# Hydroxylation of Nine Simple Steroid Ketones (Mono-oxo-5α-androstanes) with Cultures of the Fungus *Calonectria decora*

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THE vast literature on the microbiological hydroxylation of steroids[1] refers almost entirely to substrates having an oxygenated substituent at C-3. We have been examining a range of micro-organisms using as substrates a series of mono- and di-oxygenated 5α-androstanes in which the positions of the substituents around the steroid nucleus have been varied systematically. The first set of experiments,[2] involving hydroxylation of mono-oxo-compounds with *Calonectria decora*, can now be described: their relevance to recent work with other alicyclic substrates[3] prompts this preliminary account of our studies.

The incubations were carried out in flasks which were swirled at 22° for the times specified (see Table). Each flask contained a culture of *Calonectria decora* growing vigorously in a corn-steep nutrient (80 ml.), to which the steroid (40 mg.) was added as a solution in ethanol or dimethyl sulphoxide (6 ml.). All the products shown are new compounds. A combination of chemical transformations and spectrometric examination established the positions and configurations of the hydroxy-groups.

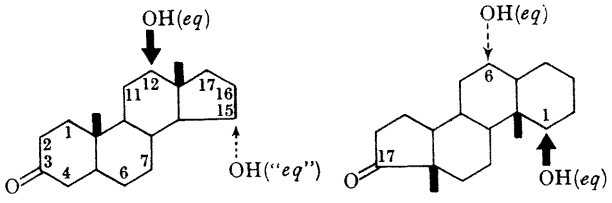
The androstanones vary considerably in their behaviour. Thus, some are easily hydroxylated while others are largely unchanged; some give complex mixtures of products, but others lead cleanly to one or two products in reasonable yield. (Allowing for recovered substrates, the yields in 5 cases are 40—80%.) The predilection for dihydroxylation is in line with previous work with *Calonectria decora*.[4] In general, two equatorial hydroxy-groups are introduced,

*Incubation of* 5α-*androstan*-x-*ones with* Calonectria decora

[In the 'result' column, the figures in brackets are percentages of the products or starting material (s.m.) isolated]

| *x* | Solvent | Time (days) | Result |
|---|---|---|---|
| 1 | $Me_2SO$ | 6 | Complex mixture (not separated) + s.m. (75) |
| 2 | $Me_2SO$ | 4 | 6α,12β-$(OH)_2$-2-one (23) + 6α,11α-$(OH)_2$-2-one (11) + s.m. (13) |
| 3 | EtOH | 2 | 12β,15α-$(OH)_2$-3-one (40) + 3β,12β,15α-$(OH)_3$ (7) + s.m. (22) |
| 4 | $Me_2SO$ | 4 | 12β,15α-$(OH)_2$-4-one (41) + 11α,15α-$(OH)_2$-4-one (40) |
| 6 | $Me_2SO$ | 6 | s.m. (90) |
| 7 | $Me_2SO$ | 7 | 12β,15α-$(OH)_2$-7-one (4) + s.m. (72) |
| 11 | EtOH | 2 | Complex mixture (separated and components identified) + s.m. (40) |
| 16 | $Me_2SO$ | 4 | 6α,11α-$(OH)_2$-16-one (26) + 1β,6α-$(OH)_2$-16-one (7) + s.m. (31) |
| 17 | EtOH | 2 | 1β,6α-$(OH)_2$-17-one (42) + s.m. (39) |

either in positions 15 and 12 (or 11) or in positions 6 and 1 (or 11 or 12). Two extreme hypotheses can be considered in attempting to rationalise these results. It may be that this micro-organism has an inherent preference for hydroxylating certain steroid positions [here 1 (or 11), 6, 12 (or 11), and 15]. A particular oxo-group's influence would then be merely to modify the relative extents to which these positions are attacked. (An oxo-group would block its own site, and might also influence the reactivities of neighbouring positions.) A radically different possibility is that the position of the oxo-group is the main factor in determining which sites are hydroxylated. For example, the results with the 3(and 4)- and 17(and 16)-oxo-5α-androstanes could be construed as indicating a geometrical relationship between the oxygen function in the starting material and the hydroxy-groups in the product. (This interpretation is similar to the enxyme–substrate model envisaged by the Upjohn group to explain their results on the hydroxylation of macrocyclic alcohols.[3]) Our experience with mono- and di-oxygenated androstanes suggests that neither extreme view is tenable, and a generalised treatment must await new experimental work.

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[1] *Inter al.*, "Microbial Transformations of Steroids," W. Charney and H. L. Herzog, Academic Press, New York, 1967, the most comprehensive of many reviews.

[2] *cf.* J. E. Bridgeman, P. C. Cherry, W. R. T. Cottrell, Sir Ewart R. H. Jones, P. W. LeQuesne, and G. D. Meakins, *Chem. Comm.*, 1966, 561; P. C. Cherry, Sir Ewart R. H. Jones, and G. D. Meakins, *ibid.*, p. 587.

[3] R. A. Johnson, M. E. Herr, H. C. Murray, and G. S. Fonken, *J. Org. Chem.*, 1968, **33**, 3217, and previous papers.

[4] A. Schubert and R. Siebert, *Chem. Ber.*, 1958, **91**, 1856.