

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

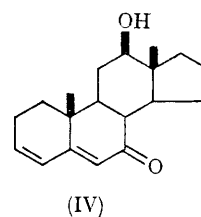
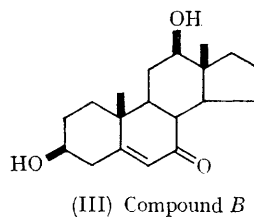
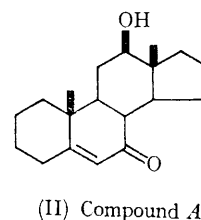
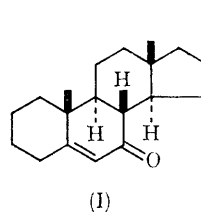
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THE direct introduction of an oxygen function into 3-deoxy-steroids and related substances is of interest, *inter alia*, in connection with simplified steroid syntheses. The multifarious microbiological oxidations of steroids have been effected almost entirely with substrates already carrying oxygen at position 3.¹ To our knowledge the only exception² 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 conversion³ 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 microorganisms. 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-one⁵ 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

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.

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⁴ D. H. R. Barton and G. P. Moss, *Chem. Comm.*, 1966, 261.

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⁶ R. F. Zürcher, *Helv. Chim. Acta*, 1963, **46**, 2054.