# Microbiological Hydroxylation. Part XVIII.[1] Introduction of 16α-, 9α-, and 3α-Hydroxy-groups into Dioxygenated 5α-Androstanes by the Fungus *Diaporthe celastrina*

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*Diaporthe celastrina*, a fungus not previously reported as being active with steroids, hydroxylates a range of dioxygenated 5α-androstanes generally by attack on the α-face. The sites of hydroxylation and the efficiencies of the processes depend on the positions and oxidation levels of the androstanes' substituents. 5α-Androstane-3,7-dione is converted into 3β,16α-dihydroxy-5α-androstan-7-one (45% yield); 3β-hydroxy-5α-androstan-17-one is hydroxylated in the 9α-position (55%), and 5α-androstane-7,17-dione gives the 3α-hydroxy-derivative (52%).

In developing the use of micro-organisms for the selective introduction of hydroxy-groups into alicyclic systems, we have studied a number of fungi not previously reported as being active with steroidal substrates.[2] Standard screening tests showed that the fungus *Diaporthe celastrina* does not hydroxylate mono-oxygenated steroids, but introduces one hydroxy-group into di-oxygenated androstanes. The indication of a marked predominance of one product from some of the diketones and hydroxy-ketones prompted the detailed investigation of the fungus reported here.

Table 1 summarises the microbiological results obtained by incubating eighteen dioxygenated androstanes, progesterone, and two trioxygenated androstanes with vegetative cultures of *D. celastrina*. The conversions in Section A are efficient and suitable for application in preparative work, whereas those in Section B give only low yields of hydroxylated products. Table 2 lists the n.m.r. spectra of the steroids, substrates, and products, involved here for which spectroscopic data have not appeared in earlier publications: the arabic serial number sequence of steroids discussed earlier is used in this Table, which contains steroids nos. 830—849. The structures of new compounds follow, as usual,[3] from a combination of spectrometric and chemical methods. For new compounds the n.m.r. signals appear in Table 2, and the other information required for their characterisation is given in Table 3. As with recent Parts of this series, and for reasons already discussed,[4] the experimental details are available only as Supplementary Publication No. SUP 21365 (9 pp.).†

The main feature of the results in Table 1 is that *D. celastrina* gives gratifyingly clean hydroxylation when certain combinations of directing groups [3] are present in the substrates. Thus with 5α-androstane 3-ketones having a second oxygenated group suitably positioned in ring B or C (see Figure 1) hydroxylation, mainly at the 16α-position, is accompanied by reduction of the 3-oxo-group to the 3β-alcohol. From this it might be thought that the dioxygenated androstanes with a 3β-hydroxy- rather than a 3-oxo-group would be as good, or even better, as substrates. However, comparison of the 3,7-diketone (clean hydroxylation, Section A) with the 3β-hydroxy-7-ketone (low utilisation, Section B) shows that reduction of the 3-oxo-group before incubation inhibits the hydroxylation process.

Hydroxylation with *D. celastrina*

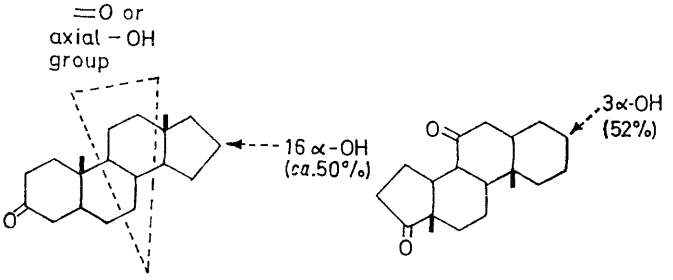



FIGURE 1

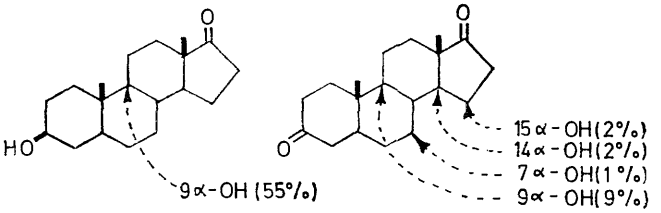



FIGURE 2

The general effect of substrate structure in determining the site of hydroxylation by certain micro-organisms [5] is

† For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1974, Index issue.

[1] Part XVII, V. E. M. Chambers, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, J. T. Pinhey, and A. L. Wilkins, preceding paper.

[2] W. Charney and H. L. Herzog, 'Microbial Transformations of Steroids,' Academic Press, New York, 1967.

[3] A. M. Bell, P. C. Cherry, I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and P. D. Woodgate, *J.C.S. Perkin I*, 1972, 2081.

[4] V. E. M. Chambers, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, and A. L. Wilkins, *J.C.S. Perkin I*, 1975, 55.

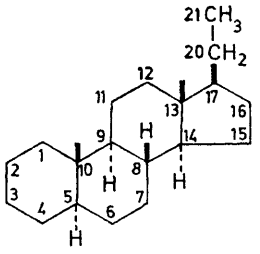
[5] V. E. M. Chambers, W. A. Denny, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, and J. Pragnell, *J.C.S. Perkin I*, 1973, 1500.

seen clearly with *D. celastrina*. When the second group of a 3,*x*-dioxygenated androstane is moved to the 17-position the hydroxylation switches from position 16 to position 9; with 7,17-disubstituted substrates, attack is directed towards the 3-position. Efficient hydroxylation at position 3, like that at position 16, requires a diketone substrate (*cf.* the 7,17-diketone and the 17β-hydroxy-7-ketone), but 9-hydroxylation (Figure 2) is much more satisfactory with the 3β-hydroxy-17-ketone than with the 3,17-diketone. (Androst-4-ene-3,17-dione and progesterone, containing the 3-oxo-$\Delta^4$-system generally regarded as favourable for selective hydroxylation,[3] resemble the saturated 3,17-diketone in giving complex mixtures; no attempt was made to isolate all the products from the conjugated ketones.) These comparisons illustrate the difficulty of predicting the outcome of hydroxylations with fungal metabolites, and show that the many other factors (*e.g.* the ease of cell-wall penetration) as well as the directing effects of the substrates' oxygenated groups must influence the processes.

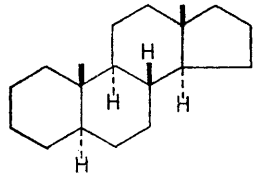
The hydroxylations in Section A suggest that *D. celestrina* has a general propensity for α-face substitution

TABLE 1

Hydroxylations with *Diaporthe celastrina*




5α-Pregnane




5α-Androstane

The substrates, all derivatives of 5α-pregnane and 5α-androstane, are indicated by trivial names, *e.g.* 9α-OH–3-CO represents 9α-hydroxy-5α-androstan-3-one. In the Products columns those oxygen functions introduced in the incubation are in bold type. The substrates were introduced as solutions in ethanol and incubated for the times (usually 4 or 6 days) specified in the Experimental section. The yields are calculated after making allowance for recovered starting material.

Section A Substrates undergoing satisfactory conversions

| Substrate | Substrate recovered | Main hydroxylation product | | | Other products | | | |
|---|---|---|---|---|---|---|---|---|
| 3,7-$(CO)_2$ | 7% | 3β, **16α-** | $(OH)_2$ | 48% | 3β, **16β-** | $(OH)_2$ | | 11% |
| 9α-OH–3-CO | 3 | 3β, **16α-** | $(OH)_2$ | 47 | **16α-** | OH | | 15 |
| 3,11-$(CO)_2$ | 0 | 3β, **16α-** | $(OH)_2$ | 55 | **16α-** | OH | | 18 |
| | | | | | 3β-OH– 11, **16-** | $(CO)_2$ | | 10 |
| 12α-OH–3-CO | 0 | 3β, **16α-** | $(OH)_2$ | 48 | **16β-** | OH | | 19 |
| 3β-OH–17-CO | 0 | **9α-** | OH | 40 | 3-CO–**9α-** | OH | | 15 |
| | | | | | **7α-** | OH | | 12 |
| 7,17-$(CO)_2$ | 0 | **3α-** | OH | 52 | 17β- | OH | | 7 |

Section B Substrates giving low yields of products

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3β-OH–7-CO | 66 | Not investigated | | | | | | |
| 11α-OH–3-CO | 28 | **16α-** | OH | 9 | | | | |
| 3,17-$(CO)_2$ | 6 | 3β, **9α-** | $(OH)_2$ | 6 | **9α-** | OH | | 3 |
| | | | | | 3β, **14α-** | $(OH)_2$ | | 2 |
| | | | | | **15α**,17β- | $(OH)_2$ | | 2 |
| | | | | | 3β,**7α-** | $(OH)_2$ | | 1 |
| 17β-OH–3-CO | 54 | 3β, **9α-**$(OH)_2$–17- | CO | 18 | 3β, **9α-** | $(OH)_2$ | | 9 |
| | | | | | **9α-**OH–17- | CO | | 4 |
| 3,17-$(CO)_2$–$\Delta^4$ | 16 | **6α-** | OH | 7 | **7α-** | OH | | 5 |
| | | | | | **9α-** | OH | | 5 |
| 17β-OH–3-CO–$\Delta^4$ | 12 | **9α-** | OH | 12 | **6β**-OH– **17-** | CO | | 3 |
| | | | | | **9α**-OH–17- | CO | | 3 |
| 3β-OH–17,17-O-O | 20 | **9α**-OH–17 | CO | 8 | **9α**-OH–3,17-$(CO)_2$ | | | 6 |
| 3β-OH–17-CO–$\Delta^5$ | 30 | 3-CO–$\Delta^4$ | | 15 | 3-CO–**7α**-OH–$\Delta^4$ | | | 5 |
| 3,20-$(CO)_2$–$\Delta^4$ | 16 | **6β,9α-** | $(OH)_2$ | 13 | | | | |
| 17β-OH–7-CO | 0 | **3β-** | OH | 26 | **3α**-OH– 17- | CO | | 16 |
| | | | | | **3α-** | OH | | 7 |
| | | | | | **3β**-OH– 17- | CO | | 5 |
| 11,17-$(CO)_2$ | 32 | **7α,** 17β- | $(OH)_2$ | 11 | **7α-** | OH | | 10 |
| 17β-OH–11-CO | 8 | **7α-** | OH | 18 | **7,** 17- | $(CO)_2$ | | 5 |
| 3β,7α-$(OH)_2$–17-CO | 70 | 3- | CO | 56 | | | | |
| 3β-OAc–7,17-$(CO)_2$–$\Delta^5$ | 2 | 3β, 17β- | $(OH)_2$ | 12 | 3β- | OH | | 9 |

TABLE 2

N.m.r. signals

The results, presented in the form used earlier,[a] were determined by examining solutions in $CDCl_3$ at 100 MHz

| No. | Compound | | $\tau_2$ | $\tau_2$ (calc.) | >CH–OR | | and other signals |
|---|---|---|---|---|---|---|---|
| 830 | 5α-Androstane-3,12,16-trione | 19 | 8·85 | 8·85 | | | |
| | | 18 | 8·78 | 8·78 | | | |
| 831 | 12α-Hydroxy-5α-androstan-3-one | 19 | 9·00 | 8·97 | H-12 | 6·17 | t (3) |
| | | 18 | 9·23 | 9·22 | | | |
| 832 | 3β-Hydroxyandrost-5-enc-7,17-dione | 19 | 8·76 | 8·76 | H-3 | 6·30 | 7 (10, 10, 5, 5) |
| | | 18 | 9·11 | 9·11 | H-6 | 4·25 | s |
| 833 | 7,17-Dioxoandrost-5-en-3β-yl acetate | 19 | 8·75 | 8·74 | H-3 | 5·23 | 7 (10, 10, 5, 5) |
| | | 18 | 9·10 | 9·13 | H-6 | 4·20 | s |
| 834 | 6α-Hydroxyandrost-4-ene-3,17-dione | 19 | 8·79 | 8·81 | H-4 | 4·28 | s |
| | | 18 | 9·02 | 9·05 | H-6 | 5·63 | 4 (9, 4) |
| 835 | 7α-Hydroxyandrost-4-ene-3,17-dione | 19 | 8·78 | 8·78 | H-4 | 4·23 | s |
| | | 18 | 9·06 | 9·05 | H-7 | 5·90 | m (8) |
| 836 | 7α-Hydroxy-5α-androstane-11,17-dione | 19 | 8·98 | 8·98 | H-7 | 5·97 | m (6) |
| | | 18 | 9·20 | 9·16 | | | |
| 837 | 9α-Hydroxy-5α-androstane-3,16-dione | 19 | 8·83 | 8·82 | | | |
| | | 18 | 9·08 | 9·07 | | | |
| 838 | 9α-Hydroxyandrost-4-ene-3,17-dione | 19 | 8·65 | 8·66 | H-4 | 4·20 | s |
| | | 18 | 9·07 | 9·03 | | | |
| 839 | 9α-Hydroxypregn-4-enc-3,6,20-trione | 19 | 8·71 | 8·69 | H-4 | 3·65 | s |
| | | 18 | 9·29 | 9·27 | | | |
| 840 | 5α-Androstane-3β,9α-diol | 19 | 9·06 | 9·06 | H-3 | 6·35 | m (15) |
| | | 18 | 9·29 | 9·27 | | | |
| 841 | 3β,17β-Dihydroxyandrost-5-en-7-one | 19 | 8·78 | 8·78 | H-3<br>H-17 | 6·35 | m (18) |
| | | 18 | 9·24 | 9·28 | H-6 | 4·29 | s |
| 842 | 7α,17β-Dihydroxy-5α-androstan-11-one | 19 | 8·99 | 9·00 | H-7 | 5·95 | m (7) |
| | | 18 | 9·32 | 9·30 | H-17 | 6·14 | t (9) |
| 843 | 9α,16α-Dihydroxy-5α-androstan-3-one | 19 | 8·87 | 8·86 | H-16 | 5·52 | m (15) |
| | | 18 | 9·26 | 9·23 | | | |
| 844 | 9α,17β-Dihydroxyandrost-4-en-3-one | 19 | 8·67 | 8·67 | H-4 | 4·16 | s |
| | | 18 | 9·19 | 9·17 | H-17 | 6·30 | t (8) |
| 845 | 12α,16α-Dihydroxy-5α-androstan-3-one | 19 | 9·00 | 8·98 | H-12 | 6·24 | m (8) |
| | | 18 | 9·23 | 9·21 | H-16 | 5·55 | m (14) |
| 846 | 15α,17β-Dihydroxy-5α-androstan-3-one * | 19 | 8·96 | 8·97 | H-15 | 5·82 | 6 (9, 9, 3) |
| | | 18 | 9·23 | 9·21 | H-17 | 6·53 | t (8) |
| 847 | 6β,9α-Dihydroxypregn-4-ene-3,20-dione | 19 | 8·50 | 8·50 | H-4 | 4·09 | s |
| | | 18 | 9·29 | 9·29 | H-6 | 5·64 | t (3) |
| 848 | 5α-Androstane-3β,9α,16α-triol | 19 | 9·07 | 9·07 | H-3 | 6·42 | m (17) |
| | | 18 | 9·28 | 9·26 | H-16 | 5·55 | m (13) |
| 849 | 5α-Androstane-3β,12α,16α-triol † | 19 | 9·20 | | | | |
| | | 18 | 9·25 | | | | |

[a] Ref. 3.
* Not fully characterised. † Solution in $(CD_3)_2SO$.

(Figure 1). This tendency can still be discerned in the less satisfactory conversions (Section B) although there are exceptions, notably the hydroxylation of 17β-hydroxy-5α-androstan-3-one which gives an epimeric mixture of 3-alcohols with the 3β-isomer predominating.

TABLE 3

Characterisation of new compounds

| Compound | M.p. (°C) (cryst. solvent) | $[\alpha]_D$ (°) * (c) | | Analyses (%) C | H |
|---|---|---|---|---|---|
| 5α-Androstane-3,12,16-trione | 208—210 (Me₂CO–hexane) | −79 (0·65) | Found<br>$C_{19}H_{26}O_3$ req. | 75·6<br>75·5 | 8·4<br>8·7 |
| 9α-Hydroxy-5α-androstan-3-one | 149—150 (MeOH) | +8 (0·9) | Found<br>$C_{19}H_{30}O_2$ req. | 78·7<br>78·6 | 10·6<br>10·4 |
| 7α-Hydroxy-5α-androstane-11,17-dione | 186—188 (Me₂CO–hexane) | +115 (1·0) | Found<br>$C_{19}H_{28}O_3$ req. | 74·9<br>75·0 | 9·3<br>9·3 |
| 9α-Hydroxy-5α-androstane-3,16-dione | 182—184 (Me₂CO–hexane) | −157 (0·35) | Found<br>$C_{19}H_{28}O_3$ req. | 74·8<br>75·0 | 9·2<br>9·3 |
| 9α-Hydroxypregn-4-ene-3,6,20-trione | 188—190 (Me₂CO–hexane) | −7 (0·1) | Found<br>$C_{21}H_{28}O_4$ req. | 73·2<br>73·2 | 8·1<br>8·2 |
| 5α-Androstane-3β,9α-diol | 164—166 (Me₂CO–hexane) | −116 (1·0) | Found<br>$C_{19}H_{32}O_2$ req. | 78·3<br>78·0 | 11·1<br>11·0 |
| 7α,17β-Dihydroxy-5α-androstan-11-one | 132—133 (Me₂CO–hexane) | +47 (0·7) | Found<br>$C_{19}H_{30}O_3$ req. | 74·7<br>74·5 | 9·7<br>9·9 |
| 9α,16α-Dihydroxy-5α-androstan-3-one | 245—246 (MeOH) | −7† (0·1) | Found<br>$C_{19}H_{30}O_3$ req. | 74·3<br>74·5 | 9·9<br>9·9 |
| 12α,16α-Dihydroxy-5α-androstan-3-one | 228—231 (MeOH) | +47 (0·25) | Found<br>$C_{19}H_{30}O_3$ req. | 74·7<br>74·5 | 9·9<br>9·9 |
| 6β,9α-Dihydroxypregn-4-ene-3,20-dione | 212—214 and 246—248 (sublimed) | +85 (0·85) | Found<br>$C_{21}H_{30}O_4$ req. | 72·8<br>72·8 | 8·6<br>8·7 |
| 5α-Androstane-3β,9α,16α-triol | 194—195 (Me₂CO–hexane) | −17† (1·0) | Found<br>$C_{19}H_{32}O_3$ req. | 73·7<br>74·0 | 10·3<br>10·5 |
| 5α-Androstane-3β,12α,16α-triol | 213—215 (MeOH) | +27 (0·1) | Found<br>$C_{19}H_{32}O_3$ req. | 74·1<br>74·0 | 10·4<br>10·5 |

* In $CHCl_3$ unless otherwise indicated. † In MeOH.

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