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THE HYDROXYLATION OF STEROIDAL RING D LACTONES BY *CEPHALOSPORIUM APHIDICOLA*

JAMES R. HANSON* and A. CHRISTY HUNTER

School of Chemistry, Physics and Environmental Sciences, University of Sussex, Brighton, Sussex, BN1, 9QJ, U.K.

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Key Word Index—*Cephalosporium aphidicola*; microbiological hydroxylation; steroid; 17a-oxa-D-homo-5α-androstan-3,17-dione.

Abstract—The microbiological hydroxylation of 17a-oxa-D-homo-5α-androstane-3,17-dione, the 3β-alcohol and the 13α-methyl analogue by *Cephalosporium aphidicola* takes place predominantly at C-7α in contrast to other hydroxylations by this organism.

INTRODUCTION

The microbiological hydroxylation and eventual cleavage of ring D of the steroids to form a δ-lactone (**1** → **2**) has often been observed [1–3]. However, there are relatively few reports of the further transformation of these lactones.

Testolactone (**2**) has been shown to be hydroxylated at C-2β by a *Penicillium sp.* [4] and at C-7α by a *Dematiaceae sp.* [5]. The microbiological hydroxylation of steroids has been rationalized in terms of a triangular relationship with defined dimensions between two binding sites and the site of hydroxylation [6, 7]. Typically, the steroids have oxygen functions at C-3 and at C-17. The conversion of ring D to a δ-lactone alters the structure of one of the binding groups. In the light of our interest [8, 9] in the microbiological hydroxylation of steroids by *Cephalosporium aphidicola*, we have examined the effect of this change on the pattern of hydroxylation by this organism. This fungus is capable of hydroxylating progesterone, firstly at C-11α and then at C-6β, whilst testosterone is hydroxylated at the C-6β position with hydroxylation, to a minor extent, occurring at the C-11α and C-14α positions.

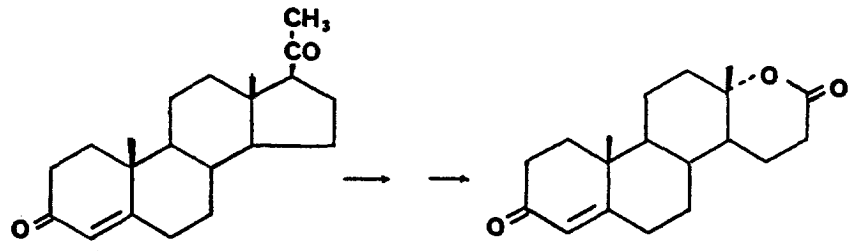
RESULTS AND DISCUSSION

The lactones were prepared in both the 13α-methyl series and in the natural 13β-methyl series in order to assess the effect of the stereochemistry of ring D. The substrates, 17a-oxa-D-homo-5α.13α-androstane-3,17-dione (**3**), 17a-oxa-D-homo-5α-androstane-3,17-dione (**7**) and the corresponding 3β-alcohol (**9**) were obtained via a Baeyer–Villiger oxidation of the relevant 3β-acetoxy-17-ketone followed by hydrolysis with methanolic K_2CO_3 and oxidation with CrO_3.

The substrates were incubated with *Cephalosporium aphidicola* for 7 days in a shake culture. The metabolites are listed in Table 1. The sites of hydroxylation were established by changes in the ^{13}C NMR spectra (see Table 2). In each case, the relevant CH_2 or CH resonance had been replaced by a CH(OH) or a C–OH signal whilst the signals for the adjacent carbons showed significant downfield shifts. Hydroxylation at the C-7α position in **5**, **8** and **12** was apparent from the downfield shift of the resonances assigned to C-6 and C-8 and the γ-gauche shielding experienced by C-5, C-9 and C-14. Hydroxylation at C-9α in **6** and **11** was revealed by a comparable pattern of β-carbon shifts for the signals assigned to C-8, C-10 and C-11 and the γ-gauche shieldings for C-1, C-5, C-7, C-12 and C-14. The hydroxylation at C-11β in **10** produced downfield shifts in the resonances assigned to C-9 and C-12 together with a γ-gauche shielding at C-8 and small effects on C-10 and C-13. The shape of the CH(OH) ^1H NMR signals [10] was consistent with the stereochemistry assigned to each of the alcohols.

The major hydroxylation of these lactones has taken place at C-7α irrespective of the geometry of ring D. This is in contrast to other hydroxylations

*Author to whom correspondence should be addressed.



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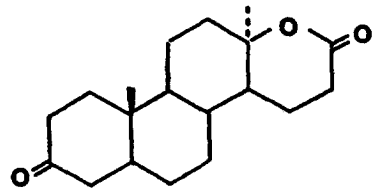

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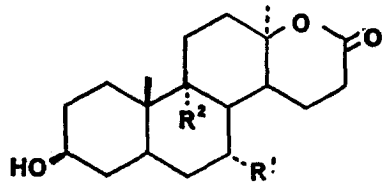

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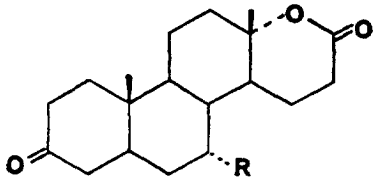

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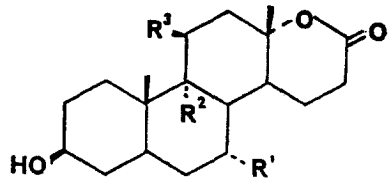
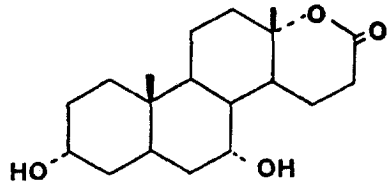


9 $R^1 = R^2 = R^3 = H$
10 $R^1 = R^2 = H, R^3 = OH$
11 $R^1 = R^3 = H, R^2 = OH$
12 $R^1 = OH, R^2 = R^3 = H$



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by this organism [8,9] which take place predominantly at C-6β, C-11α or C-14α. However, C-7α corresponds to C-11α in the reverse mode of binding in which the ring D lactone takes the place of ring A. There was also a very marked tendency for reduction to occur at C-3 suggesting that these steroids were being handled in a different way by the organism.

Table 1. Metabolites of steroidal ring D lactones

Substrate	Product	% Yield
17a-Oxa-D-homo-5α,13α-androstane-3,17-dione (**3**)		
	3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**4**)	32
	3β,7α-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**5**)	24
	3β,9α-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**6**)	4
17a-Oxa-D-5α-androstane-3,17-dione (**7**)		
	starting material	22
	7α-hydroxy-17a-oxa-D-homo-5α-androstane-3,17-dione (**8**)	1.5
	3β-hydroxy-17a-oxa-D-homo-5α-androstan-17-one (**9**)	1
	3α,7α-dihydroxy-17a-oxa-D-homo-5α-androstan-17-one (**13**)	8.5
3β-Hydroxy-17a-oxa-D-homo-5α-androstan-17-one		
	starting material	8.5
	3β,11β-dihydroxy-17a-oxa-D-homo-5α-androstan-17-one (**10**)	8.4
	3β,9α-dihydroxy-17a-oxa-D-homo-5α-androstan-17-one (**11**)	1
	3β,7α-dihydroxy-17a-oxa-D-homo-5α-androstan-17-one (**12**)	17.5

EXPERIMENTAL

General experimental and fermentation conditions have been described previously [11].

Preparation of substrates—Baeyer–Villiger oxidation

3β-Acetoxy-5α,13α-androstan-17-one [12] (1.8 g) in CH_2Cl_2 (100 ml) was treated with *m*-chloroperbenzoic acid (1.2 g) and TsOH (300 mg) at room temp. for 5 days. The soln. was diluted with CH_2Cl_2 and washed with aq. Na_2SO_3, aq. $NaHCO_3$, H_2O, brine and dried. The solvent was evaporated and the residue chromatographed on silica. Elution with 10% EtOAc–petrol gave the starting material (650 mg) whilst elution with 15% EtOAc–petrol gave 3β-acetoxy-17a-oxa-D-homo-5α,13α-androstan-17-one (370 mg) which crystallized from EtOAc–petrol as needles, mp 144–145° (Found: C, 72.3; H, 9.2. $C_{21}H_{32}O_4$ requires C, 72.4; H, 9.3%). IR υ_{max} cm^{-1} 1726, 1709; ^1H NMR δ_H0.76 (3H, *s*, H-19), 1.38 (3H, *s*, H-18), 2.02 (3H, *s*, OAc), 4.69 (1H, *tt*, J = 5 and 11 Hz, H-3). Elution with 40% EtOAc–petrol gave 3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**4**) (150 mg) which crystallized from EtOAc–petrol as needles, mp 149.5–151° (Found: C, 70.5; H, 9.8. $C_{19}H_{30}O_3.H_2O$ requires C, 70.3; H, 9.9%), IR υ_{max} cm^{-1} 3222, 1708; ^1H NMR δ_H0.75 (3 H, *s*, H-19), 1.37 (3H, *s*, H-18), 3.60 (1H, *tt*, J = 5 and 11 Hz, H-3).

Hydrolysis of 3β-acetoxy-17a-oxa-D-homo-5α,13α-androstan-17-one

The 3β-acetate (360 mg) in MeOH (20 ml) was treated with K_2CO_3 (0.4 g) in H_2O (2 ml) for 3 hr at room temp. Acetic acid (0.5 ml) was added and the soln. was concentrated, diluted with H_2O and the product recovered in EtOAc. The extract was dried and the solvent evap. to give 3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (271 mg) identical to the sample described above.

Oxidation of 3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one

The 3β-alcohol (**4**) (411 mg) in Me_2CO (30 ml) was treated with the 8N CrO_3 reagent at O° until the orange colour persisted. The mixture was left for 30 min. MeOH (1 ml) was added and the soln.

Table 2. ^{13}C NMR Data for the steroidal ring D lactones (determined in $CDCl_3$ at 75 MHz)

Carbon No.	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	36.92	36.80	37.02	29.46	38.01[a]	37.82[a]	36.74	36.84	29.67	36.83	32.57
2	37.28[a]	30.90[a]	31.21	31.04	37.74[a]	37.64[a]	31.30	31.05	31.04	31.24	29.79
3	210.60	71.18	71.03	70.70	211.01	212.41	71.01	73.38	70.60	70.94	65.59
4	43.33	37.84	37.35	37.90	44.12	43.66	37.79	37.28	37.86	37.33	37.51
5	42.15[b]	43.35[b]	36.97	34.26	45.75	38.28	44.81	46.66	35.88	36.54	31.40
6	27.50	28.30	36.54	28.13	28.42	35.35	28.25	28.66	28.14	36.49	36.59
7	29.57	31.30[a]	65.49	26.03	30.09	64.43	30.58	30.96	27.99	65.54	64.91
8	31.93	32.97	36.09	36.07	37.60	44.20	37.89	32.89	39.86	41.87	45.92
9	50.71	52.27	45.12	74.49	52.33	41.50	53.08	56.35	74.44	45.36	42.69
10	34.74	35.59	35.62	40.33	35.47	36.40	35.48	35.56	40.08	35.55	36.50
11	16.31	17.31	20.32	24.61	22.01	21.71	22.00	66.89	24.46	21.76	21.75
12	37.61[a]	38.78	38.20	33.49	39.00	38.35	39.28	47.19	34.12	38.80	39.75
13	81.34	82.52	83.02	82.68	82.91	83.74	83.35	82.65	83.16	83.50	83.56
14	44.78[b]	43.93[b]	37.17	37.43	45.93	40.41	46.28	47.98	39.85	40.94	41.62
15	19.63	20.46	16.88	17.10	19.64	19.84	19.78	19.54	19.63	19.26	19.67
16	23.86	24.91	25.10	24.96	28.32	28.27	28.62	27.77	28.70	28.57	29.18
17	171.00	172.19	172.04	172.19	171.16	172.30	171.55	171.62	171.43	171.48	171.06
18	27.73	28.83	28.77	28.60	19.96	19.81	20.09	23.25	19.34	19.95	20.21
19	10.41	12.24	11.34	14.43	11.15	10.25	12.13	15.22	14.26	11.14	10.42

[a,b] these assignments may be interchanged in vertical columns.

was concentrated, H_2O was added and the product recovered in EtOAc. The extract was washed with aq. $NaHCO_3$, H_2O and dried. The solvent was evap. to give 17a-oxa-D-homo-5α, 13α-androstane-3,17-dione (**3**) (393 mg) which crystallized from EtOAc as cubes, mp 198–199° (Found: C, 74.8; H, 9.0. $C_{19}H_{28}O_3$, requires C, 75.0; H, 9.3%), IR υ_{max} cm^{-1} 1723, 1714; ^1H NMR δ_H 0.89 (3H, *s*, H-19), 1.33 (3H, *s*, H-18).

Under similar conditions, the Baeyer–Villiger oxidation of 3β-acetoxy-5α-androstan-17-one gave 3β-acetoxy-17a-oxa-D-homo-5α-androstan-17-one, mp 151–152° (lit., [13] 149.5–153°) from which 3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**9**) mp 168° (lit., [13] 169°) was obtained by hydrolysis. Oxidation with CrO_3, as above, gave 17a-oxa-D-homo-5α-androstane-3,17-dione (**7**), mp 173° (lit., [1] 171–172°).

Incubation of steroids with C. aphidicola

(a) 17a-oxa-D-homo-5α,13α-androstan-3,17-dione (**3**)(400 mg) in EtOH (20 ml) was evenly distributed between 20 flasks (100 ml medium in 250 ml) of *C. aphidicola* 3 days after inoculation. After a further 7 days, the mycelium was filtered and the broth was extracted with EtOAc. The extract was dried and the solvent was evaporated to give a residue which was chromatographed on silicagel. Elution with 40% EtOAc–petrol gave 3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**4**) (134 mg), identified by its IR and ^1H NMR spectrum. Elution with 60% EtOAc–petrol gave 3β,9α-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**6**) (16 mg) as a gum, (M^+ 322.216 $C_{19}H_{30}O_4$ requires 322.214), IR υ_{max} cm^{-1} 3305, 3295, 1734; ^1H NMR δ_H0.89 (3H, *s*, H-19), 1.41 (3H, *s*, H-18), 3.59 (1H, *tt*, J = 4.8 and 11 Hz, H-3). Elution with EtOAc gave 3β, 7α-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**5**) (100 mg), which crystallized from EtOAc as cubes, mp 262–265° (Found: C 70.5; H, 9.4. $C_{19}H_{30}O_4$ requires C, 70.8; H, 9.4%), IR υ_{max} cm^{-1} 3424, 3329, 1731; ^1H NMR δ_H 0.75 (3H, *s*, H-19), 1.41 (3H, *s*, H-18), 3.70 (1H, *tt*, J = 5.4 and 11 Hz, H-3), 4.14 (1H, *br.s.*, H-7).

(b) Under similar conditions, 17a-oxa-D-homo-5α,13α-androstane-3,17-dione (**7**) (1 g) gave the starting material (221 mg) on elution with 50% EtOAc–petrol. Elution with 90% EtOAc–petrol gave 3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**9**) (14 mg), identified by its NMR spectra. Elution with EtOAc gave 7α-hydroxy-17a-oxa-D-homo-5α-androstane3-17-dione (**8**) (26 mg) which crystallized from EtOAc as needles, mp 211–212° (lit., [5] 210–212°), IR υ_{max} cm^{-1} 3395, 1729; ^1H NMR 0.98 (3H, *s*, H-19), 1.33 (3H, *s*, H-18), 4.03 (1H, *br.s.*, H-7). Further elution with EtOAc gave 3α.7α-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**12**) (89 mg) which crystallized from EtOAc as fine needles, mp 244° (Found: C, 70.7; H, 9.5. $C_{19}H_{30}O_4$ requires C, 70.8; H, 9.4%), IR υ_{max} cm^{-1} 3375, 1728; ^1H NMR δ_H0.77 (3H, *s*, H-19), 1.24 (3H, *s*, H-18), 4.12 (1H, *br.s.*, H-7), 4.28 (1H, *t*, J = 2.6 Hz, H-3β).

(c) Under similar conditions, 3β-hydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**9**) (1.2 g) gave the starting material (107 mg) on elution with 40% EtOAc–petrol. Elution with 50% EtOAc–petrol gave 3β,11β-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**10**) (107 mg), which crystallized from EtOAc–petrol as needles, mp. 237–239° (Found: C,69.5; H, 9.3. $C_{19}H_{30}O_4$.0.5 H_2O requires C, 68.9; H,9.4%), IR υ_{max} cm^{-1} 3583, 3485, 1742. ^1H NMR δ_H 1.00 (3H, *s*, H-19), 1.51 (3H, *s*, H-18), 3.60 (1H, *tt*, J = 5 and 11 Hz, H-3α), 4.45 (1H, *br.s.*, J = 3 Hz, H-11α). Elution with 55% EtOAc–petrol gave 3β,9α-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**11**) (14 mg), which crystallized from EtOAc–petrol as needles, mp 225° (Found: C, 70.7; H, 9.3. $C_{19}H_{30}O_4$ requires C, 70.8; H, 9.4%), IR υ_{max} cm^{-1} 3474, 3376, 1729; ^1H NMR δ_H0.91 (3H, *s*, H-19), 1.30 (3H, *s*, H-18) 3.60 (1H, *tt*, J = 4.8 and 10.8 Hz, H-3α). Elution with EtOAc gave 3β,7α-dihydroxy-17a-oxa-D-homo-5α,13α-androstan-17-one (**12**) (220 mg) which crystallized from EtOAc as needles, mp 287° (decomp.) (lit., [5] 295°), IR υ_{max} cm^{-1} 3418, 3386, 1723; ^1H NMR δ_H 0.78 (3H, *s*, H-19), 1.30 (3H, *s*, H-18), 3.65 (1H, *tt*, J = 5 and 11 Hz, H-3α), 4.00 (1H, *br.s.*, H-7β).

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