The anaerobic metabolism of deoxycholic acid by *Pseudomonas* sp. NCIB 10590 was studied. The metabolic pathway was similar to that operating under aerobic conditions with 12β-hydroxyandrosta-1,4-dien-3,17-dione as the major neutral product and 12α-hydroxy-5β-cholan-3-oxo-24-oic acid as the major acidic product. Four metabolites which are not produced during aerobic metabolism were isolated and evidence is presented for the following structures: 9α-hydroxyandrost-1-en-3,17-dione, 12α,17β-dihydroxyandrosta-1,4-dien-3-one; 3β,12β-dihydroxy-5β-androstan-17-one and 3-hydroxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione. The mechanism of formation and significance of the phenolic secosteroid is discussed.

In previous publications[1,2] we reported the transformation of deoxycholic acid by *Pseudomonas* sp. NCIB 10590 in mineral salts medium to a mixture of neutral and acidic steroids. The major neutral product, 12β-hydroxyandrosta-1,4-dien-3,17-dione (**3**) and the major acidic product 12α-hydroxypregna-1,4-dien-3-one-20-carboxylic acid (**12**) were identified. These intermediates predominated under both aerobic and anoxic conditions. Under aerobic conditions phenolic compounds were also detected but were not identified.

It has been proposed that the microbial transformations of bile acids may be implicated in the aetiology of breast[3] and colon cancer[4] especially those transformations which occur under anaerobic conditions where potassium nitrate,[5] phenazine methosulphate[6] or menadione[7] can be utilised instead of molecular oxygen as terminal electron acceptor. We have shown that possible carcinogenic compounds such as phenols can be produced from deoxycholic acid by *Pseudomonas* sp. NCIB 10590 under aerobic conditions and thus we have extended the study to include transformation under strict anaerobic conditions with potassium nitrate as terminal electron acceptor. This represents a good model system for studying bile acid metabolism under conditions similar to that in the gut where the carcinogenic process, leading to colon cancer is proposed to be mediated by bacteria.

Here we report the structures of several compounds which are not produced under either aerobic or anoxic conditions and these include a phenolic secosteroid.

RESULTS AND DISCUSSION

A resting cell suspension of *Pseudomonas* sp. NCIB 10590 was incubated in the presence of sodium deoxycholate in a mineral satls medium with potassium nitrate as terminal electron acceptor. After 6 weeks the fermentation was terminated by direct extraction with ethyl acetate and the products separated into neutral and acidic fractions. The metabolites differed in the nature of the substituent at C_{12}, the degree of unsaturation of the A ring, and in the case of the acids, length of the side chain.

Ten neutral compounds were isolated and of these 12β-hydroxyandrost-4-en-3,17-dione (**2**), 12β-hydroxyandrosta-1,4-dien-3,17-dione (**3**), 12α-hydroxyandrost-4-en-3,17-dione (**4**), 12α-hydroxyandrosta-1,4-dien-3,17-dione (**5**), 12β,17β-dihydroxyandrost-4-en-3-one (**6**) and 12β,17β-dihydroxyandrosta-1,4-dien-3-one (**7**) have been previously characterised.[2]

The four (Fig. 1) remaining neutral steroids (**8–11**) did not display many common spectral characteristics and thus structural elucidation is described separately.

The mass spectrum of **8** showed an intense ion (base peak) at *m/e* 122 (100%) which is typical for either a steroidal 1,4-dien-3-one or a 1-en-3-one A ring structure.[8] The nature of the A ring oxidation state was resolved by the UV spectrum (λ max, 231 nm, di-β-substituted α, β-unsaturated ketone in a 6-membered ring, double bond exocyclic) which is typical for a 1-en-3-one A ring structure;[9] IR spectrum (1690 and 1615 cm^{-1}, αβ-unsaturated ketone) and PMR spectrum (two vinylic protons in the range 5.91–6.76 δ).

Compound **8** could not be oxidised but was easily acetylated and reduced on TLC. This suggested the presence of an OH group and an unconjugated ketone group. This was confirmed by the IR spectrum which displayed a significant peak (3460 cm^{-1}) in the OH group region and CO region (1740 cm^{-1}). Failure to oxidise the OH group with Jones' chromic reagent[10] indicated that it occupied a tertiary position on the steroid molecule. The mass spectrum in addition to the base peak at *m/e* 122 also showed a low intensity ion at M^+-18 (H_2O, 12%) and a low intensity ion at *m/e* 135 (39%) which are typical of the 9α-hydroxysteroids.[8] The tertiary nature of the OH group was emphasised by the PMR spectrum which did not display any proton peaks due to a C atom carrying a OH group. This is again typical of 9α-hydroxysteroids.[11] Compound **8** was therefore assigned the structure 9α-hydroxyandrost-1-en-3,17-dione.

Compound **9** appeared from its TLC and GLC behaviour to be similar in nature to **7**. The mass spectrum showed an intense ion (base peak) at *m/e* 122 (100%) indicating a 1,4-dienone A ring structure. Compound **9** was readily oxidised to the known[1] trione indicating a 3,12,17 oxygen substitution pattern.

Compound **9** was acetylated but could not be reduced on TLC and was therefore classified as a 12,17-diol. This was confirmed by the IR spectrum which displayed two

†Present address. PHLS Centre for Applied Microbiology and Research, Bacterial Metabolism Research Laboratory, Porton Down, Salisbury, Wiltshite SP4 0JG, England.

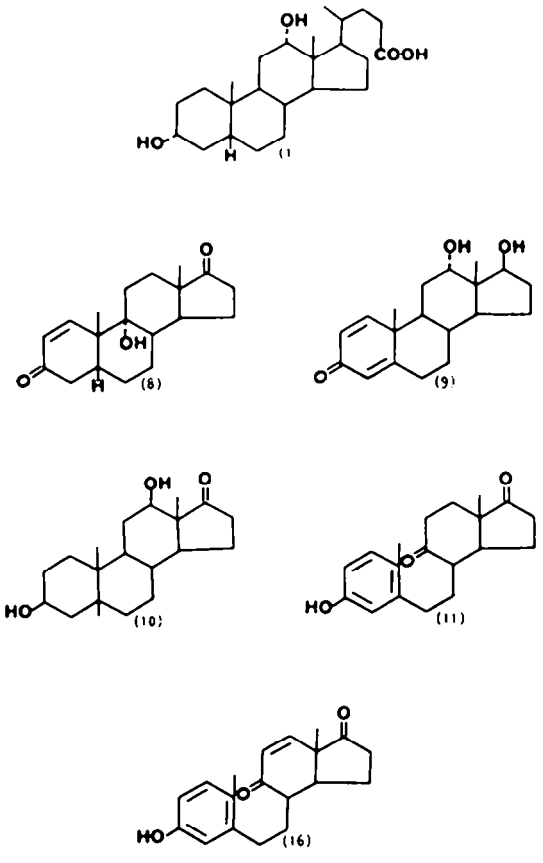

Fig. 1. Previously uncharacterised metabolites of deoxycholic acid (1) produced by *Pseudomonas* sp. NCIB 10590 under anaerobic conditions.

significant peaks (3480 and 3350 cm $^{-1}$) in the OH group region.

A PMR spectrum could not be obtained due to lack of material, thus the position and stereochemistry of the OH groups was inferred from the mass spectrum. An intense ion (with respect to the M$^+$) at M$^+$-18 (H_2O, 72%) was indicative of a 12α-axial, OH group and a low intensity ion at M$^+$-36 ($2H_2O$, 15%) was indicative of a 17β-equatorial group. This is in accordance with Zeitz and Spitellers observation[12] that in the mass spectra of steroids, loss of the elements of water from the molecular ion occurs more readily with axial OH groups than with equatorial OH groups.

Compound **9** was therefore assigned the structure 12α, 17β-dihydroxyandrosta-1,4-dien-3-one.

The mass spectrum of **10** gave many peaks characteristic of a dihydroxy saturated A-ring androstane[12] and contained the following characteristic major ions at *m/e* 41, 100%; *m/e* 55, 96%; *m/e* 67, 56%; *m/e* 81, 60%; *m/e* 97, 98%; *m/e* 107, 55%; *m/e* 121, 46%; *m/e* 133, 24%; *m/e* 147, 30%; *m/e* 201, 20%; *m/e* 232, 31%; *m/e* 262, 17%; *m/e* 270, 22% (M$^+$-36, $2H_2O$), *m/e* 273, 28%; *m/e* 288, 62% (M$^+$-18, H_2O) and *m/e* 306, 87% (M$^+$) which is almost identical to that of 3β, 12β-dihydroxy-5α-androstan-17-one.[12]

Confirmation of the saturated nature of this compound was provided by the IR spectrum which did not show evidence for a α,β-unsaturated ketone structure and the PMR spectrum which did not display any vinylic protons. The oxidation product of **10** was identical to the known trione 5β-androstan-3,12,17-trione synthesised from 12β-hydroxy-5β-androstan-3,17-dione indicating the same oxygen substitution pattern. Compound **10** was acetylated and reduced easily and on mild acetylation three products were obtained, presumably two monoacetate and one diacetate derivative. These results suggested the presence of two OH groups and one unconjugated ketone group. This was confirmed by IR and mass spectral evidence. The stereochemistry of the OH groups was deduced from the PMR spectrum: the chemical shift values and splitting patterns at 3.64 δ and 3.79 δ accorded with published values[13] for a 3α-proton and a 12α-proton in a 3β,17β-dihydroxy steroid. In the case of the 12α-proton the dihedral (Karplus)[14] angles with the 11β and 11α-protons are about 180° and 60° respectively giving rise to a four line multiplet (J = 10 Hz and 5 Hz).

Compound **10** was therefore assigned the structure 3β,12β-dihydroxy-5β-androstan-17-one.

The remaining neutral product, **11**, was recognised as a phenolic compound from its UV spectrum λ max 283 nm which underwent a bathochromic shift on addition of a base to λ max 292 nm. The shift was reversed on addition of acid. The compound when sprayed with Folin-Ciocalteau reagent on TLC and developed in an atmosphere of ammonia, gave a characteristic blue colour which is specific for phenolic OH groups.[15] The mass spectrum showed a molecular ion at *m/e* 300 (M$^+$, 39%) and a base peak at *m/e* 134 (100%) which is typical of 9,10-secosteroids.[16] The TMS-ether derivative gave a molecular ion at *m/e* 372 showing the presence of one OH group. In this instance the base peak of the spectrum was at *m/e* 206 showing that the OH group was attached to the A-ring. Compound **11** was acetylated and reduced easily but could not be oxidised indicating the presence of an OH group and unconjugated ketone. This was confirmed by IR which showed two peaks consistent with a D ring ketone (1740 cm $^{-1}$) and a C ring ketone (1705 cm^{-1}) and one peak corresponding to an OH function (3360 cm $^{-1}$). Peaks at 1608, 1585 and 1500 (aromatic ring), 1280 and 1250 (phenol) and at 855 and 800 cm $^{-1}$ (*o,m*-substituted phenol ring) gave further confirmation of a phenolic structure.

Compound **11** has therefore been assigned the structure 3-hydroxy-9,10-seco-1,3,5(10)-androstatrien-9,17-dione.

Four acidic metabolites were isolated and were identified as 12α-hydroxypregna-1,4-dien-3-one-20-carboxylic acid (**12**), 12α-hydroxychola-1,4-dien-3-one-24-oic acid (**13**), 12α-hydroxychol-4-en-3-one-24-oic acid (**14**) and 12α-hydroxy-5β-cholan-3-one-24-oic acid (**15**) which have been previously characterised.[1,2]

The anaerobic metabolism of deoxycholic acid (**1**) by *Pseudomonas* sp. NCIB 10590 was very similar to that under aerobic conditions; the major products possessing a 1,4-dienone A ring structure. A significant difference between the two conditions is that aerobically complete metabolism of the bile acid occurs in 24 hr whilst under anaerobic conditions complete metabolism does not occur after 6 weeks fermentation. However the results reported here do show that *Pseudomonas* sp. NCIB 10590 is capable of metabolising deoxycholic acid under strict anaerobic conditions even though the organism cannot grow under these conditions. Although complete metabolism is retarded the organism still produces compounds with an oxidised A ring of the 4-en and 1,4-dien-3-one structure. The side chain is also sequentially degraded to give bisnor acids (C_{22}) and androstane (C_{19}) steroids. The retardation

of metabolism noted under strict anaerobic conditions is probably effected by inhibition of induction of enzymes which require molecular oxygen such as 9α-hydroxylase. Under aerobic conditions the bile acid nucleus is degraded initially by cleavage of the allylic bond at C_9–C_{10} and involves hydroxylation at C_9 followed by a reverse *trans* aldol rearrangement to give secosteroids.[17] Secosteroids are then degraded via hexahydroindandione derivatives to compounds which can be incorporated into the intermediate metabolic pathways of bacteria.

Because 9α-hydroxylation is an oxygen requiring step it is unlikely that **8** was produced by this mechanism. Of interest is that **8** did not possess an OH group at C_{12} which is present on deoxycholic acid (**1**). Aerobic studies involving a deoxycholic acid intermediate 12β-hydroxyandrosta-1,4-dien-3,17-dione (**3**) revealed that the OH group at C_{12} is also removed under these conditions. Thus it seems unlikely that the OH group at C_{12} is involved in 9α-hydroxylation under anaerobic conditions by a process of transhydroxylation. However the secosteroid 3-hydroxy-1,3,5(10),11-androstatetraen-9,17-dione (**10**) produced under aerobic conditions possessed a double bond at C_{11}–C_{12} after removal of the OH group. It is therefore postulated that the double bond migrates to C_9–C_{11} and this allows the addition of water at C_9 followed by a reverse aldol type reaction to give the secosteroid **11** (Fig. 2).

In conclusion *Pseudomonas* sp. NCIB 10590 is capable of producing aromatic secosteroids from deoxycholic acid under anaerobic conditions, and a postulated pathway is given in Fig. 3. Aromatic steroids have been implicated in the aetiology of colon and breast cancer and thus it is necessary to elucidate the mechanism of formation more fully. It would also be of value to study such reactions by bacteria indigenous to the human intestinal tract, to determine whether or not the flora may be involved in the enterohepatic cycle of human hormones since it has also been shown that *Pseudomonas* sp. NCIB 10590 can produce the male sex hormone testosterone from lithocholic acid.[18]

EXPERIMENTAL

UV spectra were recorded in MeOH solns on a Pye-Unicam SP1800 recording spectrophotometer and IR spectra on KBr discs on a Pye-Unicam SP1200 spectrophotometer. The NMR spectra were recorded on a Jeol 220 operating at 220 MHz at 30° from solns in $CDCl_3$. Mass spectra were obtained using a Dupont 21–491 series mass spectrometer. Data reduction was performed on a Dupont 21–0948 data system.

Analysis by GLC was performed at 260° using 3% OV-17 on 80/100 mesh 'supelcoport' in a 1.5 M × 3 mm silanised glass column. Retention times were measured relative to 5α-cholestane at a flow rate of 30 cm^3 min^{-1} N_2 in a Hewlett-Packard HP 5470 instrument. Silyl ethers were prepared by dissolving the previously dried sample in bis-trimethylsilylacetamide and heating at 60° for 20 min. All acidic compounds were methylated prior to GLC.

TLC analysis was performed on 0.25 mm layers of Kiesel gel GF_{254} DC-Fertigplatten (E. Merck, Darmstadt) in the solvent systems MeOH : CH_2Cl_2, 1 : 9(1) or 2.2.4-trimethyl pentone : ethyl acetate : glacial AcOH 45 : 45 : 10(2). UV absorbing components were detected by observation under light of 254 nm wavelength and all components were finally visualised by spraying the plate with anisaldehyde and heating at 110° for 10 min.[19]

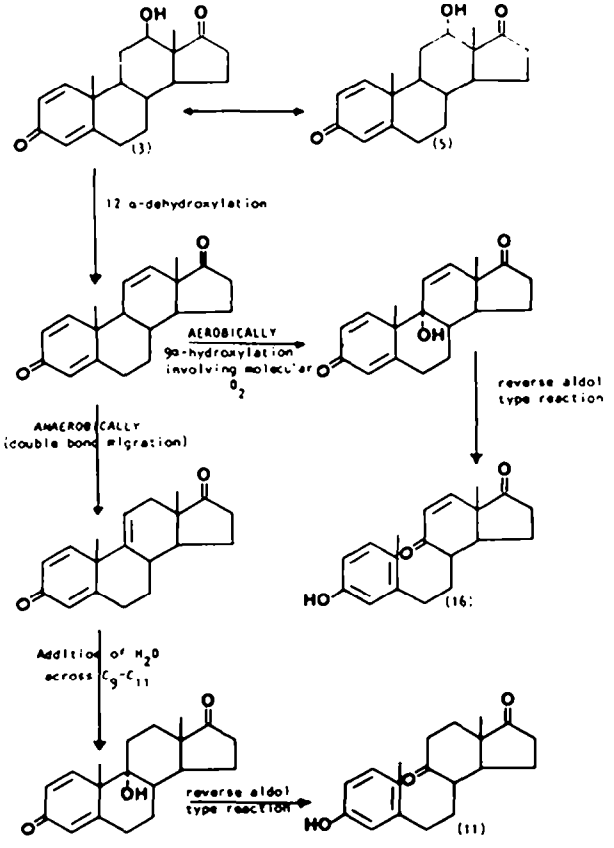

Fig. 2. Proposed mechanism of 9α-hydroxylation (followed by secosteroid formation) under anaerobic conditions.

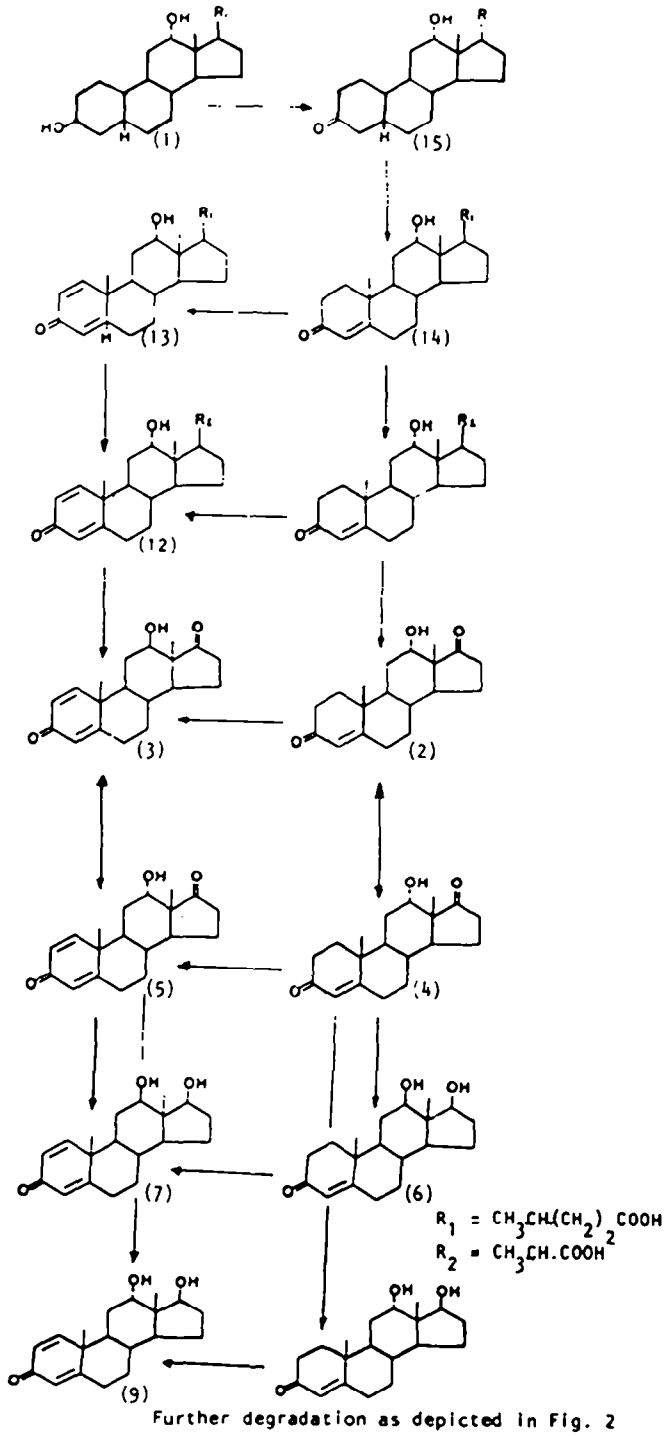

Fig. 3. Postulated pathway of deoxycholic acid degradation by Pseudomonas sp. NCIB 10590 under anaerobic conditions. Compounds isolated during this study are identified by arabic numerals.

Trienone steroids were prepared by treating an acetone soln of the hydroxysteroid with Jones chromic reagent at 4°. Reactions on TLC were performed by overspotting with Jones[10] chromic reagent (oxidation), acetyl chloride (acetylation) and with potassium borohydride (reduction).

The cells obtained by centrifugation of 4 l. of *Pseudomonas* sp. NCIB 10590 culture were used to inoculate 4 l. of medium of composition g/litre distilled water: sodium deoxycholate, 1.0; K_2HPO_4, 0.7; KH_2PO_4, 0.3; KNO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.0025; $ZnSO_4 \cdot 7H_2O$, 0.0025; $MnSO_4 \cdot 4H_2O$, 0.0025; final pH 7.2. This gave a resting cell suspension of the Pseudomonad with a final density of 1×10^9 cells ml^{-1}. The culture medium was steamed for 30 min immediately before inoculation to remove dissolved O_2. The top of the fermentation bottle was loosened (to allow gaseous exchange) prior to incubation at 28° for 6 weeks under 90% H_2: 10% CO_2 (with Pd catalyst to remove residual O_2). The fermentation was terminated by direct extraction of the metabolites into an equal volume of EtOAc and gave, after drying over $MgSO_4$ and evaporation, 1.85 g of tarry residue.

Table 1. Yield of metabolites with respect to starting material after 6 weeks anaerobic incubation

Number	Metabolites	Yield (%)
2	12β-hydroxyandrost-4-en-3,17-dione	0.31
3	12β-hydroxyandrosta-1,4-dien-3,17-dione	1.54
4	12α-hydroxyandrost-4-en-3,17-dione	0.15
5	12α-hydroxyandrosta-1,4-dien-3,17-dione	0.85
6	12β,17β-dihydroxyandrost-4-en-3-one	0.23
7	12β,17β-dihydroxyandrosta-1,4-dien-3-one	0.77
8	9α-hydroxyandrost-1-en-3,17-dione	0.54
9	12α,17β-dihydroxyandrosta-1,4-dien-3-one	0.23
10	3β,12β-dihydroxy-5β-androstan-17-one	0.16
11	3-hydroxy-9,10-seco-1,3,5(10)-androstatrien-9,17-dione	0.04
12	12α-hydroxypregna-1,4-dien-3-one-20-carboxylic acid	2.21
13	12α-hydroxychola-1,4-dien-3-one-24-oic acid	2.08
14	12α-hydroxychol-4-en-3-one-24-oic acid	1.58
15	12α-hydroxy-5β-cholan-3-oxo-24-oic acid	2.90

The residue was separated into acidic (1.23 g) and neutral (572 mg) fractions. The acids were methylated using BF_3/MeOH and separated by preparative TLC in solvent system II yielding residual deoxycholic acid (700 mg) and a series of fractions from which steroids 12–15 were crystallised. The neutral fraction was separated in solvent system I giving steroids 2–11. Metabolite yield is given in Table 1.

9α-Hydroxyandrost-1-en-3,17-dione (**8**). Recrystallisation of **8** from MeOH/CH_2Cl_2 gave white crystals (28 mg) m.p. 141° IR (KBr disc) 1615, 1690, 1740, 3460 cm^{-1}; λ max 231 nm (ξ 10,200); NMR ($CDCl_3$) δ 0.97, 1.22 (6H, s, C_{18} and C_{19} protons), 2.13–2.21 (2H, M, C_{16} protons), 5.91 (1H, d, J = 10 Hz, C_2 proton), 6.76 (1H, d, J = 10 Hz, C, proton); mass spectrum M$^+$ 302 (100%), 284 (12%), 135 (39%), 122 (80%); GLC R_f 1.75 (ov-17); TLC R_f 0.91, after oxidation R_f 0.91, after acetylation R_f 1.01, after reduction R_f 0.33.

12α,17β-Dihydroxyandrosta-1,4-dien-3-one (**9**). Recrystallisation of **9** from MeOH/CH_2Cl_2 gave white spikey crystals (12 mg). IR (KBr disc) 1600, 1610, 1660, 3350, 3480 cm^{-1}; max 244 nm; mass spectrum M$^+$ 302 (31%), 284 (72%), 266 (15%), 122 (100%); GLC R_f 3.0 (ov-17); TLC R_f 0.14; after oxidation R_f 0.85, after acetylation R_f 0.98, after reduction R_f 0.14.

3β,12β-Dihydroxy-5β-androstan-17-one (**10**). Recrystallistion of **10** from MeOH/CH_2Cl_2 gave white needles (8 mg) m.p. IR (KBr disc) 1725, 3420, 3505 cm^{-1}; λ max 292 nm (ξ 120); NMR ($CDCl_3$) δ 0.93, 0.96 (6H, s, C_{18} and C_{19} protons), 2.38–2.56 (2H, M, C_{16} protons), 3.64 (1H, M, C_3 proton), 3.79 (1H, 4-line M, J = 5, 10 Hz, C_{12} proton); mass spectrum M$^+$ 306 (87%), 288 (62%), 270 (22%); GLC R_f 1.20 (ov-17); TLC R_f 0.50, after oxidation R_f 0.94, after acetylation R_f 1.20, after reduction R_f 0.20.

3-Hydroxy-9,10-seco-1,3,5(10)-androstatrien-9,17-dione (**11**). Recrystallisation of **11** from MeOH/CH_2Cl_2 gave yellow crystals (2 mg), m.p. 122°; IR (KBr disc) 800, 855, 1250, 1280, 1500, 1585, 1608, 1705, 1749, 3360 cm^{-1}; λ max 218 nm, 283 nm; mass spectrum M$^+$ 300 (39%), 134 (100%), 122 (63%); mass spectrum (TMS-ether) M$^+$ 372 (36%), 206 (100%), 194 (25%); GLC R_f 1.24 (ov-17); TLC R_f 0.86, after oxidation R_f 0.86, after acetylation R_f 1.22, after reduction R_f 0.44.

3-Hydroxy-9,10-seco-1,3,5(10),11-androstatetraene-9,17-dione (**12**). *Pseudomonas* sp. NCIB 10590 was cultured aerobically with 12β-hydroxyandrosta-1,4-diene-3,17-dione (0.02%) as substrate in mineral salts medium (1000 ml) for 48 hr. 2% n-propanol (which causes accumulation of secosteroids) was added to the medium and incubation was continued for 3 days. Extraction of the culture with EtOAc gave residual substrate (35 mg) plus four major products, namely 12β-hydroxyandrost-4-en-3,17-dione (5 mg), 12α-hydroxyandrosta-1,4-diene-3,17-dione (12 mg), 12β,17β-dihydroxyandrosta-1,4-diene-3-one (3 mg) and a secosteroid (**16**). Recrystallisation of **12** from MeOH/CH_2Cl_2 gave white crystals (20 mg), m.p. 124°; IR (KBr disc) 1260, 1300, 1505, 1585, 1605, 1660, 1730, 1750 and 3400 cm^{-1}; λ max 223 nm, 283 nm; mass spectrum M$^+$ 298 (49%), 134 (100%); GLC R_f 1.47 (ov-17); TLC R_f 0.92, after oxidation R_f 0.92; after acetylation R_f 1.38, after reduction R_f 0.51.

Acknowledgements—R. W. Owen was in receipt of a Liverpool Education Research Assistantship during this study. We should like to thank M. H. Thompson of PHLS Centre for Applied Microbiology and Research, Bacterial Metabolism Research Laboratory, Porton Down, Salisbury, Wiltshire SP4 OJG for the mass spectra obtained on equipment funded by the Cancer Research Campaign.

REFERENCES

[1]P. J. Barnes, J. D. Baty, R. F. Bilton and A. N. Mason, *Tetrahedron* **32**, 89 (1976).
[2]R. F. Bilton, A. N. Mason and M. E. Tenneson, *Ibid.* **37**, 2509 (1981).
[3]M. J. Hill, P. Goddard and R. E. O. Williams, *Lancet ii*, 472 (1971).
[4]V. C. Aries, J. S. Crowther, B. S. Drasar, M. J. Hill and R. E. O. Williams, *Gut* **10**, 334 (1969).
[5]P. J. Barnes, R. F. Bilton, F. Fernandez, M. J. Hill and A. N. Mason, *Biochem. Soc. Trans.* **3**, 299 (1975).
[6]V. C. Aries, P. Goddard and M. J. Hill, *Biochimica et Biophysica Acta* **248**, 482 (1971).

[7]P. Goddard, F. Fernandez, B. West, M. J. Hill and P. J. Barnes, *J. Med. Microbiol* **8**, 429 (1975).
[8]H. Budziekiewicz, *Biochemical Applications of Mass Spectrometry* (Edited by G. R. Waller), Chap. 10, pp. 251–289. Wiley-Interscience, New York (1972).
[9]L. Dorfman, *Chem. Rev.* **53**, 47 (1953).
[10]A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.* 2555 (1953).
[11]R. M. Jankov, *Glus. Hem. Drus. Beograd.* **42**, 655 (1977).
[12]E. Zeitz and G. Spiteller, *Tetrahedron* **30**, 585 (1974).
[13]J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, E. R. H. Jones, A. Kasal, G. D. Meakins, Y. Morisawa, E. E. Richards and P. D. Woodgate, *J. Chem. Soc.* (C) 250 (1970).
[14]M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).
[15]I. E. Bush, *Br. Med. Bull.* **10**, 232 (1954).
[16]Z. V. Zaretskii, *Mass Spectrometry of Steroids*, Chap. 3, Wiley, New York (1976).
[17]R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.* **83**, 4631 (1961).
[18]M. E. Tenneson, R. F. Bilton and A. N. Mason, *Febs Letters* **91**(1), 140 (1978).
[19]V. C. Aries, J. S. Crowther, B. S. Drasar, M. J. Hill and R. E. O. Williams, *Gut* **10**, 334 (1969).