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The microbiological hydroxylation of 4β-hydroxy-4α-methyl-5α-androstanes by *Cephalosporium aphidicola*

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Abstract

Some 4β-hydroxy-4α-methyl-5α-androstanes were synthesized and their microbiological hydroxylation by *Cephalosporium aphidicola* was examined in order to explore the possibility of a biosynthetically patterned methylcarbinol: vicinal glycol biotransformation; however the substrates were hydroxylated mainly at C-7. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Microbiological hydroxylation; *Cephalosporium aphidicola*; Steroids; 4β-hydroxy-4α-methyl-5α-androstanes

1. Introduction

The biosynthesis of the diterpenoid aphidicolin (**1**) by the fungus, *Cephalosporium aphidicola*, involves the efficient hydroxylation of the equatorial 17-methyl of 3α,16β,18-trihydroxyaphidicolane to afford the 16β,17-glycol of aphidicolin [1]. The transformation of a methylcarbinol to a vicinal glycol exemplified by this step, is difficult to achieve regiospecifically by chemical means. Consequently we have explored [2] the possibility of using this organism for a biosynthetically-directed biotransformation to achieve this reaction using steroidal methylcarbinols as test substrates. 4β,17β-Dihydroxy-4α-methyl-5α-androstane (**2**) and 4β,17β-dihydroxy-4α,17α-dimethyl-5α-androstane (**3**) were potential substrates since not only is there a formal similarity between these steroids binding in both their normal and reverse modes when compared to aphidicolin but also the methyl groups at C-4 are equatorial substituents.

2. Results and discussion

The substrates (**2**) and (**3**) were prepared from testosterone (**4**) and 17α-methyltestosterone (**13**). The addition of methylmagnesium iodide to steroidal ketones shows a marked preference for the formation of the tertiary alcohol with an equatorial methyl group [3] although this has not been examined previously with steroidal 4-ketones as substrates. The steroidal 4-ketones were prepared via the 4-enes. 17β-Hydroxyandrost-4-ene (**5**) [4], free from double bond isomers, has become readily available from testosterone (**4**) by using a selective reduction with sodium borohydride in a mixture of trifluoroacetic acid, acetic acid, acetonitrile and dichloromethane [5].

Hydroboration of 17β-acetoxyandrost-4-ene (**6**) [6] and oxidation of the borane with alkaline hydrogen peroxide gave a mixture of 4α,17β-dihydroxy-5α-androstane (**7**) and 4β,17β-dihydroxy-5β-androstane (**8**). However the protecting acetoxyl group at C-17 had been hydrolysed. Oxidation of the borane with chromium trioxide [7] to avoid the alkaline conditions and form the 4-ketone directly was not successful. Attempts to circumvent the hydrolysis of the 17β-acetate by oxidizing the borane with sodium perborate [8] were also unsuccessful. Hence the 17β-hydroxyl group was protected as its sterically more hindered 17β-pivaloate (**9**). The mixture of 17β-pivaloyloxy-4α- and 4β-alcohols obtained from the hydroboration, was oxidized with chromium trioxide to a mixture of 17β-pivaloyloxy-5α- and 5β-androstan-4-ones, (**10**) and (**11**), without further purification. This mixture was then epimerized at C-5 to afford the more

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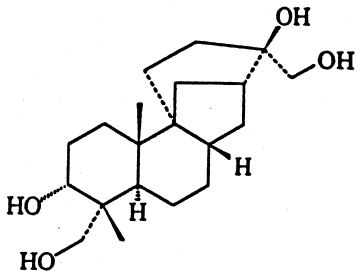

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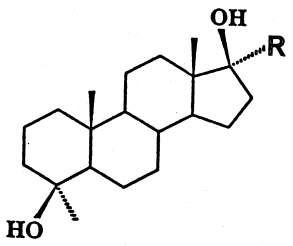

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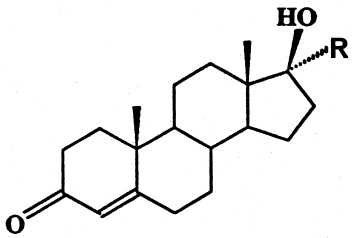

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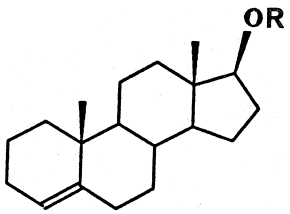

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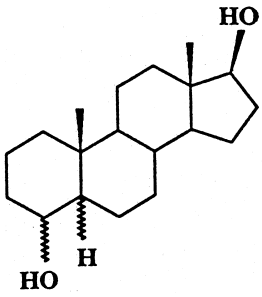
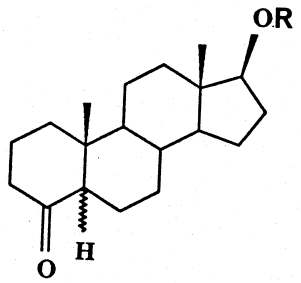
9 R = Piv



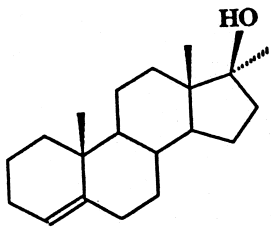
7 4α-OH,5α-H
8 4β-OH,5β-H



10 5α-H,R = Piv
11 5β-H,R = Piv
12 5α-H,R = H

Table 1. Incubation of steroids with *C. aphidicola*

Substrate	Product	% Yield
4β,17β-Dihydroxy-4α-methyl-5α-androstane (**2**)	starting material	20
	4β-hydroxy-4α-methyl-5α-androstan-17-one (**16**)	37
	4β,7α-dihydroxy-4α-methyl-5α-androstan-17-one (**17**)	10
	4β,15α,17β-trihydroxy-4α-methyl-5α-androstane (**19**)	7
4β,17β-Dihydroxy-4α,17α-dimethyl-5α-androstane (**3**)	starting material	16
	4β,7α,17β-trihydroxy-4α,17α-dimethyl-5α-androstane (**18**)	15



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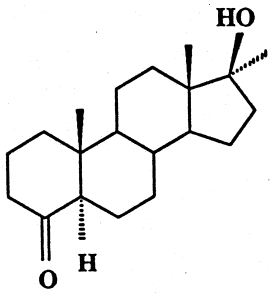

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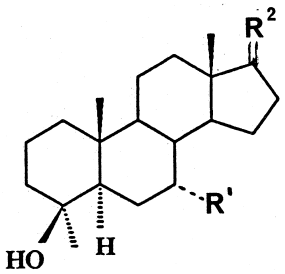

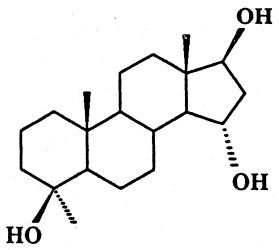

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16 R^1 = H, R^2 = O
17 R^1 = OH, R^2 = O
18 R^1 = OH, R^2 = α-Me, β-OH

Table 2. ^{13}C NMR spectra of 4β-hydroxy-4α-methylandrostanes (determined in $CDCl_3$ at 75 MHz)

	Compound					
Carbon	**2**	**3**	**16**	**17**	**18**	**19**
1	38.74	38.81	38.69	38.47	38.45	39.37
2	18.11	18.16	18.07	18.03	17.93	18.56
3	40.91	40.97	40.97	41.20	40.82	41.43
4	72.17	72.20	72.12	72.13	72.03	71.32
5	53.84	53.93	53.79	45.94	45.81	54.27
6	20.05	20.15	20.33	29.23	28.75	20.31
7	31.94	32.13	31.22	66.90	67.14	33.18
8	35.08	35.96	34.62	38.73	39.88	35.34
9	55.84	55.82	55.80	47.53	47.21	56.16
10	36.67	36.72	36.77	36.95	36.70	37.07
11	20.42	20.51	19.76	19.55	19.75	21.03
12	36.71	39.05	36.71	35.76	38.45	37.58
13	42.82	45.42	47.68	47.46	45.20	44.63
14	51.23	50.93	51.65	45.94	44.55	59.30
15	23.31	23.20	21.71	21.31	22.45	72.14
16	30.53	31.66	35.85	31.13	31.06	43.14
17	81.94	81.72	221.45	221.10	81.47	78.67
18	11.11	14.10	13.80	13.48	13.53	13.16
19	14.05	13.98	14.02	12.95	12.78	14.41
4-Me	30.83	30.86	30.87	30.78	30.24	31.05
17-Me		25.78			25.43	

stable 5α-androstan-4-one (**12**) by refluxing with methanolic sodium hydroxide. The C-5 epimers may be readily distinguished by the position of the H-19 resonance in the ^1H NMR spectrum [9]. The pivaloyloxy group was hydrolysed at the same time. Treatment of the ketone with methylmagnesium iodide in ether gave 4β,17β-dihydroxy-4α-methyl-5α-androstane (**2**). We were unable to detect any of the 4β-methyl isomer.

A similar sequence was applied to 17α-methyltestosterone (**13**) except that there was no need to protect the tertiary 17β-hydroxyl group. Thus the ring A unsaturated ketone was reduced to the 4-ene (**14**) with sodium borohydride in a mixture of trifluoroacetic acid, acetic acid, acetonitrile and dichloromethane and thence via hydroboration, oxidation and isomerization, converted to the 4-ketone (**15**). Reaction with methylmagnesium iodide gave 4β,17β-dihydroxy-4α,17α-dimethyl-5α-androstane (**3**).

The stereochemistry at C-4 followed from an examination of the ^1H NMR spectra of the 4β-hydroxy-4α-methyl-5α-androstanes. Whilst there was no n.O.e. enhancement of the C-4 methyl group signal on irradiation of the C-10 methyl group signal, the trans-

annular 1:3-diaxial interaction with a 4β-hydroxyl group produced a significant downfield shift ($\Delta\delta$ 0.24 ppm) in the position of the C-10 methyl group signal when compared to 17β-hydroxy-5α-androstane [9].

The substrates were incubated with *C. aphidicola* for 8 days and the metabolites were then isolated and separated by chromatography. The results are tabulated (see Table 1). The metabolites were identified from their ^{1}H and ^{13}C NMR spectra (see Table 2).

The major metabolite from the incubation of 4β,17β-dihydroxy-4α-methyl-5α-androstane (**2**) was the 17-ketone (**16**). The H-17α signal at δ_H 3.61 had disappeared from the ^{1}H NMR spectrum whilst the ^{13}C NMR spectrum (see Table 2) showed a new carbonyl resonance at δ_C 221.45 ppm. The second metabolite to be isolated from the column, was assigned the structure of 4β,7α-dihydroxy-4α-methyl-5α-androstan-17-one (**17**). The H-17α signal had disappeared and a carbonyl signal had appeared at δ_C 221.1 ppm. The ^{13}C NMR spectrum also contained a CH(OH) resonance at δ_C 66.70 ppm in place of a methylene carbon. Compared to the starting material, the signals assigned to C-6 and C-8 had moved downfield ($\Delta\delta$ 8.81 and 3.65 ppm respectively) whilst there were γ-gauche upfield shifts for the signals assigned to C-5, C-9 and C-14 ($\Delta\delta$ 7.90, 8.31 and 5.29 ppm respectively). Consequently the new alcohol was located at C-7. The ^{1}H NMR spectrum showed a CH(OH) signal at δ_H 4.09 (doublet, J 2.5 Hz) resembling that of a 7α-alcohol [10]. The third metabolite to be isolated was assigned the structure of 4β,15α,17β-trihydroxy-4α-methyl-5α-androstane (**19**). Compared to the starting material, the ^{13}C NMR spectrum revealed downfield shifts for the signals assigned to C-14 and C-16 ($\Delta\delta$ 8.07 and 12.61 ppm, respectively) whilst there was a γ-gauche upfield shift for the signal assigned to C-17 ($\Delta\delta$ 3.27 ppm). The ^{1}H NMR spectrum showed a new signal at δ_H 4.27 ppm as a triplet (J, 9 Hz) of doublets (J, 3 Hz) consistent with the presence of a 15α-alcohol.

Incubation of 4β,17β-dihydroxy-4α,17α-dimethyl-5α-androstane (**3**) gave the corresponding 7α-alcohol (**18**). The presence of the 7α-alcohol was established by the downfield shifts of the ^{13}C NMR resonances of C-6 and C-8 ($\Delta\delta$ 8.6 and 3.9 ppm, respectively) and the γ-gauche shieldings for C-5, C-9 and C-14 ($\Delta\delta$ 8.12, 8.61 and 6.38 ppm, respectively) when compared to the starting material. The H-7β proton resonance (δ_H 3.95 ppm) showed a comparable multiplicity to other 7α-alcohols [10].

In conclusion the transformations which we have observed with these substrates have followed the typical pattern of steroidal hydroxylations by this organism [11, 12] rather than a biosynthetically patterned pathway. It may be that the systems responsible for steroidal transformations are more readily accessible to the exogenous substrates and are present throughout the fermentation whilst those involved in aphidicolin biosynthesis may only be present for a limited period, for example during the idiophase, of the fermentation.

3. Experimental

^{1}H NMR spectra were recorded in deuteriochloroform at 300 or 500 MHz. ^{13}C NMR spectra were determined at 75 MHz. IR Spectra were recorded as nujol mulls. Chromatography was carried out on silica, Merck 9385. Light petroleum refers to the fraction, b.p. 60–80°. Extracts were dried over sodium sulfate. Jones reagent refers to a solution of chromium trioxide (26.72 g) in conc. sulfuric acid (23 cm^3) diluted to 100 cm^3 with water. *Cephalosporium aphidicola* was cultured as described previously [13].

3.1. 17β-Pivaloyloxyandrost-4-en-3-one

Testosterone (**3**) (5 g) in dry pyridine (50 cm^3) was treated with pivaloyl chloride (5 g) at room temp. overnight. The mixture was poured into dil. HCl (200 cm^3) and the steroid was recovered in EtOAc. The extract was washed with dil. HCl, aq. $NaHCO_3$, H_2O and dried. The solvent was evap. to give 17β-pivaloyloxy-androst-4-en-3-one (4.53 g) which crystallized from petrol as prisms, m.p. 155–157° (found: C, 77.6; H,10.1; $C_{24}H_{36}O_3$ requires C, 77.4; H, 9.7%), IR, ν_{max} 1722, 1679, 1617 cm^{-1}; ^{1}H NMR δ_H 0.85 (3H, s, H-18), 1.20 (9H, s OPiv), 1.25 (3H, s, H-19), 4.57 (1H, t, J 8.5 Hz, H-17), 5.73 (1H, s, H-4).

3.2. 17β-Pivaloyloxyandrost-4-ene (**9**)

$NaBH_4$ (1.3 g) was gradually added to a stirred mixture of CF_3CO_2H (8 cm^3), HOAc (8 cm^3) and CH_3CN (8 cm^3) which was cooled in an ice-bath. 17β-Pivaloyloxyandrost-4-en-3-one (2.4 g) in CH_2Cl_2 (40 cm^3) was added to the mixture which was then stirred for 6 h (TLC control). Sat. aq. $NaHCO_3$ was added to neutralize the mixture. The steroid was extracted with CH_2Cl_2. The extract was thoroughly washed with H_2O and dried. The solvent was evap. to give 17β-pivaloyloxyandrost-4-ene (**9**)-(1.84 g) which crystallized from MeOH as needles, m.p. 114–116° (found: C, 80.4; H, 11.1; $C_{24}H_{38}O_2$ requires C, 80.4; H, 10.7%), IR, ν_{max} 1733 cm^{-1}; ^{1}H NMR δ_H 0.82 (3H, s, H-18), 1.02 (3H, s, H-19), 1.19 (9H, s, OPiv), 4.57 (1H, t, J 8.5 Hz, H-17), 5.27 (1H, br.s. H-4).

3.3. Preparation of 17β-hydroxy-5α-androstan-4-one (**12**)

17β-Pivaloyloxyandrost-4-ene (3 g) in THF (100 cm^3) was treated with 1 M borane in THF

($20\ cm^3$) at 0° for 4 h under N_2. H_2O ($10\ cm^3$) was added dropwise at 0° followed by 10%, aq. NaOH ($30\ cm^3$) and 30% aq. H_2O_2 ($30\ cm^3$). The mixture was stirred for 1 h. Na_2SO_3 (6 g) was then added followed by HOAc ($3\ cm^3$), H_2O ($150\ cm^3$), dil. HCl ($150\ cm^3$) and EtOAc ($150\ cm^3$). The mixture was stirred for a further 15 min and then the organic layer was separated, washed with H_2O and dried. The solvent was evap. to give a residue which was taken up in Me_2CO ($75\ cm^3$) and treated with the Jones reagent ($3\ cm^3$) dropwise. The mixture was stirred for 45 min and then MeOH was added until the soln. remained a green colour. After 30 min the solvents were evap. and the residue was suspended in H_2O. The steroids were extracted with EtOAc. The extract was washed with aq $NaHCO_3$ and H_2O. The solvent was evap to give a gum (1.5 g) which was dissolved in MeOH ($50\ cm^3$). This soln. was treated with NaOH (8 g) in H_2O ($20\ cm^3$) under N_2 and heated under reflux for 1 h. The soln was cooled and neutralized with dil HCl. The MeOH was evap. and the steroids were recovered in EtOAc. The extract was washed with aq $NaHCO_3$, H_2O and dried. The solvent was evap to give 17β-hydroxy-5α-androstan-4-one (1 g) which was recrystallized from petrol as needles, m.p. 121° (lit. [14], 125°), IR ν_{max} 3526, 1712 cm^{-1}; ^1H NMR δ_H 0.74 and 0.75 (each 3H, s, H-18 and H-19), 3.61 (1H, t, J 8.5 Hz, H-17).

3.4. 4β,17β-Dihydroxy-4α-methyl-5α-androstane (2)

17β-Hydroxy-5α-androstan-4-one (1 g) in dry THF ($30\ cm^3$) was treated with 3 M methylmagnesium iodide in Et_2O ($8\ cm^3$) under N_2 at room temp. overnight. 10% Aq. NH_4Cl ($100\ cm^3$) was added and the mixture was left to stand for 1 h. The steroid was extracted with EtOAc and the extract was washed with brine and dried. The solvent was evap. to give 4β,17β-dihydroxy-4α-methyl-5α-androstane (**2**) (0.7 g) which was recrystallized from MeOH as needles, m.p. 186–189° (found: C, 78.4; H, 11.4. $C_{20}H_{34}O_2$ requires C, 78.4; H, 11.7%); IR, ν_{max} 3383 cm^{-1}. ^1H NMR δ_H 0.73 (3H, s, H-18), 1.03 (3H, s, H-19), 1.15 (3H, s, 4-Me), 3.61 (1H, t, J 8.5 Hz, H-17).

3.5. 17β-Hydroxy-17α-methylandrost-4-ene (14)

$NaBH_4$ (1.3 g) was gradually added to a stirred mixture of CF_3CO_2H ($8\ cm^3$), HOAc ($8\ cm^3$) and CH_3CN ($8\ cm^3$) which was cooled in an ice-bath. 17β-Hydroxy-17α-methylandrost-4-en-3-one (2.4 g) in CH_2Cl_2 ($40\ cm^3$) was added to the mixture which was then stirred for 4 h. (TLC control). Sat. aq. $NaHCO_3$ was added to neutralize the mixture. The steroid was extracted with CH_2Cl_2. The extract was thoroughly washed with H_2O and dried. The solvent was evap. to give 17β-hydroxy-17α-methylandrost-4-ene (**14**) (2 g) which crystallized from petrol as needles, m.p. 98–100° (found: C, 82.8; H, 11.4; $C_{20}H_{32}O$ requires C, 83.3; H, 11.2%), IR, ν_{max} 3361 cm^{-1}; ^1H NMR δ_H 0.88 (3H, s, H-18), 1.03 (3H, s, H-19), 1.20 (3H, s, H-20), 5.27 (1H, br s, H-4).

3.6. 17β-Hydroxy-17α-methyl-5α-androstan-4-one (15)

17β-Hydroxy-17α-methylandrost-4-ene (3 g) in dry THF ($100\ cm^3$) was treated with 1 M borane in THF ($20\ cm^3$) at 0° for 6 h under N_2. H_2O ($10\ cm^3$) was added dropwise at 0° followed by 10% aq. NaOH ($30\ cm^3$) and 30% aq. H_2O_2 ($30\ cm^3$). The mixture was stirred for 1 h. Na_2SO_3 (6 g) was then added followed by HOAc ($3\ cm^3$), H_2O ($150\ cm^3$), dil. HCl ($150\ cm^3$) and EtOAc ($150\ cm^3$). The mixture was stirred for a further 15 min and then the organic layer was separated, washed with H_2O and dried. The solvent was evap. to give a residue which was taken up in Me_2CO ($75\ cm^3$) and treated with the Jones reagent ($3\ cm^3$) dropwise. The mixture was stirred for 45 min and then MeOH was added until the soln. remained a green colour. After 30 min the solvents were evap. and the residue was suspended in H_2O. The steroids were extracted with EtOAc. The extract was washed with aq. $NaHCO_3$ and H_2O. The solvent was evap. to give a gum (1.5 g) which was dissolved in MeOH ($50\ cm^3$). This soln. was treated with NaOH (8 g) in H_2O ($20\ cm^3$) under N_2 and heated under reflux for 1 h. The soln. was cooled and neutralized with dil HCl. The MeOH was evap. and the steroids were recovered in EtOAc. The extract was washed with aq $NaHCO_3$, H_2O and dried. The solvent was evap. to give 17β-hydroxy-17α-methyl-5α-androstan-4-one (1.36 g) which was recrystallized from EtOAc: petrol as needles, m.p. 149–151° (found: C, 77.2; H, 10.5. $C_{20}H_{32}O_2$ requires C, 76.7; H, 10.5%) IR ν_{max} 3344, 1711 cm^{-1}; ^1H NMR δ_H 0.76 (3H, s, H-19), 0.85 (3H, s, H-18), 1.22 (3H, s, H-20).

3.7. 4β,17β-Dihydroxy-4α,17α-dimethyl-5α-androstane (3)

17β-Hydroxy-17α-methyl-5α-androstan-4-one (0.8 g) in dry THF ($30\ cm^3$) was treated with 3 M methylmagnesium iodide in Et_2O ($8\ cm^3$) under N_2 at room temp. overnight. 10% Aq. NH_4Cl ($100\ cm^3$) was added and the mixture was left to stand for 1 h. The steroid was extracted with EtOAc and the extract was washed with brine and dried. The solvent was evap. to give 4β,17β-dihydroxy-4α,17α-dimethyl-5α-androstane (**3**) (0.3 g) which was recrystallized from MeOH as needles, m.p. 155–157° (found: C, 78.6; H, 11.5. $C_{21}H_{36}O_2$ requires C, 78.8; H, 11.3%); IR, ν_{max}

3400 cm^{-1}; ^1H NMR δ_H 0.84 (3H, s, H-18), 1.03 (3H, s, H-19), 1.15 (3H, s, 4-Me), 1.20 (3H, s, H-20).

3.8. Biotransformation of 4β,17β-dihydroxy-4α-methyl-5α-androstane (2)

The substrate (**2**) (970 mg) in DMSO (25 cm^3) and EtOH (5 cm^3) was evenly distributed between 50 flasks of a 3 day old culture of *C. aphidicola*. After a further 8 days, the mycelium was filtered and the broth was extracted with EtOAc. The extract was dried and the solvent was evap. to give a residue which was chromatographed on silica. Elution with 10% EtOAc:petrol gave 4β-hydroxy-4α-methyl-5α-androstan-17-one (**16**) (360 mg) which crystallized from EtOAc:petrol as prisms, m.p. 196–198° (found: C, 78.9; H, 10.2. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%); ^1H NMR δ_H 0.86 (3H, s, H-18), 1.05 (3H, s, H-19), 1.17 (3H, s, Me-4). Elution with 13% EtOAc:petrol gave the starting material (190 mg). Elution with 45% EtOAc:petrol gave 4β,7α-dihydroxy-4α-methyl-5α-androstan-17-one (**17**) (93 mg) which crystallized from EtOAc:petrol as prisms, m.p. 203–205° (found: C, 74.9; H, 10.1. $C_{20}H_{32}O_3$ requires C, 75.0; H, 10.1%); ^1H NMR δ_H 0.87 (3H, s, H-18), 1.07 (3H, s, H19), 1.21 (3H, s, Me-4), 4.09 (1H, d, J 2.5 Hz, H-7). Further elution gave 4β,15α,17β-trihydroxy-4α-methyl-5α-androstane (**19**) (72 mg) which crystallized from EtOAc:petrol as prisms, m.p. 263–265° (found: C, 74.2; H, 10.9. $C_{20}H_{34}O_3$ requires C, 74.5; H, 10.6%); IR, ν_{max} 3422 cm^{-1}; ^1H NMR δ_H (C_5D_5N) 0.98 (3H, s, H-19), 1.30 (3H, s, H-18), 1.31 (3H, s, Me-4), 4.10 (1H, t, J 9 Hz, H-17α), 4.27 (1H, t, d J 9 and 3 Hz, H-15β).

3.9. Biotransformation of 4β,17β-dihydroxy-4α,17α-dimethyl-5α-androstane (3)

The substrate (**3**) (740 mg) in DMSO (25 cm^3) and EtOH (5 cm^3) was evenly distributed between 50 flasks of a 3 day old culture of *C. aphidicola*. The fermentation was continued for a further 8 days. The mycelium was filtered and the broth was extracted with EtOAc. The extract was dried and the solvent evaporated to give a residue which was chromatographed on silica. Elution with 12% EtOAc:petrol gave the starting material (120 mg). Elution with 25% EtOAc: petrol gave 4β,7α,17β-trihydroxy-4α,17α-dimethyl-5α-androstane (**18**) (110 mg) which crystallized from $CHCl_3$ as prisms, m.p. 168–170° (found: C, 70.8; H, 10.5. $C_{21}H_{36}O_3$. H_2O requires C, 71.1; H, 10.8%), IR, ν_{max} 3495 cm^{-1}; δ_H 0.84 (3H, s, H-18), 1.04 (3H, s, H-19), 1.15 (3H, s, Me-4), 1.22 (3H, s, H-20), 3.95 (1H, d, J 2 Hz, H-7β).

References

Ackland, M. J., Hanson, J. R., Yeoh, B. L., & Ratcliffe, A. H. (1985). *Journal of the Chemical Society Perkin Transactions, 1,* 2705.

Hanson, J. R., in *Phytochemical Diversity; A Source of New Industrial Products*, eds. R. Thomas, S. Wrigley, M. Hayes and E. Chrystal. The Royal Society of Chemistry, Cambridge, 1997, p. 210.

Kirk, D. N. and Hartshorn, M. P., *Steroid Reaction Mechanisms.* Elsevier, Amsterdam, 1968, p. 147.

Crastes de Paulet, A. and Bascoul, J., *Bulletin de la Societe Chimique.* France, 1966, p. 939.

Winterfeldt, E., Tibtamm, H., Hofmeister, H. and Laurent, H. (Schering A. G.), German Patent D.E. 3,909,770. *Chemical Abstracts*, 1991, 114P, 82262.

Hanson, J. R., Hitchcock, P. B., Liman, M. D., & Nagaratnam, S. (1995). *Journal of the Chemical Society Perkin Transactions, 1,* 2183.

Brown, H. C., & Garg, C. P. (1961). *Journal of the American Chemical Society, 83,* 2951.

Kabalka, G. W., Shoup, T. M., & Goudgaon, N. M. (1989). *Tetrahedron Letters, 30,* 1483.

Zurcher, R. F. (1963). *Helvetica Chimica Acta, 46,* 2054.

Bridgeman, J. E., Cherry, P. C., Clegg, A. S., Evans, J. M., Jones, Sir Ewart R. H., Kumar, V., Meakins, G. D., Morisawa Y. and Richards, E. E., *Journal of the Chemical Society C*, 1970, 250.

Hanson, J. R., Nasir, H., & Parrez, A. (1996). *Phytochemistry, 42,* 411.

Boynton, J., Hanson, J. R., & Hunter, A. C. (1997). *Phytochemistry, 45,* 951.

Hanson, J. R., & Nasir, H. (1993). *Phytochemistry, 33,* 831.

Heusler, K., Kalvoda, J., Anner, G., & Wettstein, A. (1963). *Helvetica Chimica Acta, 46,* 352.