Our deep gratitude is expressed to Mr. M. Kodama, Tohoku University, for his cooperation in some experiments, to Prof. J. B. Stothers, University of Western Ontario, for NMR measurement, and to Prof. C. Djerassi, Stanford University, for mass spectra.

Department of Chemistry,
Tohoku University,
Sendai.

Shô Itô (伊東 椒)
Michiharu Kato (加藤紀元)
Koichi Shibata (柴田耕一)
Tetsuo Nozoe (野副鉄男)

Received June 25, 1963
Revised July 23, 1963

[Chem. Pharm. Bull. 11 (10) 1340 ~ 1342]

UDC 547.918 : 615.711.5

Hydroxylation of Digitoxigenin Derivatives by *Absidia orchidis*

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

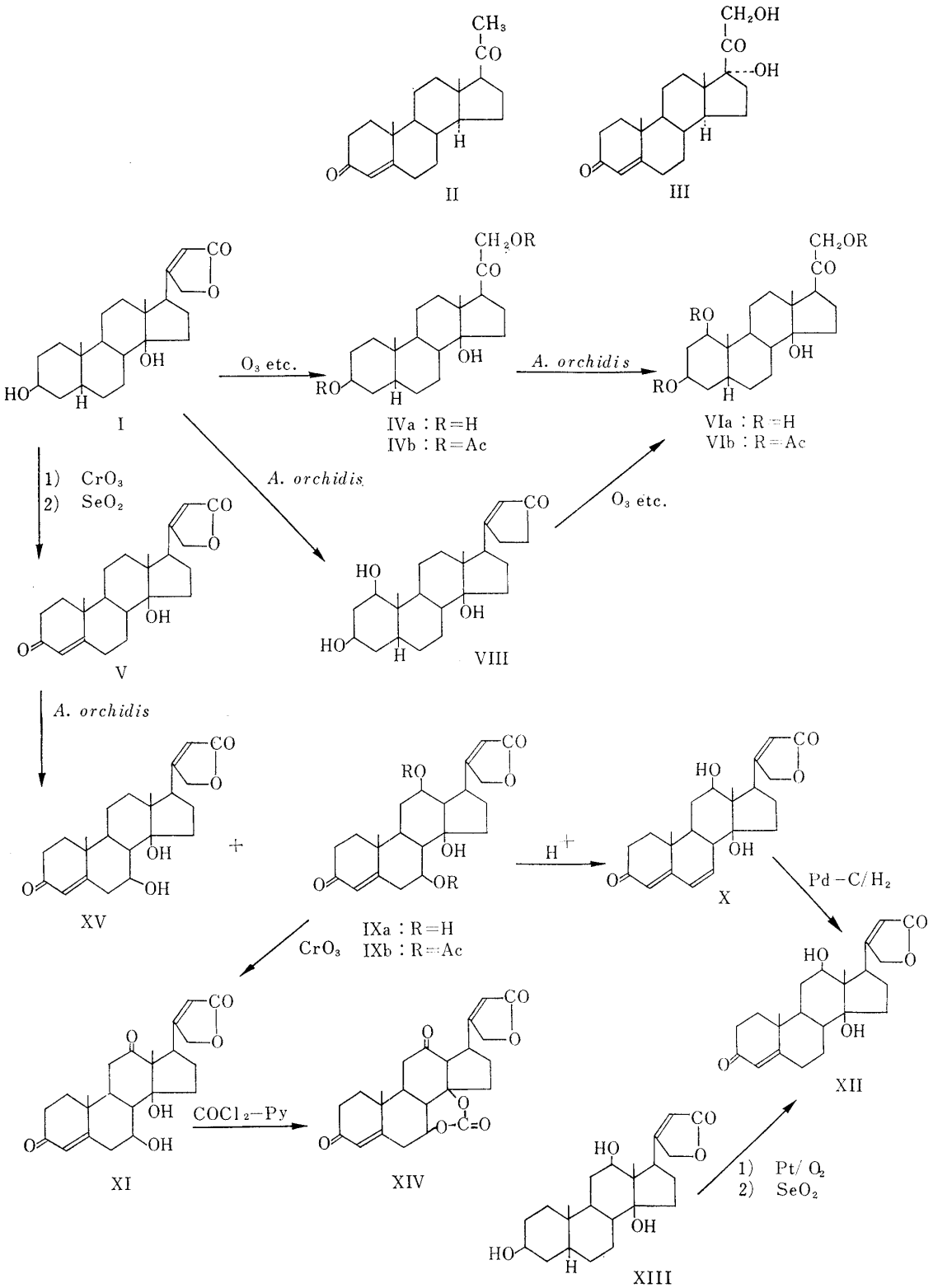
A. orchidis was grown for 66 hours with shaking on a nutrient medium containing glucose, peptone and corn steep liquor, the mycelium harvested, washed and suspended in distilled water. The substrate (IVb) dissolved in methanol was added to this mycelium suspension and incubation was continued for a further 48 hours. After usual treatment of the fermentation filtrate, a monohydroxylated product (VIa), $C_{21}H_{34}O_5$, m.p. 252~263°, was obtained. As this substance gave a triacetate (VIb), $C_{27}H_{40}O_8$, 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 1β-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 progesterone[4] gave one of them predominantly. This compound (IXa), $C_{23}H_{30}O_6$, m.p. 287~290°, $[\alpha]_D^{25}$ +66.7° (pyridine), UV : λ_{max}^{EtOH} 226 mμ*1 (log ε 4.32), gave a diacetate (IXb) $C_{27}H_{34}O_8$, 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), $C_{23}H_{28}O_5$, m.p. 279~283°, UV: λ_{max}^{EtOH} 283 mμ (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 mμ and that of unsaturated lactone at 217 mμ.

1) Y. Nozaki, T. Okumura : Agr. Biol. Chem., **25**, 515 (1961); Y. Nozaki : *Ibid.*, **25**, 884; 879 (1961); H. Ishii, Y. Nozaki, T. Okumura, D. Satoh : This Bulletin, **11**, 156 (1963).
2) Y. Nozaki, H. Ishii, T. Okumura : Collection of Lectures Commemorating the Inauguration of the New Shionogi Research Laboratory Building, p. 227 (1963).
3) Y. Nozaki, E. Masuo, H. Ishii, T. Okumura, D. Satoh : Ann. Rept. Shionogi Research Lab., **11**, 9 (1961); O. Hanc, A. Capek, B. Kakac : Folia Microbiologica, **6**, 392 (1961).
4) Y. Nozaki, E. Masuo, D. Satoh : Agr. Biol. Chem., **26**, 399 (1962).

and that no hydroxyl was present in ring A. Oxidation of IXa with chromium trioxide in acetone gave a diketone (XI), $C_{23}H_{28}O_6$, m.p. 272~275°, $[\alpha]_D^{26.5}$ +111.8° (pyridine), the infrared 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), $C_{23}H_{30}O_5$, m.p. 263~269°, UV : λ_{max}^{EtOH} 226 mμ*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 12β-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.), $[\alpha]_D^{26.5}$ +69.1° (pyridine) from XI.

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

Research Laboratory,
Shionogi & Co., Ltd.,
Fukushima-ku, Osaka.

Tamotsu Okumura (奥村 保)
Yoshio Nozaki (野崎義雄)
Daisuke Satoh (佐藤大助)

Received July 29, 1963

[Chem. Pharm. Bull 11 (10) 1342 ~ 1343]

UDC 615.711.76 : 582.892

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 report[1] 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 Rf value on paper chromatogram [for example, Rf=0.27; solvent : *sec*-butanol-pyridine-water (10:3:4) ; location reagent : Dragendorff reagent].

1) Arzneimittel Forschung, **9**, 305 (1959).