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MICROBIOLOGICAL 7- AND 15-HYDROXYLATIONS OF C-19 STEROIDS

G. Defaye, M. J. Luche and E. M. Chambaz
Biochimie Endocrinienne, C.E.R.M.O., Université Scientifique et Médicale, BP 53, Centre de tri, 38041 Grenoble Cedex, France

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SUMMARY

Microbiological reactions with *Fusarium* were used in an attempt to obtain 15α-hydroxy products from C-19 steroid structures. The hydroxylation specificity appeared strongly oriented by the nature of the substrate, introducing a 15α-hydroxyl group into a saturated compound such as 3β-hydroxy-5α-androstan-17-one (isoandrosterone), whereas 3β-hydroxy-5-androsten-17-one (DHA) was mostly 7α-hydroxylated. Identification of the various hydroxylation products led to the synthesis of 7α and 7β-hydroxy-isoandrosterone, 7α and 7β-hydroxy-DHA and the corresponding triols by chemical routes and their subsequent characterization. These observations point to the potential pitfalls related to substrate-directed stereospecificity in the use of biological hydroxylations of steroids.

INTRODUCTION

A number of the typical steroid metabolites excreted by the human newborn have been characterized as having 5-ene-polyhydroxylated C-19 and C-21 structures [1–3]. Identification of several of these metabolites is impaired by the lack of appropriate model compounds. This is especially the case for the 15-hydroxylated, C-19 steroid structures, as active 15α-hydroxylation has been demonstrated in the foetal compartment [4], and may represent an interesting enzymic marker in sexual differentiation [5]. On the other hand, 15 hydroxylation may represent an interesting substitution for coupling of steroid structures to antigenic proteins and the subsequent obtention of specific steroid antibodies [6].

In contrast to tedious chemical synthesis, an elegant approach would involve the use of microbiological, stereospecific hydroxylations to obtain 15-hydroxylated model compounds [7]. *Fusarium moniliformis, Fusarium linii* or *Penicillium* have yielded 15α-hydroxylated derivatives of C-18 and C-19 steroids [8–10]. *Fusarium lateritium* has been used to introduce a 15β-hydroxyl group into corticosterone [11].

The aim of this work was to obtain 15α-hydroxylated compounds from such 5-ene-C-19 steroids as dehydroisoandrosterone (DHA) by using *Fusarium* as the biological hydroxylating agent. During the course of this study, it appeared that the hydroxylation specificity was different when the corresponding saturated steroid (isoandrosterone) was used as substrate. Identification of the various hydroxylated products generated in these biological reactions led to the chemical synthesis of a number of reference structures. This paper describes several novel compounds and reports complementary characteristics for a number of already known products obtained in the course of this study.

EXPERIMENTAL

Melting points were determined on a Tottoli apparatus (Büchi). Infrared and ultraviolet spectra were obtained on a Beckmann DBT and a Beckmann Acculab 4 instruments, respectively. Nuclear magnetic resonance spectra were obtained (at 60 MHz) on a Jeol C-60-HL or (at 250 MHz) a Cameca 250 spectrometer, with deuteriochloroform as solvent and tetramethylsilane as the internal standard. Optical rotations were measured with a Perkin–Elmer 141 polarimeter, in chloroform, unless another solvent is mentioned. Gas chromatography was carried out with a Carlo Erba Model 2400 T instrument; the column (5.85 m) was packed with 1% OV-101 or 1% OV-17; retention time values were referred as methylene units [13]. TLC were carried out on Merck silica gel F 254. Mass spectra were obtained on a AEI MS-30 spectrometer coupled to a Girdel gas chromatograph. Acetylation were performed in a mixture acetic anhydride–pyridine (1:4, v/v) for 18 h at room temperature. *Fusarium graminearum* was purchased from the Central Bureau von Schimmel Culture (Baarn, Netherlands). All compounds gave satisfactory microanalysis.

Microbiological hydroxylations with Fusarium *graminearum*. The culture medium consisted of 1% glucose, 1% bactopeptone (Difco), and 0.5% yeast extract (Difco) in distilled water and it was sterilized for 20 min at 110°C. A culture of *Fusarium graminearum* on agar was transferred to the medium (100 ml) and incubated for 2 days with stirring at 27°C then transferred to 1 l. of medium and incubated for 2 additional days at 27°C. At that time, 120 mg of steroid dissolved in

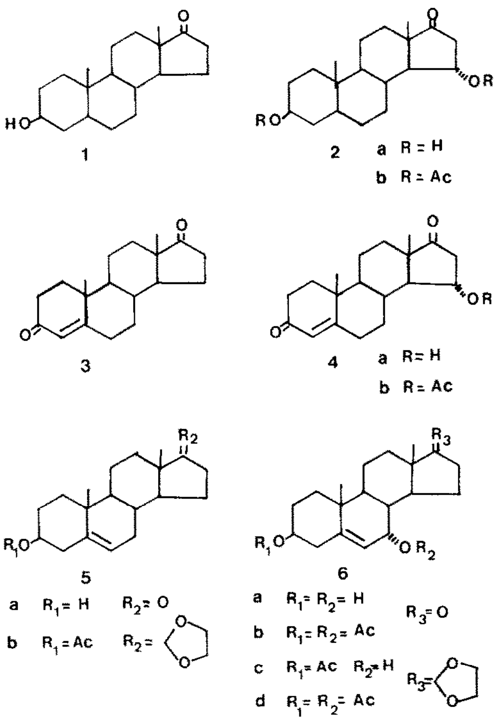

Fig. 1. Structures 1–6.

acetone (5 ml) was added and the mixture was incubated for 3 days at 27°C. The suspension was filtered and the filtrate extracted with dichloromethane. The organic layer was washed with water and then evaporated to dryness.

3β,15α-Dihydroxy-5α-androstan-17-one (**2**a). Incubation of 200 mg of isoandrosterone (**1**) with *Fusarium graminearum* yielded 200 mg of crude product that contained about 30% of starting material. The mixture was chromatographed on a column of silica gel; ether eluted isoandrosterone and then ethyl acetate–ether (4:6, v/v) eluted 82 mg of 3β,15α-dihydroxy-5α-androstan-17-one (**2**a), m.p. 160–162°C; $[\alpha]_D + 122°$ (*c* 0.6). 3β,15α-diacetoxy-5α-androstan-17-one (**2**b), m.p. 145–147°C; $[\alpha]_D + 115$ (*c* 0.7); n.m.r. (250 MHz): 0.85 (3H, *s*, Me-19); 0.94 (3H, *s*, Me-18), 2.03 and 2.04 (3H, *s*, CH_3—CO), 3.13 (2H, *d* of *d*, CH_2-16, *J* 16a, 16b 18 Hz, *J* 16a, 15b = *J* 16b, 15a 8 Hz), 4.68 (1H, *m*, *J* 15, 16 8 Hz).

Hydrogenation of 3β,15α-dihydroxy-5-androsten-17-one. Hydrogenation of 3β,15α-dihydroxy-5-androsten-17-one (0.1 mg) in ethanol (2 ml) for 15 min over 10% Pd-C gave a mixture of compound (**2**a) and starting material; compound (**2**) was identified by gas chromatography and mass spectrometry (as trimethylsilyl derivative).

15α-Hydroxy-4-androstene-3,17-dione (**4**a). Prepared by microbiological hydroxylation with *Penicillium* (ATCC 11598) according to M. D. Stern *et al.* [9]; m.p. 198–200°C (lit. [9] 200–202°), $[\alpha]_D + 220°$ (*c* 0.5); acetate (**4**b), m.p. 170–172°C, $[\alpha]_D + 200°$ (*c* 0.5); n.m.r. (250 MHz): 1.01 (3H, *s*, Me-18), 1.27 (3H, *s*, Me-19), 2.07 (3H, *s* CH_3—CO), 3.17 (2H, *d* of *d*, H_2-16), 5.25 (1H, *m*, H-15β), 5.76 (1H, *s*, H-4).

3β,7α-Dihydroxy-5-androsten-17-one (**6**a). Dehydroisoandrosterone (**5**a) (200 mg) gave, after incubation with *Fusarium graminearum*, 200 mg of a mixture that was purified by thin-layer chromatography (3 plates, silica gel, ethyl acetate–ether, 4:6, v/v). Three fractions were obtained, namely: dehydroisoandrosterone (40 mg): 3β,7α-dihydroxy-5-androsten-17-one (**6**a) (75 mg); and 5-androsten-3β,7α,17β-triol (10 mg). Compound (**6**a) had m.p. 183–184°C (lit. [12] 181.5–183.5°), $[\alpha]_D - 73$ (*c* 0.5) (lit. [12] − 70.7°). Compound (**6**b) had m.p. 175–176°C (lit. [12] 168–170°), $[\alpha]_D - 168°$ (*c* 0.3) (lit. [12] −178.5); n.m.r. (250 MHz): 0.88 (3H, *s*, Me-18), 1.04 (3H, *s*, Me-19), 2.06 (6H, *s*, CH_3—CO), 4.67 (1H, *m*, H-3), 5.10 (1H, *t*, *J* 4.5 Hz, H-7), 5.49 (1H, *d*, *J* 4.5 Hz, H-6).

3β-Acetoxy-17-ethylenedioxy-5-androsten-7-one (**8**). To 100 ml of dichloromethane (dried over P_2O_5) was added at 0°C, under nitrogen, 6.4 ml of pyridine (0.08 mol) and 4 g of chromium trioxide (0.04 mol). The solution, which turned dark red, was brought to room temperature and 0.9 g (0.025 mol) of 3β-acetoxy-17-ethylene-dioxy-androst-5-ene was added. The mixture was stirred overnight, dichloromethane and water were added, and the organic layer was separated. The latter was washed with aqueous sodium hydrogen carbonate and then water. Evaporation of the solvent gave 0.8 g of compound (**8**). Purification on a column of silica gel (eluant: dichloromethane–ether 95:5, v/v) afforded compound (**8**), m.p. 177–178°C (lit. [6] 175–177°), $[\alpha]_D - 106$ (*c* 0.5), $[\alpha]_D - 144$ (*c* 1.1, 1,4-dioxane), (lit. [6] −574, 1,4-dioxane).

3β-Acetoxy-17-ethylenedioxy-5-androsten-7α(and 7β)-ol. Compound (**8**) (1.5 g) was dissolved in methanol and sodium borohydride (2.5 g) and boric acid (2.5 g) were added. After 5 h, conventional processing afforded a mixture of the 7α (and 7β)-hydroxy com-

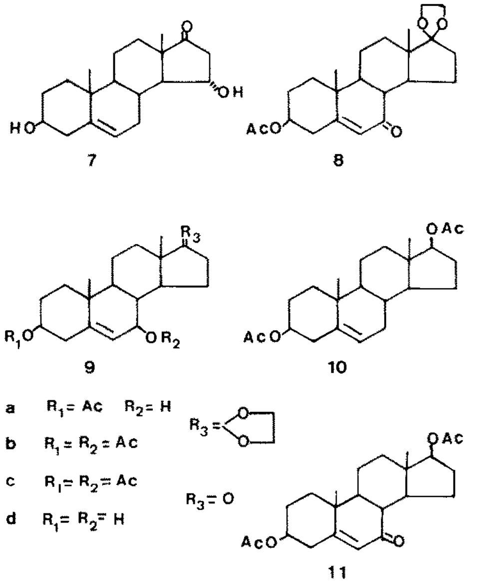

Fig. 2. Structures 7–11.

pounds, which were separated by chromatography on a column of silica gel eluted with pentane–ether (1:1, v/v). The 7β derivative was eluted first, followed by compound 7α. (**9a**) (565 mg), m.p. 144–146°C, $[\alpha]_D$ − 48.5° (*c* 0.3); n.m.r. (60 MHz): 0.88 (3H, *s*, Me-18); 1.07 (3H, *s*, Me-19), 2.05 (3H, *s*, CH_3—CO), 3.9 (4H, *s*, acetal), 4.66 (1H, *m*, H-3), 5.32 (1H, *m*, H-6); compound (**9b**) had m.p. 191–192°C; n.m.r. (60 MHz): 0.9 (3H, *s*, Me-18), 1.11 (3H, *s*, Me-19), 2.05 (6H, *s*, CH_3—CO), 3.9 (4H, *s*, acetal), 4.6 (1H, *m*, H-3), 5.08 (1H, *d*, *J* 6 Hz, H-7), 5.25 (1H, H-6). Compound (**6c**) (120 mg) had m.p. 166–168°C, $[\alpha]_D$ − 122° (*c* 0.7); n.m.r. (60 MHz): 0.86 (3H, *s*, Me-18), 1.01 (3H, *s*, Me-19), 2.05 (3H, *s*, CH_3—CO), 3.9 (4H, *s*, acetal), 4.60 (1H, *m*, H-3), 5.65 (2H, *d*, *J* 5 Hz H-6). Compound (**6d**) had m.p. 187–189°C; n.m.r. (60 MHz): 0.87 (3H, *s*, Me-18), 1.05 (3H, *s*, Me-19), 2.10 and 2.01 (6H, 2*s*, CH_3—CO), 3.9 (4H, *s*, acetal), 4.60 (1H, *m*, H-3), 5.0 (1H, *d*, H-7), 5.60 (1H, *d*, H-6).

3β,7β-Diacetoxy-5-androsten-17-one. Compound (**9b**) (575 mg) was dissolved in acetone containing 15% of water and 30 mg of *p*-toluenesulphonic acid was added. The solution was stirred for 4 h at 0°C. After extraction with diethyl ether and conventional processing, 520 mg of compound (**9c**) was obtained, m.p. 170–172°C (lit. [12] 161–163°), $[\alpha]_D$ + 108° (*c* 1.2) (lit. [12] +105°); n.m.r. (250 MHz; 0.92 (3H, *s*, Me-18), 1.13 (3H, *s*, Me-19), 2.04 and 2.09 (6H, 2*s*, CH_3—CO), 4.61 (1H, *m*, H-3), 5.18 (*q*, 1H, *J* 7,8 9.5 Hz *J* 6,7 1.5 Hz, H-7), 5.29 (1H, *d*, *J* 6,7 1.5 Hz, H-6).

3β,7β-Dihydroxy-5-androsten-17-one. A solution of the diacetoxy compound (**9c**) (30 mg) in 2 ml of M NaOH in methanol was kept for 1 h. Water was added and the precipitate collected; crystallization from acetone gave 17 mg of (**9d**), m.p. 214–215°C (lit. [12] 214–216°), $[\alpha]_D$ + 71° (*c* 0.4) (lit. [12] +67.5°).

3β,7α-Diacetoxy-5-androsten-17-one. Treatment of (**6d**) as for (**9c**) gave (**6b**); m.p. 175–176° (identical in all respects with the compound obtained previously). Saponification gave (**6a**), m.p. 183–184°.

3β,17β-Diacetoxy-5-androsten-7-one. To a stirred solution of 3β,17β-diacetoxy-androst-5-ene (**10**), (0.9 g) in acetic acid (3.5 ml) and acetic anhydride (2 ml) kept below 25°C sodium chromate (0.8 g) was added portion wise and left over-night. After water was added the precipitate was collected; crystallization from methanol gave 3β,17β-diacetoxy-5-androsten-7-one (**11**) (650 mg), m.p. 217–218°C (lit. [14] 218–219°), $[\alpha]_D$ − 128° (*c* 0.8) (lit. [14] −135°); n.m.r. (60 MHz): 0.81 (3H, *s*, Me-18), 1.23 (3H, *s*, Me-19), 2.10 (6H, *s*, CH_3—CO), 4.65–4.85 (2H, *m*, H-3, H-17), 5.73 (1H, *s*, H-6); EtOH λ_{max} 233 nm ($\epsilon = 9580$).

5-Androsten-3.β,7α(and 7β),17β-triol. Compound (**11**) (500 mg) dissolved in 20 ml of dry diethyl ether was added dropwise to 20 ml of diethyl ether containing 200 mg of lithium aluminium hydride and the mixture was stirred for 3 h at 20°C. After addition of ethyl acetate, conventional treatment gave a mixture of hydroxy compounds, which were separated by thin-layer chromatography on silica gel with ether as eluant. 5-Androsten-3β,7β,17β-triol (**13a**) had m.p. 247–248°C (lit. [14] 236°), $[\alpha]_D$ + 33° (*c* 0.4, MeOH) (lit. [14] +26°); triacetate, m.p. 210–211°, $[\alpha]_D$ + 52° (*c* 0.5, $CHCl_3$); n.m.r. (60 MHz): 0.81 (3H, *s*, Me-18), 1.06 (3H, *s*, Me-19), 2.03 (9H, *s*, CH_3—CO), 4.6 (1H, *m*, H-3), 5.06 (1H, *d*, *J* 6 Hz, H-17), 5.62 (1H, *d*, H-6).

5-Androsten-3β,7α,17β-triol (**12a**) had m.p. 271–273°C (lit. [15] 264–267°), $[\alpha]_D$ − 48° (*c* 0.7, MeOH); triacetate (**12b**), m.p. 262–263°, $[\alpha]_D$ − 60° (*c* 0.5, $CHCl_3$); n.m.r. (60 MHz): 0.80 (3H, *s*, Me-18), 1.02 (3H, *s*, Me-19), 2.02 (9H, *s*, CH_3—CO), 4.6 (1H, *m*, H-3), 5.0 (1H, H-7), 5.6 (1H, H-6).

3β-Acetoxy-17-ethylenedioxy-5α-androstan-7-one (**14**). Hydrogenation of (**8**) (500 mg) in ethanol (20 ml) ethyl acetate (2 ml) over 10% Pd-C (30 mg) gave 3β-acetoxy-17-ethylenedioxy-5α-androstan-7-one (**14**), m.p. 195–196°C, $[\alpha]_D$ − 75° (*c* 0.5); n.m.r. (60 MHz): 0.85 (3H, *s*, Me-18); 1.11 (9H, *s*, Me-19), 2.05 (3H, *s*, CH_3—CO), 3.87 (4H, *s*, acetal), 4.60 (3H, *m*, H-3).

3β,7α(and 7β)-Dihydroxy-5α-androstan-17-one. Reduction of compound (**14**) (0.9 g) with lithium aluminium hydride (0.3 g) in diethyl ether (50 ml) for 3 h at room temperature gave a mixture of 7α and 7β-dihydroxy compounds, which was acetylated. 3β,7ξ-diacetoxy-17-ethylenedioxyandrostane (0.8 g) was dissolved in acetone (40 ml), and water (10 ml) and *p*-toluenesulphonic acid (80 mg) were added. After 5 h, the same treatment as previously described gave the compound. Separation by chromatography on silica gel (eluant dichloromethane–ether (85:5, v/v) gave first the α isomer then the β. 3β,7α-Diacetoxy-5α-androstan-17-one had m.p. 182–183°C, $[\alpha]_D$ + 25.9° (*c* 0.6). n.m.r. (250 MHz): 0.87 (3H, *s*, Me-18), 0.88 (3H, *s*, Me-19), 2.04 and 2.10 (6H, 2*s*, acetoxy), 4.73 (1H, *m*, H-3), 5.05 (1H, *d*, H-7). 3β,7β-Diacetoxy-5α-androstan-17-one had m.p. 145–146°C, $[\alpha]_D$ + 158° (*c* 0.5). n.m.r. (250 MHz): 0.92 (6H, *s*, Me-18, Me-19),

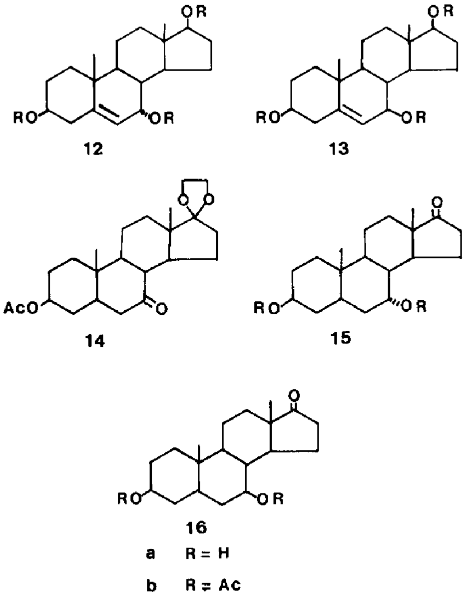

Fig. 3. Structures 12–16.

2.04 and 2.05 (6H, 2*s*, CH_3—CO), 4.67 (2H, *m*, H-3, H-7). 3β,7α-Dihydroxy-5α-androstan-17-one: m.p. 193–194°C (lit. [17] 194–195°), $[\alpha]_D$ + 57° (*c* 0.4) (lit. [16] +58°). 3β,7β-Dihydroxy-5α-androstan-17-one: m.p. 240–241°C (lit. [17] 241–243°).

RESULTS AND DISCUSSION

1. *Action of* Fusarium graminearum *on isoandrosterone*

Fusarium linii was first used in an attempt to obtain the 15α-hydroxy derivative of isoandrosterone. Under the incubation conditions used, the starting steroid was recovered unchanged. Therefore, *Fusarium graminearum* was used for subsequent studies.

Incubation of isoandrosterone (**1a**) with *Fusarium graminearum* yielded a monohydroxylated isoandrosterone whose mass spectrum exhibited a molecular ion at *m/e* 306 (free compound) and 450 (as its trimethylsilyl ether). Its n.m.r. spectrum showed that the proton of the additional hydroxyl group was coupled to three other protons. Oxidation yielded a triketone having a U.V. spectrum characteristic of a β-diketone ($\epsilon \simeq$ 14,000 at 275 nm in ethanolic KOH). These data led to the conclusion that the additional hydroxyl group had been introduced at the 15-position of isoandrosterone, as might have been expected [10]. To establish the final identification as 15α-hydroxy isoandrosterone (**2a**) [18], 15α-hydroxyandrostenedione (**4**) was prepared by microbiological hydroxylation of androstenedione (**3**) with *Penicillium* [9]. The n.m.r. constants, chemical shifts, and coupling constants for the C-15 and C-16 protons were identical for (**2**b) and (**4**b) (Table 1). Authentic 15α-hydroxy-DHA [19] was hydrogenated to 15α-hydroxy isoandrosterone in the presence of palladium on charcoal, and the resulting product appeared identical to compound (**2a**) upon gas-chromatographic analysis (as trimethylsilyl derivatives) on two different phases (Table 2).

Table 1. n.m.r. Parameters of 3β,15α-diacetoxy-5α-androstan-17-one (**2**b) and 15-acetoxy-4-androstene-3-17-dione (**4**b)

	Chemical shifts		Coupling constants		
	CH_2 [16]	CH [15]	J_{15-16}	J_{16-16}	J_{14-15}
2b	*3.12	5.20	8	18.5	8.5
4b	*3.17	5.25	8	18	8.5

* Irradiation at 5.20 and 5.25 ppm respectively converts this quadruplet into a doublet.

2. *Action of* Fusarium graminearum *on DHA*

On the basis of the foregoing result, showing that 15α-hydroxylation occurred when isoandrosterone was used as substrate, identical conditions were applied in an attempt to obtain 15α-hydroxy-DHA from DHA, although 7α-hydroxylation has been mentioned as occurring under similar conditions [15]. In this instance, two products could be isolated. One of them was identified as a dihydroxy-androsten-17-one (molecular ion at *m/e* 448 as the trimethyl-silyl derivative); the other product was the androstenetriol (**12a**), which was obtained also by borohydride reduction of (**6a**). Compound (**6a**) was definitely different from authentic 15α-hydroxy-DHA [19] and its final identification as 3β,7α-dihydroxy-5-androsten-17-one

Table 2. Gas chromatographic retention parameters (expressed as methylene units [13]) of the hydroxylated steroids obtained in this work, on OV-1 and OV-17 stationary phases and their molecular ions (M^+)

	OV-1	OV-17	M^+
7α-hydroxy-DHA-di-TMS	25.84	27.49	448
7β-hydroxy-DHA-di-TMS	26.35	26.64	448
15α-hydroxy-DHA-di-TMS	27.36	28.95	448
15α-hydroxy-DHA-MO-di-TMS	27.95		477
7α-hydroxy-*iso*-A-di-TMS	26.32	29.05	450
7β-hydroxy-*iso*-A-di-TMS	27.62	29.27	450
15α-hydroxy-*iso*-A-di-TMS	27.46	29.05	450
15α-hydroxy-*iso*-A-BO-di-TMS	34.22	36.97	553
3β,7α,17β-trihydroxy-A-5-ene-tri-TMS	26.33	26.48	522
3β,7β,17β-trihydroxy-A-5-ene-tri-TMS	27.72	28.02	522
3β,15α,17β-trihydroxy-A-5-ene-tri-TMS	28.00	28.06	522
15α-hydroxy-A-4-en-3,17-dione-TMS	27.72	31.50	

Trivial names and abbreviations used in the table are as follows: 7α-hydroxy-DHA: 3β,7α-dihydroxy-5-androsten-17-one; 7β-hydroxy-DHA: 3β,7β-dihydroxy-5-androsten-17-one; 15α-hydroxy-DHA: 3β,15α-dihydroxy-5-androsten-17-one; 7α-hydroxy-iso-A: 3β,7α-dihydroxy-5α-androstan-17-one; 7β-hydroxy-iso-A: 3β,7β-dihydroxy-5α-androstan-17-one; 15α-hydroxy-iso-A: 3β,15α-dihydroxy-5α-androstan-17-one; 3β,7α,17β-trihydroxy-A-5-ene: 5-androstene-3β,7α,17β-triol; 3β,7β,17β-trihydroxy-A-ene: 5-androstene-3β,7β,17β-triol; 3β,15α,17β-trihydroxy-A-5-ene: 5-androstene-3β,15α,17β-triol; 15α-hydroxy-4-androstene-3, 17-dione: 15α-hydroxy-4-androstene-3, 17-dione; TMS, di-TMS and tri-TMS are respectively the mono, di and tri-trimethylsilyl ethers, MO-di-TMS and BO-di-TMS are the methyloxime di-trimethylsilyl ethers and the benzyloxime, di-trimethylsilyl ethers.

(**6a**) was secured after chemical synthesis of 7α- and 7β-hydroxy-DHA as model compounds.

3. *Chemical synthesis of 7α-(and 7β)-hydroxy-DHA*

After derivatization of DHA to its 3-acetoxy-17-ethylene acetal (**5b**), an allylic oxidation was performed with chromium trioxide–pyridine complex in dichloromethane [20], and led to compound (**8**) in 80% yield. This result contrasts with the poor yield observed by Condom *et al.* using sodium chromate in acetic anhydride–acetic acid [6]. Reduction of **8** with sodium borohydride in boric acid-buffered methanol yielded a mixture of the 7α-(**6c**:30%) and 7β-(**9a**:70%) hydroxy derivatives, which were isolated by thin-layer chromatography on silica gel. After acetylation to remove by-products from the allylic alcohol, compounds **6c** and **9a** were deacetalated by acid hydrolysis. A subsequent saponification step yielded 7α-(**6a**) and 7β-(**9d**)-hydroxy-DHA.

4. *5Androsten-3β,7α(and 7β),17β-triol*

The foregoing procedure was repeated but starting from 5-androsten-3β,17β-diol (**10a**). The diacetoxy derivative **10b** was oxidized by sodium chromate [21] to the 7-keto product, which was then reduced by lithium aluminium hydride to yield the 7α-(30%) and 7β-(70%)-hydroxy compounds. The two products were isolated as acetoxy derivatives (**12b** and **13b**) by thin-layer chromatography. A saponification step then led to the androstene-triols **12a** and **13a**.

5. *7α(and 7β)-hydroxy-isoandrosterone*

As, in our hands, *Fusarium graminearum* apparently exhibited a different hydroxylation specificity when the substrate was DHA (7α) or isoandrosterone (15α), we synthesized 7α-(and 7β)-hydroxyisoandrosterone with the aim of looking at these compounds as possible minor products of incubation with isoandrosterone. As the hydrogenation of (**6a**) and (**9d**) was not possible without hydrogenolysis of the 7-hydroxyl group, derivative (**8**) was hydrogenated to the corresponding saturated ketone, and then reduced by treatment with borohydride in buffered medium. The major (65%) and least polar product isolated after thin-layer chromatography was the 7α-hydroxy derivative, as observed in the case of cholestan-7-one (22). Removal of the 17-ethylene acetal group and subsequent saponification gave 7α- and 7β-hydroxy-isoandrosterone. Using these compounds as standards, no evidence was obtained showing the introduction of a 7-hydroxyl group when isoandrosterone was used as substrate with *Fusarium graminearum*.

These results demonstrate that microbiological enzymic systems, which offer a practical and elegant route for stereospecific modifications of steroids may give unexpected results because of substrate-directed stereospecificity as, for example, in hydroxylation reactions. On the basis of these observations and previous ones obtained with other steroid substrates, this potential pitfall should not be overlooked when stereospecific hydroxylation reactions are attempted.

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