Microbiological Transformations. Part 5.[1] Microbiological Transformations of 17a-Aza- and 17-Aza-D-homoandrost-5-ene Derivatives with the Fungus *Cunninghamella elegans*

By **Trevor A. Crabb,* Philip J. Dawson,** and **Roger O. Williams,** Department of Chemistry, Portsmouth Polytechnic, Portsmouth, Hampshire PO1 2DT

The products obtained from the incubation of 17a-aza- and 17-aza-D-homoandrost-5-ene derivatives with *Cunninghamella elegans* are largely those resulting from allylic oxidation or epoxidation of the Δ^5-bond. The substrates are predominantly monohydroxylated whereas androst-5-ene derivatives under the same conditions show a tendency towards dihydroxylation.

In a previous paper [2] in this series the transformation of Δ^4- and Δ^5-androstene derivatives by *Cunninghamella elegans* has been described. The work contained in the present paper is concerned with the microbiological transformation of 3β-acetoxy- and 3β-hydroxy-17a-aza-D-homoandrost-5-en-17-one (2) and (3),[3,4] *N*-acetyl-17a-aza-D-homoandrost-5-en-3β-yl acetate (9),[3] *N*-acetyl-17-aza-D-homoandrost-5-en-3β-yl acetate (22),[3] and 17a-aza-D-homoandrost-4-ene-3,17-dione (27) [3] to assess the influence of an amide function in ring D on the course of microbiological transformation by the same fungus.

The results of the incubations carried out under comparable conditions to those employed for the androstene derivatives [2] are summarised in Table 1 and structural assignments were made largely on the ^{1}H n.m.r. data summarised in Table 2.

The configuration of the epoxide (7) was confirmed by synthesis of the α- and β-epoxides of (3). Reaction between 3β-hydroxy-17a-aza-D-homoandrost-5-en-17-one (3) and *m*-chloroperbenzoic acid gave a mixture of two compounds in the ratio of *ca.* 5 : 1 (by n.m.r.). Crystallisation gave the major product (31). N.m.r. analysis of the residue (from the mother-liquors) showed it to contain mainly the minor product which was identical with the product (7) of incubation of (2). The chemical shifts of the two epoxides, together with the coupling constants of the C-6 protons, are given in Table 2. The major product of the reaction was assumed to be the 5α,6α-epoxide [5-8] and since the incubation product was identical with the minor product, it was considered to be the 5β,6β-epoxide (7). This was confirmed by comparison of the n.m.r. chemical shifts and coupling constants of the epoxides (7) and (31) (Table 2) with reported values for steroidal epoxides.[9-11]

The assignments of the allylic alcohols (5) and (6) were confirmed by comparison of the $J_{6,7\xi}$ values with those in 3β,7β-hydroxy- and 3β,7α-hydroxycholestenes ($J_{6,7\xi}$ 1.5 in 3β,7β-diol; $J_{6,7\xi}$ 5.5 in 3β,7α-diol).[11]

The structure of the 3,6,17-trione (29) was confirmed by sodium borohydride reduction to (32) and that of (30) by epoxidation of 17a-aza-D-homoandrost-4-ene-3,17-dione (27) (Scheme), to give a mixture of 4ξ,5-epoxy-17a-aza-D-homo-5ξ-androstane-3,17-diones (33), (34); these were then separated, purified, and reduced to give easily identifiable mixtures. The ^{1}H n.m.r. data of these compounds (30), (35), (36), and (37) are summarised in Table 3. Comparison of the n.m.r. data with those (Table 2) for (30) confirms the assigned stereochemistry.

TABLE 1

Transformation of 17a-aza-D-homoandrostenes by *Cunninghamella elegans*

Aza-steroid	Products
3β-Acetoxy-17a-aza-D-homoandrost-5-en-17-one (2)	3β-OH (3) 0.2% 3β-OH, 7-(C=O) (4) 1.7% 3β,7β-$(OH)_2$ (5) 6.7% 3β,7α-$(OH)_2$ (6) 6.7% 3β-OH, 5,6β-epoxy (7) 4.3%
3β-Hydroxy-17a-aza-D-homoandrost-5-en-17-one (3)	3β-OH, 7-(C=O) (4) 1.6% 3β,7β-$(OH)_2$ (5) 5% 3β,7α-$(OH)_2$ (6) 5% 3β,OH, 5,6β-epoxy (7) 2.4%
17α-Aza-D-homoandrost-5-en-3β-yl acetate (9)	3β,7β-$(OH)_2$ (11) 0.5% 3β,7α-$(OH)_2$ (12) 8.8% 1β,3β-$(OH)_2$ (13) 4.5% 1β,3β,7β-$(OH)_3$ (14) 0.6% 1β,3β-$(OH)_2$, 5,6β-epoxy (19) 1.5%
N-Acetyl-17-aza-D-homoandrost-5-en-3β-yl acetate (22)	3β-OH, 7-(C=O) (24) 4.3% 3β,7β-$(OH)_2$ (25) 10.3% 3β,7α-$(OH)_2$ (26) 26.2%
17a-Aza-D-homoandrost-4-ene-3,17-dione (27)	11α-OH (28) 0.5% 3,6-$(C=O)_2$ (29) 6.8% 3α-OH, 4α,5α-epoxy (30) 0.5%

TABLE 2

^{1}H N.m.r. spectra ($CDCl_3$) of 17a-aza- and 17-aza-D-homoandrostanes

Derivatives of (1)	Chemical shifts (δ) 3α-H	6-H	18-H	19-H	C_xHOH	Δδ Values [a] 18-H	19-H	Coupling constants (J/Hz) and half-widths ($W_{\frac{1}{2}}$/Hz) 6-H	C_xHOH
3β-OAc (2)	4.60	5.40	1.17	1.00				$W_{\frac{1}{2}} = 10$	
3β-OH (3)	3.54	5.36	1.17	1.00				$W_{\frac{1}{2}} = 10$	
3β-OH, 7-(C=O) (4)	3.68	5.73	1.19	1.22		0.02	0.22 [b]	$J_{4,6\beta} = -1.1$	
3β,7β-$(OH)_2$ (5)	3.57	5.29	1.19	1.04	3.94	0.02	0.04 [b]	$J_{6,7\alpha} = 2.5$ $J_{4\beta,6} = -2.5$	$J_{7\alpha,8\beta} = 6.8$
3β,7α-$(OH)_2$ (6)	3.59	5.63	1.17	0.98	4.04	0.00	−0.02 [b]	$J_{6,7\beta} = 5.0$ $J_{4\beta,6} = -1.7$	$J_{6,7\beta} = 5.0$
3β-OH, 5,6β-epoxy (7)	3.71	3.14	1.13	1.00		−0.04	0.00 [b]	$J_{6\alpha,7\beta} = 3.0$	
3β-OH, 5,6α-epoxy (31)	3.92	2.95	1.11	1.05		−0.06	0.05 [b]	$J_{6\beta,7\beta} = 4.5$	
Derivatives of (8)									
3β-OAc (9)	4.61	5.37	1.43	0.99				$W_{\frac{1}{2}} = 10$	
3β-OH (10)	3.52	5.35	1.44	0.97				$W_{\frac{1}{2}} = 10$	
3β,7β-$(OH)_2$ (11)	3.55	5.27	1.46	1.02	3.89	0.02	0.05 [c]	$W_{\frac{1}{2}} = 7$	$W_{\frac{1}{2}} = 13$
3β,7α$(OH)_2$ (12)	3.57	5.61	1.45	0.97	3.98	0.01	0.00 [c]	$J_{6,7\beta} = 5.1$ $J_{4\beta,6} = -1.5$	$W_{\frac{1}{2}} = 12$ $J_{1\alpha,2\beta} = 11.0$
1β,3β-$(OH)_2$ (13)	3.50	5.54	1.44	1.01	3.60	0.00	0.04 [c]	$J_{6,7\beta} = 5.5$	$J_{1\alpha,2\alpha} = 5.5$
1β,3β,7β-$(OH)_3$ (14)	3.76	5.41	1.46	1.04	3.59	0.02	0.07 [c]	$J_{6,7\alpha} = 2.0$ $J_{4\beta,6} = -2.0$	$J_{1\alpha,2\beta} = 8.5$ $J_{1\alpha,2\alpha} = 4.4$
					3.88				$W_{\frac{1}{2}} = 13$
1β,3β-$(OH)_2$, 5,6β-epoxy (19)	3.68	3.11	1.39	1.05	3.54	−0.05	0.08 [c]	$J_{6\alpha,7\beta} = 2.8$	$J_{1\alpha,2\beta} = 12.0$ $J_{1\alpha,2\alpha} = 4.2$
3β,7β-$(OAc)_2$ (15)	4.60	5.23	1.46	1.05	5.09	0.03	0.06 [d]	$W_{\frac{1}{2}} = 6$	$W_{\frac{1}{2}} = 12$
3β,7α-$(OAc)_2$ (16)	4.67	5.59	1.42	0.98	5.09	−0.01	−0.01 [d]	$J_{6,7\beta} = 4.4$	$W_{\frac{1}{2}} = 11$
1β,3β-$(OAc)_2$ (17)	4.65	5.61	1.43	1.11	4.66	0.00	0.12 [d]	$J_{6,7\beta} = 5.0$	$J_{1\alpha,2\beta} = 11.4$ $J_{1\alpha,2\alpha} = 4.2$
1β,3β,7β-$(OAc)_3$ (18)	4.67	5.39	1.46	1.16	4.65	0.03	0.17 [d]	$W_{\frac{1}{2}} = 6$	$J_{1\alpha,2\beta} = 11.1$ $J_{1\alpha,2\alpha} = 3.8$
					5.07				$W_{\frac{1}{2}} = 12$
1β,3β-$(OAc)_2$, 5.6β-epoxy (20)	4.93	3.11	1.39	1.07	4.85	0.04	0.08 [d]	$J_{6\alpha,7\beta} = 2.5$	$J_{1\alpha,2\beta} = 9.4$ $J_{1\alpha,2\alpha} = 3.3$
Derivatives of (21)									
3β-OAc (22)	4.60	5.37	0.84	1.01				$W_{\frac{1}{2}} = 10$	
3β-OH (23)	3.53	5.35	0.85	1.00				$W_{\frac{1}{2}} = 10$	
3β-OH, 7(C=O) (24)	3.67	5.69	0.84	1.23		−0.01 [e]	0.23	$W_{\frac{1}{2}} = 3$	
3β,7β-$(OH)_2$ (25)	3.56	5.29	0.87	1.04	3.86	0.02 [e]	0.04	$W_{\frac{1}{2}} = 6$	$W_{\frac{1}{2}} = 13$
3β,7α-$(OH)_2$ (26)	3.59	5.61	0.85	0.99	3.96	0.00 [e]	−0.01	$J_{6,7\beta} = 5.0$	$W_{\frac{1}{2}} = 14$
Derivatives of (27)	4-H	18-H	19-H	C_xHOH		18-H	19-H	4-H	C_xHOH
(27)	5.75	1.21	1.18					$W_{\frac{1}{2}} = 3$	
11α-OH (28)	5.75	1.22	1.32	3.90		0.01	0.14 [f]	$W_{\frac{1}{2}} = 3$	$J_{9\alpha,11\beta} = 10.5$ $J_{11\beta,12\alpha} = 10.5$ $J_{11\beta,12\beta} = 4.0$
3,6-(C=O)$_2$ (29)		1.20	0.95			−0.01	−0.23 [f]		
3α-OH, 4α,5α-epoxy (30)	3.24	1.18	1.02	4.05		−0.03	−0.16 [f]	$J_{3\beta,4\beta} = 4.3$	$W_{\frac{1}{2}} = 15$

[a] Minus sign (Δδ) represents an upfield shift. [b] Δδ relative to (3). [c] Δδ relative to (10). [d] Δδ relative to (9). [e] Δδ relative to (23). [f] Δδ relative to (27).

Comparison of the results obtained on the microbiological transformations of the aza-steroidal systems (2), (3), (22), and (27) described in this paper with those reported [2] for related Δ^4- and Δ^5-androstene derivatives shows predominant 7-oxidation for both systems.

Unusually the *N*-acetyl-17a-azasteroid (9) underwent *inter alia* some 1β-hydroxylation to give (13) and some dioxygenation to give (14) and (19). Such 1-hydroxylation was not noted for the androstene derivative or for the *N*-acetyl-17-aza-analogue (22).

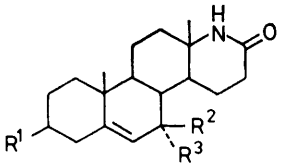
EXPERIMENTAL

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

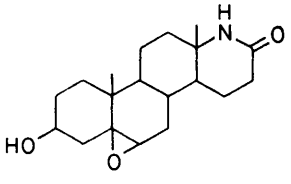
3β-Acetoxy- and 3β-hydroxy-17a-aza-D-homoandrost-5-en-17-one (2) and (3), 3β-acetoxy-*N*-acetyl-17a-aza-D-homoandrost-5-ene (9), 3β-acetoxy-*N*-acetyl-17-aza-D-homoandrost-5-ene (22) and 17a-aza-D-homoandrost-4-ene-3,17-dione (27) were prepared according to published procedures.[3,4]

Incubation of 3β-*Acetoxy*-17*a-aza*-D-*homoandrost*-5-*en*-17-*one* (2) [3,4] *with* Cunninghamella elegans.—The lactam (2) (8.0 g), in ethanol (800 ml), was added to the fungus in the nutrient medium (80 l, 400 flasks), and incubated for three days. The broth extract (4.18 g) was chromatographed over alumina (Woelm, neutral, activity IV, 335 g).

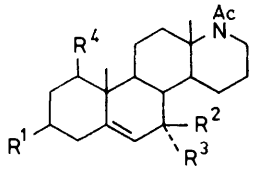
Elution with ether–methanol (99 : 1) yielded an oily fraction (1.265 g) which was crystallised, from methanol, to give 5,6β-epoxy-3β-hydroxy-17a-aza-D-homo-5β-androstan-17-one (7) (320 mg). Ether–methanol (19 : 1) yielded a semi-solid fraction (1.425 g) which was crystallised, from ethyl acetate, to give a mixture of 3β,7β-dihydroxy-17a-aza-D-homoandrost-5-en-17-one (5) and 3β,7α-dihydroxy-17a-aza-D-homoandrost-5-en-17-one (6) (741 mg), in approximately equal proportions. The combined mother-liquors were chromatographed (p.l.c.) and yielded, after extraction of the various fractions, 3β-hydroxy-17a-aza-D-homoandrost-5-en-17-one (3) (14 mg), 3β-hydroxy-17a-aza-D-



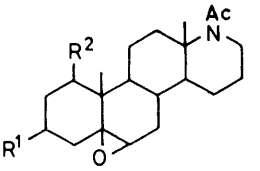
(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = OAc, R^2 = R^3 = H$
(3) $R^1 = OH, R^2 = R^3 = H$
(4) $R^1 = OH, R^2R^3 = O$
(5) $R^1 = OH, R^2 = OH, R^3 = H$
(6) $R^1 = OH, R^2 = H, R^3 = OH$



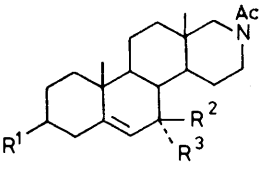
(7)



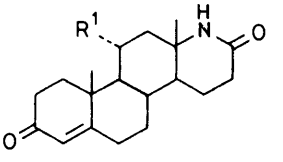
(8) $R^1 = R^2 = R^3 = R^4 = H$
(9) $R^1 = OAc, R^2 = R^3 = R^4 = H$
(10) $R^1 = OH, R^2 = R^3 = R^4 = H$
(11) $R^1 = OH, R^2 = OH, R^3 = R^4 = H$
(12) $R^1 = OH, R^2 = R^4 = H, R^3 = OH$
(13) $R^1 = R^4 = OH, R^2 = R^3 = H$
(14) $R^1 = R^2 = R^4 = OH, R^3 = H$
(15) $R^1 = R^2 = OAc, R^3 = R^4 = H$
(16) $R^1 = R^3 = OAc, R^2 = R^4 = H$
(17) $R^1 = R^4 = OAc, R^2 = R^3 = H$
(18) $R^1 = R^2 = R^4 = OAc, R^3 = H$



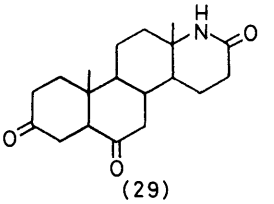
(19) $R^1 = R^2 = OH$
(20) $R^1 = R^2 = OAc$



(21) $R^1 = R^2 = R^3 = H$
(22) $R^1 = OAc, R^2 = R^3 = H$
(23) $R^1 = OH, R^2 = R^3 = H$
(24) $R^1 = OH, R^2R^3 = O$
(25) $R^1 = OH, R^2 = OH, R^3 = H$
(26) $R^1 = OH, R^2 = H, R^3 = OH$



(27) $R^1 = H$
(28) $R^1 = OH$



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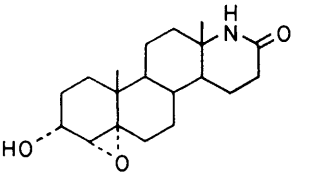

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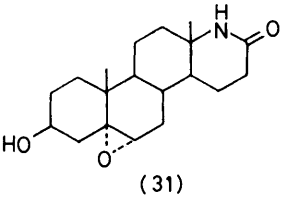

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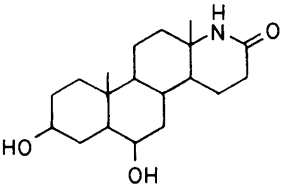


(32)

homoandrost-5-ene-7,17-dione (4) (126 mg), and a further amount of (5) and (6) (248 mg; total 989 mg) which was separated by p.l.c. over silica gel.

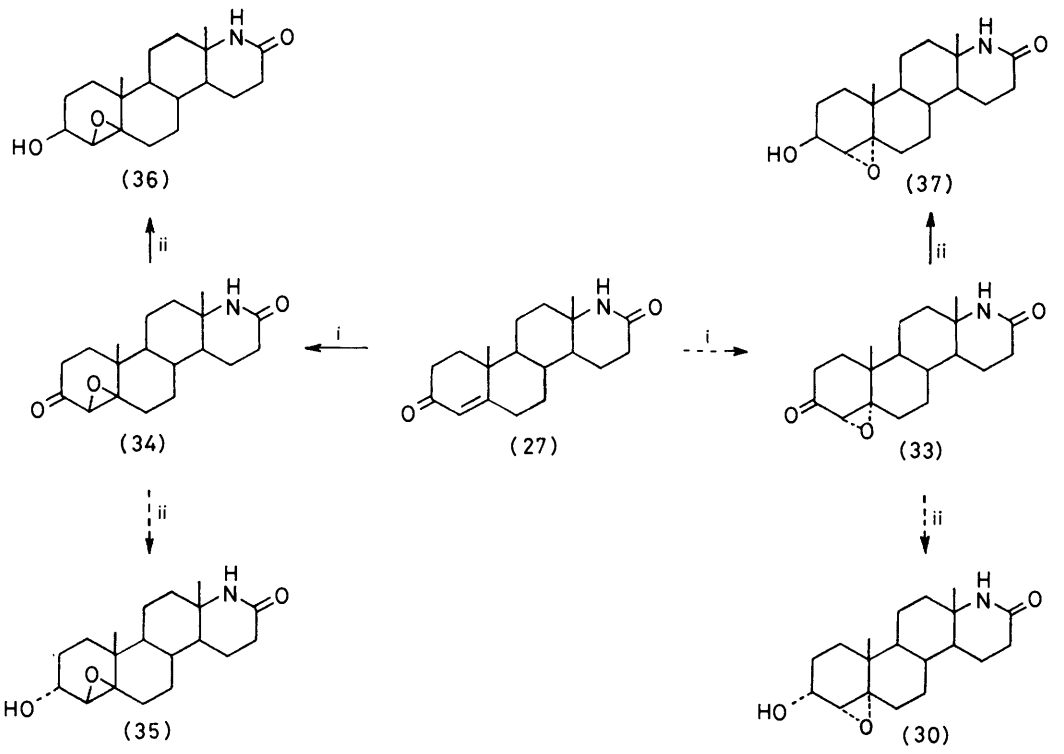
3β-Hydroxy-17a-aza-D-homoandrost-5-en-17-one (3) was obtained as crystals (14 mg, 0.2%) from ethyl acetate, m.p. 295—297 °C (lit.,[4] 292—295 °C; lit.,[3] 295—297°), M^+, 303 ($C_{19}H_{29}NO_2$), $R_F = 0.35$.

3β-*Hydroxy*-17*a*-*aza*-D-*homoandrost*-5-*ene*-7,17-*dione* (4) was obtained as crystals (126 mg, 1.7%) from ethyl acetate, m.p. 303—305 °C (Found: C, 71.6; H, 8.8; N, 4.4. $C_{19}H_{27}NO_3$ requires C, 71.9; H, 8.6; N, 4.4%), $\lambda_{max.}$ 238 nm (ε 10 400); M^+, 317; $R_F = 0.24$.

5,6β-*Epoxy*-3-*hydroxy*-17*a*-*aza*-D-*homo*-5β-*androstan*-17-*one* (7) was obtained as crystals (320 mg, 4.3%) from methanol, m.p. 247—252 °C; recrystallisation from ethyl acetate raised the melting point to 262—265 °C (Found: C, 71.7; H, 9.3; N, 4.1. $C_{19}H_{29}NO_3$ requires C, 71.4; H, 9.2; N, 4.4%); M^+, 319; $R_F = 0.20$.

3β,7β-*Dihydroxy*-17*a*-*aza*-D-*homoandrost*-5-*en*-17-*one* (5) was obtained as crystals (*ca.* 450 mg, *ca.* 6.7%) from ethyl acetate, m.p. 257—260 °C (Found: C, 71.6; H, 9.2; N, 4.5. $C_{19}H_{29}NO_3$ requires C, 71.4; H, 9.2; N, 4.4%). ^{1}H N.m.r. (C_5D_5N): δ 3.88 (sept. *J* 9.8, 4.9 Hz, 3α-C*H*OH) 5.68 (s, $W_{\frac{1}{2}}$ 5 Hz, 6-H), 1.14 (s, 18-H_3), 0.96 (s, 19-H_3), 4.12 (m, $W_{\frac{1}{2}}$ 16 Hz, 7α-C*H*OH); M^+, 319; $R_F = 0.11$.

3β,7α-*Dihydroxy*-17*a*-*aza*-D-*homoandrost*-5-*en*-17-*one* (6) was obtained as crystals (*ca.* 450 mg, *ca.* 6.7%) from ethyl acetate, m.p. 247—250 °C (Found: C, 71.5; H, 9.4; N, 4.3. $C_{19}H_{29}NO_3$ requires C, 71.4; H, 9.2; N, 4.4%), ^{1}H n.m.r. (C_5D_5N): δ 3.80 (sept. *J* 9.8, 4.9 Hz, 3α-C*H*OH), 5.85 (d, *J* 4.8 Hz, 6-H), 1.18 (s, 18-H_3), 1.02 (s, 19-H_3), and 4.25 (m, $W_{\frac{1}{2}}$ 12 Hz, 7β-C*H*OH); M^+, 319; $R_F = 0.06$.



SCHEME *Reagents:* i, H_2O_2–NaOH–MeOH; ii, $NaBH_4$–MeOH; —— major product, – – – minor product

TABLE 3

^1H N.m.r. spectra ($CDCl_3$) of 3-oxo- and 3ξ-hydroxy-4ξ,5-epoxy-17a-aza-D-homo-5ξ-androstan-17-ones

Compound	C-3	4ξ,5ξ-epoxide	Chemical shifts (δ) 3ξ-H	4ξ-H	18-H	19-H	Coupling constants (*J*/Hz) and half-widths ($W_{\frac{1}{2}}$/Hz) 3-H	$W_{\frac{1}{2}}$
(33)	keto	α		3.08	1.20	1.06		$W_{\frac{1}{2}} = 2$
(34)	keto	β		3.02	1.19	1.16		$W_{\frac{1}{2}} = 2$
(30)	α-OH	α	4.05	3.24	1.18	1.02	*m*	$J_{3\beta,4\beta} = 4.3$
(37)	β-OH	α	4.00	2.96	1.18	1.10	$J_{3\alpha,4\alpha} = J_{3\alpha,4\beta} = 8.4$	$W_{\frac{1}{2}} = 3$
(35)	α-OH	β	3.99	2.88	1.18	0.99	$J_{3\beta,4\alpha} = J_{3\beta,4\beta} = 7.9$	$W_{\frac{1}{2}} = 3$
(36)	β-OH	β	4.08	3.18	1.18	1.03	$W_{\frac{1}{2}} = 15$	$J_{3\alpha,4\alpha} = 4.5$

Incubation of 3β-*Hydroxy*-17*a*-*aza*-D-*homoandrost*-5-*en*-17-*one* (3) *with* Cunninghamella elegans.—3β-Hydroxy-17a-aza-D-homoandrost-5-en-17-one (3) (2.5 g), in ethanol (250 ml), was added to the fungus in the nutrient medium (25 l, 125 flasks), and incubated for three days. P.l.c. of the broth extract (0.65 g), extraction, and crystallisation (ethyl acetate), yielded starting material (3) (104 mg, 4.2%), 3β-hydroxy-17a-aza-D-homoandrost-5-ene-7,17-dione (4) (41 mg, 1.6%), 5,6β-epoxy-3β-hydroxy-17a-aza-D-homo-5β-androstan-17-one (7) (62 mg, 2.4%), and a mixture of 3β,7β-dihydroxy-17a-aza-D-homoandrost-5-en-17-one (5) and 3β,7α-dihdroxy-17a-aza-D-homoandrost-3-en-17-one (6) (263 mg, 10.0%).

5,6α-*Epoxy- and* 5,6β-*Epoxy*-3β-*hydroxy*-17*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (31) *and* (7).—3β-*Hydroxy*-17*a*-*aza*-D-*homoandrost*-5-*en*-17-*one* (3) (2.0 g) was stirred, in chloroform (250 ml), and the mixture was cooled to 0 °C. This was treated with a solution of *m*-chloroperbenzoic acid (1.40 g, excess) in chloroform (40 ml), pre-cooled to the same temperature. The mixture was allowed to warm to room temperature, with stirring. After 1.5 h, the reaction mixture was further diluted with chloroform (100 ml). Excess of *m*-chloroperbenzoic acid was destroyed by successive washing with sodium sulphite solution (10% w/v; 100 ml), saturated aqueous sodium hydrogencarbonate (2 × 50 ml), and water. The organic layer was dried (sodium sulphate) and the solvent removed, under reduced pressure. Analysis of the residue, by ^1H n.m.r., gave the epoxide ratio (α : β) as *ca.* 5 : 1, the α-isomer predominating. Fractional crystallisation from ethyl acetate yielded almost pure 5,6α-epoxide. Recrystallisation from ethyl acetate yielded 5,6α-*epoxy*-3β-*hydroxy*-7*a*-*aza*-D-*homo*-5α-*androstan*-17-*one* (31) as white crystals (1.05 g, 50%), m.p. 283—285 °C (Found: C, 71.2 H, 9.3; N, 4.2. $C_{19}H_{29}NO_3$ requires C, 71.4; H, 9.2; N, 4.4%), δ($CDCl_3$) 3.92 (sept, *J* 10.8, 5.4 Hz, 3α-C*H*OH), 2.95 (d, *J* 4.5 Hz, 6β-*H*), 1.11 (s, 18-H_3), and 1.05 (s, 19-H_3); M^+, 319; $R_F = 0.19$.

Evaporation of the mother-liquors, from the fractional

crystallisation, gave a mixture of the two epoxides, the 5,6β-epoxy-3β-hydroxy-17a-aza-D-homo-5α-androstan-17-one predominating.

3β,7α-*Diacetoxy-17a-aza-D-homoandrost-5-en-17-one*.—3β-Acetoxy-17a-aza-D-homoandrost-5-en-17-one (2) (3.0 g) and cuprous bromide (3.0 g) were refluxed in acetic acid (50 ml), in an atmosphere of nitrogen, whilst t-butyl perbenzoate (7.5 ml) in acetic acid (30 ml) was added over a period of 15 min. The mixture was refluxed for a further 15 min and then cooled and poured into chloroform (300 ml). The mixture was then filtered and washed successively with water (3 × 50 ml), aqueous sodium carbonate (5% w/v; 3 × 100 ml), and water (2 × 50 ml) and then dried (Na_2SO_4). Removal of the solvent left a very viscous oil (4.0 g), to which was added ethyl acetate (5 ml). The solution was allowed to stand overnight at room temperature. The precipitate was filtered off and crystallised from ethyl acetate to give the expected [12] 3β,7α-*diacetoxy-17a-aza-D-homoandrost-5-en-17-one* (0.43 g, 12%), m.p. 211—212 °C (Found: C, 68.6; H, 8.1; N, 3.7. $C_{23}H_{33}NO_5$ requires C, 68.5; H, 8.2; N, 3.5%), ^{1}H n.m.r.: δ 4.68 (sept, *J* 10.4, 5.2 Hz, 3α-C*H*OAc), 5.60 (d, *J* 5.0 Hz, 6-H), 1.17 (s, 18-H_3), 1.01 (s, 19-H_3), 5.17 (m, $W_{\frac{1}{2}}$ 11 Hz, 7β-C*H*OAc), 2.04 (s, 3β-OCOCH_3), and 2.06 (s, 7α-OCOCH_3); M^+, 403.

3β,7α-*Dihydroxy-17a-aza-D-homoandrost-5-en-17-one* (6).—3β,7α-Diacetoxy-17a-aza-D-homoandrost-5-en-17-one (0.35 g) and potassium hydroxide (0.10 g) were refluxed in methanol (10 ml) for 2 h. After neutralisation (acetic acid) the mixture was poured into water (50 ml) and extracted with chloroform (3 × 25 ml). Removal of the solvent and crystallisation from ethyl acetate gave the desired product (0.14 g, 51%), m.p. 235—238 °C, R_F = 0.06. Recrystallisation of a small sample from ethanol gave white crystals, m.p. 248—251 °C.

Incubation of N-*Acetyl-17a-aza-D-homoandrost-5-en-3β-yl Acetate* (9) *with* Cunninghamella elegans.—*N*-Acetyl-17a-aza-D-homoandrost-5-en-3β-yl acetate (9) (3.0 g), in ethanol (300 ml), was added to the fungus, in the nutrient medium (30 l, 150 flasks), and incubated for 3 d. The broth extract (1.75 g) was chromatographed over alumina (Woelm, neutral, activity II, 200 g), with ether–methanol (99 : 1) as eluant, to give the following hydroxylated derivatives: (i) N-*acetyl-17a-aza-D-homoandrost-5-ene-3β,7β-diol* (11) as needles (14 mg, 0.5%) from ethyl acetate, m.p. 272—274 °C (Found: C, 72.8; H, 9.7; N, 3.9. $C_{21}H_{33}NO_3$ requires C, 72.6; H, 9.6; N, 4.0%); M^+, 347; R_F = 0.45. Acetylation yielded *N*-acetyl-17a-aza-D-homoandrost-5-en-3β,7β-diyl diacetate (15) (^{1}H n.m.r. Table 2); (ii) N-*acetyl-17a-aza-D-homoandrost-5-ene-3β,7α-diol* (12) as needles (245 mg, 8.8%) from methanol, m.p. 316—318 °C (Found: C, 72.6; H, 9.8; N, 4.2. $C_{21}H_{33}NO_3$ requires C, 72.6; H, 9.6; N, 4.0%); M^+, 347; R_F = 0.34. Acetylation yielded *N*-acetyl-17a-aza-D-homoandrost-5-ene-3β,7α-diyl diacetate (16) (^{1}H n.m.r. Table 2); (iii) N-*acetyl-17a-aza-D-homoandrost-5-ene-1β,3β-diol* (13) as crystals (125 mg, 4.5%) from methanol, m.p. 321—323 °C (Found: C, 72.3; H, 9.4; N, 4.3. $C_{21}H_{33}NO_3$ requires C, 72.6; H, 9.6; N, 4.0%); M^+, 347; R_F = 0.24. Acetylation yielded *N*-acetyl-17a-aza-D-homoandrost-5-ene-1β-3β-diyl diacetate (17) (^{1}H n.m.r. Table 2); (iv) N-*acetyl-5,6β-epoxy-17a-aza-D-homo-5β-androstane-1β,3β-diol* (19) as crystals (43 mg, 1.5%) from methanol, m.p. 258—261° (Found: C, 69.5; H, 9.3; N, 3.7. $C_{21}H_{33}NO_4$ requires C, 69.4; H, 9.2; N, 3.9%); M^+, 363; R_F = 0.20. Acetylation yielded *N*-acetyl-5,6β-epoxy-17a-aza-D-homo-5β-androstane-1β,3β-diyl diacetate (20) (^{1}H n.m.r. Table 2); (v) N-*acetyl-17a-aza-D-homoandrost-5-ene-1β,3β,7β-triol* (14) as crystals (18 mg, 0.6%) from methanol, m.p. 278—280 °C (Found: C, 69.2; H, 9.2; N, 4.0. $C_{21}H_{33}NO_4$ requires C, 69.4; H, 9.2; N, 3.9%); M^+, 363; R_F = 0.16. Acetylation yielded *N*-acetyl-17a-aza-D-homoandrost-5-ene-1β,3β,7β-triyl triacetate (18) (^{1}H n.m.r. Table 2).

Incubation of N-*Acetyl-17-aza-D-homoandrost-5-en-3β-yl Acetate* (22) *with* Cunninghamella elegans.—*N*-Acetyl-17-aza-D-homoandrost-5-en-3β-yl acetate (22) (0.50 g), in ethanol (50 ml), was incubated with the fungus in the nutrient medium (5 l, 25 flasks), for 3 d.

The broth extract (0.35 g) was chromatographed over alumina (Woelm, neutral, activity II; 50 g). Elution with ether–methanol (99 : 1) yielded, in order of decreasing R_F value, N-*acetyl-3β-hydroxy-17-aza-D-homoandrost-5-en-7-one* (24) as crystals (20 mg, 4.3%) from ethyl acetate, m.p. 240—243 °C (Found: C, 72.8; H, 8.9; N, 4.3. $C_{21}H_{31}NO_3$ requires C, 73.0; H, 9.0; N, 4.1%); δ(C_5D_5N) 3.87 (sept, *J* 10.0, 5.0 Hz, 3α-C*H*OH), 5.87 (6-*H*), 0.83 (18-H_3), 1.10 (s, 19-H_3), and 2.10 (17-NCOCH_3); $\lambda_{max.}$ 237 nm (ε = 13 600); $\nu_{max.}$ ($CHCl_3$) 3 610 and 1 670 cm^{-1}; M^+, 345; R_F = 0.52; N-*acetyl-17-aza-D-homoandrost-5-ene-3β,7β-diol* (25) as crystals (48 mg, 10.3%) from ethyl acetate, m.p. 247—249 °C (Found: C, 72.7; H, 9.4; N, 4.2. $C_{21}H_{33}NO_3$ requires C, 72.6; H, 9.6; N, 4.0%); δ(C_5D_5N) 3.88 (m, $W_{\frac{1}{2}}$ 23 Hz, 3α-C*H*OH), 5.70 (6-H), 0.89 (18-H_3), 0.95 (s, 19-H_3), 2.11 (17-NCOCH_3), and 4.07 (t, *J* 7.0 Hz, 7α-C*H*OH); M^+, 347; R_F = 0.35; N-*acetyl-17-aza-D-homoandrost-5-ene-3β,7α-diol* (26) as crystals (122 mg, 26.2%) from ethyl acetate, m.p. 217—220 °C (Found: C, 72.4; H, 9.6; N, 4.3. $C_{21}H_{33}NO_3$ requires C, 72.6; H, 9.6; N, 4.0%); δ(C_5D_5N) 3.80 (m, $W_{\frac{1}{2}}$ 23 Hz, 3α-C*H*OH), 5.88 (6-H), 0.89 (18-H_3), 1.00 (s, 19-H_3), 2.11 (17-NCOCH_3), and 4.17 (m, $W_{\frac{1}{2}}$ 20 Hz, 7β-C*H*OH), M^+, 347; R_F = 0.28.

Incubation of 17a-*aza-D-homoandrost-4-ene-3,17-dione* (27) *with* Cunninghamella elegans.—17a-Aza-D-homoandrost-4-ene-3,17-dione (27) (4.0 g) in ethanol (400 ml) was added to the fungus in the nutrient medium (40 l, 200 flasks)—no precipitate observed—and incubated for 3 d. The broth extract (5.23 g) was chromatographed over alumina (Woelm, neutral, activity III, 530 g). Elution with ether–methanol (49 : 1) yielded, in decreasing order of R_F value, unchanged starting material (1.68 g, 42%), R_F = 0.31; 17*a-aza-D-homo-5α-androstane-3,6,17-trione* (29) as crystals (286 mg, 6.8%) from methanol, m.p. 317—319 °C (Found: C, 71.7; H, 8.8; N, 4.6. $C_{19}H_{27}NO_3$ requires C, 71.9; H, 8.6; N, 4.6%); $\nu_{max.}$ ($CHCl_3$): 3 390, 1 720, and 1 712 cm^{-1}; M^+, 317; R_F = 0.24; 4α,5-*epoxy-3α-hydroxy-17a-aza-D-homo-5α-androstan-17-one* (30) as crystals (22 mg, 0.5%) from methanol, m.p. 206—209 °C (Found: C, 71.2; H, 9.4; N, 4.0. $C_{19}H_{29}NO_3$ requires C, 71.4; H, 9.2; N, 4.4%); M^+, 319; R_F = 0.12; 11α-*hydroxy-17a-aza-D-homoandrost-4-ene-3,17-dione* (28) as crystals (19 mg, 0.5%) from methanol, m.p. 190—193 °C (Found: C, 72.1; H, 8.8; N, 4.3. $C_{19}H_{27}NO_3$ requires C, 71.9; H, 8.6; N, 4.4%), $\lambda_{max.}$ 239 nm (ε 13 700); $\nu_{max.}$ ($CHCl_3$) 3 610, 3 390, and 1 650 cm^{-1}; M^+, 317; R_F = 0.07.

3β,6β-*Dihydroxy-17a-aza-D-homo-5α-androstan-17-one* (32).—17a-Aza-D-homo-5α-androstane-3,6,17-trione (29) (20 mg) was reduced with sodium borohydride (20 mg) in methanol (2 ml). After 1 h, at room temperature, the solvent was evaporated (nitrogen), the residue being washed well (water) and dried (*in vacuo*). Crystallisation from ethyl acetate yielded 3β,6β-*dihydroxy-17a-aza-D-homo-5α-androstan-17-one* (32) (10 mg, 50%), m.p. 310—312 °C (Found:

C, 75.3; H, 9.5; N, 4.4. $C_{19}H_{29}NO_2$ requires C, 75.2; H, 9.6; N, 4.6%); ^{1}H n.m.r.: δ 3.69 (sept, *J* 10.0, 5.0 Hz, 3α-C*H*OH), 1.20 (s, 18-H_3), 1.04 (s, 19-H_3), and 3.89 (m, $W_{\frac{1}{2}}$ 8 Hz, 6α-C*H*OH); M^+, 321.

4ξ,5-Epoxy-17a-aza-D-homo-5ξ-androstane-17-diones (33) and (34).—17a-Aza-D-homoandrost-4-ene-3,17-dione (27) (1.0 g) was dissolved in methanol (100 ml) and cooled to 6 °C. Hydrogen peroxide (100 vol; 6.8 ml) was also cooled and added. This was followed by a cooled aqueous solution of sodium hydroxide (4N; 4.5 ml). After 3 h, the methanol was removed (*in vacuo*, with gentle warming) and the residue was dissolved in ethyl acetate, filtered, washed (water, 50 ml), and dried (sodium sulphate). Removal of the solvent left a residue (0.72 g) which was purified by p.l.c. to give *4α,5-epoxy-17a-aza-D-homo-5α-androstane-3,17-dione* (33) as crystals (12 mg, 1%) from ethyl acetate, m.p. 258—260 °C (Found: C, 72.1; H, 8.4; N, 4.6. $C_{19}H_{27}NO_3$ requires C, 71.9; H, 8.6; N, 4.4%); δ 3.08 (s, $W_{\frac{1}{2}}$ 2 Hz, 4β-H), 1.20 (s, 18-H_3), and 1.06 (s, 19-H_3); R_F = 0.38; and *4β,5-epoxy-17a-aza-D-homo-5β-androstane-3,17-dione* (34) as crystals (116 mg, 11%) from ethyl acetate, m.p. 264—266 °C (Found: C, 71.7; H, 8.5; N, 4.3. $C_{19}H_{27}NO_3$ requires C, 71.9; H, 8.6; N, 4.4%); δ 3.02 (s, $W_{\frac{1}{2}}$ 2 Hz, 4α-H), 1.19 (s, 18-H_3), and 1.15 (s, 19-H_3); R_F = 0.32.

4α,5-Epoxy-3ξ-hydroxy-17a-aza-D-homo-5α-androstan-17-ones (30) and (37).—4α,5-Epoxy-17a-aza-D-homo-5α-androstane-3,17-dione (33) (8 mg) was dissolved in methanol (0.5 ml) and reduced with sodium borohydride (10 mg), at room temperature, for 1 h. Evaporation of the solvent (nitrogen) left a residue which was washed well (water) and dried (*in vacuo* at 60 °C). Separation of the products was not attempted. ^{1}H N.m.r. analysis of the product mixture gave δ 4.05 (m, 3β-C*H*OH), 3.24 (d, *J* 4.3 Hz, 4β-*H*), 1.18 (s, 18-H_3), and 1.02 (s, 19-H_3), attributed to 4α,5-epoxy-3α-hydroxy-17a-aza-D-homo-5α-androstan-17-one (30); and δ 4.00 (t, *J* 8.4 Hz, 3α-C*H*OH), 2.96 (s, $W_{\frac{1}{2}}$ 3 Hz, 4β-H), 1.18 (s, 18-H_3), and 1.10 (s, 19-H_3), attributed to 4α,5-epoxy-3β-hydroxy-17a-aza-D-homo-5α-androstan-17-one (37). The ratio (3β : 3α) of the two isomers was *ca.* 5 : 1.

4β,5-Epoxy-3ξ-hydroxy-17a-aza-D-homo-5β-androstan-17-ones (35) and (36).—4β,5-Epoxy-17a-aza-D-homo-5β-androstane-3,17-dione (34) (30 mg) was dissolved in methanol (2 ml). Sodium borohydride (20 mg) was added and the reaction was treated as above. ^{1}H N.m.r. analysis of the unseparated product mixture gave δ 3.99 (t, *J* 7.9 Hz, 3β-C*H*OH), 2.88 (s, $W_{\frac{1}{2}}$ 3 Hz, 4α-H), 1.18 (s, 18-H_3), and 0.99 (s, 19-H_3), attributed to 4β,5-epoxy-3α-hydroxy-17a-aza-D-homo-5β-androstan-17-one (35) and δ 4.08 (m, $W_{\frac{1}{2}}$ 15 Hz, 3α-C*H*OH), 3.18 (d, *J* 4.5 Hz, 4α-H), 1.18 (s, 18-H_3), 1.03 (s, 19-H_3), attributed to 4β,5-epoxy-3β-hydroxy-17a-aza-D-homo-5β-androstan-17-one (36). The ratio (3β : 3α) of the two isomers was *ca.* 11 : 9.

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