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# MICROBIOLOGICAL TRANSFORMATIONS OF 3β-ACETOXY-17a-AZA-D-HOMOANDROST-5-EN-17-ONE AND 3β-ACETOXY-ANDROST-5-EN-17-ONE WITH THE FUNGUS *CUNNINGHAMELLA ELEGANS*

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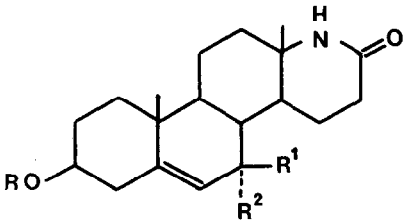
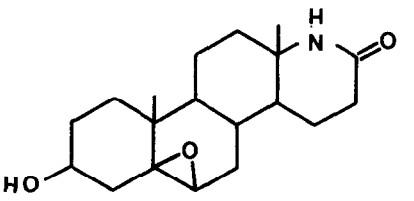
In contrast to the vast amount of information available concerning microbial transformations of steroids[1,2], there is a marked lack of data on transformations of heterocyclic steroidal systems[3]. As part of a programme aimed at assessing the influence of a ring-nitrogen atom on the course of microbial transformation of such steroid-like substrates, 3β-acetoxy-17a-aza-D-homoandrost-5-en-17-one (1) was incubated with *Cunninghamella elegans* Lendner (CBS 167.53), selected after a broad screening exercise. The lactam (1) was prepared from the oxime of 3β-acetoxy-androst-5-en-17-one (7) by a simple Beckmann rearrangement[4].

The cultures of *C. elegans* were grown at 25° in 200 ml aliquots of nutrient medium[5] for three days. A solution of the lactam (1) in ethanol was added (100 mg $l^{-1}$ of nutrient medium) and incubated for a further three days. Products were isolated by sequential column (alumina)




(1) R=Ac, $R^1$=$R^2$=H

(2) R=$R^1$=$R^2$=H

(3) R=$R^1$=H, $R^2$=OH

(4) R=$R^2$=H, $R^1$=OH

(5) R=H, $R^1R^2$=O

(13) R=Ac, $R^1$=H, $R^2$=AcO

(6)

and preparative layer (silica-gel) chromatography of the broth extract ($CH_2Cl_2$): structures were assigned to these largely from a consideration of the n.m.r. -CHOH and olefinic proton resonances, together with the chemical shifts of the angular methyl groups (in relation to the parent compound[6]) - see Table 1. The Δδ values obtained from the aza-steroids were similar to those reported for their steroidal analogues[6].

The products isolated from the incubation of (1) with C. elegans were:

3β-hydroxy-17a-aza-D-homoandrost-5-en-17-one (2, 0.2%)

3β,7α-dihydroxy-17a-aza-D-homoandrost-5-en-17-one (3, ≈ 6.7%)

3β,7β-dihydroxy-17a-aza-D-homoandrost-5-en-17-one (4, ≈ 6.7%)

3β-hydroxy-17a-aza-D-homoandrost-5-en-7,17-dione (5, 1.7%)

5β,6β-epoxy-3β-hydroxy-17a-aza-D-homoandrost-5-en-17-one (6, 4.3%)

Unchanged starting material also present in the extract was not isolated. Similar incubation of the readily available 3β-hydroxy derivative (2) also yielded compounds (3)-(6).

Table 1. Relevant Chemical Shifts (p.p.m. from $Me_4Si$ in $CDCl_3$ solution) of the Products from the Action of C. elegans on 3β-Acetoxy-17a-aza-D-homoandrost-5-en-17-one (1)

| Compound | 3α H δ | 6 H δ | 18-$CH_3$ δ | 19-$CH_3$ δ | 7 H δ | 3α H Δδ | 6 H Δδ | 18-$CH_3$ Δδ | 19-$CH_3$ Δδ |
|---|---|---|---|---|---|---|---|---|---|
| (2) | 3.54 | 5.36 | 1.17 | 1.00 | - | - | - | - | - |
| (3) | 3.59 | 5.63 | 1.17 | 0.98 | 4.03 | 0.05 | 0.27 | 0.00 | -0.02 |
| (4) | 3.57 | 5.29 | 1.19 | 1.04 | 3.94 | 0.03 | -0.07 | 0.02 | 0.04 |
| (5) | 3.68 | 5.73 | 1.19 | 1.22 | - | 0.14 | 0.37 | 0.02 | 0.22 |
| (6) | 3.71 | 3.14 | 1.13 | 1.00 | - | 0.17 | -2.22 | -0.04 | 0.00 |

Δδ values relative to (2); minus sign represents an upfield shift

The structures and stereochemistry of the allylic alcohols (3) and (4) were confirmed by alkaline hydrolysis of synthesised[7] 3β,7α-diacetate (13) to (3) and the characteristic[8] chemical shift for the C-6 proton and the 6,7-vicinal coupling constants ($J_{6,7\beta}$=5.0 Hz for (3) and $J_{6,7\alpha}$=2.5 Hz for (4)). Similarly, compound (6) was assigned the β-epoxide stereochemistry by comparison of its n.m.r. parameters with those of the synthesised[9] 5β,6β- and 5α,6α-epoxides. The C-6 proton absorbed at lower field with a smaller vicinal coupling constant[10] in (6) than in the α-epoxide.

For comparison purposes, 3β-acetoxyandrost-5-en-17-one (7), a near-carbocyclic analogue of (1), was also incubated (250 mg $l^{-1}$) with C. elegans to give, after a similar work-up procedure to that described, five steroidal products.

From microbial transformation of (7) were obtained:

3β,7α-dihydroxy-androst-5-en-17-one (8, 26.8%)

3β,7β-dihydroxyandrost-5-en-17-one (9, 9.0%)

3β-hydroxy-androst-5-en-7,17-dione (10, 5.4%)

3β,14α-dihydroxy-androst-5-en-7,17-dione (11, 1.1%)

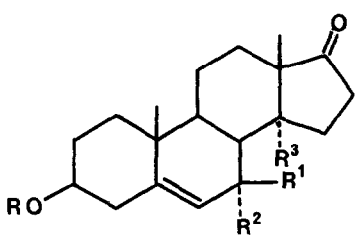
5β,6β-epoxy-3β,12α-dihydroxyandrost-5-en-17-one (12, 4.1%)

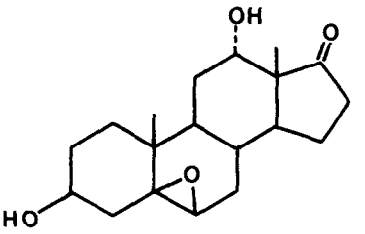
The n.m.r. data summarised in Table 2 is completely consistent with the proposed structures.

Table 2. Relevant Chemical Shifts (p.p.m. from $Me_4Si$ in $CDCl_3$ solution) of the Products from the Action of C. elegans on 3β-Acetoxy-androst-5-en-17-one (7)

| Compound | 3α H δ | 6 H δ | 18-$CH_3$ δ | 19-$CH_3$ δ | CHOH δ | 3α H Δδ | 6 H Δδ | 18-$CH_3$ Δδ | 19-$CH_3$ Δδ |
|---|---|---|---|---|---|---|---|---|---|
| (14) | 3.54 | 5.39 | 0.89 | 1.04 | - | - | - | - | - |
| (8) | 3.59 | 5.66 | 0.89 | 1.03 | 3.98 | 0.05 | 0.27 | 0.00 | -0.01 |
| (9) | 3.57 | 5.32 | 0.90 | 1.08 | 3.98 | 0.03 | -0.07 | 0.01 | 0.04 |
| (10) | 3.70 | 5.76 | 0.90 | 1.23 | - | 0.16 | 0.37 | 0.01 | 0.19 |
| (11) | 3.70 | 5.76 | 1.00 | 1.25 | - | 0.16 | 0.37 | 0.11 | 0.21 |
| (12) | 3.76 | 3.15 | 0.86 | 1.04 | 4.21 | 0.22 | -2.24 | -0.03 | 0.00 |

Δδ values relative to (14); minus sign represents an upfield shift




(7) R=Ac, $R^1$=$R^2$=$R^3$=H

(8) R=$R^1$=$R^3$=H, $R^2$=OH

(9) R=$R^2$=$R^3$=H, $R^1$=OH

(10) R=$R^3$=H, $R^1R^2$=O

(11) R=H, $R^1R^2$=O, $R^3$=OH

(14) R=$R^1$=$R^2$=$R^3$=H

(12)

These results show that, under the conditions employed, the steroidal lactam (1) undergoes mono-oxygenation whereas the steroidal ketone (7) shows a tendency for di-oxygenation.

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