Microbiological Transformations. Part 4.[1] Microbiological Transformations of 5α-Androstan-17-ones and of 17a-Aza-D-homo-5α-androstan-17-ones with the Fungus *Cunninghamella elegans*

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The microbiological transformation of 5α-androstan-17-one, and the 3β-acetoxy- and 3α-hydroxy-derivatives, by *Cunninghamella elegans* is dominated by 1β,7-dihydroxylation or 7-monohydroxylation. 3α-Acetoxy-5α-androstan-17-one undergoes predominant 6β,11β-dihydroxylation. 17a-Aza-D-homo-5α-androstan-17-one and the 3α-acetoxy-derivative undergo predominant monohydroxylation at 6β or 7α, in contrast to the 3β-acetoxy-derivative which, although undergoing similar monohydroxylation, gives good yields of 9α-monohydroxylated products.

As part of a study of the microbiological transformations of aza-steroidal derivatives, 17a-aza-D-homo-5α-androstan-17-one (1), 3β-acetoxy-17a-aza-D-homo-5α-androstan-17-one (2), and 16-aza-5α-androstan-17-one (19) were selected as substrates for incubation with *Cunninghamella elegans*. For comparison purposes the carbocylic analogues 5α-androstan-17-one (23), 3β-acetoxy-5α-androstan-17-one (27), 3α-hydroxy-5α-androstan-17-one (34), and 3α-acetoxy-5α-androstane (41) were incubated under the same conditions with the same fungus. The results of the incubation of 3α-acetoxy-17a-aza-D-homo-5α-androstan-17-one (3) and 3α-acetoxy-5α-androstan-17-one (37) have been reported.[2] Incubations of all the substrates were carried out for 3 d at 25 °C under conditions previously described and the results are summarised in Tables 1 and 2. Assignments were based largely on the ^{1}H n.m.r. angular methyl group chemical-shift changes [3-5] and the chemical shifts and coupling constants of the C*H*OH protons (Tables 3—5). In certain cases assignments were confirmed by acetylation or by oxidation of the product and the ^{1}H n.m.r. spectra of these derivatives are included in the Tables.

DISCUSSION

5α-Androstan-17-one (23) is 1β,7α-dihydroxylated by *C. elegans* (*cf.* the 1β,6α-dihydroxylation of the same substrate by *Calonectria decora* [6]) and it is tempting to think in terms of the Jones' model, with binding of the C=O group to the enzyme surface and hydroxylation at *ca.* 6.0 and 7.3 Å from this group. This is, in part, supported by the non-conversion of 3α-acetoxy-5α-androstane (41) by *C. elegans*. 3β-Acetoxy-5α-androstan-17-one (27) undergoes predominant 7-mono-oxygenation with accompanying hydrolysis of the 3-acetoxy-group. Dihydroxylation does not now occur to any great extent, possibly because, having achieved the dihydroxy-status, there is no great driving force for the substitution of additional hydroxy-functions. The minor product, however, like the product from 5α-androstan-17-one (23),

TABLE 1

Transformation of 5α-androstane derivatives by *Cunninghamella elegans*

5α-Androstane	Substrate recovered	Main product [a]	Other products [a]
5α-Androstan-17-one (23)	50%	1β,7α-$(OH)_2$ (26) 14%	1β-OH, 7-(C=O) (24) 6%
3α-Acetoxy-5α-androstan-17-one (27)	0%	3β,7β-$(OH)_2$ (30) 31%	3β,7α-$(OH)_2$ (33) 3% 3β-OH, 7-(C=O) (29) 3%
3α-Acetoxy-5α-androstan-17-one (37) [b]	15%	3α-OAc, 6β,11β-$(OH)_2$ (38) 22%	3α-OAc, 6β-OH, 11-(C=O) (39) 1%; 1β,3α-$(OH)_2$ (40) 8%
3α-Hydroxy-5α-androstan-17-one (34)	0%	3α,7β-$(OH)_2$ (35) 43%	3α,7α-$(OH)_2$ (36) 10%

[a] Yields are based on the amount of substrate transformed by the fungus. [b] Results on (37) have been reported previously (ref. 2.)

TABLE 2

Transformation of 17a-aza-D-homoandrostan-17-ones and of 16-aza-5α-androstan-17-one by *Cunninghamella elegans*

Aza-steroid	Substrate recovered	Main product(s) [a]	Other products [a]
17α-Aza-D-homo-5α-androstan-17-one (1)	33%	7α-OH (12) 35%	6β-OH (13) 12%
3β-Acetoxy-17a-aza-D-homo-5α-androstan-17-one (2)	7%	3β-OAc, 9α-OH (7) 24% 3β-OAc, 6β-OH (5) 12%	3β-OAc, 7α-OH (8) 3% 3β,6β-$(OH)_2$ (10) 3β,9α-$(OH)_2$ (9) 3β,11α-$(OH)_2$ (11) } 21% [c]
3α-Acetoxy-17a-aza-D-homo-5α-androstan-17-one (3) [b]	16%	3α-OAc, 6β-OH (15) 36% 3α-OAc, 7α-(OH) (16) 16%	3α,11α-$(OH)_2$ (17) 3α,11β-$(OH)_2$ (18) } 2%
16-aza-5α-androstan-17-one (19)	24%	7α,11α-$(OH)_2$ (20) 19% 1β,7α-$(OH)_2$ (21) 19% 6β,11α-$(OH)_2$ (22) 19%	

[a] % Yields are based on the amount of substrate transformed by the fungus. [b] Results on (3) have been reported previously (ref. 2). [c] Tentative assignment of structures to these three products.

TABLE 3

^{1}H N.m.r. spectra of derivatives of 17a-aza-D-homo-5α-androstan-17-one (1)

Derivatives of (1)	Solvent	Obs. methyl frequencies C-19 (δ)	C-18 (δ)	Obs. methyl shifts (Δδ) [a]		Lit. methyl shifts (Δδ) [b]		C-3 (δ) [c]	C-*n* (δ) [c]
(1)	$CDCl_3$	0.76	1.13	—		—			
	C_5D_5N	0.69	1.07	—		—			
3β-OAc (2)	$CDCl_3$	0.81	1.14	—		—		4.67 (21)	—
	C_5D_5N	0.69	1.07	—		—		4.80 (18)	—
3β-OH (4)	$CDCl_3$	0.78	1.11	—		—		3.60 (21)	
	C_5D_5N	0.74	1.08	—		—			—
3β-OAc, 6β-OH (5)	$CDCl_3$	1.03	1.16	+0.23	+0.02 [d]	+0.23	+0.04	4.70 (20)	3.85 (9)
	C_5D_5N	1.23	1.09	+0.54	+0.03	+0.55	+0.04	4.89 (23)	3.95 (10)
3β-OAc, 6-(C=O) (6)	$CDCl_3$	0.77	1.16	−0.03	+0.02 [d]	−0.05	+0.02	4.65 (19)	
3β-OAc, 9α-OH (7)	$CDCl_3$	0.93	1.13	+0.13	−0.01 [d]	+0.13	+0.03	4.68 (21)	—
	C_5D_5N	0.91	1.15	+0.22	+0.09	+0.23	+0.08	4.90 (24)	—
3β-OAc, 7α-OH (8)	$CDCl_3$	0.81	1.13	+0.01	−0.01 [d]	0.00	+0.01	4.68 (21)	4.03 (8)
3β-OH, 9α-OH (9)	$CDCl_3$	0.93	1.16	+0.15	+0.05 [e]	+0.13	+0.03	3.60 (23)	—
3β-OH, 6β-OH (10)	$CDCl_3$	1.03	1.15	+0.25	+0.04 [e]	+0.23	+0.04	3.60 (23)	3.86 (10)
3β-OH, 11α-OH (11)	$CDCl_3$	0.98	1.18	+0.20	+0.07 [e]	+0.12	+0.03	3.60 (23)	3.66 (21)
7α-OH (12)	$CDCl_3$	0.73	1.11	−0.03	−0.02 [f]	0.00	+0.01		4.03 (8)
	C_5D_5N	0.77	1.15	+0.08	+0.08	+0.09	+0.06		4.25 (9)
6β-OH (13)	$CDCl_3$	0.98	1.14	+0.22	+0.01 [f]	+0.23	+0.04		3.83 (8)
	C_5D_5N	1.23	1.10	+0.54	+0.03	+0.55	+0.04		4.02 (8)
6β-OAc (14)	$CDCl_3$	0.96	1.21	+0.20	+0.08 [f]	+0.16	+0.06		5.12 (8)

[a] A positive value represents a downfield shift. [b] Refs. 3, 4, and 5. [c] δ Value is followed in parenthesis by width at half-height ($W_{\frac{1}{2}}$, Hz). [d] Methyl shift relative to 3β-OAc. [e] Methyl shift relative to 3β-OH. [f] Methyl shift relative to (1).

TABLE 4

^{1}H N.m.r. spectra of derivatives of 16-aza-5α-androstan-17-one (19)

Derivatives of (19)	Solvent	Obs. methyl frequencies (δ) C-19	C-18	Obs. methyl shifts (Δδ) [a] C-19	C-18	Lit. methyl shifts (Δδ) [b] C-19	C-18	C-*n* (δ) [c]	
(19)	$CDCl_3$	0.81	1.00	—	—	—	—	—	
	C_5D_5N	0.73	1.01	—	—	—	—	—	
								C-7	C-11
7α,11α-$(OH)_2$ (20)	$CDCl_3$	0.91	1.00	+0.11	0.00 [d]	+0.12	+0.04	3.78 (7.5)	2.75 (*J* 11.2, 11.2, 6.0)
								C-7	C-1
1β,7α-$(OH)_2$ (21)	$CDCl_3$	0.86	1.01	+0.05	+0.01 [d]	+0.05	+0.01	3.78 (7.5)	3.48 (*J* 10.8, 5)
	C_5D_5N	1.13	1.17	+0.40	+0.16 [d]	+0.36	+0.08	3.97 (8)	3.65 (15)
								C-6	C-11
6β,11α-$(OH)_2$ (22)	$CDCl_3$	1.07	1.00	+0.26	+0.00 [d]	+0.35	+0.07	3.95 (8.8)	2.49 (*J* 11.0, 11.0, 6.1),

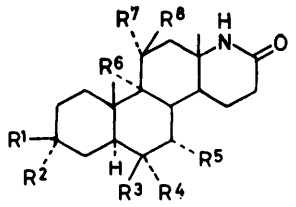
[a] A positive value represents a downfield shift. [b] Lit. Δδ values from refs. 3, 4, and 5. [c] δ Value is followed in parenthesis by width at half-height ($W_{\frac{1}{2}}$, Hz). [d] Shifts relative to (4).

TABLE 5

^{1}H N.m.r. spectra of derivatives of 5α-androstan-17-one (23)

Derivatives of (23)	Solvent	Obs. methyl frequencies (δ) C-19	C-18	Obs. methyl shifts (Δδ) [a]		Lit. methyl shifts (Δδ) [b]		C-3 (δ) [c]	C-*n* (δ) [c]	
(23)	$CDCl_3$	0.80	0.85	—	—	—	—	—	—	
	C_5D_5N	0.73	0.76							
1β-OH, 7-(C=O)	$CDCl_3$	1.12	0.86	+0.32	+0.01 [d]	+0.33	+0.01		3.40 (16)	
(24)	C_5D_5N	1.19	0.80	+0.46	+0.04	+0.51	+0.05		3.50 (14)	
1,7-$(C=O)_2$ (25)	$CDCl_3$	1.43	0.87	+0.63	+0.02 [d]	+0.66	+0.03			
									C-1	C-7
1β,7α-$(OH)_2$ (26)	$CDCl_3$	0.85	0.85	+0.05	0.00 [d]	+0.05	+0.01		3.47 (15)	3.97 (6)
	C_5D_5N	1.09	0.88	+0.29	+0.03 [d]	+0.36	+0.08		3.66 (13)	4.07 (7)
3β-OAc (27)	$CDCl_3$	0.85	0.85					4.70 (21)		
	C_5D_5N	0.73	0.76					4.82 (19)		
3β-OH (28)	$CDCl_3$	0.82	0.84					3.55 (21)		
	C_5D_5N	0.78	0.78					3.73 (22)		
3β-OH, 7-(C=O) (29)	$CDCl_3$	1.11	0.86	+0.29	+0.02 [e]	+0.28	+0.01	3.70 (20)		
	C_5D_5N	1.03	0.79	+0.25	+0.01	+0.24	+0.03	3.78 (20)		
3β,7β-$(OH)_2$ (30)	$CDCl_3$	0.84	0.87	+0.02	+0.03 [e]	+0.03	+0.03	3.58 (22)	3.58	
	C_5D_5N	0.84	0.84	—	—	—	—	3.70 (24)	3.70	
3,7-$(C=O)_2$ (31)	$CDCl_3$	1.29	0.88	+0.49	+0.03 [d]	+0.52	+0.05			
	C_5D_5N	1.19	0.76	+0.44	0.00 [d]	+0.37	+0.01			
3β,7β-$(OAc)_2$ (32)	$CDCl_3$	0.90	0.86	+0.05	+0.01 [f]	+0.02	+0.02	4.63 (20.5)		
3β,7α-$(OH)_2$ (33)	$CDCl_3$	0.85	0.85	−0.03	−0.01 [e]	0.00	+0.01	3.53 (21)	4.00 (8)	
3α-OH (34)	$CDCl_3$	0.79	0.85	—	—	—	—	4.07 (7)		
	C_5D_5N	0.80	0.80	—	—	—	—	4.22 (8)		
3α,7β-$(OH)_2$ (35)	$CDCl_3$	0.83	0.88	−0.02	+0.03 [g]	+0.03	+0.03	4.07 (8)	3.48 (20)	
3α,7α-$(OH)_2$ (36)	$CDCl_3$	0.78	0.84	−0.01	−0.01 [g]	0.00	+0.01	4.02	3.93	

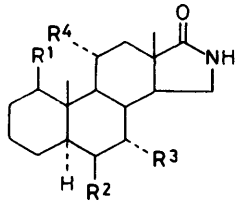
[a] A positive value represents a downfield shift. [b] Lit. Δδ values from refs. 3, 4, and 5. δ Value is followed in parenthesis by width at half height ($W_{\frac{1}{2}}$, Hz). [d] Methyl shifts relative to (23). [e] Methyl shifts relative to 3β-OH. [f] Methyl shifts relative to 3β-OAc [g] Methyl shifts relative to 3α-OH.



(1)

R^n = H unless stated otherwise

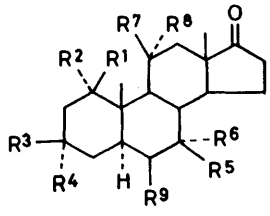
(2) R^1 = OAc
(3) R^2 = OAc
(4) R^1 = OH
(5) R^1 = OAc, R^3 = OH
(6) R^1 = OAc, R^3R^4 = O
(7) R^1 = OAc, R^6 = OH
(8) R^1 = OAc, R^5 = OH
(9) R^1 = R^6 = OH
(10) R^1 = R^3 = OH
(11) R^1 = R^7 = OH
(12) R^5 = OH
(13) R^3 = OH
(14) R^3 = OAc
(15) R^2 = OAc, R^3 = OH
(16) R^2 = OAc, R^5 = OH
(17) R^2 = R^7 = OH
(18) R^2 = R^8 = OH



(19)

R^n = H unless stated otherwise

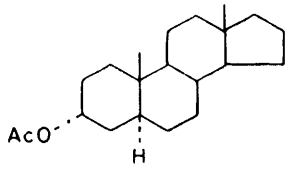
(20) R^3 = R^4 = OH
(21) R^1 = R^3 = OH
(22) R^2 = R^4 = OH



(23)

R^n = H unless stated otherwise

(24) R^1 = OH, R^5R^6 = O
(25) R^1R^2 = R^5R^6 = O
(26) R^1 = R^6 = OH
(27) R^3 = OAc
(28) R^3 = OH
(29) R^3 = OH, R^5R^6 = O
(30) R^3 = R^5 = OH
(31) R^3R^4 = R^5R^6 = O
(32) R^3 = R^5 = OAc
(33) R^3 = R^6 = OH
(34) R^4 = OH
(35) R^4 = R^5 = OH
(36) R^4 = R^6 = OH
(37) R^4 = OAc
(38) R^9 = R^7 = OH
(39) R^9 = OH, R^7R^8 = O
(40) R^1 = R^4 = OH



(41)

preserves the 1β,7α-dihydroxy-pattern. Whereas 7β-monohydroxylation also dominates the transformation of 3α-hydroxy-5α-androstan-17-one (34) the presence of a 3α-acetoxy-group in 3α-acetoxy-5α-androstan-17-one (37) causes a drastic change in the substitution pattern. The major product is now that resulting from 6β,11β-dihydroxylation and, unlike the reaction involving the 3β-acetoxy-5α-androstan-17-one (27), no hydrolysis of the acetoxy-function occurs. Possibly, the presence of the axial 3α-acetoxy-function favours approach of the molecule to the enzyme surface in the ' capsized ' mode, when hydroxylation occurs at the axial 11β- and 6β-positions *syn* to the angular methyl group rather than at the equatorial 7β- or axial 7α-position, as in the other androstanes.

Unlike 5α-androstan-17-one (23), which undergoes 1β,7α-dihydroxylation, 17a-aza-D-homo-5α-androstan-17-one (1) undergoes monohydroxylation at the 6β- and 7α-positions. This also occurs with 3α-acetoxy-17a-aza-D-homo-5α-androstan-17-one (3), but there is also some 11α- and 11β-monohydroxylation with accompanying hydrolysis of the 3α-acetoxy-function. If the dihydroxylation of 3α-acetoxy-5α-androstan-17-one (37) is sequential, with 11β-hydroxylation occurring after 6β-hydroxylation, then the transformations of this compound and of its 17a-aza-D-homo-derivative (3) may not be too different, with hydroxylation of the aza-steroid stopping at the monohydroxylation stage. The predominant β-attack of 3α-acetoxy-17a-aza-D-homo-5α-androstan-17-one (3) may, as in the case of 3α-acetoxy-5α-androstan-17-one (37), be a consequence of the presence of the axial 3α-acetoxy-group. 9α-Hydroxylation predominates the transformation of 3β-acetoxy-17a-aza-D-homo-5α-androstan-17-one (2), but the remaining products are similar to those from 3α-acetoxy-17a-aza-D-homo-5α-androstan-17-one (3).

EXPERIMENTAL

General experimental details and incubation procedure are as given previously.[2]

Incubation of 17*a-Aza*-D-*homo*-5α-*androstan*-17-*one* (1) *with* Cunninghamella elegans.—17a-Aza-D-homo-5α-androstan-17-one [7] (2.5 g), dissolved in ethanol (250 ml), was incubated for 3 d at 25 °C with *Cunninghamella elegans* grown in the nutrient medium (63 flasks). Extraction gave the mycelial and broth extracts (3.9 g and 1.5 g, respectively) which were combined and chromatographed over neutral alumina (Woelm, activity IV, 600 g). Elution with ether gave the starting material (831 mg). Elution with ether–methanol (2—5%) gave 6β-*hydroxy*-17*a-aza*-D-*homo*-5α-*androstan*-17-*one* (13) (216 mg), m.p. (acetone) 259—261 °C (Found: C, 74.5; H, 10.4; N, 4.6. $C_{19}H_{31}NO_2$ requires C, 74.7; H, 10.2; N, 4.6%); $\nu_{max.}$ ($CHCl_3$) 3 652, 3 601, 3 382, and 1 645 cm^{-1}; *m/e* 305 (M^+), 290 (M^+ — Me), and 272 (M^+ — Me — H_2O) and 7α-*hydroxy*-17*a-aza*-D-*homo*-5α-*androstan*-17-*one* (12) (615 mg), m.p. (acetone) 251—253 °C (Found: C, 74.8; H, 10.2; N, 4.5. $C_{19}H_{31}NO_2$ requires C, 74.7; H, 10.2; N, 4.6%); $\nu_{max.}$ ($CHCl_3$) 3 655, 3 662, 3 382, and 1 641 cm^{-1}; *m/e* 305 (M^+), 290 (M^+ — CH_3), and 272 (M^+ — Me — H_2O).

3β-*Acetoxy*-17*a-aza*-D-*homo*-5α-*androstan*-17-*one* (2).—This compound was prepared from 3β-acetoxy-5α-androstan-17-one [8] by published procedures.[9,10]

3β-*Hydroxy*-17*a-aza*-D-*homo*-5α-*androstan*-17-*one* (4).—A solution of 3β-acetoxy-17a-aza-D-homo-5α-androstan-17-one

(2.0 g) in methanol (30 ml) was boiled under reflux with potassium hydroxide (0.45 g) for 1 h. The solution was cooled and acidified with glacial acetic acid, and the resultant solution was concentrated and the crude product recrystallised from methanol to give 3β-*hydroxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (4) as white plates (1.2 g, 68.5%), m.p. 299—301 °C (Found: C, 74.9; H, 10.0; N, 4.5. $C_{19}H_{31}NO_2$ requires C, 74.8; H, 10.2; N, 4.6%).

Incubation of 3β-*Acetoxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (2) *with* Cunninghamella elegans.—3β-Acetoxy-17a-aza-D-homo-5α-androstan-17-one (4.00 g), dissolved in ethanol (500 ml), was incubated for 3 d at 25 °C with *Cunninghamella elegans* grown in the nutrient medium (100 flasks). Extraction gave the mycelial and broth extracts (3.87 g and 1.52 g, respectively). The combined broth and mycelial extracts, dissolved in chloroform, were chromatographed over neutral alumina (Woelm, activity IV, 600 g). The first fraction eluted with ether gave starting material (284 mg). Elution with ether–methanol (5%) gave fractions identified as 3β-hydroxy-17a-aza-D-homo-5α-androstan-17-one (4) (130 mg), m.p. (acetone–hexane) 300 °C; *m/e* 305 (M^+), 290 (M^+ — Me), and 272 (M^+ — Me — H_2O); 3β-*acetoxy*-6β-*hydroxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (5) (460 mg), m.p. (acetone) 266—268 °C (Found: C, 69.6; H, 9.0; N, 3.95. $C_{21}H_{33}NO_4$ requires C, 69.4; H, 9.15; N, 3.85%); $\nu_{max.}$ 1 727, 1 642, 3 601, and 3 658 cm^{-1}; *m/e* 363 (M^+), 348 (M^+ — Me), 288 (M^+ — Me — $MeCO_2H$), and 270 (M^+ — Me — $MeCO_2H$ — H_2O); and 3β-*acetoxy*-9α-*hydroxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (7) (929 mg), m.p. (acetone) 280—282 °C (Found: C, 69.3; H, 9.3; N, 3.75. $C_{21}H_{33}NO_4$ requires C, 69.4; H, 9.15; N, 3.85%); $\nu_{max.}$ 1 728 and 1 640 cm^{-1}; *m/e* 363 (M^+), 330 (M^+ — Me — H_2O).

Later fractions of 3β-acetoxy-9α-hydroxy-17a-aza-D-homo-5α-androstan-17-one (7) were mixed with 3β-acetoxy-7α-hydroxy-17a-aza-D-homo-5α-androstan-17-one (8), $\nu_{max.}$ 1 728 and 1 640 cm^{-1}.

Ether–methanol (9 : 1) eluted a mixture of three monohydroxylated derivatives of 3β-hydroxy-17a-aza-D-homo-5α-androstan-17-one (4) present in an approximately 1 : 1 : 1 ratio. On the basis of the ^1H n.m.r. spectrum ($CDCl_3$) (Table 4) of the mixture, the structures of the products were tentatively assigned as 3β,6β-*dihydroxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (10), 3β,9α-*dihydroxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (9), and 3β,11α-*dihydroxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (11).

Incubation of 16-*Aza*-5α-*androstan*-17-*one* (19) *with* Cunninghamella elegans.—16-Aza-5α-androstan-17-one [11] (1.8 g) dissolved in ethanol (100 ml) was incubated with *Cunninghamella elegans* grown in the nutrient medium (44 flasks) for 3 d at 25 °C. Extraction gave the mycelial and broth extracts (6.4 g and 1.9 g, respectively) which were combined and chromatographed over neutral alumina (Woelm, activity IV, 200 g).

The three component mixture was purified by preparative t.l.c. [5 20 × 20 cm plates, 2 × $CHCl_3$–Me_2CO–MeOH (25 : 4 : 1)]. The band of highest R_F yielded 7α,11α-dihydroxy-16-aza-5α-androstan-17-one (20) as an oil, *m/e* 307 (M^+) when extracted with acetone–methanol (1 : 1). The middle band yielded 1β,7α-dihydroxy-16-aza-5α-androstan-17-one (21) as an oil, *m/e* 307 (M^+), while the band of lowest R_F gave a mixture of 6β,11α- (22) and 7α,11α-dihydroxy-16-aza-5α-androstan-17-one (20) as an oil, *m/e* 307 (M^+).

Incubation of 5α-*Androstan*-17-*one* (23) *with* Cunninghamella elegans.—5α-Androstan-17-one (4.0 g), dissolved in ethanol (500 ml), was incubated at 25 °C for 3 d with *Cunninghamella elegans* grown in the nutrient medium (100 flasks). Extraction gave the mycelial and broth extracts (4.7 g and 2.5 g, respectively) which were combined and chromatographed over neutral alumina (Woelm, activity III, 600 g) to give, on elution with ether, 1β-hydroxy-5α-androstane-7,17-dione (24) (121 mg), m.p. (ethyl acetate) 177—179 °C (lit.,[12] m.p. 180—182 °C) and 1β,7α-*dihydroxy*-5α-*androstan*-17-*one* (26) (318 mg), m.p. (acetone) 217—219 °C (Found: C, 74.5; H, 10.05. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%); $\nu_{max.}$ ($CHCl_3$) 3 599, 3 436, and 1 740 cm^{-1}; *m/e* 306 (M^+) 288 (M^+ — H_2O), and 270 (M^+ — $2H_2O$).

Oxidation of 1β-hydroxy-5α-androstane-7,17-dione (24) (52 mg) by Jones reagent gave 5α-androstane-1,7,17-trione (25) (35.6 mg) as a white, crystalline solid from acetone–hexane, m.p. 238—240 °C (lit.,[12] m.p. 235—237 °C).

Incubation of 3β-*Acetoxy*-5α-*androstan*-17-*one* (27) *with* Cunninghamella elegans.—3β-Acetoxy-5α-androstan-17-one (4.40 g), dissolved in ethanol (400 ml), was incubated with *Cunninghamella elegans* (110 flasks) for 3 d at 25 °C. Extraction gave the mycelial and broth extracts (5.0 g and 4.4 g, respectively), which were combined and chromatographed over neutral alumina (Woelm, activity III, 600 g).

Elution with ether gave 3β-hydroxy-5α-androstan-7,17-dione (29) (112 mg), m.p. (acetone–hexane) 198—200 °C (lit.,[13] m.p. 202—204 °C); $\nu_{max.}$ 1 738, 1 709, 3 602, and 3 604; *m/e* 304 (M^+), 286 (M^+ — H_2O), and 271 (M^+ — H_2O — Me); 3β,7β-dihydroxy-5α-androstan-17-one (30) (901 mg), m.p. (acetone) 237—238 °C (lit.,[14] m.p. 241—243 °C); $\nu_{max.}$ ($CHCl_3$) 1 733, 3 599, and 3 410 cm^{-1}; *m/e* 306 (M^+), 291 (M^+ — Me), 288 (M^+ — H_2O), and 273 (M^+ — H_2O — Me); and 3β,7α-dihydroxy-5α-androstan-17-one (33) (110 mg), m.p. (acetone) 196—198 °C (lit.,[13] m.p. 194—195 °C); $\nu_{max.}$ ($CHCl_3$) 1 738, 3 602, and 3 415 cm^{-1}; *m/e* 306 (M^+), 288 (M^+ — H_2O), and 270 (M^+ — $2H_2O$).

Oxidation of 3β,7β-dihydroxy-5α-androstan-17-one (30) (90 mg) by Jones reagent gave 5α-androstane-3,7,17-trione (25) (52 mg) as a white, crystalline solid from acetone–hexane, m.p. 235—237 °C (lit.,[14] m.p. 239—241 °C).

Acetylation of 3β,7β-dihydroxy-5α-androstan-17-one (30) (60 mg) gave the crude diacetate as a viscous oil which was recrystallised from methanol to give 3β,7β-diacetoxy-5α-androstan-17-one (32) (34 mg) as a white solid, m.p. 144—146 °C (lit.,[14] 142—145 °C).

Incubation of 3α-*Hydroxy*-5α-*androstan*-17-*one* (34) *with* Cunninghamella elegans.—3α-Hydroxy-5α-androstan-17-one (4.8 g), dissolved in ethanol (500 ml), was incubated for 3 d at 25 °C with *Cunninghamella elegans* grown in the nutrient medium (120 flasks). Extraction gave the broth and mycelial extracts (6.5 g and 2.1 g, respectively). The broth extract was recrystallised from acetone to give 3α,7β-dihydroxy-5α-androstan-17-one (35) (2.07 g) as white prisms, m.p. 190—192 °C (lit.,[13] m.p. 201—202 °C). The mother liquor from the recrystallisation was concentrated and combined with the mycelial extract and chromatographed over neutral alumina (Woelm, activity III, 200 g) to give, on elution with ether, 3α,7α-dihydroxy-5α-androstan-17-one (36) (484 mg), m.p. (acetone–hexane) 159.5—161.5 °C (Found: C, 74.7; H, 9.7. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.8%).

Oxidation of 3α,7β-dihydroxy-5α-androstan-17-one (35) (152 mg) by Jones reagent gave 5α-androstan-3,7,17-trione (25) (108 mg) recrystallised from acetone–hexane, m.p. 235—236 °C (lit.,[14] m.p. 239—241 °C).

[0/1008 *Received*, 30*th June*, 1980]

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