Institute of Applied Microbiology,
University of Tokyo,
Yayoi-cho, Bunkyo-ku, Tokyo
Kyosuke Tsuda (津田恭介)

Takamine Research Laboratory,
Sankyo Co., Ltd.,
Nishi-Shinagawa, Shinagawa-ku, Tokyo
Masaaki Kawamura (河村正明)
Ryoichi Hayatsu (早津了一)

February 14, 1958

UDC 547.92 : 576.8.095

Oxidation of Steroids by Microörganisms. 19-Hydroxylation of Reichstein's Compound S by *Corticium sasakii*

It has been announced that enzymatic hydroxylation of steroids at 19-position is effected in adrenal homogenate,[1] but there is no report that this reaction was conducted by microörganisms.[2] In the present paper we wish to report microbiological hydroxylation of Reichstein's compound S (4-pregnene-17α,21-diol-3,20-dione) (I) at the 19-position by *Corticium sasakii.*

In a previous communication,[3] Hasegawa, Takahashi, Nishikawa, and Hagiwara reported that Corticium had effected the transformation of (I) into hydrocortisone (II), 11-epihydrocortisone (III), and an unidentified monohydroxy compound S (IV), which showed the following constants: m.p. 233~236°, $[\alpha]_D^{27}$ $+127°$(dioxane); $+144°$(EtOH); λ_{max}^{EtOH} 243.5 mμ (ε 15,500).

We found that (IV) was identical with 19-hydroxy compound S, which was produced by incubation of steroids in adrenal homogenate.[4] The structure of (IV) was established by the following reactions.

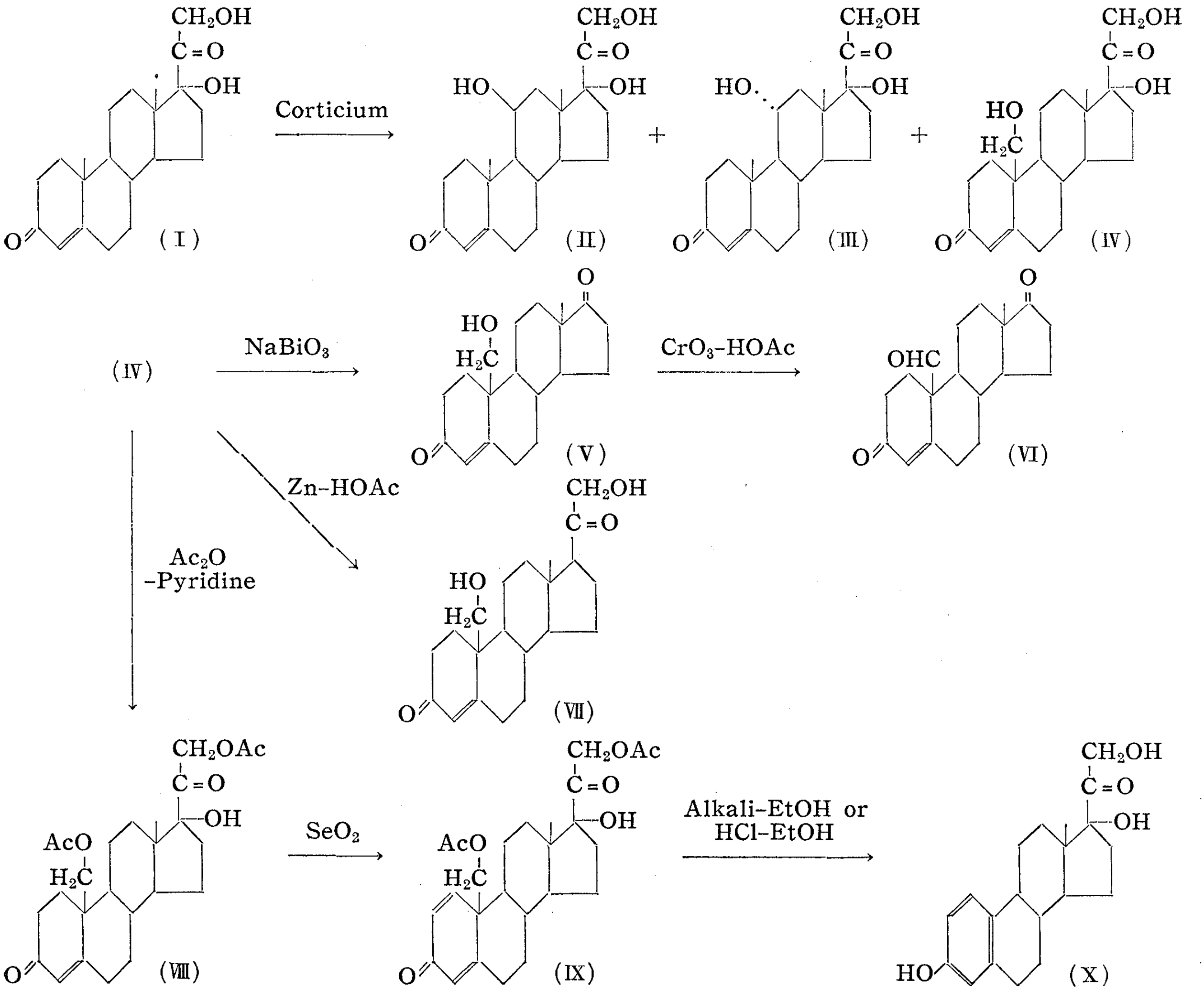
Oxidation of (IV) with sodium bismuthate gave 19-hydroxy-4-androstene-3,17-dione[5] (V), m.p. 165~167°, $[\alpha]_D^{24}$ $+182°$($CHCl_3$); λ_{max}^{Nujol} μ: 3.05(OH), 5.78(17, C=O), 6.04(3, C=O), 6.19(Δ^4). *Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.65. Found: C, 75.34; H, 8.50. Oxidation of (V) with chromium trioxide in acetic acid yielded 19-oxo-4-androstene-3,17-dione[5] (VI), m.p.* 129~133°, $[\alpha]_D^{20}$ $+269°$($CHCl_3$); λ_{max}^{EtOH} 244 mμ (ε 11,900), $\lambda_{max}^{CS_2}$ μ: 5.75 (17, C=O), 5.81(10-CHO), 5.96(3, C=O), 6.18(Δ^4). *Anal.* Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.84; H, 8.30.

The structure of (VI) was supported by the fact that the contribution of the 10-aldehyde group to the molecular rotation of this compound (ΔMD $+228°$) showed good agreement with ΔMD calculated from other 19-oxosteroids in the literature.[6]

---

1) A.S. Meyer: Experientia, **11**, 99(1955); A. Zaffaroni: Chem. & Ind. (London), **1955**, 534; M. Hayano, *et al.*: Arch. Biochem. (Biophys.), **55**, 289(1955); H. Levy, *et al.*: *Ibid.*, **55**, 290(1955); A. Wettstein, *et al.*: Helv. Chim. Acta, **38**, 1257(1955); **39**, 2062(1956).
2) cf. S.H. Eppstein, *et al.*: Vitamins and Hormones, **14**, 359(1956).
3) T. Hasegawa, T. Takahashi, M. Nishikawa, H. Hagiwara: Bull. Agr. Chem. Soc. Japan, **21**, 390(1957).
4) R. Neher, A. Wettstein: Helv. Chim. Acta, **39**, 2062(1955); cf. H. Levy, *et al.*: Arch. Biochem. (Biophys.), **55**, 290(1955).
5) A.S. Meyer: Experientia, **11**, 99(1955).
6) G.W. Barber, M. Ehrenstein: J. Org. Chem., **20**, 1253(1955).

* A.S. Meyer also obtained this substance as an amorphous product. Although its m.p. was not reported, the infrared spectrum ($\lambda_{max}^{CS_2}$) was in good agreement with that of our sample.



Reduction of (IV) with zinc and acetic acid,[7] gave 19-hydroxydesoxycorticosterone[8] (VII), m.p. 157°, $\lambda_{max}^{CH_2Cl_2}$ μ: 2.80, 2.90(OH), 5.88(20, C=O), 6.02(3, C=O), 6.20(Δ^4). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.52; H, 8.59. With acetic anhydride and pyridine, (VII) gave the 19,21-diacetate, m.p. 122~124°, $[\alpha]_D^{18}$ +215°(CHCl$_3$); $\lambda_{max}^{CS_2}$ μ: 5.75(acetate), 5.8. (20, C=O), 5.97(3, C=O), 6.20(Δ^4), 8.18(C-O-C of acetate). *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.60; H, 8.01.

With acetic anhydride and pyridine, (IV) gave the 19,21-diacetate (VIII). Dehydrogenation of (VIII) with selenium dioxide in *tert*-amyl alcohol gave 1,4-pregnadiene-17,19,21-triol-3,20-dione 19,21-diacetate (IX), m.p. 179~181°, $[\alpha]_D^{17}$ +79°(dioxane); λ_{max}^{EtOH} 243 mμ (ε 15,500); λ_{max}^{Nujol} μ: 2.87(OH), 5.71(acetate), 5.80(20, C=O), 6.00(3, C=O), 6.17, 6.21($\Delta^{1,4}$), 8.16 (C-O-C of acetate), 11.21($\Delta^{1,4}$). *Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 67.01; H, 7.08.

The ultraviolet spectrum of (IX) in ethanolic alkaline solution,[9] however, showed a maximum at 300 mμ, which is a typical absorption of 3-hydroxyestra-1,3,5(10)-triene[10] (i.e. estrone and estradiol).

Actually, with ethanolic potassium hydroxide or with ethanolic hydrochloric acid, (IX)

---

[7] J.K. Norymberski: J. Chem. Soc., **1956**, 517.
[8] F.W. Kahnt, R. Neher, A. Wettstein: Helv. Chim. Acta, **38**, 1237(1955); G.W. Barber, M. Ehrenstein: J. Org. Chem., **19**, 1758(1954).
[9] A.S. Meyer: J. Org. Chem., **20**, 1240(1955).
[10] L. Dorfman: Chem. Revs., **53**, 47(1953).

yielded 3,17,21-trihydroxy-19-norpregna-1,3,5(10)-trien-20-one (X), m.p. 225.5~229° (decomp.), $[\alpha]_D^{18}$ +80°(dioxane); λ_{max}^{EtOH} 280 mμ (ε 2,200); λ_{max}^{KBr} μ: 2.90(OH), 5.87(20, C=O), 6.21 (C=C). (All melting points are uncorrected.)

The writers appreciate the valuable advice and encouragement of Prof. Y. Asahina, Dr. S. Kuwada, Dr. K. Sato, and Dr. T. Matsukawa. They wish to thank Messrs. T. Hasegawa and T. Takahashi for their help in the culture of Corticium which they discovered to effect the 11β-hydroxylation of steroids. Thanks are also due to Dr. M. Hayano and Dr. A. Saito for their helpful advice.

Research Laboratories,
Takeda Pharmaceutical Industries, Ltd.,
Juso-nishino-cho, Higashiyodogawa-ku, Osaka.

Masamoto Nishikawa (西川正元)
Hikoichi Hagiwara (萩原彦一)

February 11, 1958