presence of dil. sulfuric acid or BF_3-etherate afforded a monoacetate (IVb), m.p. 160~161°, $[\alpha]_D$ +3.9°, on standing at room temperature.

Oxidation of (III) in acetone solution with ca. two equiv. moles of Jones's reagent[4] (the same reagent was used for all oxidation processes in this work) gave 10β-formyl-3,5-cyclo-5α-estrane-6,17-dione (Va), m.p. 224~225°, $[\alpha]_D$ +108.5°; with the excess oxidizing agent both (III) and (Va) were converted into the corresponding 10β-carboxylic acid (Vb), m.p. 260~262°, $[\alpha]_D$ +118.7°. Direct oxidation of (II) regulating the amount of the oxidation agent in a similar manner gave the compound (Va) or (Vb) in high yield.

The monoacetate (IVb) was smoothly oxidized into the corresponding 10β-formyl derivative (VIa), m.p. 148~150°, $[\alpha]_D$ −228.3°, but resisted to further oxidation even with the excess oxidizing agent to give a low yield of the 10β-carboxylic acid (VIb), m.p. 253° (decomp.), $[\alpha]_D$ −79.3°.

New substances encountered in this work mentioned above were characterized by ultraviolet, infrared, and nuclear magnetic resonance spectra confirmatory of the structures presented, and satisfactory analytical data were obtained for all compounds. The authors are currently undertaking studies on the preparation of C_{19}-norsteroids starting from the substances prepared here and the results will be reported shortly.

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July 13, 1962

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UDC 547.831.7 : 581.15 : 582.282.12

Mutagenic Activity of 4-Hydroxyaminoquinoline 1-Oxide

4-Nitroquinoline 1-oxide (4NQO) and its related compounds are calling attention due to the powerful mutagenic action.[1~3] Although, up to date, some postulations have been presented to explain this activity, the mechanism has not been established unequivocally. In the present communication it is indicated that 4-hydroxyaminoquinoline 1-xide (4HAQO), one of the microbial reduction products of 4NQO[4] has mutagenic activity on *Aspergillus niger*.

The spore suspension of *A. niger* strain W. prepared by the similar manner as previously reported[1] was treated with various concentrations of 4HAQO·HCl at 28° for 24 hours. The aliquots of the suspension were plated on agar medium composed of 5% glucose, 0.5% peptone, 0.2% yeast, extract and 0.1% K_2HPO_4, and incubated for 72 hours at 28°. The appeared colonies were picked up at random on agar slants

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having the same composition as described above and incubated for one week at 28°. Then the morphological properties of isolated progenies were observed. The results summarized in Table I clearly indicate that 4HAQO is mutagenic to the microörganisms. The morphological types of mutants appeared to be restrict type floccose type, light type, sterile type, yellow mycelium type, brown type, sclerotium type and their intermediate types. They were of almost the same types as those induced by 4NQO [1]

It is noteworthy that *A. niger*, which is one of the most sensitive microörganisms to 4NQO and easily be mutated by 4NQO treatment, can reduce 4NQO only to 4HAQO, while *E. coli* for which 4NQO exhibited only slight mutagenic activity,[5] can reduce 4HAQO to 4-aminoquinoline 1-oxide.[4,6] So it may be possible to consider that the microbial reduction of 4NQO to 4HAQO is one of the necessary step for the mutagenic activity of 4NQO. The finding presented here may offer clues to account for many points remaining cloudy by the mechanism, which include that the substitution reaction of 4NQO with SH compounds plays the primary role for the biological action of 4NQO.[1,7] However, it does not account for the induction of mutation of tobacco mosaic virus by 4NQO on the experimental conditions where the reduction of 4NQO is not plausible.[3] Therefore, further study will be needed to establish the mechanism.

Table I. Effect of 4HAQO Treatment on the Morphorogical Properties of *A. niger*

4HAQO (γ/cc.)	0	12.5	25	50	100
Survival per cent	100	approx. 100	66.5	34.1	17.7
Number isolated	323	180	112	236	197
Mutant obtained	0	0	4	25	26

Thanks are due to Dr. Ken'ichi Takeda, Director of our laboratory and Prof. emeritus Eiji Ochiai, University of Tokyo for their encouragement. The author also appreciates the supply of 4HAQO•HCl for Dr. Ryôzo Maeda of this laboratory and Prof. Masakazu Hamana, University of Kyushu. The work described here was partially supported from the Ministry of Education Tokyo Japan.

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Received June 8, 1962

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Addendum: According to the private communication from Prof. Hideya Endo at the Cancer Institute of Kyushu University, his recent independent works found that 4HAQO, as well as 4NQO, caused the bacteriophage induction in lysogenic bacteria and the formation of the characteristic intranuclear inclusions in tissue culture cells while 4-aminoquinoline N-oxide was shown to have no effects. These findings were consistent with our results, suggesting that 4NQO might eventually act through its reduced compound, 4HAQO.