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THE MICROBIOLOGICAL HYDROXYLATION OF SOME STEROIDS WITH A CORTICAL SIDE CHAIN BY *CEPHALOSPORIUM APHIDICOLA*

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Key Word Index—*Cephalosporium aphidicola*; hyphomycetes; microbiological hydroxylation; steroid; 21-hydroxypregna-4-en-3,20-dione.

Abstract—Hydroxylation of pregna-4-en-3-ones at C-11α by the fungus *Cephalosporium aphidicola* is shown to be affected by the nature of the pregnane side chain whilst hydroxylation at C-6β is unaffected.

INTRODUCTION

In our studies on the microbiological hydroxylation of steroids by the fungus *Cephalosporium aphidicola*, we have noted a number of differences between the hydroxylation of progesterone and testosterone [1, 2]. Whereas progesterone (**1**) was efficiently hydroxylated first at C-11α and then at C-6β, testosterone (**2**) was hydroxylated at C-6β and the hydroxylation at C-11α was only a minor transformation. On the other hand testosterone was hydroxylated at C-14α, a transformation which was not observed with progesterone. Hydroxylation of progesterone at C-17α led to further hydroxylation at C-12β. In the light of these differences, it was of interest to see how the hydroxylation pattern was modified by the oxidation of the pregnane side chain which is a characteristic of the cortical hormones. In mammals oxidation of C-11 is an important transformation of the cortical steroids leading to cortisol and cortisone. A number of other studies, exemplified by Refs [3]–[6], have been reported on the microbiological transformation of cortical steroids. The major transformations which have been reported involve hydroxylation at C-6 and C-11, reduction of the Δ^4-double bond and cleavage of the side chain.

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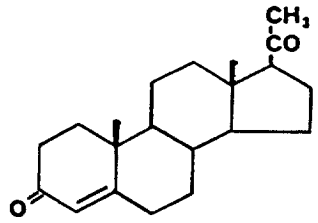
RESULTS AND DISCUSSION

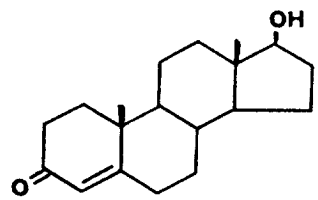
In order to examine the effect of oxidation of the side chain,the microbiological hydroxylation of 21-hydroxyprogesterone (deoxycorticosterone) (**3**), 17α,21-dihydroxyprogesterone (cortexolone) (**7**) and 16α,17α-epoxyprogesterone (**9**) by *C. aphidicola* was compared to the results obtained earlier with progesterone [1]. The substrates were incubated with the fungus in shake culture for 7 days. The results are tabulated (see Table 1).

The sites of hydroxylation were established from changes (β-deshielding and γ-gauche shielding effects) in the ^{13}C NMR spectra (see Table 2) [7]. The stereochemistry of the hydroxylation followed from the characteristic shape and multiplicity of the CH(OH) resonances and the effect of the additional hydroxyl group on the position of the H-18, H-19 and H-21 resonances in the ^1H NMR spectra [8].

Hydroxylation of 21-hydroxyprogesterone (**3**) proceeded efficiently at C-6β and was accompanied by some reduction of the C-20 ketone to the alcohol. In the previous work [1] on progesterone (**1**) this microbial reduction was shown to give the 20(*R*) alcohol. In contrast to the transformation of progesterone, hydroxylation at C-11α was a secondary transformation. However, when a 17α-hydroxyl group was introduced as in 17α,21-dihydroxyprogesterone (**7**), hydroxylation proceeded inefficiently and only at C-12β rather than at C-11α or C-6β. Interestingly in the previous study [1] with pro-



1



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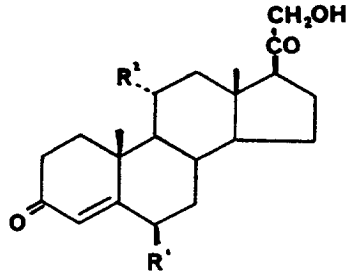
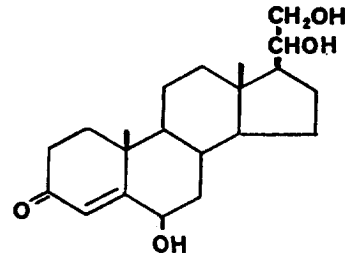


3 $R^1 = R^2 = H$
4 $R^1 = OH$, $R^2 = H$
6 $R^1 = R^2 = OH$



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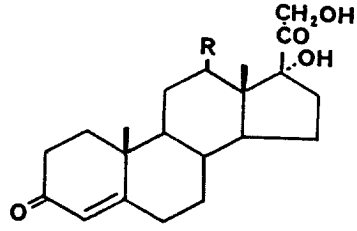
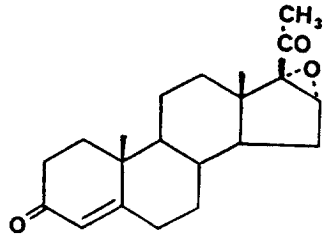


7 R = H
8 R = OH



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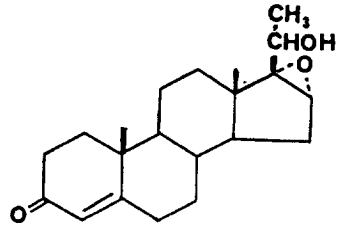

10 R = H
11 R = OH

gesterone, it was only 17α-hydroxyprogesterone that underwent hydroxylation at C-12β. However, when the 17α-alcohol was replaced by a 16α,17α-epoxide as in (**9**), hydroxylation took place at C-6β and was accompanied by reduction of C-20.

In the Brannon-Jones model for steroid microbial hydroxylations, attack on ring B (C-6/7) and ring C (C-11) are linked in terms of "reverse" and "normal" binding [9, 10]. In this series the binding which leads to the hydroxylation at C-11α in *C. aphidicola*

Table 1. Hydroxylation of cortical steroids by *Cephalosporium aphidicola*

Substrate	Product	Yield %
Deoxycorticosterone (**3**)		
	starting material (**3**)	10.7
	6β,21-dihydroxypregna-4-en-3,20-dione (**4**)	6.4
	6β,20*S*,21-trihydroxypregna-4-en-3-one (**5**)	9.0
	6β,11α,21-trihydroxypregna-4-en-3,20-dione (**6**)	10.0
Cortexolone (**7**)		
	starting material (**7**)	38.0
	12β,17α,21-trihydroxypregna-4-en-3,20-dione (**8**)	1.5
16α,17α-Epoxyprogesterone (**9**)		
	starting material (**9**)	8.2
	16α,17α-epoxy-20R-hydroxypregna-4-en-3-one (**10**)	1.5
	6β,20*R*-dihydroxy-16α,17α-epoxypregna-4-en-3-one (**11**)	21.0

is sensitive to the extent of the oxidation of the pregnane side chain whereas the binding which leads to oxidation at C-6β is relatively independant of variations in this part of the molecule.

EXPERIMENTAL

General experimental details have been described previously [11]. Except where stated NMR spectra were determined in $CDCl_3$. Petrol refers to the fraction b.p. 60–80°. Extracts were dried over Na_2SO_4. *Cephalosporium aphidicola* was grown on shake culture (100 cm^3 medium) in 250 cm^3 conical flasks as described previously.

Incubation of steroids with C. aphidicola

(*a*). Three days after inoculation deoxycorticosterone (21-hydroxypregna-4-en-3,20-dione (**3**) (1.5 g) in EtOH (50 cm^3) was evenly distributed between 50 flasks of *C. aphidicola*. After a further 7 days, the mycelium was filtered and the broth extracted with EtOAc. The extract was dried and the solvent evaporated to give a gum which was chromatographed on silica. Elution with 50% EtOAc:petrol gave the starting material (160 mg). Elution with 60% EtOAc:petrol gave 6β,21-dihydroxypregna-4-en-3,20-dione (**4**) (100 mg) which was crystallized from EtOAc:petrol as needles, m.p. 210–211° (lit., [12] 211–212°), IR ν_{max} 3511, 3349, 1706, 1676, 1634 cm^{-1}. ^1H NMR δ_H 0.72 (3H, *s*, H-18), 1.39 (3H, *s*, H-19), 3.29 (1H, *t*, *J* 4.6 Hz, H-17α), 4.19 and 4.21 (1H each, *s*, H-21), 4.37 (1H, *br.s.*, H-6), 5.82 (1H, *s*, H-4). Elution with 80% EtOAc:petrol gave 6β, 20*S*, 21-trihydroxypregna-4-en-3-one (**5**) (140 mg) as a gum (Found M^+ 348.230, $C_{21}H_{32}O_4$ requires 348.230), IR ν_{max} 3343 (*br*), 1696 cm^{-1}, ^1H NMR 0.85 (3H, *s*, H-18), 1.39 (3H, *s*, H19), 3.38 (1H, *dq*, *J* 6.4 and 9 Hz, H-20), 3.66 (2H, *d*, *J* 6.4 Hz, H-21), 4.35 (1H, *br.s.*, H-6α), 5.82 (1H, *s*, H-4). Elution with EtOAc gave 6β,11α,21-trihydroxypregna-4-en-3,20-dione (**6**) (162 mg) which crystallized from EtOAc:petrol as needles, m.p. 249° (lit., [12] 248–251°), IR ν_{max} 3441, 3374, 1697, 1658 cm^{-1}, ^1H NMR (C_5D_5N), 0.79 (3H, *s*, H-18), 1.79 (3H, *s*, H-19), 4.32 (1H, *dt*, *J* 4.2 and 10.3 Hz, H-11β), 4.41 and 4.50 (1H each, *s*, H-21), 4.54 (1H, *br.s.*, H-6α), 6.07 (1H, *s*, H-4).

Table 2. ^{13}C NMR signals of the substrates and metabolites (determined at 75 MHz in $CDCl_3$)

Carbon No.	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	35.0	37.50	37.47	41.24	35.2	35.01	35.49	35.36	36.91
2	33.4	34.61	34.65	37.50	33.4	33.19	33.81	33.81	34.12
3	197.5	200.71	201.04	202.71	197.4	199.71	199.29	200.00	200.90
4	123.0	126.86	126.73	128.86	123.0	123.54	123.93	123.86	125.92
5	170.4	168.29	168.95	172.81	170.4	170.69	170.51	171.27	170.02
6	31.8	73.35	75.51	75.09	31.9	31.90	31.45	31.38	72.27
7	31.5	38.74	38.92	42.23	32.1	32.57	32.56	32.35	38.23
8	34.8	30.15	30.00	31.43	35.2	34.98	33.19	33.62	28.06
9	52.9	53.77	52.68	62.09	53.0	51.79	53.79	53.73	53.91
10	38.0	38.36	38.43	42.40	38.1	38.38	38.56	38.52	38.23
11	20.4	21.28	21.24	70.78	20.3	30.01	20.30	20.37	20.59
12	37.6	38.74	39.83	53.04	30.1	71.30	31.08	32.63	32.71
13	43.5	45.19	43.04	47.39	47.0	52.70	41.49	41.63	42.12
14	55.3	56.46	55.50	58.25	49.9	48.43	44.76	44.65	43.11
15	23.9	24.85	24.95	27.10	23.2	23.19	27.26	27.04	27.18
16	22.3	23.32	24.95	25.67	33.4	33.74	60.30	60.31	60.14
17	57.4	59.54	54.01	61.11	88.4	85.93	70.61	72.53	72.62
18	13.1	13.93	12.93	17.27	14.5	9.66	15.11	15.65	15.78
19	16.7	19.95	19.94	22.96	17.0	17.68	17.13	17.05	19.19
20	209.6	210.54	74.90	213.20	211.3	211.03	204.65	64.00	64.43
21	68.6	69.82	6.6.82	72.50	65.9	65.92	25.84	19.83	19.49

(*b*). Under similar conditions cortexolone (17α,21-dihydroxy-pregna-4-en-3,20-dione (**7**) (1.5 g) gave after chromatography in 50% EtOAc:petrol, the starting material (571 mg). Further elution with 60% EtOAc:petrol gave 12β,17α,21-trihydroxy-pregna-4-en-3,20-dione (**8**) (23 mg) which crystallized from EtOAc:petrol as needles, m.p. 175–179° (lit., [13] 177–181°), IR ν_{max} 3584, 3487, 3415, 1712, 1661 cm^{-1}, ^1H NMR δ_H 0.69 (3H, *s*, H-18), 1.26 (3H, *s*, H-19), 4.16 (1H, *dd*, *J* 5.2 and 10.1 Hz, H-12α), 4.30 and 4.68 (each 1H, *d*, *J* 19 Hz, H-21), 5.82 (1H, *s*, H-4).

(*c*). Under similar conditions 16α,17α-epoxy-progesterone (**9**) (2 g) gave on chromatography in 20% EtOAc:petrol, the starting material (164 mg). Further elution with 50% EtOAc:petrol gave 16α,17α-epoxy-20*R*-hydroxypregna-4-en-3-one (**10**) (36 mg) as a gum (Found M^+ 330.219, $C_{21}H_{30}O_3$ requires 330.219), IR ν_{max} 3583, 1663 cm^{-1}; ^1H NMR δ_H 0.92 (3H, *s*, H18), 1.10 (3H, *d*, *J* 6.4 Hz, H-21), 1.19 (3H, *s*, H-19), 3.2 (1H, *br.s.*, H-16), 4.30 (1H, *q*, *J* 6.4 Hz, H-20), 5.71 (1H, *s*, H-4). Further elution with 60% EtOAc:petrol gave 6β,20*R*-dihydroxy-16α,17α-epoxypregna-4-en-3-one (**11**) (440 mg) which crystallized from EtOAc:petrol as needles, m.p. 253–255° (Found: C, 72.5; H, 8.7. $C_{21}H_{30}O_4$ requires C, 72.8; H, 8.7%), IR ν_{max} 3467 (*br*), 1707 cm^{-1}; ^1H NMR δ_H 0.95 (3H, *s*, H-18), 1.10 (3H, *d*, *J* 6.4 Hz, H-21), 1.39 (3H, *s*, H-19), 3.30 (1H, *s*, H-16), 4.37 (2H, *m*, H-6α and H-20), 5.81 (1H, *s*, H-4).

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