Microbial 1 β -Hydroxylation of Digitoxigenin

Sir:

In recent years cardiac aglycones of *Digitalis* have been studied by several investigators as the substrates in the microbial transformations of steroids and thus far hydroxylations of digitoxigenin at positions 12 β ^{1,2)}, 16 β ³⁾, 6 β ⁴⁾, 11 α ⁴⁾, 7 β ⁵⁾, and 5 β ⁶⁾ have been described. In continuing our studies on the microbial conversion of cardiac aglycones, we now wish to report the formation of 1 β -hydroxydigitoxigenin (=acovenosigenin A⁷⁾) from digitoxigenin by a strain of *Absidia orchidis* (Vuill.) Hagem.

Incubation of digitoxigenin (I) for 5 days with *A. orchidis* grown in a medium containing glucose, peptone and corn steep liquor (30 mg of I per 100 ml of broth) and extraction of the resultant fermentation liquor (pH 6.2) with chloroform afforded, after chromatography on alumina, a monohydroxydigitoxigenin C₂₃H₃₄O₅ (II), m.p. 276~282°C (decomp.) (17.1% of added I); $[\alpha]_D^{30} + 3.2 \pm 2^\circ$ (methanol); $\lambda_{\max}^{\text{Nujol}}$ 2.84, 2.92, 5.56, 5.77, 6.12 μ ; diacetate (III), m.p. 220~223°C; $[\alpha]_D^{28} + 1.2 \pm 2^\circ$ (acetone). The position in which a hydroxyl group was introduced could be reasonably inferred to be C-1 since the oxidation of II with Pt and O₂ followed by dehydration in boiling glacial acetic acid gave 1 β -digitoxigenone (IV), m.p. 220~232°C;

$\lambda_{\max}^{\text{EtOH}}$ 235m μ (log ϵ 3.91)*, identified by comparison with an authentic specimen⁸⁾. Moreover, II was identical in every respect with an authentic sample of acovenosigenin A kindly furnished to us by Prof. T. Reichstein. In addition to II, the presence of 7 β -hydroxydigitoxigenin (V) and 5 β -hydroxydigitoxigenin (=periplogenin) (VI) in chloroform-methanol (100:1) eluates was assumed by paper chromatographic analysis. R_F 0.48, 0.34, 0.30 (benzene-ethylene dichloride (3:7)**); R_F 0.70, 0.55, 0.50 (toluene-butanol (3:1)**); acetates: R_F 0.52, 0.18, 0.32 (*n*-heptane-methyl ethylketone (1:1)**), which were in good agreement with the R_F values of authentic samples of V, VI and II and with those of their acetates, respectively.

From the fractions eluted with chloroform-methanol (50:1) and (25:1) were obtained two unknown dihydroxylated derivatives of I, C₂₃H₃₄O₆, with m.p. 244~254°C (decomp.) and m.p. 249~250°C (decomp.), respectively. Prolonged incubation of I with *A. orchidis* resulted in the formation of increased amounts of the dihydroxylated derivatives. The determination of structures and configurations of these two compounds is now being made and will be submitted for publication in near future.

The authors wish to express their sincere thanks to Dr. D. Satoh of this laboratory for

1) A. Gubler and Ch. Tamm, *Helv. Chim. Acta*, **41**, 297 (1958).

2) M. Okada, et al., *Chem. Pharm. Bull.*, **8**, 530 (1960).

3) H. Nawa, et al., *Nature*, **184**, 469 (1959).

4) E. Titus, et al., *J. Biol. Chem.*, **235**, 3399 (1960).

5) H. Ishii, et al., *Yakugaku Zasshi*, **80**, 1150 (1960).

6) H. Ishii, *ibid.*, **81**, 153 (1961).

7) J. v. Euw and T. Reichstein, *Helv. Chim. Acta*, **33**, 485 (1950).

* Calculated by subtracting the absorption of unsaturated lactone ring at 218 m μ from the U. V. of III, $\lambda_{\max}^{\text{EtOH}}$ 221 m μ (log ϵ 4.36).

** Formamide-acetone (1:4) mixture was used as the stationary phase for these mobile phases.

8) D. Satoh and T. Wada, *Yakugaku Zasshi*, **9**, 1314 (1960).

his helpful suggestions and encouragement in carrying out this work.

Yoshio NOZAKI
Tamotsu OKUMURA

Research Laboratory,
Shionogi & Co., Ltd.
192, Imafuku
Amagasaki, Hyogo.

Received April 10, 1961