Biotransformation of androst-4-ene-3,17-dione by some fungi

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The incubations of androst-4-ene-3,17-dione with *Aspergillus candidus* MRC 200634, *Aspergillus tamarii* MRC 72400, *Aspergillus wentii* MRC 200316 and *Mucor hiemalis* MRC 70325 for 5 days are reported. *A. candidus* MRC 200634 mainly hydroxylated androst-4-ene-3,17-dione at C-11α, C-15α and C-15β whilst *A. wentii* MRC 200316 hydroxylated it mainly at C-6β. *A. tamarii* MRC 72400 showed predominately a Baeyer–Villiger monooxygenase activity. *M. hiemalis* MRC 70325 hydroxylated the substrate at C-14α and reduced most of it at C-17.

Keywords: androst-4-ene-3,17-dione, *Aspergillus*, *Mucor*, biotransformation

Microbial steroid biotransformations have been widely used to prepare more valuable and functionalised compounds such as steroid drugs and hormones due to the high regio- and stereo-selectivities of these reactions. Efforts are still being made to improve the efficiency of fungal steroid biotransformations and to find new useful reactions and microorganisms.[1]

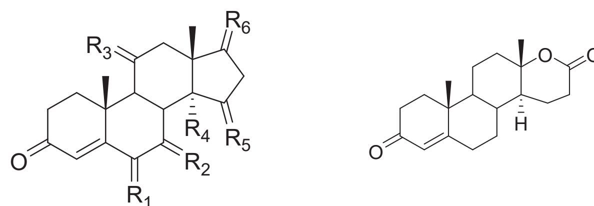
Androst-4-ene-3,17-dione **1**, a 4-en-3-oxo-steroid, is an important metabolite in androgen metabolism and is also used as a starting material for the preparation of pharmaceutically important androgens, anabolic drugs and other compounds, such as spironolactone.[2]

A number of microorganisms have been used for the biotransformation of androst-4-ene-3,17-dione **1**.[1,3] Most metabolites obtained from the microbial biotransformations of androst-4-ene-3,17-dione **1** are either biologically active compounds or important intermediates in the synthesis of steroidal drugs.[4]

In the present work, androst-4-ene-3,17-dione **1** was incubated with *Aspergillus candidus* MRC 200634, *Aspergillus tamarii* MRC 72400, *Aspergillus wentii* MRC 200316 and *Mucor hiemalis* MRC 70325 for 5 days in order to investigate its metabolism.

The structures of the metabolites (Fig. 1), which were known were established by comparing their ^{1}H NMR and ^{13}C NMR spectra (Table 1) with those of the starting material and the literature values.[5–11] All of the compounds retained the resonances assigned to the 4-ene-3-keto moiety of **1**.

Incubation of androst-4-ene-3,17-dione **1** with *A. candidus* MRC 200634 for 5 days afforded six metabolites (Table 2) identified as 17β-hydroxyandrost-4-en-3-one **2**,



1 $R_1 = R_2 = R_3 = R_5 = H_2, R_4 = H, R_6 = O$ **9**
2 $R_1 = R_2 = R_3 = R_5 = H_2, R_4 = H, R_6 = \beta OH, \alpha H$
3 $R_1 = R_2 = R_3 = R_5 = H_2, R_4 = OH, R_6 = O$
4 $R_1 = R_2 = R_5 = H_2, R_3 = \beta H, \alpha OH, R_4 = H, R_6 = O$
5 $R_1 = R_2 = R_3 = H_2, R_4 = H, R_5 = \beta OH, \alpha H, R_6 = \beta OH, \alpha H$
6 $R_1 = R_2 = R_5 = H_2, R_3 = \beta H, \alpha OH, R_4 = H, R_6 = \beta OH, \alpha H$
7 $R_1 = R_2 = R_3 = H_2, R_4 = H, R_5 = \beta H, \alpha OH, R_6 = \beta OH, \alpha H$
8 $R_1 = R_2 = R_5 = H_2, R_3 = \beta OH, \alpha H, R_4 = H, R_6 = O$
10 $R_1 = \beta OH, \alpha H, R_2 = R_3 = R_5 = H_2, R_4 = H, R_6 = O$
11 $R_1 = R_2 = R_3 = R_5 = H_2, R_4 = OH, R_6 = \beta OH, \alpha H$
12 $R_1 = R_3 = R_5 = H_2, R_2 = \beta H, \alpha OH, R_4 = H, R_6 = \beta OH, \alpha H$
13 $R_1 = R_2 = R_3 = R_5 = H_2, R_4 = H, R_6 = \beta COCH_3, \alpha H$

Fig. 1 Chemical structures for compounds **1–13**.

Table 1 ^{13}C NMR data determined in $CDCl_3$ for compounds **1–12**

Carbon atom	1	2	3	4	5	6	7	8	9	10	11	12
1	35.62	35.51	35.62	37.03	35.72	37.32	35.54	34.88	35.25	37.07	35.57	35.88
2	33.84	33.81	33.83	33.84	33.92	34.10	33.77	33.70	33.61	34.13	33.80	33.80
3	199.33	199.85	199.54	200.29	199.68	200.65	200.01	199.71	199.19	200.42	200.03	199.46
4	123.07	123.68	124.07	124.67	123.89	124.30	123.47	122.44	123.81	126.44	123.64	126.50
5	170.35	171.68	170.02	170.56	171.17	171.74	171.84	171.93	169.55	168.40	171.39	168.67
6	32.50	32.72	32.26	33.31	32.65	33.56	32.74	31.78	32.15	72.66	32.48	40.88
7	31.20	31.41	25.50	30.18	31.00	31.05	32.02	31.40	30.17	37.07	28.43	67.63
8	35.07	38.57	37.85	34.48	31.42	35.22	35.18	30.86	37.72	29.31	38.76	39.65
9	53.78	53.76	46.75	59.04	54.22	59.04	53.82	56.55	52.25	53.51	46.62	44.85
10	38.58	36.28	38.59	39.97	38.75	39.96	38.58	39.24	38.27	37.99	38.65	38.45
11	20.24	20.52	19.04	68.58	20.52	68.68	20.41	67.80	21.62	20.18	19.59	20.33
12	30.68	35.56	24.42	42.76	37.82	43.46	36.51	40.85	38.74	31.14	32.48	35.24
13	47.45	42.69	52.48	47.98	42.19	48.26	44.08	46.69	82.67	47.98	46.84	42.66
14	50.76	50.32	80.67	49.94	55.10	49.69	58.08	52.25	45.43	50.77	83.27	45.13
15	21.68	23.22	30.19	21.65	69.06	23.17	72.11	21.61	19.61	21.65	29.56	22.62
16	35.69	30.21	33.02	35.72	43.34	30.30	42.27	35.25	28.32	35.84	26.01	30.07
17	220.43	81.44	218.37	218.81	81.07	80.83	78.41	219.41	171.24	220.90	78.41	81.30
18	13.64	11.00	17.25	14.61	13.70	12.23	12.53	15.79	19.88	13.72	14.87	10.87
19	17.31	17.30	17.81	18.28	17.28	18.30	17.38	20.97	17.19	19.51	17.12	16.95

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Table 2 Metabolite yields following chromatography

Fungus	Metabolite	Yield (%)
A. candidus MRC 200634	17β-Hydroxyandrost-4-en-3-one **2**	7
	14α-Hydroxyandrost-4-ene-3,17-dione **3**	5
	11α-Hydroxyandrost-4-ene-3,17-dione **4**	8
	15β,17β-Dihydroxyandrost-4-en-3-one **5**	10
	11α,17β-Dihydroxyandrost-4-en-3-one **6**	3
	15α,17β-Dihydroxyandrost-4-en-3-one **7**	13
A. tamarii MRC 72400	17β-Hydroxyandrost-4-en-3-one **2**	5
	11β-Hydroxyandrost-4-ene-3,17-dione **8**	4
	17a-Oxa-D-homo-androst-4-ene-3,17-dione **9**	60
A. wentii MRC 200316	14α-Hydroxyandrost-4-ene-3,17-dione **3**	8
	6β-Hydroxyandrost-4-ene-3,17-dione **10**	68
M. hiemalis MRC 70325	14α-Hydroxyandrost-4-ene-3,17-dione **3**	10
	14α,17β-Dihydroxyandrost-4-en-3-one **11**	60
	7α,17β-Dihydroxyandrost-4-en-3-one **12**	6

14α-hydroxyandrost-4-ene-3,17-dione **3**, 11α-hydroxyandrost-4-ene-3,17-dione **4**, 15β,17β-dihydroxyandrost-4-en-3-one **5**, 11α,17β-dihydroxyandrost-4-en-3-one **6** and 15α,17β-dihydroxyandrost-4-en-3-one **7**. In the ^{13}C NMR spectrum of **2**, the resonance attributed to the 17-ketone (δ_C 220.43 ppm) of **1** was replaced by that for a CH(OH) (δ_C 81.44 ppm). The metabolite **3** showed a new carbon atom resonance at δ_C 80.67 ppm in its ^{13}C NMR spectrum whilst it did not show any new resonance in the range 3–5 ppm in its ^{1}H NMR spectrum, indicating the presence of a tertiary hydroxyl group. The downfield shifts for C-8 ($\Delta\delta_C$ 2.78 ppm) and C-15 ($\Delta\delta_C$ 8.51 ppm) and γ-gauche upfield shifts for C-7 ($\Delta\delta_C$ 5.70 ppm) and C-16 ($\Delta\delta_C$ 2.67 ppm) compared to **1** were in accordance with the presence of a 14α-hydroxyl group. The metabolite **4** had characteristic resonances at δ_H 4.08 ppm (1H, dt, J = 5.0 and 10.0 Hz) and δ_C 68.58 ppm, which were consistent with the presence of an 11α-hydroxyl group.[5,6] The ^{13}C NMR spectrum of **4** showed downfield shifts for C-9 ($\Delta\delta_C$5.26 ppm) and C-12 ($\Delta\delta_C$ 12.08 ppm) and a γ-carbon upfield shift for C-8 ($\Delta\delta_C$ 0.59 ppm), which were further consistent with the presence of an 11α-hydroxyl group.[6] The metabolite **5** had new resonances at δ_H 4.20 ppm (1H, ddd, J = 7.8, 5.7 and 2.5 Hz) and δ_C 69.06 ppm, which were in accordance with the presence of a 15β hydroxyl group.[7] Its ^{13}C NMR spectrum, on comparison to **1**, showed downfield shifts for C-14 ($\Delta\delta_C$ 4.34 ppm) and C-16 ($\Delta\delta_C$ 7.65 ppm) whereas it showed a γ-gauche upfield shift for C-8 ($\Delta\delta_C$ 3.65 ppm). These changes were further in accordance with the presence of a 15β hydroxyl group. The ^{13}C NMR spectrum of **5** lacked the C-17 resonance of **1** at δ_C 220.43 ppm and had a new carbon atom resonance at δ_C 81.07 ppm, suggesting the presence of a 17β-hydroxyl group. The metabolite **6** had new resonances at δ_H 3.99 ppm (1H, dt, J = 5.0 and 10.0 Hz) and δ_C 68.68 ppm, whilst the ^{13}C NMR spectrum of **6** showed downfield shifts for C-9 ($\Delta\delta_C$ 5.26 ppm) and C-12 ($\Delta\delta_C$ 12.78 ppm), indicating the presence of an 11α-hydroxyl group. The lack of the C-17 resonance of **1** at δ_C 220.43 ppm and the presence of a new carbon atom resonance at δ_C 80.83 ppm suggested the presence of a 17β-hydroxyl group. The metabolite **7** showed characteristic resonances at δ_H 4.10 ppm (1H, dt, J = 4.0 and 10.0 Hz) and δ_C 72.11 ppm, and demonstrated downfield shifts for C-14 ($\Delta\delta_C$ 7.32 ppm) and C-16 ($\Delta\delta_C$ 6.58 ppm) in its ^{13}C NMR spectrum, indicating the presence a 15α-hydroxyl group.[8] The ^{13}C NMR spectrum of **7** lacked the C-17 resonance of **1** at δ_C 220.43 ppm and had a new carbon atom resonance at δ_C 78.41 ppm, indicating the presence of a 17β-hydroxyl group.

Incubation of androst-4-ene-3,17-dione **1** with *A. tamarii* MRC 72400 for 5 days afforded three metabolites (Table 2) identified as 17β-hydroxyandrost-4-en-3-one **2,** 11β-hydroxyandrost-4-ene-3,17-dione **8** and 17a-oxa-D-homo-androst-4-ene-3,17-dione **9**. The structure of **2** was identified by comparison of its NMR spectra with those of a previously isolated metabolite. The metabolite **8** had characteristic new resonances at δ_H4.46 ppm (1H, m) and δ_C 67.80 ppm, and showed downfield shifts for C-9 ($\Delta\delta_C$ 2.77 ppm) and C-12 ($\Delta\delta_C$ 10.17 ppm) and a γ-carbon upfield shift for C-8 ($\Delta\delta_C$ 4.21 ppm) in its ^{13}C NMR spectrum, which were consistent with the presence of an 11β-hydroxyl group.[9] The metabolite **9** showed a downfield shift ($\Delta\delta_C$ 35.22 ppm) for the C-13 resonance, which was in accordance with the insertion of an oxygen atom adjacent to this position on ring D. This was coupled with downfield shifts ($\Delta\delta_H$ 0.46 ppm and $\Delta\delta_C$ 6.24 ppm) for the 18-methyl resonance. The lack of C-17 resonance of **1** at δ_C 220.43 ppm and the presence of a new resonance at δ_C171.24 ppm suggested the formation of a lactone for ring D.[10]

Incubation of androst-4-ene-3,17-dione **1** with *A. wentii* MRC 200316 for 5 days afforded two metabolites (Table 2) identified as 6β-hydroxyandrost-4-ene-3,17-dione **10** and 14α-hydroxyandrost-4-ene-3,17-dione **3**. The metabolite **10** showed new resonances at δ_H 4.36 ppm (1H, t, J = 3 Hz) and δ_C 72.66 ppm, and demonstrated a downfield shift for C-7 ($\Delta\delta_C$ 5.87 ppm) and a γ-carbon upfield shift for C-8 ($\Delta\delta_C$ 5.76 ppm) in its ^{13}C NMR spectrum, revealing the presence of a 6β-hydroxyl group.[7] The structure of **3** was identified by comparison of its NMR spectra with those of a previously isolated metabolite.

Incubation of androst-4-ene-3,17-dione **1** with *M. hiemalis* MRC 70325 for 5 days afforded three metabolites (Table 2) identified by their NMR spectra as 14α-hydroxyandrost-4-ene-3,17-dione **3**, 14α,17β-dihydroxyandrost-4-en-3-one **11** and 7α,17β-dihydroxyandrost-4-en-3-one **12**. The metabolite **11** showed a new carbon atom resonance at δ_C 83.27 ppm in its ^{13}C NMR spectrum whereas it did not show any new signal in its ^{1}H NMR spectrum. The ^{13}C NMR spectrum of **11** had downfield shifts for C-8 ($\Delta\delta_C$ 3.69 ppm) and C-15 ($\Delta\delta_C$ 7.88 ppm) whereas it had γ-gauche upfield shifts for C-7 ($\Delta\delta_C$ 2.77 ppm) and C-16 ($\Delta\delta_C$ 9.68 ppm), which indicated the presence of a 14α-hydroxyl group. The ^{13}C NMR spectrum of **11** lacked the C-17 resonance of **1** at δ_C 220.43 ppm and had a new methine carbon atom resonance at δ_C 78.41 ppm, consistent with the presence of a 17β-hydroxyl group. The metabolite **12** had NMR resonances at δ_H 3.97 ppm (1H, bs) and δ_C 67.63 ppm, and compared to **1** the ^{13}C NMR spectrum of **12** showed downfield shifts for C-6 ($\Delta\delta_C$ 8.38 ppm) and C-8 ($\Delta\delta_C$ 4.58 ppm) and γ-carbon upfield shifts for C-9 ($\Delta\delta_C$ 8.93 ppm) and C-14 ($\Delta\delta_C$ 5.63 ppm), which were consistent with the presence of a 7α-hydroxyl group. The lack of the C-17 resonance of **1** at δ_C 220.43 ppm and the presence of a new carbon atom resonance at δ_C 81.30 ppm indicated the presence of a 17β-hydroxyl group.

As can be seen from Table 2, *A. candidus* MRC 200634 mainly hydroxylated androst-4-ene-3,17-dione **1** at C-11α, C-15α and C-15β, accompanied by a minor hydroxylation at C-14α. In a previous work, *A. candidus* MRC 200634 had been shown to hydroxylate pregn-4-ene-3,20-dione **13** at C-11α and C-15β, accompanied by a minor hydroxylation at C-14α.[12] These results suggested that *A. candidus* MRC 200634 might have metabolised androst-4-ene-3,17-dione **1** in a different way since the substrate lacked the C-17 side chain of pregn-4-ene-3,20-dione **13**.

A. tamarii MRC 72400 showed a high Baeyer-Villiger mono-oxygenase (BVMO) activity on androst-4-ene-3,17-dione **1,** accompanied by a minor hydroxylation at C-11β and a minor reduction at C-17. In previous work, *A. tamarii* MRC 72400 had also shown a high BVMO activity on 17β-hydroxyandrost-4-en-3-one **2**.[13] Although *A. tamarii* is known for its high BVMO activity on pregn-4-ene-3,20-dione **13**, another *A. tamarii* strain showed low BVMO activities on androst-4-ene-3,17-dione **1** and 17β-hydroxyandrost-4-en-3-one **2**.[14]

A. wentii MRC 200316 mainly hydroxylated androst-4-ene-3,17-dione **1** at C-6β, accompanied by a minor hydroxylation at C-14α. In previous work, 17β-hydroxyandrost-4-en-3-one **2** was hydroxylated by *A. wentii* MRC 200316 in the same way in lower yields.[15] In that work, however, the same fungus only hydroxylated pregn-4-ene-3,20-dione **13** at C-11α in a high yield.

M. hiemalis MRC 70325 hydroxylated androst-4-ene-3,17-dione **1** predominantly at C-14α and reduced most of the substrate at C-17. This was accompanied by a minor hydroxylation at C-7α. In previous work, 17β-hydroxyandrost-4-en-3-one **2** was metabolised by *M. hiemalis* MRC 70325 in a similar way in higher yields.[16] In another study, a different *M. hiemalis* strain hydroxylated pregn-4-ene-3,20-dione **13** predominantly at C-14α. This was accompanied by some minor hydroxylations at various carbon atoms.[17]

In short, *A. candidus* MRC 200634, *A. tamarii* MRC 72400, *A. wentii* MRC 200316 and *M. hiemalis* MRC 70325 metabolised androst-4-ene-3,17-dione **1** in different ways. *A. tamarii* MRC 72400 showed predominantly BVMO activity on androst-4-ene-3,17-dione **1** whereas the rest of the fungi hydroxylated it at different sites.

Experimental

General experimental details

Androst-4-ene-3,17-dione **1** was purchased from Sigma-Aldrich (Istanbul, Turkey). Solvents which were of analytical grade, potato dextrose agar (PDA) and agar for PDA slopes and ingredients for liquid medium were purchased from Merck (Istanbul, Turkey). 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 a *p*-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 Perkin-Elmer Spectrum Two spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in deuteriochloroform with tetramethylsilane as an internal standard reference at 300 MHz and 75 MHz, respectively, with a Varian Mercury 300 spectrometer. Chemical shifts are given in ppm (δ scale), coupling constants (J) are given in Hz. Melting points were determined by an Electrothermal IA 9200 melting point apparatus and are uncorrected.

General fermentation details

A. candidus MRC 200634, *A. tamarii* MRC 72400, *A. wentii* MRC 200316 and *M. hiemalis* MRC 70325 were obtained from TUBITAK, 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 liquid medium for *A. candidus* MRC 200634 was prepared by mixing sucrose (30 g), $NaNO_3$ (3 g), K_2HPO_4 (1 g), KCl (0.5 g), $MgSO_4·7H_2O$ (0.5 g) and $FeSO_4·7H_2O$ (0.01 g) in distilled water (1 L).[18] The liquid medium for *A. tamarii* MRC 72400 was prepared by dissolving malt extract (30 g) in distilled water (1 L).[14] The liquid medium for *A. wentii* MRC 200316 was prepared by mixing sucrose (15 g), glucose (15 g), polypeptone (5 g), $MgSO_4·7H_2O$ (0.5 g), KCl (0.5 g), K_2HPO_4 (1 g) and $FeSO_4·7H_2O$ (0.01 g) in distilled water (1 L) and the pH was adjusted to 7.2.[19] The liquid medium for *M. hiemalis* MRC 70325 was prepared by mixing glucose (20 g), peptone (10 g) and yeast extract (10 g) in distilled water (1 L).[17] Each medium was evenly distributed among 10 culture flasks of 250 ml capacity (100 mL in each) and autoclaved for 20 minutes at 121 °C. Spores freshly obtained from PDA slopes were transferred aseptically into each flask containing sterile medium in a biological safety cabinet. After a three-day cultivation on a rotary shaker at an appropriate temperature and rotation speed for each fungus, androst-4-ene-3,17-dione **1** (1 g) dissolved in DMF (10 mL) was evenly distributed aseptically among the flasks. The biotransformation of the substrate by each fungus was carried out in 10 flasks for 5 days. The fungal mycelium was then 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 which was then chromatographed on silica gel. All biotransformation experiments were performed in duplicate and run with a control flask containing non-inoculated sterile medium and the substrate. After 5 days of incubation, the control was harvested and analysed by TLC. No metabolites were detected in controls.

Incubation of androst-4-ene-3,17-dione **1** (1 g) with *A. candidus* MRC 200634 for 5 days on a rotary shaker (150 rpm) at 28 °C afforded a brown gum (2071 mg), which was then chromatographed on silica gel. Elution with 30% ethyl acetate in *n*-hexane afforded the unreacted starting material (180 mg), which was identified by comparison of its ^{1}H and ^{13}C NMR spectra with those of an authentic sample.

Elution with 40% ethyl acetate in *n*-hexane afforded 17β-hydroxyandrost-4-en-3-one **2** (71 mg, 7%), which was crystallised from acetone as prisms; m.p.158–159 °C (lit.[20] 154–155 °C); IR (ν_{max}/cm^{-1}): 3210, 1660 and 1620; δ_H 0.81 (3H, s, 18-H), 1.18 (3H, s, 19-H), 3.66 (1H, t, J = 8.5 Hz, 17α-H), 5.71 (1H, s, 4-H).

Elution with 50% ethyl acetate in *n*-hexane afforded 14α-hydroxyandrost-4-ene-3,17-dione **3** (53 mg, 5%), which was crystallised from methanol as plates; m.p. 261–262 °C (lit.[21] 255–260 °C); IR (ν_{max}/cm^{-1}) 3460, 1735 and 1660; δ_H 1.03 (3H, s, 18-H), 1.21 (3H, s, 19-H), 5.73 (1H, s, 4-H).

Elution with 70% ethyl acetate in *n*-hexane afforded 11α-hydroxyandrost-4-ene-3,17-dione **4** (85 mg, 8%), which was crystallised from acetone as needles; m.p. 242–243 °C (lit.[22] 240–241 °C); IR (ν_{max}/cm^{-1}) 3430, 1735 and 1665; δ_H 0.94 (3H, s, 18-H), 1.33 (3H, s, 19-H), 4.08 (1H, dt, J = 5.0 and 10.0 Hz, 11β-H), 5.74 (1H, s, 4-H).

Further elution with 70% ethyl acetate in *n*-hexane afforded 15β,17β-dihydroxyandrost-4-en-3-one **5** (106 mg, 10%), which was crystallised from acetone as needles; m.p. 200–201 °C (lit.[7] 201–205 °C); IR (ν_{max}/cm^{-1}) 3435 and 1660; δ_H 1.05 (3H, s, 18-H), 1.23 (3H, s, 19-H), 3.54 (1H, t, J = 8.5 Hz, 17α-H), 4.20 (1H, ddd, J = 7.8, 5.7 and 2.5 Hz, 15α-H), 5.74 (1H, s, 4-H).

Elution with pure ethyl acetate afforded 11α,17β-dihydroxyandrost-4-en-3-one **6** (32 mg, 3%), which was crystallised from acetone as cubes; m.p. 172–173 °C (lit.[7] 168–172°C); IR (ν_{max}/cm^{-1}) 3390, 1660 and 1610; δ_H 0.80 (3H , s, 18-H) 1.33 (3H , s, 19-H), 3.68 (1H, t, J = 8.5 Hz, 17α-H), 3.99 (1H, dt, J = 5.0 and 10.0 Hz, 11β-H), 5.72 (1H, s, 4-H).

Further elution with pure ethyl acetate afforded 15α,17β-dihydroxyandrost-4-en-3-one **7** (138 mg, 13%), which was crystallised from acetone as needles; m.p. 95–96 °C (lit.[8] 93–94 °C); IR (ν_{max}/cm^{-1}) 3395 and 1665; δ_H 0.80 (3H, s, 18-H), 1.20 (3H, s, 19-H), 3.88 (1H, t, J = 8.5 Hz, 17α-H), 4.10 (1H, dt, J = 4.0 and 10.0 Hz, 15β-H), 5.72 (1H, s, 4-H).

Incubation of androst-4-ene-3,17-dione **1** (1 g) with *A. tamarii* MRC 72400 for 5 days on a rotary shaker (180 rpm) at 24 °C afforded a brown gum (2023 mg), which was then chromatographed on silica gel. Elution with 30% ethyl acetate in *n*-hexane afforded the unchanged starting material (105 mg), which was identified by comparison of its ^{1}H and ^{13}C NMR spectra with those of an authentic sample.

Elution with 40% ethyl acetate in *n*-hexane afforded 17β-hydroxyandrost-4-en-3-one **2** (51 mg, 5%), which was identified by comparison of its ^{1}H and ^{13}C NMR spectra with those of a previously isolated metabolite.

Elution with 50% ethyl acetate in *n*-hexane afforded 11β-hydroxyandrost-4-ene-3,17-dione **8** (42 mg, 4%), which was crystallised from methanol as prisms; m.p. 194–195 °C, (lit.[9] 196–197 °C); IR (ν_{max}/cm^{-1}) 3420, 1740 and 1655; δ_H 1.18 (3H, s, 18-H), 1.47 (3H, s, 19-H), 4.46 (1H, m, 11α-H), 5.70 (1H, s, 4-H).

Elution with 60% ethyl acetate in *n*-hexane afforded 17a-oxa-D-homo-androst-4-ene-3,17-dione **9** (634 mg, 60%), which was

crystallised from methanol as needles; m.p. 205–206 °C (lit.[23] 209–210 °C); IR (v_{max}/cm^{-1}) 1720 and 1665; δ_H 1.17 (3H, s, 19-H), 1.36 (3H, s, 18-H), 5.76 (1H, s, 4-H).

Incubation of androst-4-ene-3,17-dione **1** (1 g) with *A. wentii* MRC 200316 for 5 days on a rotary shaker (150 rpm) at 27 °C afforded a brown gum (2299 mg), which was then chromatographed on silica gel.

Elution with 50% ethyl acetate in *n*-hexane afforded 6β-hydroxyandrost-4-ene-3,17-dione **10** (718 mg, 68%), which was crystallised from acetone as prisms; m.p. 195–196 °C; (lit.[21] 190–193 °C); IR (v_{max}/cm^{-1}) 3450, 1730 and1665; δ_H 0.92 (3H, s, 18-H), 1.37 (3H, s, 19-H), 4.36 (1H, t, J = 3 Hz, 6α-H), 5.83 (1H, s, 4-H).

Further elution with 50% ethyl acetate in *n*-hexane afforded 14α-hydroxyandrost-4-ene-3,17-dione **3** (84 mg, 8%), which was identified by comparison of its ^{1}H and ^{13}C NMR spectra with those of a previously isolated metabolite.

Incubation of androst-4-ene-3,17-dione **1** (1 g) with *M. hiemalis* MRC 70325 for 5 days on a rotary shaker (100 rpm) at 26 °C afforded a brown gum (2378 mg), which was then chromatographed on silica gel. Elution with 30% ethyl acetate in *n*-hexane afforded the unconverted starting material (50 mg), which was identified by comparison of its ^{1}H and ^{13}C NMR spectra with those of an authentic sample.

Elution with 50% ethyl acetate in *n*-hexane afforded 14α-hydroxyandrost-4-ene-3,17-dione **3** (106 mg, 10%), which was identified by comparison of its ^{1}H and ^{13}C NMR spectra with those of a previously isolated metabolite.

Elution with 60% ethyl acetate in *n*-hexane afforded 14α,17β-dihydroxyandrost-4-en-3-one **11** (638 mg, 60%), which was crystallised from ethyl acetate as prisms; m.p. 184–185 °C (lit.[21] 181–185 °C); IR (v_{max}/cm^{-1}) 3435, 1660 and 1615; δ_H 0.93 (3H, s, 18-H), 1.22 (3H, s, 19-H), 4.32 (1H, t, J = 8.5 Hz, 17α-H), 5.74 (1H, s, 4-H).

Elution with 70% ethyl acetate in *n*-hexane afforded 7α,17β-dihydroxyandrost-4-en-3-one **12** (64 mg, 6%), which was crystallised from dichloromethane as plates, m.p. 213-214 °C (lit.[7] 205–206 °C); IR (v_{max}/cm^{-1}) 3360, 1660 and 1610; δ_H 0.79 (3H, s, 18-H), 1.20 (3H, s, 19-H), 3.70 (1H, t, J = 8.5 Hz, 17α-H), 3.97 (1H, bs, 7β-H), 5.79 (1H, s, 4-H).

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References

1 M.V. Donova and O.V. Egorova, *Appl. Microbiol. Biotechnol.,* 2012, **94**, 1423.
2 A. Malaviya and J. Gomes, *Appl. Biochem. Biotechnol.,* 2009, **158**, 374.
3 P. Fernandes, A. Cruz, B. Angelova, H.M. Pinheiro and J.M.S. Cabral, *Enzyme Microb. Tech*., 2003, **32**, 688.
4 M. Heidary and Z. Habibi, *J. Mol. Catal. B: Enzym*., 2016, **126**, 32.
5 D.N. Kirk, H.C. Toms, C. Douglas, K.A. White, K.E. Smith, S. Latif and R.W.P. Hubbard, *J. Chem. Soc*., *Perkin Trans. 2*, 1990, 1567.
6 J.W. Blunt and J.B. Stothers, *Org. Magn. Reson*., 1977, **9**, 439.
7 P.C. Peart, W.F. Reynolds and P.B. Reese, *J. Mol. Catal. B: Enzym*., 2013, **95**, 70.
8 P.C. Peart, K.P. McCook, F.A. Russell, W.F. Reynolds and P.B. Reese, *Steroids*, 2011, **76**, 1317.
9 M.T. Yazdi, H. Arabi, M.A. Faramarzi, Y. Gasemi, M. Amini, S. Shokravi and F.A. Mohseni, *Phytochemistry*, 2006, **65**, 2205.
10 J. R. Hanson and A.C. Hunter, *Phytochemistry*, 1998, **49**, 2349.
11 T. Janeczko, J. Dmochowska-Gładysz, E. Kostrzewa-Susłow, A. Białońska and Z. Ciunik, *Steroids*, 2009, **74**, 657.
12 K. Yildirim and A. Kuru, *J. Chem. Res.,* 2015, **39**, 546.
13 K. Yildirim, A. Uzuner and E.Y. Gulcuoglu, *Collect. Czech. Chem. Commun*., 2011, **76**, 743.
14 D.R. Brannon, J. Martin, A.C. Oehlschlager, N.N. Durham and L.H. Zalkow, *J. Org. Chem*., 1965, **30**, 760.
15 K. Yildirim, I. Kupcu and F. Gulsan, *Z. Naturforsch., C: J. Biosci*., 2010, **65**, 688.
16 K. Yildirim, H. Saran, O.F. Dolu and A. Kuru, *J. Chem. Res.,* 2013, **37**, 566.
17 T.N.H. Antoniou, B.P.G. Curran and K.E. Smith, *Microbiol.,* 1994, **140**, 1633.
18 M.L. Mascotti, A.A. Orden, F.B. Bisogno, G. Gonzalo and M. Kurina-Sanz, *J. Mol. Catal. B: Enzym*., 2012, **82**, 32.
19 M. Miyazawa, T. Takahashi, K. Sakata and I. Horibe, *J. Chem. Technol. Biotechnol*., 2008, **83**, 1006.
20 M. Nagasawa, H. Hashiba, N. Watanabe, M. Bae, G. Tamura and K. Arima, *Agr. Biol. Chem*., 1970, **34**, 801.
21 J.R. Hanson, H. Nasir and A. Parvez, *Phytochemistry*, 1996, **42**, 411.
22 M.I. Choudhary, S. Sultan, M.T.H. Khan, A. Yasin, F. Shaheen and A.U. Rahman, *Nat. Prod. Res.,* 2004, **18**, 529.
23 T. Kołek, A. Szpineter and A. Świzdor, *Steroids*, 2008, **73**, 1441.