SHORT PAPER

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The microbiological hydroxylation of 17-chloroandrosta-4,16-dien-3-one by *Cephalosporium aphidicola*†

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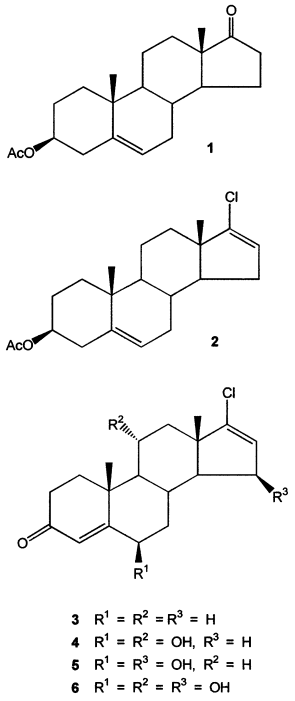
17-Chloroandrosta-4,16-dien-3-one is hydroxylated at C-6β, C-11α and C-15β by the fungus *Cephalosporium aphidicola*.

Keywords: Microbiological hydroxylation, chloro-steroids, *Cephalosporium aphidicola*

The regiospecificity of the microbiological hydroxylation of the steroids has been rationalised in terms of the location of existing hydroxyl groups and carbonyl groups on the carbon skeleton which may act as binding groups. These groups serve to orient the steroid within the hydroxylase and place a C-H bond close to the oxidant co-enzyme.[1,2] We have been studying the sites of microbiological hydroxylation by the fungus *Cephalosporium aphidicola*, in the context of building a predictive model for this organism. In prior work[3] we have shown that the organism will hydroxylate testosterone and a number of androstanes predominantly at C-6β with a minor hydroxylation occurring at C-11α and C-14α. In contrast, progesterone was hydroxylated first at C-11α on ring C and then at C-6β.[4] The position of the hydroxylation on ring C appeared to be affected by the ring D substituent. In this paper we report the effect of replacing the C-17 binding group (typically a carbonyl or a hydroxyl group) by an alkenyl chloride. A polar group of this kind might act as a hydrogen-bond acceptor.

The substrate, 17-chloroandrosta-4,16-dien-3-one (**3**) was prepared by treating 3β-acetoxyandrost-5-en-17-one (**1**) with phosphorus oxychloride and dimethylformamide to give the 17-chloro-16-ene **2**.[5] The 3β-acetate was hydrolysed to give the 3β-alcohol which was then oxidized under Oppenauer conditions to give 17-chloroandrosta-4,16-dien-3-one (**3**).

Incubation of 17-chloroandrosta-4,16-dien-3-one (**3**) with *Cephalosporium aphidicola* for 10 days gave three metabolites which were separated by chromatography. The metabolites were identified as 17-chloro-6β,11α-dihydroxyandrosta-4,16-dien-3-one (**4**), 17-chloro-6β,15β-dihydroxyandrosta-4,16-dien-3-one (**5**) and 17-chloro-6β,11α,15β-trihydroxyandrosta-4,16-dien-3-one (**6**). The location and stereochemistry of the hydroxyl groups was established by comparison of the changes in the ^{13}C NMR spectra[6,7] (see Table 1) and from the ^{1}H NMR spectra.[8] The 6α-H signals appeared within the typical range (δ_H 4.3 - 4.5) as a broad singlet. There was a characteristic downfield shift of the 19-H signal (Δδ *ca* 0.2 ppm) associated with a diaxial interaction with the 6β-hydroxyl group. The 11β-H signal (δ_H 4.14 and 4.19) appeared as a triplet (*J* 10.5 Hz) of doublets (*J* 5.5 Hz) corresponding to two diaxial couplings and one axial: equatorial coupling. The 15α-H signal (δ_H 4.57) was a narrow signal showing only small couplings to H-14α and H-16. The 18-H signal showed a significant downfield shift (Δδ 0.35



3 $R^1 = R^2 = R^3 = H$
4 $R^1 = R^2 = OH$, $R^3 = H$
5 $R^1 = R^3 = OH$, $R^2 = H$
6 $R^1 = R^2 = R^3 = OH$

Table 1 ^{13}C NMR data determined at 75 MHz in $CDCl_3$

Carbon atom	Compound 3	4	5	6
1	33.9	38.7	36.6	38.5
2	33.4	34.4	34.1	34.4
3	199.4	200.7	200.7	200.9
4	124.5	127.2	126.4	127.3
5	170.7	167.9	168.0	167.6
6	32.6	73.1	72.6	72.8
7	31.2	37.1	37.3	36.7
8	35.5	27.7	25.5	25.3
9	54.0	59.8	53.9	60.0
10	38.7	39.0	38.4	39.7
11	20.6	68.7	20.2	68.1
12	34.2	45.4	34.1	44.7
13	47.4	47.9	47.8	48.0
14	55.0	54.3	57.4	57.0
15	30.5	30.4	71.8	71.7
16	124.1	124.9	126.6	127.3
17	144.4	143.4	151.3	150.2
18	15.0	16.5	19.2	22.6
19	17.2	20.1	20.2	21.0

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.

ppm) arising from the transannular interaction with the 15β-hydroxyl group.

The hydroxylations at C-6β and C-11α are typical hydroxylations by this fungus. Although hydroxylation occurred at the allylic C-15β position, there was no detectable hydroxylation at C-14α. In prior work with *Calonectria decora* and *Rhizopus nigricans* 17-halosteroids were not metabolized.[9] In the present study we have shown that the presence of the 17-chlorine has little effect on the transformation of rings B and C by *Cephalosporium aphidicola*. However, the 17-alkenyl chloride has modified the transformation of ring D. Hydroxylation occurred at the allylic C-15β position and not at C-14α, which is hydroxylated when there is a C-17 carbonyl group.

Experimental

Silica for chromatography was Merck 9385. Light petroleum refers to the fraction b.p.60–80°. ^{1}H and ^{13}C NMR spectra were determined at 300 and 75 MHz respectively of solutions in deuteriochloroform. IR spectra were determined as nujol mulls. Extracts were dried over sodium sulfate.

Preparation of 17-chloroandrosta-4,16-dien-3-one (**3**): 3β-Acetoxy-17-chloroandrosta-5,16-diene[5] (2 g) in methanol (50 cm^3) was treated with 10% aqueous potassium carbonate (20 cm^3) at room temperature for 2 h. The solution was neutralized with acetic acid and the methanol was evaporated. The residue was extracted with ethyl acetate and the extract was washed with water and brine and then dried. The solvent was evaporated to give crude *3β-hydroxy-17-chloroandrosta-5,16-diene* (1.6 g), m.p. 150–152°C, ν_{max}/cm^{-1} 3423, 1627; δ_H 0.90 (3H, s, H-18), 1.05 (3H, s, H-19), 1.05–2.30 (19H, overlapping multiplets), 3.50 (1H, tt, *J* 5.0 and 11.1 Hz, H-3), 5.39 (1H, d, *J* 4.9 Hz, H-6) and 5.63 (1H, br.s., H-16).

The crude product was dissolved in toluene (20 cm^3) containing cyclohexanone (8 cm^3) and a portion of the toluene (8 cm^3) was distilled from the mixture. A solution of aluminium isopropoxide (750 mg) in dry toluene (7 cm^3) was added dropwise over 10 min. A further portion of the toluene (17 cm^3) was slowly distilled over a period of 1 h. The solution was cooled and a saturated aqueous solution of potassium tartrate (10 cm^3) was added. The mixture was steam distilled with the regular addition of water, until 125 cm^3 of distillate had been collected. The remaining mixture was then extracted with chloroform. The extract was washed with aqueous sodium hydrogen carbonate, then water and then dried. The solvent was evaporated to give a residue which was chromatographed on silica. Elution with 15% ethyl acetate : light petroleum gave 17-chloroandrosta-4,16-dien-3-one (**3**) (800 mg) which crystallised from ethyl acetate : light petroleum as needles, m.p. 123–125°C (Found: M$^+$ 305.166 $C_{19}H_{26}{}^{35}ClO$ [(M + H)$^+$] requires 305.167); ν_{max}/cm^{-1} 1685, 1663, 1600; Δδ 0.92 (3H, s, H-18), 1.22 (3H, s, H-19), 0.90–2.50 (18H, overlapping multiplets), 5.63 (1H, dd, *J* 1.6 and 3.0 Hz, H-16), 5.74 (1H. s, H-4).

Incubation with Cephalosporium aphidicola.: The fungus was grown on shake culture (80 cm^3 medium per 250 cm^3 conical flask) as described previously.[3,4] After 3 days 17-chloroandrosta-4,16-dien-3-one (**3**) (700 mg) in ethanol (25 cm^3) was evenly distributed between 12 flasks, The fermentation was continued for a further 10 days. The mycelium was filtered off and the broth was acidified to pH 2. The metabolites were recovered in ethyl acetate. The extract was dried and the solvent evaporated to give a residue (700 mg) which was chromatographed on silica. Elution with 55% ethyl acetate : light petroleum gave *17-chloro-6β,11α-dihydroxyandrosta-4,16-dien-3-one* (**4**) as a gum (120 mg). (Found: M$^+$ 336.151 $C_{19}H_{25}{}^{35}ClO_3$ requires M$^+$ 336.149); ν_{max}/cm^{-1} 3386,1683,1613,1598; δ_H 0.97 (3H, s, H-18), 1.43 (3H, s, H-19), 0.80–2.60 (15H, overlapping multiplets), 4.14 (1H, td, *J* 10.6 and 5.4 Hz, H-11β), 4.39 (1H, br.s., H-6α), 5.65 (1H, dd, *J* 1.6 and 3.0 Hz, H-16) and 5.84 (1H, s, H-4). Further elution with 90% ethyl acetate : light petroleum gave *17-chloro-6β,15β-dihydroxyandrosta-4,16-dien-3-one* (**5**) as a gum (50 mg) (Found: M$^+$ 336.151 $C_{19}H_{25}{}^{35}ClO_3$ requires M$^+$ 336.149); ν_{max}/cm^{-1} 3383, 1664, 1613, 1598; δ_H 1.26 (3H, s, H-18), 1.43 (3H, s, H-19), 0.80–2.60 (15H overlapping multiplets), 4.34 (1H, br.s, H-6α), 4.57 (1H, t, *J* 2.1 Hz, H-15α), 5.80 (1H, s, H-4) and 5.84 (1H, d, *J* 2.1 Hz, H-16). Elution with ethyl acetate gave *17-chloro-6β,11α,15β-trihydroxyandrosta-4,16-dien-3-one* (**6**) as a gum (30 mg). (Found: M$^+$ 352.143 $C_{19}H_{25}{}^{35}ClO_4$ requires 352.144), ν_{max}/cm^{-1} 3390, 1664, 1613, 1598; δ_H 1.26 (3H, s, H-18), 1.43 (3H, s, H-19),0.80–2.70 (14H, overlapping multiplets), 4.19 (1H, m (td), H-11β), 4.41 (1H, br.s, H-6α). 4.59 (1H, narrow multiplet, H-15α), 5.85 (1H, s, H-4), 5.87 (1H, d, *J* 2.8 Hz, H-16).

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