Microbial transformation of epiandrosterone by *Aspergillus sydowii*

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Incubation of epiandrosterone with *Aspergillus sydowii* MRC 200653 afforded ten metabolites. The fungal dehydrogenation of epiandrosterone is reported for the first time. The formation of the major metabolite, 6β-hydroxyandrost-4-ene-3,17-dione, involved first dehydrogenation to give a 4-ene and then hydroxylation at C-6β. Small amounts of the substrate were hydroxylated at C-1α, C-7α, C-7β and C-11α.

Keywords: epiandrosterone, *Aspergillus sydowii*, dehydrogenation, biotransformation

The remarkable regio- and stereoselectivities of microbial steroid biotransformations have been used for a long time in order to synthesise more valuable steroidal drugs and hormones. There continue to be many attempts to perform more effective microbial steroid biotransformations and to detect new, useful microorganisms and reactions.[1] *Aspergillus* species are well known for their mycotoxin formation. They have been used in studies of fundamental eukaryotic genetics and in biotechnological exploration.[2] These species are widely found in soil, water and decaying materials. A few *Aspergillus* species are regarded as pathogenic organisms for humans and animals.[3]

Aspergillus sydowii is considered to be a mesophilic soil and marine saprophyte, a food contaminant and a potential opportunistic pathogen for humans.[4] There has been only one steroid biotransformation by *A. sydowii* reported in the literature.[5] In the present work, epiandrosterone **1** was incubated with *A. sydowii* MRC 200653 in order to investigate its metabolism by the fungus.

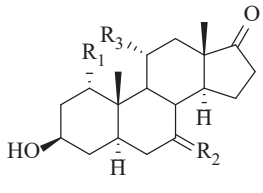
Incubation of epiandrosterone **1** with *A. sydowii* MRC 200653 for five days yielded 10 metabolites (Table 1). The first metabolite was identified as 5α-androst-1-ene-3,17-dione **2** (Fig. 1). The ^{1}H NMR spectrum of **2** had a downfield shift (Δ 0.21 ppm) for the 19-methyl group and demonstrated characteristic 1,2-double bond resonances[6] at δ_H 5.86 ppm (1H, d, J = 10.0 Hz) and δ_H 7.15 ppm (1H, d, J = 10.0 Hz). The ^{13}C NMR spectrum of **2** showed resonances at δ_C 157.82 and δ_C 127.63 (Table 2), confirming the presence of a 1,2-double bond. In addition to this, the ^{13}C NMR spectrum of **2** lacked the C-3 resonance of **1** at δ_C 71.06 ppm and showed a new resonance at δ_C 200.00 ppm, indicating the presence of a new carbonyl group conjugated with a 1,2-double bond. The C-17 resonance of **1** was retained at δ_C 220.57 ppm. Both the melting point and the ^{1}H NMR data for **2** were comparable with the values reported in the literature.[6]

The second metabolite was identified as androsta-1,4-dien-3,17-dione **3**. The ^{1}H NMR spectrum of **3** showed a significant downfield shift (Δ 0.41 ppm) for the 19-methyl group and had characteristic resonances[7] at δ_H 6.10 ppm (1H, s), δ_H 6.24 ppm (1H, d, J = 10.0 Hz) and δ_H 7.10 ppm (1H,d, J = 10.0 Hz), which were assigned to the 4-H, 2-H and 1-H protons, respectively. These resonances were in accordance with the presence of 1,2- and 4,5-double bonds. The ^{13}C NMR spectrum of **3** lacked the C-3 resonance of **1** at δ_C 71.06 ppm and showed a new resonance at δ_C 184.85 ppm, confirming the presence of a new carbonyl group conjugated with two double bonds. The C-17 resonance of **1** was maintained at δ_C 220.07 ppm. The ^{1}H and ^{13}C NMR data for **3** were consistent with the literature.[7]

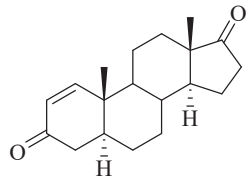
The third metabolite was identified as 17β-hydroxyandrost-4-en-3-one **4**. The ^{1}H NMR spectrum of **4** lacked the 3α-H resonance (1H, tt, J = 5.0 and 11.0 Hz) of **1** at δ_H 3.59 ppm, demonstrated a significant downfield shift (Δ 0.35 ppm) for

Table 1 Metabolite yields following chromatography

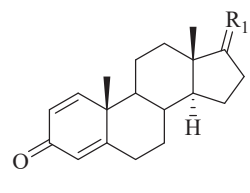
Substrate	Metabolite	Yield/%
Epiandrosterone **1**		
	5α-Androst-1-ene-3,17-dione **2**	2
	Androsta-1,4-diene-3,17-dione **3**	5
	17β-Hydroxyandrost-4-en-3-one **4**	2
	17β-Hydroxyandrosta-1,4-dien-3-one **5**	3
	6β-Hydroxyandrost-4-en-3,17-dione **6**	32
	6β,17β-Dihydroxyandrost-4-ene-3-one **7**	2
	3β,11α-Dihydroxy-5α-androstan-17-one **8**	2
	1α,3β-Dihydroxy-5α-androstan-17-one **9**	5
	3β,7β-Dihydroxy-5α-androstan-17-one **10**	3
	3β,7α-Dihydroxy-5α-androstan-17-one **11**	2



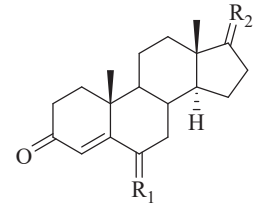
1 $R_1 = R_3 = H, R_2 = H_2$
8 $R_1 = H, R_2 = H_2, R_3 = OH$
9 $R_1 = OH, R_2 = H_2, R_3 = H$
10 $R_1 = H, R_2 = \beta OH, \alpha H, R_3 = H$
11 $R_1 = H, R_2 = \beta H, \alpha OH, R_3 = H$



2



3 $R_1 = O$
5 $R_1 = \beta OH, \alpha H$



4 $R_1 = H_2, R_2 = \beta OH, \alpha H$
6 $R_1 = \beta OH, \alpha H, R_2 = O$
7 $R_1 = R_2 = \beta OH, \alpha H$

Fig. 1 Chemical structures of compounds **1–11**.

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Table 2 ^{13}C NMR data of compounds **1–11** determined in $CDCl_3$

Carbon atom	1	2	3	4	5	6	7	8	9	10	11
1	36.87	157.82	155.54	35.61	156.05	36.88	36.28	38.41	72.74	36.77	36.63
2	31.35	127.63	127.54	33.85	127.33	34.04	34.08	31.63	38.36	31.35	31.31
3	71.06	200.00	184.85	199.72	186.44	200.58	200.80	70.72	66.36	70.91	71.00
4	37.98	40.85	123.97	123.73	123.73	126.20	126.01	38.41	37.80	37.56	37.53
5	44.76	44.28	168.32	171.51	169.40	168.31	168.90	44.94	37.25	42.03	37.06
6	28.32	27.32	32.21	33.07	32.73	72.40	72.57	28.80	28.15	38.76	35.70
7	30.83	30.08	31.06	31.44	32.73	37.09	36.90	30.68	30.44	74.76	66.78
8	34.97	35.17	34.98	35.55	35.47	29.27	29.65	34.13	34.92	42.88	39.06
9	54.35	51.28	52.15	53.81	52.42	53.48	53.58	60.34	46.86	52.49	46.07
10	35.57	38.97	43.41	38.59	43.59	37.95	37.96	37.07	39.56	35.10	36.00
11	20.43	20.46	22.01	20.55	22.43	20.13	20.46	68.56	19.78	20.70	20.28
12	31.47	31.44	32.47	36.32	36.23	31.11	37.96	42.82	31.28	31.50	31.12
13	47.76	47.77	47.62	42.72	43.02	47.55	42.77	47.98	47.77	48.27	47.57
14	51.34	50.08	50.28	50.37	50.00	50.72	50.32	50.18	51.24	51.01	45.81
15	21.72	21.70	21.83	23.25	23.45	21.59	23.16	21.65	21.75	24.92	21.33
16	35.80	35.79	35.56	30.20	30.28	35.69	30.17	35.84	35.78	36.01	36.63
17	221.50	220.57	220.07	81.31	81.47	220.85	81.46	220.16	221.63	221.00	221.10
18	13.76	13.88	13.72	10.99	11.10	13.66	11.07	14.49	13.72	14.04	13.50
19	12.25	13.03	18.61	17.32	18.63	19.40	19.37	12.70	12.87	12.43	11.20

the 19-methyl group, and had a characteristic resonance[8] at δ_H 5.72 ppm (1H, s), suggesting the presence of a 4-en-3-keto moiety in ring A. The ^{13}C NMR spectrum of **4** showed new resonances at δ_C 199.72 ppm (C-3), δ_C 171.51 ppm (C-5) and δ_C 123.73 ppm (C-4), further suggesting the presence of a 4-en-3-keto moiety. The ^{13}C NMR spectrum of **4** also lacked the C-17 resonance of **1** at δ_C 221.50 ppm and showed a new resonance at δ_C 81.31 ppm, indicating the presence of a 17β-hydroxyl group.

The fourth metabolite was identified as 17β-hydroxyandrosta-1,4-dien-3-one **5**. The ^1H NMR spectrum of the metabolite showed a significant downfield shift (Δ 0.41 ppm) for the 19-methyl group and had characteristic resonances[7] at δ_H 6.08 ppm (1H, s), δ_H 6.21 ppm (1H, dd, J = 1.8 and 10.0 Hz) and δ_H 7.05 ppm (1H, d, J = 10.0 Hz), suggesting the presence of 1,2- and 4,5-double bonds in ring A. The ^{13}C NMR spectrum of **5** lacked the C-3 resonance of **1** at δ_C 71.06 ppm and showed a new resonance at δ_C 186.44 ppm, indicating the presence of a new carbonyl group conjugated with two double bonds. The ^{13}C NMR spectrum of **5** also lacked the C-17 resonance of **1** at δ_C 221.50 ppm and had a new resonance at δ_C 81.47, confirming the presence of a 17β-hydroxyl group. The ^1H and ^{13}C NMR data for **5** were consistent with the literature.[7]

The fifth metabolite was identified as 6β-hydroxyandrost-4-ene-3,17-dione **6**. The ^1H NMR spectrum of **6** showed a significant downfield shift (Δ 0.55 ppm) for the 19-methyl group and had a new resonance at δ_H 5.82 ppm (1H, bs). The ^{13}C NMR spectrum of **6** lacked the C-3 resonance of **1** at δ_C 71.06 ppm and showed a new resonance at δ_C 200.58 ppm. These results indicated the presence of a 4-en-3-keto moiety. NMR spectra of **6** had characteristic resonances[8,9] at δ_H 4.36 ppm (1H, bs) and δ_C 72.40 ppm, suggesting the presence of a 6β-hydroxyl group. The ^{13}C NMR spectrum of **6** showed a downfield shift for C-7 (Δ 6.26 ppm), whereas it showed a γ-gauche upfield shift for C-8 (Δ 5.70 ppm), further suggesting that a 6β-hydroxylation had taken place. The C-17 resonance of **1** remained at δ_C 220.85 ppm.

The sixth metabolite was identified as 6β,17β-dihydroxyandrost-4-en-3-one **7**. The NMR spectra of **7** lacked the C-3 resonance of **1** at δ_C 71.06 ppm and revealed a downfield shift (Δ 0.54 ppm) for the 19-methyl group, together with a new resonance at δ_H 5.81 ppm (1H, bs), indicating the presence of a 4-en-3-keto moiety in ring A. The NMR spectra of **7** also contained new resonances at δ_H 4.35 ppm (1H, bs) and δ_C 72.57 ppm, which are typical of a 6β-hydroxyl group. The ^{13}C NMR spectrum of **7** showed a downfield shift for C-7 (Δ 6.07 ppm), whereas it showed a γ-gauche upfield shift for C-8 (Δ 5.32 ppm), suggesting the presence of a 6β-hydroxyl group. The ^{13}C NMR spectrum of **7** also lacked the C-17 resonance of **1** at δ_C 221.50 ppm and had a new resonance at δ_C 81.46, confirming the presence of a 17β-hydroxyl group.

The seventh metabolite was identified as 3β,11α-dihydroxy-5α-androstan-17-one **8**. The NMR spectra of **8** had new resonances at δ_H 3.96 ppm (1H, dt, J = 5.0 and 10.0 Hz) and δ_C 68.56 ppm, suggesting the presence of an 11α-hydroxyl group.[10] The ^{13}C NMR spectrum of **8** showed a downfield shift for C-9 (Δ 5.99 ppm), whereas it showed a γ-gauche upfield shift for C-8 (Δ 0.84). These results further suggested that an 11α-hydroxylation had taken place. The 3α-H resonance (1H, tt, J = 5.0 and 11.0 Hz) of **1** remained at δ_H 3.57 ppm.

The eighth metabolite was identified as 1α,3β-dihydroxy-5α-androstan-17-one **9**. The ^1H NMR spectrum of **9** had characteristic resonances[11] at δ_H 3.83 ppm (1H, bs) and δ_H 4.03 ppm (1H, tt, J = 5.0 and 11.0 Hz), indicating the presence of a 1α-hydroxyl group and a 3β-hydroxyl group, respectively. The ^{13}C NMR spectrum of **9** showed downfield shifts for C-2 (Δ 7.01 ppm) and C-10 (Δ 3.99 ppm), whereas it showed γ-gauche upfield shifts for C-5 (Δ 7.51 ppm) and C-9 (Δ 7.49 ppm), confirming the presence of a 1α-hydroxyl group. The ^1H NMR spectrum of **9** showed a significant downfield shift (Δ 0.44 ppm) for the 3α-H resonance (1H, tt, J = 5.0 and 11.0 Hz) of **1**, suggesting the presence of a 1α-hydroxyl group in close proximity. Both the melting point[12] and the ^1H NMR data[13] for **9** were comparable with the values reported in the literature.

The ninth metabolite was identified as 3β,7β-dihydroxy-5α-androstan-17-one **10**. NMR spectra of **10** showed new resonances at δ_H 3.45 ppm (1H, m) and δ_C 74.76 ppm, which are typical of a 7β-hydroxyl group.[14] The ^{13}C NMR spectrum of **10** showed downfield shifts for C-6 (Δ 10.44 ppm) and C-8 (Δ 7.91 ppm), whereas it showed γ-gauche upfield shifts for C-5 (Δ 2.73 ppm) and C-9 (Δ 1.86 ppm). These shifts are consistent with the presence of a 7β-hydroxyl group. The ^1H NMR spectrum of **10** had a resonance (1H, tt, J = 5.0 and 11.0 Hz)

at δ_H 3.60 ppm, indicating that the 3β-hydroxyl group was retained.

The tenth metabolite was identified as 3β,7α-dihydroxy-5α-androstan-17-one **11**. NMR spectra of **11** had characteristic resonances[10] at δ_H 3.95 ppm (1H, bs) and δ_C 66.78 ppm, indicating the presence of a 7α-hydroxyl group. The ^{13}C NMR spectrum of **11** showed downfield shifts for C-6 (Δ 7.38 ppm) and C-8 (Δ 4.09 ppm), whereas it showed γ-gauche upfield shifts for C-5 (Δ 7.70 ppm) and C-9 (Δ 8.28 ppm), further indicating the presence of a 7α-hydroxyl group. The 3α-H resonance (1H, tt, J = 5.0 and 11.0 Hz) of **1** was maintained at δ_H 3.58 ppm.

As can be seen from Table 1, most of epiandrosterone **1** was dehydrogenated and then hydroxylated mainly at C-6β by *A. sydowii* MRC 200653, whereas some of the remaining substrate was hydroxylated at C-1α, C-7α, C-7β and C-11α.

According to the literature, the dehydrogenation of steroids by some fungal species has been observed.[5,7,15–22] Dehydrogenation in ring A of steroids is widely used in the production of corticosteroids.[23] Synthetic steroidal drugs with a 1,2-double bond have a greater affinity for the glucocorticoid receptors, and the presence of a 1,2-double bond together with a 3-keto-4-ene moiety gives these compounds higher therapeutic potency by reducing their rate of metabolic degradation.[7,23] Introduction of a 1,2-double bond into the steroids is very rare in fungi although it is very common in bacteria.[24] Only a few fungi, such as *Phycomyces blakesleeanus*,[15] *Nectria haematococca*,[16] *Trichoderma hamatum*,[17] *Cephalosporium aphidicola*,[18] *Fusarium lini*,[7,18] *Fusarium oxysporum*,[19] *Acremonium strictum*[20] and *Aspergillus niger*,[21] were known to perform this type of dehydrogenation. In this work, *A. sydowii* carried out the introduction of both a 1,2-double bond and a 4,5-double bond into the substrate simultaneously as well as separately. Furthermore, this work is the first epiandrosterone incubation affording dehydrogenated metabolites as far as fungal steroid biotransformations are concerned.

Most of the dehydrogenated material was hydroxylated only at C-6β, whilst a minor hydroxylation of the remaining epiandrosterone **1** occurred at C-1α, C-7α, C-7β and C-11α. In the previous work, *A. sydowii* dehydrogenated dehydroepiandrosterone predominantly and then hydroxylated it at C-6β, whilst the remaining dehydroepiandrosterone was hydroxylated only at C-7α or C-7β.[5] In this work, the 1α-hydroxylation of epiandrosterone **1** by *A. sydowii* could have been due to the hydration of the 1,2-double bond, as in the testosterone catabolism by *Steroidobacter denitrificans*.[25]

Experimental

Epiandrosterone **1** was purchased from Sigma-Aldrich (Istanbul, Turkey). Solvents were of analytical grade and were purchased from Merck (Istanbul). Potato dextrose agar (PDA) and agar for PDA slopes and ingredients for liquid media were also purchased from Merck. The steroids were separated by column chromatography on silica gel 60 (Merck 107734), eluting with increasing concentrations of ethyl acetate in *n*-hexane. Thin layer chromatography (TLC) was carried out with 0.2 mm thick Merck Kieselgel 60 F_{254} TLC plates using ethyl acetate/*n*-hexane (1:1) as eluent. TLC plates were dipped into an anisaldehyde/H_2SO_4 reagent and heated to 120 °C for 3 minutes in order to visualise the spots. Infrared spectra were recorded using a PerkinElmer Spectrum Two spectrometer. ^1H NMR spectra were recorded in deuteriochloroform with tetramethylsilane as internal reference at 300 MHz with a Varian Mercury 300 spectrometer unless otherwise specified. ^{13}C NMR spectra were recorded in deuteriochloroform at 75 MHz with a Varian Mercury 300 spectrometer unless otherwise specified. Chemical shifts are given in ppm (δ scale) and coupling constants (J) are given in Hz. Melting points were determined by an Electrothermal IA 9200 melting point apparatus and are uncorrected.

A. sydowii MRC 200653 was obtained from the TÜBİTAK Marmara Research Center, Food Science and Technology Research Institute, Culture Collection Unit, Kocaeli, Turkey. Stock cultures were maintained at 4 °C on PDA slopes. The biotransformation experiment was performed in duplicate and run with control flasks containing non-inoculated sterile medium and the substrate. After 5 days of incubation, all controls were harvested and analysed by TLC. No metabolites were detected in the controls.

Biotransformation of epiandrosterone **1**

The liquid medium for *A. sydowii* MRC 200653 was prepared by mixing malt extract (30 g) and peptone (3 g) in 1 L of artificial seawater containing $CaCl_2.2H_2O$ (1.36 g), $MgCl_2.6H_2O$ (9.68 g), KCl (0.61 g), NaCl (30 g), Na_2HPO_4 (0.014 mg), Na_2SO_4 (3.47 g), $NaHCO_3$ (0.17 g), KBr (0.1 g), $SrCl_2.6H_2O$ (0.04 g) and H_3BO_3 (0.03 g).[26] The final pH of the medium was adjusted to 8.0 by the addition of 3M KOH. The medium was evenly distributed among ten culture flasks of 250 mL capacity (100 mL in each) and autoclaved for 20 minutes at 121 °C. Spores freshly obtained from a PDA slope were transferred aseptically to each flask containing sterile medium in a biological safety cabinet. After cultivation at 32 °C for three days on a rotary shaker (150 rpm), epiandrosterone **1** (1 g) dissolved in 10 mL of dimethylformamide (DMF) was evenly distributed aseptically among the flasks and the biotransformation was carried out in ten flasks for five days under the same conditions. The fungal mycelium was separated from the broth by filtration under vacuum, and the mycelium was rinsed with ethyl acetate (500 mL). The broth was extracted three times each with 1 L of ethyl acetate. The organic extract was dried over anhydrous sodium sulfate, and the solvent evaporated *in vacuo* to give a brown gum (2847 mg) which was then chromatographed on silica gel. Elution with 20% ethyl acetate in *n*-hexane yielded 5α-androst-1-ene-3,17-dione **2** (20 mg, 2%), which crystallised from ethyl acetate as needles, m.p. 141–142 °C (lit.[6] 139.5–140.5 °C), ν_{max}/cm^{-1} 1740 and 1671; δ_H 0.93 (3H, s, 18-H), 1.04 (3H, s, 19-H), 5.86 (1H, d, J = 10.0 Hz, 2-H), 7.15 (1H, d, J = 10.0 Hz, 1-H).

Elution with 30% ethyl acetate in *n*-hexane yielded the unreacted starting material (155 mg), which was identified by comparison of its ^1H and ^{13}C NMR spectra with those of an authentic sample.

Further elution with 30% ethyl acetate in *n*-hexane yielded androsta-1,4-dien-3,17-dione **3** (49 mg, 5%), which crystallised from acetone–*n*-hexane as needles, m.p. 143–144 °C (lit.[27] 141–142 °C), ν_{max}/cm^{-1} 2920, 2860, 1738 and 1660; δ_H 0.94 (3H, s, 18-H), 1.24 (3H, s, 19-H), 6.10 (1H, s, 4-H), 6.24 (1H, d, J = 10.0 Hz, 2-H), 7.10 (1H, d, J = 10.0 Hz, 1-H).

Elution with 40% ethyl acetate in *n*-hexane yielded 17β-hydroxyandrost-4-en-3-one **4** (20 mg, 2%), which crystallised from acetone as prisms, m.p. 157–158 °C (lit.[28] 154–155 °C), ν_{max}/cm^{-1} 3200, 1655 and 1615; δ_H 0.80 (3H, s, 18-H), 1.18 (3H, s, 19-H), 3.65 (1H, t, J = 8.5 Hz, 17α-H), 5.72 (1H, s, 4-H).

Further elution with 40% ethyl acetate in *n*-hexane yielded 17β-hydroxyandrosta-1,4-dien-3-one **5** (31 mg, 3%), which crystallised from acetone as needles, m.p. 172–73 °C (lit.[28] 169–170 °C), ν_{max}/cm^{-1} 3520, 1660, 1620 and 1600; δ_H 0.82 (3H, s, 18-H), 1.24 (3H, s, 19-H), 3.64 (1H, t, J = 8.5 Hz 17α-H), 6.08 (1H, s, 4-H), 6.21 (1H, dd, J = 1.8 and 10.0 Hz, 2-H), 7.05 (1H, d, J = 10.0 Hz, 1-H).

Elution with 50% ethyl acetate in *n*-hexane yielded 6β-hydroxyandrost-4-ene-3,17-dione **6** (333 mg, 32%), which crystallised from acetone as prisms, m.p. 193–194 °C (lit.[29] 190–193 °C), ν_{max}/cm^{-1} 3420, 1735 and 1670; δ_H 0.93 (3H, s, 18-H), 1.38 (3H, s, 19-H), 4.36 (1H, bs, 6α-H), 5.82 (1H, bs, 4-H).

Elution with 60% ethyl acetate in *n*-hexane yielded 6β,17β-dihydroxyandrost-4-en-3-one **7** (21 mg, 2%), which crystallised from ethyl acetate as prisms, m.p. 213–214 °C (lit.[29] 215–220 °C), ν_{max}/cm^{-1} 3495, 1660 and 1630; δ_H 0.83 (3H, s, 18-H), 1.37 (3H, s, 19-H), 3.64 (1H, t, J = 8.5 Hz, 17α-H), 4.35 (1H, bs, 6α-H), 5.81 (1H, bs, 4-H).

Further elution with 60% ethyl acetate in *n*-hexane yielded 3β,11α-dihydroxy-5α-androstan-17-one **8** (22 mg, 2%), which crystallised from acetone as plates, m.p. 105–106 °C (lit.[10] 103–106 °C), ν_{max}/cm^{-1}

3473 and 1740; δ_H 0.92 (3H, s, 18-H), 1.01 (3H, s, 19-H), 3.57 (1H, tt, J = 5.0 and 11.0 Hz, 3α-H), 3.96 (1H, dt, J = 5.0 and 10.0 Hz, 11β-H).

Elution with 70% ethyl acetate in *n*-hexane yielded 1α,3β-dihydroxy-5α-androstan-17-one **9** (53 mg, 5%), which crystallised from ethyl acetate as prisms, m.p. 204–205 °C (lit.[12] 200–201 °C), ν_{max}/cm^{-1} 3495, 3430 and 1730; δ_H 0.86 (3H, s, 18-H), 0.83 (3H, s, 19-H), 3.83 (1H, bs, 1β-H), 4.03 (1H, tt, J = 5.0 and 11.0 Hz, 3α-H).

Elution with 80% ethyl acetate in *n*-hexane yielded 3β,7β-dihydroxy-5α-androstan-17-one **10** (32 mg, 3%), which crystallised from acetone as cubes, m.p. 238–239 °C (lit.[14] 240–244 °C), ν_{max}/cm^{-1} 3425 and 1740; δ_H 0.86 (3H, s, 18-H), 0.89 (3H, s, 19-H), 3.45 (1H, m, 7α-H), 3.60 (1H, tt, J = 5.0 and 11.0 Hz, 3α-H).

Further elution with 80% ethyl acetate in *n*-hexane yielded 3β,7α-dihydroxy-5α-androstan-17-one **11** (21 mg, 2%), which crystallised from acetone as prisms, m.p. 197–198 °C (lit.[10] 194 °C), ν_{max}/cm^{-1} 3600, 3420 and 1740; δ_H 0.84 (3H, s, 18-H), 0.87 (3H, s, 19-H), 3.58 (1H, tt, J = 5.0 and 11.0 Hz, 3α-H), 3.95 (1H, bs, 7β-H).

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