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Hydroxylation of Digitoxigenin Derivatives by *Absidia orchidis*

In the previous paper¹⁾ we reported the hydroxylation of digitoxigenin (I) at 1 β , 5 β , and 7 β positions by *Absidia orchidis* (VUILL.) HAGEM, a microorganism known to hydroxylate progesterone (II) at 6 β , 7 α , and 11 α ,²⁾ and Reichstein's substance S (III) at 6 β , 11 α , and 11 β positions.³⁾ In order to investigate the relationships between the structure of substrate molecules and the positions to be hydroxylated, we carried out the transformation of 3 β ,14,21-trihydroxy-14 β -pregnan-20-one (IVa) and 4,5-dehydrodigitoxigenone (V) using this microorganism with the results that the former compound is hydroxylated at 1 β and the latter mainly at 7 β and 12 β positions.

A. orchidis was grown for 66 hours with shaking on a nutrient medium containing glucose, peptone and corn steep liquor, the mycelium harvested, washed and suspended in distilled water. The substrate (IVb) dissolved in methanol was added to this mycelium suspension and incubation was continued for a further 48 hours. After usual treatment of the fermentation filtrate, a monohydroxylated product (VIa), C₂₁H₃₄O₅, m.p. 252~263°, was obtained. As this substance gave a triacetate (VIb), C₂₇H₄₀O₈, m.p. 158~162°, $[\alpha]_D^{25}$ -0.4° (pyridine), the newly introduced hydroxyl was considered to be a secondary or a primary one, and proved to be at 1 β -position on identification of VIb with ketol triacetate derived from acovenosigenin A (VIII).

While the usual bioconversion performed by us of 4,5-dehydrodigitoxigenone (V) with *A. orchidis* produced several kinds of product, the use of suspensions of mycelium preincubated with progesterone⁴⁾ gave one of them predominantly. This compound (IXa), C₂₃H₃₀O₆, m.p. 287~290°, $[\alpha]_D^{25}$ +66.7° (pyridine), UV: $\lambda_{\max}^{\text{EtOH}}$ 226 m μ *¹ (log ϵ 4.32), gave a diacetate (IXb) C₂₇H₃₄O₈, m.p. 267~268°, $[\alpha]_D^{25}$ +52.1° (pyridine), showing that both of the newly introduced hydroxyls are secondary or primary. Treatment of IXa with 1% hydrochloric acid in acetone afforded a $\Delta^{4,6}$ -3-ketone (X), C₂₃H₂₈O₅, m.p. 279~283°, UV: $\lambda_{\max}^{\text{EtOH}}$ 283 m μ (log ϵ 4.40). This fact and the unsuccessful reduction of IXa with zinc dust in acetic acid indicated that one of the hydroxyls introduced into ring B was at 7-position

*¹ The appearance of absorption band at this wave length is due to overlapping of an absorption for α,β -unsaturated ketone at 241 m μ and that of unsaturated lactone at 217 m μ .

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and that no hydroxyl was present in ring A. Oxidation of IXa with chromium trioxide in acetone gave a diketone (XI), $C_{23}H_{28}O_8$, m.p. $272\sim 275^\circ$, $[\alpha]_D^{26.5} +111.8^\circ$ (pyridine), the infra-red spectrum of which exhibited a new absorption band for six-membered ring ketone in addition to the bands for conjugated ketone. Treatment of this diketone with dilute acid in a similar way as described above gave a compound having ultraviolet absorption

maximum for $\Delta^{4,6}$ -3-ketone. The C-7 hydroxyl of IXa was not oxidized under the above-mentioned condition. These results suggested that another new hydroxyl introduced may be located at 11 or 12-position. Partial hydrogenation of X with paradium-charcoal resulted in selective saturation of 6,7-double bond giving a Δ^4 -3-ketone (XII), $C_{23}H_{30}O_6$, m.p. 263~269°, UV: $\lambda_{\max}^{EtOH}$ 226 m μ *¹ (log ϵ 4.32). XII was shown to be identical with the Δ^4 -3-ketone derived from digoxigenin (XIII) by comparison of their melting points, infrared spectra and mobilities in TLC. In this way the new hydroxyl introduced into ring C was clarified to be at 12 β -position. The configuration of the hydroxyl at 7-position of IXa was confirmed to be β by preparing a cyclocarbonate (XIV), $C_{24}H_{26}O_7$, m.p. 294~300° (decomp.), $[\alpha]_D^{25} +69.1^\circ$ (pyridine) from XI.

Furthermore, we isolated a small amount of 7 β -hydroxy derivative (XV) in the microbiological transformation of V. The structure investigations on other by-products are now being made.

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Choline in *Panax ginseng* C. A. MEYER

Roots of *Panax ginseng* C. A. MEYER has been used as a valuable Chinese drug from ancient times, but pharmacologically effective components of this drug have been yet actually unknown.

Taking a great interest in Petkov's report¹⁾ that alcoholic extract of ginseng roots has a marked hypotonic action on blood pressure which is completely suppressed by atropine, the authors attempted to separate this effective components in parallel with the hypotonic tests on rabbits.

Effective hypotonic substances were extracted with methanol or ethanol. This extracts, obtained with yield of 20~23% weight from the crude drug, were dissolved in water and passed through the column of IRC-50(H-type). On treatment of this column it can be presumed that this effective substances are chemically basic. On treatment of this column with diluted hydrochloric acid, the hypotonic fraction was collected into early eluted fraction. This fraction was neutralized and concentrated. The saturated solution of Reinecke salt was added, and the Reineckate of the effective quaternary ammonium base was obtained at 0.4~0.5% weight of the crude drug. This Reineckate was purified with alumina-celite 535 (1:1) chromatography and a pure Reineckate(I), m.p. 262~265° (decomp.), was obtained. Following the usual procedure, I was decomposed with silver sulfate and treated with bariumchloride, colorless and hygroscopic crystals of this chloride were obtained. The infrared spectra of this chloride and choline chloride were indicated in Figs. 1 and 2, respectively. This chloride and choline chloride have a same R_f value on paper chromatogram [for example, R_f=0.27; solvent: *sec*-butanol-pyridine-water (10:3:4); location reagent: Dragendorff reagent].

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