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

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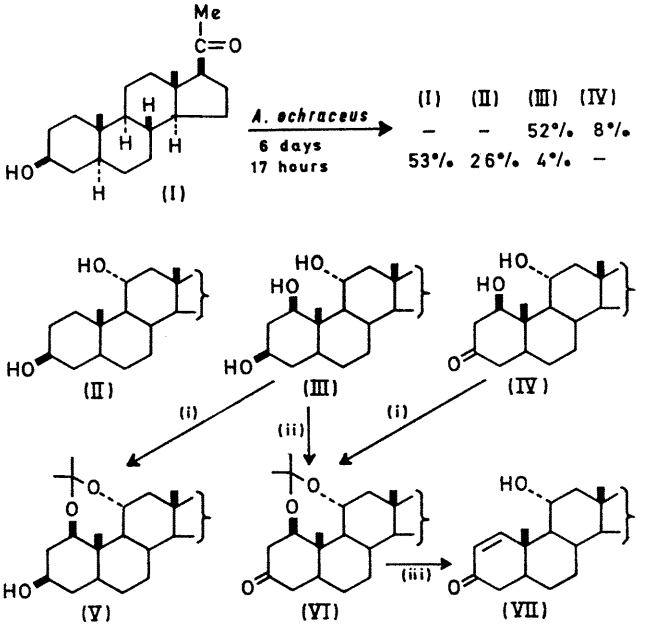
Summary Incubation of 3β-hydroxy-5α-pregnan-20-one with the fungus *Aspergillus ochraceus* gives the 1β,11α-dihydroxy-derivative in 52% yield.

THE extensive literature on the fungus *Aspergillus ochraceus* shows that it generally introduces one hydroxy-group into the steroid nucleus, producing 11α-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 microbiologically) inhibits entry of a second group into a position which brings the two groups into close proximity. Thus, 11α- or 1β-hydroxylation by *Absidia orchidis* have been regarded as mutually exclusive.[3c]

We now report that incubation of 3β-hydroxy-5α-pregnan-20-one (I) with *A. ochraceus* gives the 1β,11α-dihydroxy-product (III) in satisfactory yield. [The conditions[3] 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 11α-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-(11α)-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 induction[7] 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, H_2SO_4–Me_2CO; ii, H_2CrO_4–Me_2CO; iii, 2N-HCl–dioxan, reflux.

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

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[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.