Microbiological Hydroxylation at Position 3 of Androst-5-en-7-one

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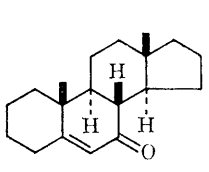
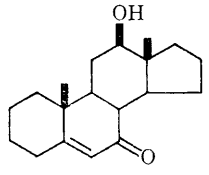
THE direct introduction of an oxygen function into 3-deoxy-steroids and related substances is of interest, *inter alia*, in connection with simplified steroid syntheses. The multifarious microbiological oxidations of steroids have been effected almost entirely with substrates already carrying oxygen at position 3.[1] To our knowledge the only exception[2] is a 3,5-cyclo-compound which was found to hydroxylate at the 11-position. With terpenoids there are two examples of hydroxylation at C-3: the conversion[3] of kaurene into giberellic acid involves introduction of a 3-hydroxyl group as part of a complex transformation, while a significant, direct 3-hydroxylation of lanosta-8,24-diene by a cell-free yeast extract has just been reported.[4]

We have examined the oxygenation of 3-deoxy-androstane derivatives using a range of micro-organisms. With *Calonectria decora* there were indications that androst-5-en-7-one (I) might be a promising substrate, particularly as the product of 3-hydroxylation, a vinylogue of a β-hydroxy-ketone, would undergo easy dehydration to a conjugated dienone.

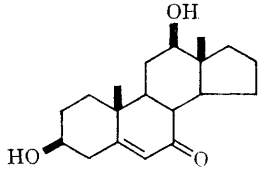
Androst-5-en-7-one, from androsta-3,5-dien-7-one[5] by partial hydrogenation on incubation for two days gave a mixture of hydroxy-ketones. In the monohydroxy-ketone fraction one compound (*A*) predominated, but the dihydroxy-ketone material consisted of several isomers, two of which (compounds *B* and *C*) were obtained pure after preparative layer chromatography. Compound (*A*) is



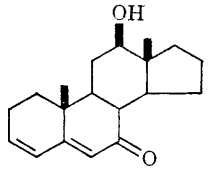
(I)



(II) Compound *A*



(III) Compound *B*



(IV)

the 12β-hydroxy-Δ^5-7-ketone (II). This structure, suggested originally from n.m.r. examination (C-19 and C-18 protons' signals at τ 8·79 and 9·26; 12α-hydrogen signal, a characteristic distorted quartet with half-height width 18 c./sec. at τ 6·57) and other spectroscopic features, was confirmed by similar study of the derived diketone. (The C-18 protons' signal of the latter at τ 8·94 is strong evidence for a 12-oxo-group.[6]) For compound (*B*) the 3β, 12β-dihydroxy-structure (III) emerged from spectroscopic examination. Support was adduced from the transformation of the diacetate with methanolic potassium hydroxide into the 12β-hydroxy-$\Delta^{3,5}$-7-ketone (IV) showing ultraviolet absorption at 2770 Å (ϵ, 21,500). Product (*C*) is provisionally formulated as the 4β,12β-dihydroxy-Δ^5-7-ketone.

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[1] For summaries see: C. Tamm, *Angew. Chem. Internat. Edn.*, 1962, **1**, 178; L. M. Kogan, *Russ. Chem. Rev.*, 1962, **31**, 294; P. H. Goll, *Process Biochem.*, 1966, 201.
[2] W. J. Wechter and H. C. Murray, *Chem. and Ind.*, 1962, 411; Y. Kurosawa, *Chem. Abs.*, 1959, **53**, 11510.
[3] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1964, 295; J. E. Graebe, D. T. Dennis, C. D. Upper, and C. A. West, *J. Biol. Chem.*, 1965, **240**, 1847.
[4] D. H. R. Barton and G. P. Moss, *Chem. Comm.*, 1966, 261.
[5] R. Beugelmans, R. H. Shapiro, L. J. Durham, D. H. Williams, H. Budzikiewicz and C. Djerassi, *J. Amer. Chem. Soc.*, 1964, **86**, 2832.
[6] R. F. Zürcher, *Helv. Chim. Acta*, 1963, **46**, 2054.