

The Preparation of 1 β ,11 α -Dihydroxy-steroids by Microbiological Hydroxylation

By A. S. CLEGG, SIR EWART R. H. JONES, G. D. MEAKINS,* and J. T. PINHEY
(Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY)

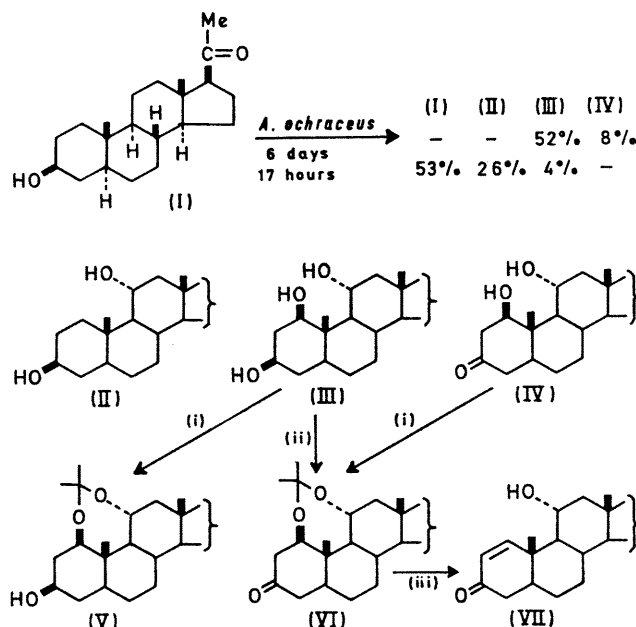
Summary Incubation of 3 β -hydroxy-5 α -pregnan-20-one with the fungus *Aspergillus ochraceus* gives the 1 β ,11 α -dihydroxy-derivative in 52% yield.

THE extensive literature on the fungus *Aspergillus ochraceus* shows that it generally introduces one hydroxy-group into the steroid nucleus, producing 11 α -hydroxy-compounds from a wide range of oxygenated substrates.¹ If access to the 11-position is impeded, or if the size of the 13-alkyl group is increased, hydroxylation occurs at alternative sites.^{1,2} With another fungus, *Calonectria decora*, there is a predilection for dihydroxylation to give products in which the hydroxy-groups are well separated from each other.³ From these and numerous similar observations it might be inferred that the presence of one oxygen group (whether originally present in the substrate or introduced micro-biologically) inhibits entry of a second group into a position which brings the two groups into close proximity. Thus, 11 α - or 1 β -hydroxylation by *Absidia orchidis* have been regarded as mutually exclusive.^{3c}

We now report that incubation of 3 β -hydroxy-5 α -pregnan-20-one (I) with *A. ochraceus* gives the 1 β ,11 α -dihydroxy-product (III) in satisfactory yield. [The conditions³ are reasonably forcing, but not unusual. Each incubation flask contained a culture of the fungus growing vigorously in a corn-steep nutrient (200 ml), to which the steroid (40 mg) was added as a solution in dimethyl sulphoxide. The flasks were swirled at 25° for 6 days.] The product (III) is readily converted into an acetonide (V) whose formulation as a 1,11-isopropylidenedioxy-compound follows from n.m.r. examination:⁴ the remarkable stability of this derivative (which is unchanged by boiling with 2N-hydrochloric acid in dioxan) is paralleled by the very strong 1,11-hydrogen bonding of the parent compound (ν_{\max} 3320 cm⁻¹). Treatment of the 3-keto-acetonide (VI) with acid afforded the known 11 α -hydroxy-diketone (VII)⁵ by the expected β -elimination of the 1-alkoxy-group.

The product (III) is formed by a sequential rather than by a concerted introduction of the two hydroxy-groups. A short hydroxylation period gives mainly the mono-(11 α)-hydroxy-derivative (II);⁶ incubation of this compound leads to the dihydroxy-product (III). The results do not fit the kinetic expression for normal consecutive reactions. For example, in parallel experiments using the starting material (I) and the monohydroxy-derivative (II),

the former gives the product (III) more quickly. We incline to the view that enzyme induction⁷ occurs in the present system, and that the rates of the reactions depend upon the speed with which the required enzymes are produced.



Reagents i, H₂SO₄-Me₂CO; ii, H₂CrO₄-Me₂CO; iii, 2N-HCl-dioxan, reflux.

The course of the hydroxylation of 3 β -hydroxy-20-oxo-5 α -pregnanes with *A. ochraceus* is profoundly influenced by structural variations in the substrate. Under the conditions used here 1 β ,11 α -dihydroxylation occurs with the 16 β -methyl derivative, the 16-17-olefin, and the related 16 α ,17 α -epoxide, but not with the 17 α -alcohol or the 16-methyl-16,17-olefin. The present work provides an easy route to 1-dehydro-3-oxo-11 α -hydroxy-compounds, and applications to the preparation of physiologically active compounds can be envisaged.

(Received, June 8th, 1970; Com. 878.)

¹ W. Charney and H. L. Herzog, "Microbial Transformations of Steroids," Academic Press, New York, 1967.

² T. Okumura, Y. Nozaki, and D. Satoh, *Chem. and Pharm. Bull. (Japan)*, 1962, 12, 1143; L. L. Smith, G. Greenspan, R. Rees, and T. Foell, *J. Amer. Chem. Soc.*, 1966, 88, 3120.

³ (a) A. Schubert and R. Siebert, *Chem. Ber.*, 1958, 91, 1856; (b) J. E. Bridgeman, J. W. Browne, P. C. Cherry, M. G. Combe, J. M. Evans, E. R. H. Jones, A. Kasal, G. D. Meakins, Y. Morisawa, and P. D. Woodgate, *Chem. Comm.*, 1969, 463; (c) V. Schwarz, M. Ulrich, and K. Syhora, *Steroids*, 1964, 4, 645.

⁴ J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, E. R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, Y. Morisawa, E. E. Richards, and P. D. Woodgate, *J. Chem. Soc. (C)*, 1970, 250.

⁵ C. Meystre, J. Kalvoda, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, 1963, 46, 2844.

⁶ O. Mancera, J. Romo, F. Sondheimer, G. Rosenkranz, and C. Djerassi, *J. Org. Chem.*, 1952, 17, 1066.

⁷ M. Shibahara, J. A. Moody, and L. L. Smith, *Biochim. Biophys. Acta*, 1970, 202, 172.