UDC 547.993

On the Constitution of Tetrodotoxin

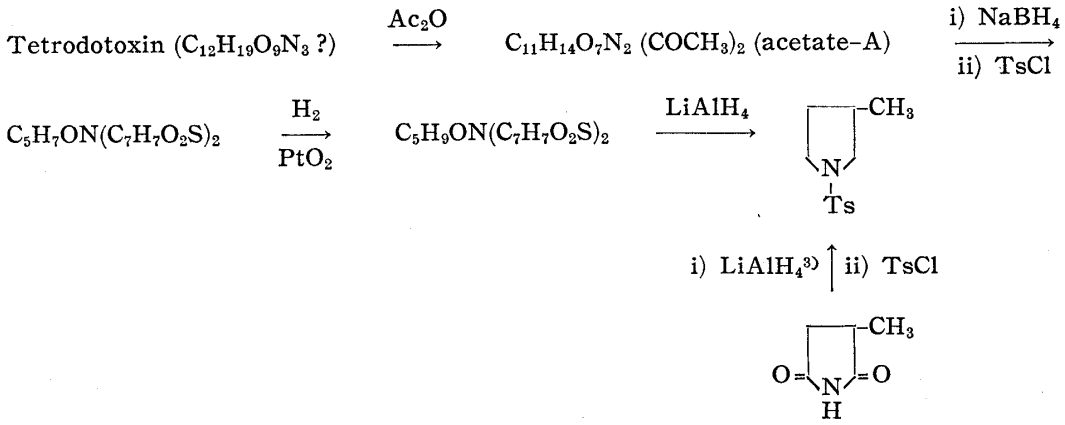
Acetylation of tetrodotoxin[1] affords two kinds of acetate, $C_{15}H_{20}O_9N_2$ (acetate-A) and $C_9H_{13}O_4N$ (acetate-B). From the measurement of their acetyl number, it is known that A is a diacetate and B, a monoacetate. Since the analytical values of both acetates indicate loss of some carbon, 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; $2CH_3CO$, 21.0. Found : C, 44.32; H, 5.29; N, 7.08; Cl, 8.42; CH_3CO, 22.01); $[\alpha]_D$ +16.7°(c=1.7, EtOH); U.V. λ_{max}^{EtOH} 235~238 mμ (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). Dibenzoate : 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°(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.



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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]

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* 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.