On the Constitution of Tetrodotoxin

Acetylation of tetrodotoxin¹⁾ affords two kinds of acetate, $C_{15}H_{20}O_9N_2$ (acetate-A) and $C_9H_{13}O_4N$ (acetate-B). From the measurement of their acetyl number, it is known that A is a diacetate and B, a monoacetate. Since the analytical values of both acetates indicate loss of some carbon, hydrogen, and nitrogen from the composition of original tetrodotoxin,^{1,2)} it may be assumed that the acetylation of tetrodotoxin is accompanied with decomposition.

The diacetate (A) is hard to crystallize in the free amine state but forms a picrate (m.p. 199~201°) and a crystalline hydrochloride of needles, m.p. 219~221° (*Anal.* Calcd. for $C_{15}H_{20}O_9N_2 \cdot HCl$: C, 44.06; H, 5.14; N, 6.85; Cl, 8.69; 2 CH_3CO , 21.0. Found: C, 44.32; H, 5.29; N, 7.08; Cl, 8.42; CH_3CO , 22.01); $[\alpha]_D +16.7^\circ$ (c=1.7, EtOH); U. V. λ_{max}^{EtOH} 235~238 m μ (ϵ 5200)).

Monoacetate (B), m.p. 150~151° (*Anal.* Calcd. for $C_9H_{13}O_4N$: C, 54.26; H, 6.58; N, 7.03; CH_3CO , 21.60; mol. wt., 199.2. Found: C, 54.33; H, 6.63; N, 6.86; CH_3CO , 21.21; mol. wt., 205).

Reduction of the diacetate (A) with sodium borohydride gives acetaldehyde and a secondary amine, C_5H_9ON , which can be purified through recrystallization of its acyl derivative. N,O-Ditosylate: Needles, m.p. 132~134° (*Anal.* Calcd. for $C_{19}H_{21}O_5NS_2$: C, 56.02; H, 5.20; N, 3.44; mol. wt., 407.37. Found: C, 55.68; H, 5.43; N, 3.31; mol. wt., 384.0). Dibenzozate: Needles, m.p. 146~148°. This ditosylate is insoluble in alkalis and is clearly a tosylate of a secondary amine. The hydrogenation of this ditosylate results in absorption of 1 mole of hydrogen and a dihydride, m.p. 122°, $[\alpha]_D -48^\circ$ (c=0.9, $CHCl_3$), is obtained. Its reduction with lithium aluminum hydride effects substitution of the O-tosyl group with hydrogen. In this manner, a simple secondary amine, $C_5H_{11}N$, has been obtained. Its N-tosylate melts at 70~72° (*Anal.* Calcd. for $C_{12}H_{17}O_2NS$: C, 60.22; H, 7.16; N, 5.86. Found: C, 59.89; H, 7.00; N, 6.07). It was found by mixed fusion to be identical with the N-tosylate, m.p. 72~73°, of *dl*- β -methylpyrrolidine prepared by the reduction of β -methylsuccinimide by the method of Blicke and others.³⁾ The infrared spectra of the two substances were in complete agreement.

These results have experimentally proved a part of the most stable skeleton in the tetrodotoxin molecule, which is easily destroyed by chemicals.

1) K. Tsuda, M. Kawamura: This Bulletin, 1, 112(1953).

2) Analytical values agree approximately with $C_{12}H_{19}O_9N_3$ but its molecular weight still remains unknown.

3) F.F. Blicke, Chi-Jung Lu: J. Am. Chem. Soc., 74, 3933(1952).

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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.