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February 14, 1958

UDC 547.92 : 576.8.095

Oxidation of Steroids by Microorganisms. 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 microorganisms.²⁾ In the present paper we wish to report microbiological hydroxylation of Reichstein's compound S (4-pregnene-17 α ,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°, $[\alpha]_D^{27} +127^\circ$ (dioxane); $+144^\circ$ (EtOH); $\lambda_{\max}^{\text{EtOH}} 243.5 \text{ m}\mu$ (ϵ 15,500).

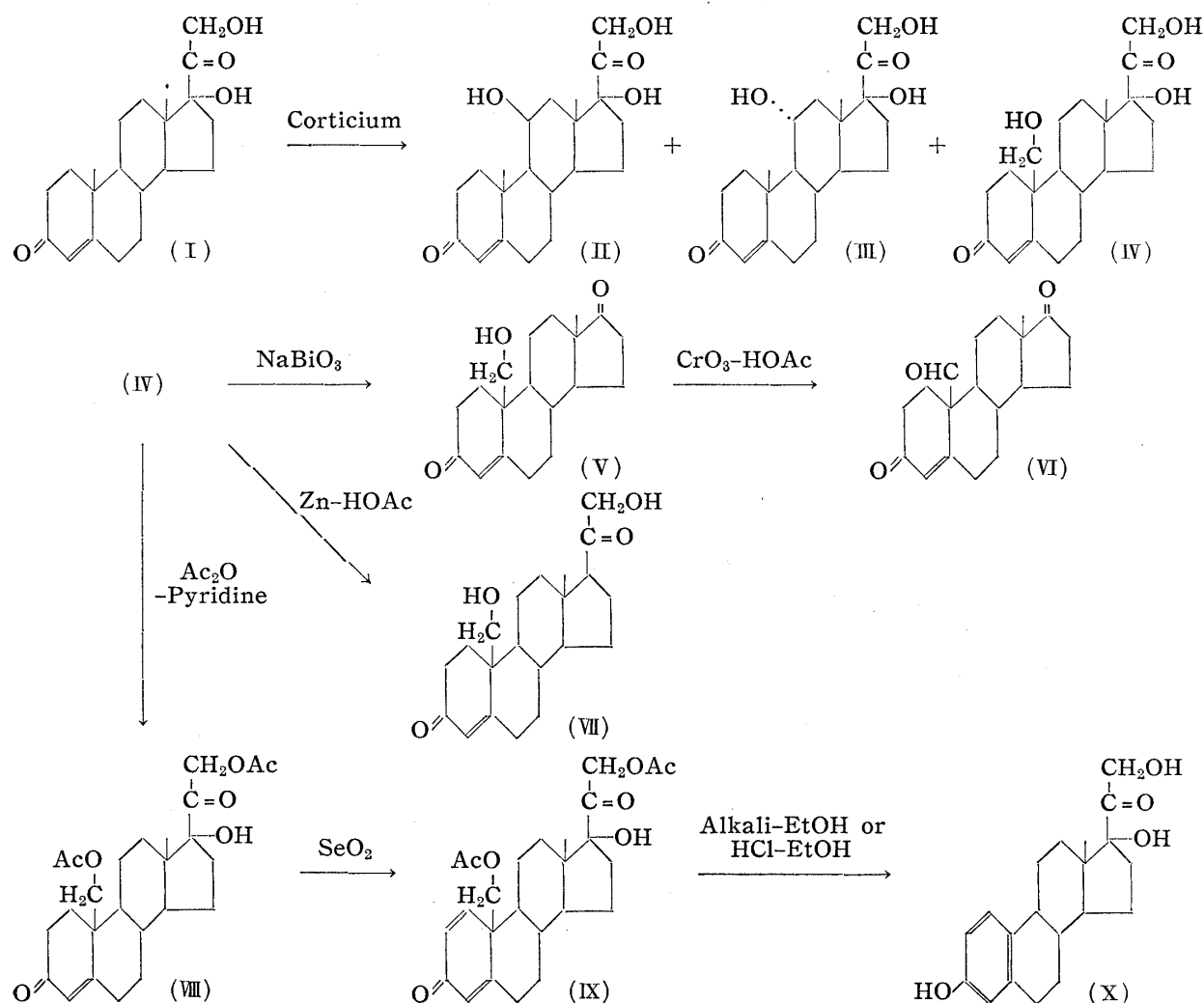
We found that (IV) was identical with 19-hydroxy compound S, which was produced by incubation of steroids in adrenal homogenate.⁴⁾ The structure of (IV) was established by the following reactions.

Oxidation of (IV) with sodium bismuthate gave 19-hydroxy-4-androstene-3,17-dione⁵⁾ (V), m.p. 165~167°, $[\alpha]_D^{24} +182^\circ$ (CHCl₃); $\lambda_{\max}^{\text{Nujol}} \mu$: 3.05 (OH), 5.78 (17, C=O), 6.04 (3, C=O), 6.19 (4). *Anal.* Calcd. for C₁₉H₂₆O₃: 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-dione⁵⁾ (VI), m.p.* 129~133°, $[\alpha]_D^{20} +269^\circ$ (CHCl₃); $\lambda_{\max}^{\text{EtOH}} 244 \text{ m}\mu$ (ϵ 11,900), $\lambda_{\max}^{\text{CS}_2} \mu$: 5.75 (17, C=O), 5.81 (10-CHO), 5.96 (3, C=O), 6.18 (4). *Anal.* Calcd. for C₁₉H₂₄O₃: 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 ($\Delta\text{MD} +228^\circ$) 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 ($\lambda_{\max}^{\text{CS}_2}$) was in good agreement with that of our sample.



Reduction of (IV) with zinc and acetic acid,⁷⁾ gave 19-hydroxydesoxycorticosterone⁸⁾ (VII), m.p. 157°, $\lambda_{\text{max}}^{\text{CH}_2\text{Cl}_2}$ μ : 2.80, 2.90(OH), 5.88(20, C=O), 6.02(3, C=O), 6.20(Δ^4). *Anal.* Calcd. for C₂₁H₃₀O₄: 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°, $[\alpha]_D^{25} +215^\circ(\text{CHCl}_3)$; $\lambda_{\text{max}}^{\text{CS}_2}$ 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 C₂₅H₃₄O₆: 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°, $[\alpha]_D^{25} +79^\circ(\text{dioxane})$; $\lambda_{\text{max}}^{\text{EtOH}}$ 243 m μ (ϵ 15,500); $\lambda_{\text{max}}^{\text{NaIol}}$ μ : 2.87(OH), 5.71(acetate), 5.80(20, C=O), 6.00(3, C=O), 6.17, 6.21(Δ^4), 8.16(C-O-C of acetate), 11.21(Δ^4). *Anal.* Calcd. for C₂₅H₃₄O₇: 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 m μ , which is a typical absorption of 3-hydroxyestra-1,3,5(10)-triene¹⁰⁾ (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.), $[\alpha]_D^{18} +80^\circ$ (dioxane); $\lambda_{\max}^{\text{EtOH}}$ 280 m μ (ϵ 2,200); $\lambda_{\max}^{\text{KBr}}$ 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 11 β -hydroxylation of steroids. Thanks are also due to Dr. M. Hayano and Dr. A. Saito for their helpful advice.

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February 11, 1958
