Summary

3-Enol acetates of some 4-en-3-oxo-steroids were oxidized to the corresponding 7-ones with *t*-butyl chromate and 3-enol methyl ethers were to the corresponding 4-ene-3,6-diones, 6β-hydroxy-4-en-3-ones or 4,6-dien-3-ones according to the reaction conditions. 3-Enol methyl ethers were prepared with methyl orthoformate in dioxane by catalysis of *p*-toluenesulfonic acid. 17α-Acetoxy-5α,6β-dichloropregnane-3,20-dione was converted into 3-methoxy-6-chloro-17α-acetoxypregna-3,5-dien-20-one in one step.

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191. **Shoji Shibata,**[*1] **Akihiro Ohta,**[*2] **and Yukio Ogihara**[*1] **:** Metabolic Products of Fungi. XXI.[*3] On Ustilaginoidins. (1).[*4] The Reactions of Ustilaginoidin A.

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An optically active red coloring matter named ustilaginoidin was isolated from the false smutted ball (*Ustilaginoidea virens* (Cooke) Takahashi[1)]) growing parasitically on spike of rice plant (in Japanese—Inekoji (稲麹)).

Yabuta and Sumiki and their coworkers[2)] proposed a molecular formula, $C_{19}H_{16}O_7$, for ustilaginoidin and assumed that it belongs to the anthraquinone pigment mainly on the basis of obtaining anthracene on zinc dust distillation. However, the earlier study of ustilaginoidin was interrupted without providing further evidences for the structure.

In relation to some other studies on the fungal anthraquinones we realized a fact that ustila ginoidin gave no characteristic color reactions of hydroxyanthraquinone, which prompted us to reexamine its chemical structure.

The acetone extracts of the false smutted ball (*Ustilaginoidea virens*) collected from the infected rice spikes were separated by $CaHPO_4$-column chromatography to give three bands. The lowest band which consisted the main portion of the extracts was eluted Ⓐ and recrystallized from dioxan to give a red pigment m.p. >300°, $[\alpha]_D$ $-384°$, which would correspond to ustilaginoidin of earlier workers and now has been designated ustilaginoidin .

Ustilaginoidin A recrystallized from dioxan gave analytical figures and molecular weight (determined by X-ray crystallographical method) consistent with $C_{28}H_{18}O_{10} \cdot 2C_4H_8O_2$, which lost dioxan on drying at 180° *in vacuo*. It is slightly soluble in bicarbonate solution and soluble in carbonate to form red solution. It gives a red color in conc. H_2SO_4, which turns into green on heating. It shows a green color with $FeCl_3$ and no remarkable coloration with magnesium acetate in alcohol.

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[*3] Part XX. S. Shibata, T. Ikekawa : This Bulletin, **11**, 368 (1963).

[*4] Presented before Ⅵth Japanese Symposium on the Chemistry of Natural Products (July, 1962). Proceeding, p. 47. (in Japanese).

1) Y. Takahashi : Bot. Mag. (Tokyo), **10**, 109 (1895).

2) T. Yabuta, Y. Sumiki : Nippon Nogei-Kagaku Kaishi, **9**, 478 (1933); *Ibid.*, **13**, 106 (1937); T. Yabuta, Y. Sumiki H. Igarashi : *Ibid.*, **13**, 110 (1937).

Ustilaginoidin A yielded tetraacetate, $C_{36}H_{26}O_{14}\cdot C_6H_6$ (from benzene), m.p. 285°, $[\alpha]^{D}$ $-46.5°$ (tetrahydrofuran), tetraethoxy-carbonate, $C_{40}H_{34}O_{18}\cdot C_2H_5OH$ (from ethanol), m.p. 131°, and tetrabenzoate, $C_{56}H_{34}O_{14}\cdot C_2H_5OH$ (from acetone-ethanol), m.p. 297°, all of which showed the presence of unblocked hydroxyls by the blue coloration with $FeCl_3$.

On methylation with dimethyl sulfate and anhydrous potassium carbonate in acetone, ustilaginoidin A afforded hexamethyl ether, $C_{34}H_{30}O_{10}$ (from ethanol), m.p. 253°, $[\alpha]_D$ $+89.5°$, which gave no coloration with $FeCl_3$, and showed yellowish green fluorescence.

The above reactions revealed that ustilaginoidin A possesses six hydroxyls, two of which are sterically hindered or hydrogen bonded.

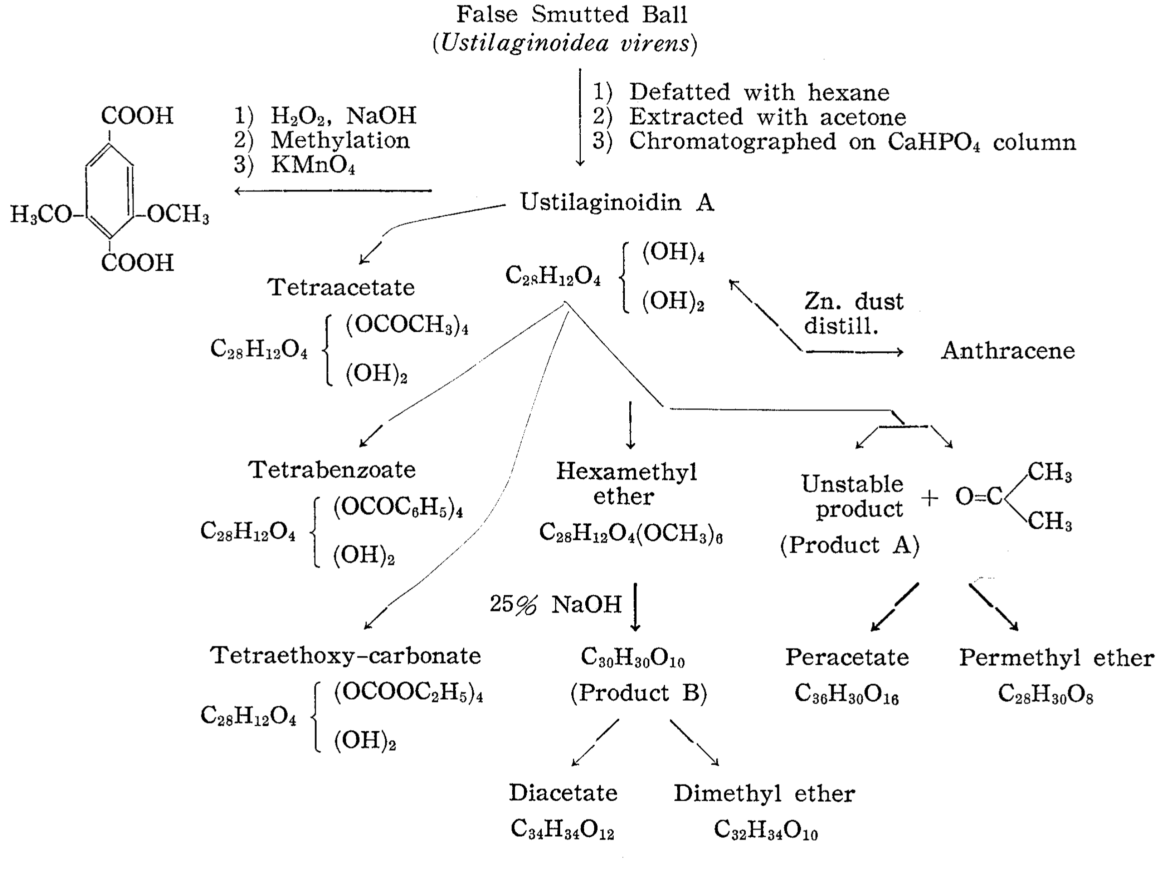
The presence of ketone group in ustilaginoidin A was proved by infrared spectra ($\nu_{C=O}$ 1645 cm^{-1} (KBr or Nujol)), though it did not react with ordinary carbonyl reagents.

On oxidation with alkaline 3% hydrogen peroxide followed by methylation and further oxidation with permanganate, ustilaginoidin A furnished 2,6-dimethoxyterephthalic acid, m.p. 280°, which was synthetically established.

Similar to the earlier result, ustilaginoidin A yielded anthracene (trinitrobenzene complex, m.p. 161°) on zinc dust distillation.

On boiling in 15% caustic alkali under N_2 stream, ustilaginoidin A was converted into an unstable polyphenolic product liberating acetone. The unstable product (Product A) which turned readily into a greenish compound on exposure to air was characterized as crystalline peracetate $C_{36}H_{30}O_{16}$, m.p. 235°, $[\alpha]_D$ $-21°$ (chloroform), partial methyl ether, $C_{26}H_{26}O_8$, m.p. 190° and permethyl ether, $C_{28}H_{30}O_8$, m.p. 202°.

Refluxing ustilaginoidin A hexamethyl ether in 25% caustic alkali under N_2 stream, a yellow product (Product B), $C_{30}H_{30}O_{10}$, m.p. 234°, was formed, which exhibited a blue color with $FeCl_3$ and yielded diacetate, $C_{34}H_{34}O_{12}$, m.p. 240°, and dimethyl ether, $C_{32}H_{34}O_{10}$, m.p. 182°, $[\alpha]_D$ $+15.8°$ (chloroform). It would be noted that in this case acetone was not separated by the action of alkali. The above reactions of ustilaginoidin A are summarized as follows :



The ultraviolet spectrum of product A peracetate showed a close similarity with those of 1-phenylnaphthalene[3] and 1,1′-binaphthalene (Fig. 1).

An inversion of optical activity from *levo* to *dextro* was observed when ustilaginoidin A was refluxed in formic acid. The orange red crystalline product, isoustilagionoidin A, m.p. >300° and $[\alpha]_D$ +378° (dioxan), showed almost the same properties as ustilaginodin A except the optical activity.

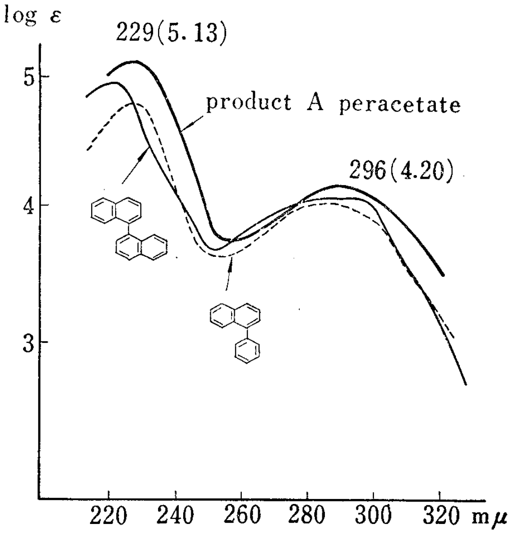


Fig. 1.

Experimental*[5]

Isolation of the Pigments of *Ustilaginoidea virens* TAKAHASHI——The fungal material, *Ustilaginoidea virens* TAKAHASHI, collected from the infected rice spikes at the rice field was pulverized and defatted by extraction with hexane. The pigments was obtained by the extraction of defatted material with Me_2CO. The crude pigments were chromatographed on a $CaHPO_4$-column using benzene and benzene-Me_2CO mixture (8:2) as the solvents. Orange red powder which was separated from the Me_2CO-benzene eluate was chromatographed again over the $CaHPO_4$ column using benzene-Me_2CO mixture to separate into 3 bands. The bottom band was eluted to obtain ustilaginoidin A.

Ustilaginoidin A——The red prisms obtained by recrystallization from dioxan do not melt over 300°, and give $[\alpha]_D$ −384° (dioxan). It forms orange red needles from tetrahydrofuran-MeOH mixture. It dissolves slightly in aq. $NaHCO_3$, and readily in aq. Na_2CO_3, NaOH solution and NH_4OH to form red solution which turns into brownish to green. The red conc. H_2SO_4 solution of ustilaginoidin A turns into green on warming. The original substance is not recovered on dilution. It is soluble in Me_2CO, AcOEt, dioxan, tetrahydrofuran, pyridine, and AcOH, but insoluble in petr. ether and H_2O. It shows no color change with $Mg(OAc)_2$ in EtOH, and a dark green color with $FeCl_3$. *Anal.* Calcd. for $C_{28}H_{18}O_{10}\cdot 2C_4H_8O_2$ (recrystallized from dioxan): C, 62.61; H, 4.93; mol. wt., 690. Found: C, 62.50, 62.73; H, 4.37, 4.69; mol. wt., 682 (by X-ray method: Tetragonal, a=12.90 Å, b=12.90 Å, c=35.87 Å; space group: D_4^4-$P_{4_1 2_1 2}$; sp. gr.: 1.51). *Anal.* Calcd. for $C_{28}H_{18}O_{10}$ (dried *in vacuo* at 180°): C, 65.37; H, 3.53. Found: C, 65.21. 65.02, 65.26; H, 3.72, 3.59, 3.68.

Tetraacetate——Ustilaginoidin A dissolved in pyridine was added with Ac_2O dropwise under shaking at room temperature, when the color of solution changed into yellow. After 15 min. standing the mixture was poured into ice water. The yellow precipitates formed were dissolved in benzene and chromatographed on $CaHPO_4$-column. Yellow prisms, m.p. 285°, $[\alpha]_D$ −46.5° (tetrahydrofuran), were obtained by recrystallization from benzene. The same acetate was obtained by refluxing ustilaginoidin A with Ac_2O. *Anal.* Calcd. for $C_{36}H_{26}O_{14}\cdot C_6H_6$ (recrystallized from benzene): C, 66.31; H, 4.21. Found: C, 66.29; H, 4.20. It gives a blue color with $FeCl_3$.

Tetra-ethoxycarbonate——Ustilaginoidin A dissolved in pyridine was added with ClCOOEt dropwise under ice-cooling. After stirring for 3 hr. the mixture was poured into ice water, and the precipitates

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*[5] All mps are not corrected.

[3] A. Ohta, K. Nei, Y. Ogihara, S. Shibata: This Bulletin, **11**, 754 (1963).

formed were purified by chromatography on $CaHPO_4$-column and by recrystallization from EtOH to give yellow crystals, m.p. 130~131°. It shows a blue color with $FeCl_3$. *Anal.* Calcd. for $C_{40}H_{34}O_{18} \cdot 2C_2H_5OH$ (recrystallized from EtOH): C, 59.06; H, 5.14. Found: C, 58.80; H, 4.81.

Tetrabenzoate——To a solution of ustilaginoidin A in pyridine benzoyl chloride was dropped under ice-cooling. As soon as the color changed to yellow, the mixture was poured into water to separate yellow precipitates, which were purified as in the case of acetate and recrystallized finally from Me_2CO-EtOH mixture to give yellow prisms, m.p. 296~297°, giving a blue color with $FeCl_3$. *Anal.* Calcd. for $C_{56}H_{34}O_{14} \cdot C_2H_5OH$ (recrystallized from EtOH): C, 71.31; H, 4.10. Found: C, 71.35; H, 3.79.

Hexamethyl Ether——A mixture of ustilaginoidin A (500 mg.) anhydr. K_2CO_3 (10 g.), and Me_2SO_4 (10 ml.) in Me_2CO (20 ml.) was allowed to stand for 4 days in the dark at 30°. The dark colored reaction mixture was added with H_2O to dissolve K_2CO_3 and then extracted with benzene. The benzene solution was chromatographed on an alumina column to separate a yellow band which was repeatedly chromatographed on alumina using benzene-Me_2CO (9:1) as the solvent. The product was finally recrystallized from EtOH to form yellow needles, m.p. 252~253°, $[\alpha]_D$ +89.5° (tetrahydrofuran), showing yellowish green fluorescence in the solution. Yield: 300 mg. It gives no coloration with $FeCl_3$. *Anal.* Calcd. for $C_{34}H_{30}O_{10}$: C, 68.22; H, 5.05; $6OCH_3$, 31.10; mol. wt. 598. Found: C, 68.27, 68.15, 67.90; H, 5.10, 5.03, 5.00; OCH_3, 30.62; mol. wt. (Rast), 523.

Zinc Dust Distillation of Ustilaginoidin A and its Acetate——On dry distillation of a mixture of ustilaginoidin A or its acetate (1 part) in Zn dust (20 parts), a yellow green fluorescent oil was obtained. The hexane-soluble portion of the product was chromatographed on alumina and, from a blue fluorescent eluate colorless crystals, m.p. ca. 180°, were obtained (Yield: 2 mg. from 30 g. ustilaginoidin A). An equal amount of trinitrobenzene was mixed with the above crystals, and the mixture was recrystallized from MeOH to form orange needles, m.p. 159°, which showed no melting point depression on admixture with anthracene trinitrobenzene complex m.p. 161°.

Oxidative Degradation of Ustilaginoidin A——The oxidation of ustilaginoidin A was attempted using $KMnO_4$, HNO_3, CrO_3 or ozon, where oxalic acid, AcOH and Me_2CO were yielded giving no remarkable evidences for the structure of original substance.

Ustilaginoidin A (500 mg.) dissolved in 1% NaOH (80 ml.) was added with 1% H_2O_2 (60 ml.), and the mixture was allowed to stand for 1 week at 30°. The reaction mixture was acidified with H_2SO_4 and extracted with Et_2O to obtain a brownish substance which gave a violet coloration with $FeCl_3$. On heating the product under diminished pressure, 2 mm.Hg, at 120°, a sublimable carboxylic acid was removed to remain a residue, which was methylated with Me_2SO_4, anhydr. K_2CO_3 in Me_2CO and treated subsequently with boiling 2*N* NaOH for 1 hr.

A methylated product thus obtained was dissolved in 2*N* Na_2CO_3 and added with 4% $KMnO_4$ on a boiling water bath. Manganese dioxide was filtered off and the filtrate was extracted with Et_2O after acidification. On removing the solvent, a colorless crystalline product was obtained which was recrystallized from boiling water to give m.p. 279~280° (decomp.). *Anal.* Calcd. for $C_{10}H_{10}O_6$: C, 53.10; H, 4.46; $2OCH_3$, 27.44. Found: C, 53.08; H, 4.40; OCH_3, 29.89. On methylation with CH_2N_2, it afforded methyl ester, leaflets, m.p. 120~122° (from MeOH·H_2O), which gave no depression of melting point on admixture with synthetic dimethyl 2,6-dimethoxytelephthalate. The latter sample was prepared by oxidation of *p*-orsellinic acid dimethyl ether with $KMnO_4$ in aq. Na_2CO_3 followed by methylation.

Alkaline Degradation of Ustilaginoidin A (Formation of Product A)——On refluxing the solution of ustilaginodin A in 15% NaOH under N_2-stream, Me_2CO was separated and proved as the 2,4-dinitrophenylhydrazone. The remaining reaction mixture was acidified with HCl after cooling to separate white precipitate. The product A thus obtained was very unstable in air turning into greenish substance.

Product A Peracetate——To the suspension of product A in Ac_2O pyridine was added gradually under N_2 stream, when heat evolved. The reaction mixture was allowed to stand 4 days, and then poured into H_2O to obtain precipitates, which were dissolved in benzene and chromatographed on $CaHPO_4$ column. A fluorescent band visualized under UV-illumination was eluted and purified by recrystallization from Me_2CO-EtOH or benzene to give colorless prisms, m.p. 235°. *Anal.* Calcd. for $C_{36}H_{30}O_{16}$: C, 60.17; H, 4.27; $8CH_3CO$, 47.91. Found: C, 60.25; H, 4.88; CH_3CO, 50.58. IR: $\nu_{\max}^{\text{Nujol}}$ 1780 cm^{-1} (phenolic acetate C=O).

Product A Methyl Ethers——A mixture of product A, Me_2SO_4 and anhydr. K_2CO_3 was allowed to stand for 2 days in a room temperature, and the precipitates formed on pouring the reaction mixture into H_2O were dissolved in benzene, which were chromatographed on alumina to separate blue and yellowish green fluorescent portions.

From the blue fluorescent band colorless needles, m.p. 190° were obtained, which were proved to be a partially methylated product A. *Anal.* Calcd. for $C_{26}H_{26}O_8$: C, 66.95; H, 5.58. Found: C, 66.84; H, 5.85.

From the yellowish green fluorescent portion, permethyl ether of product A, m.p. 202°, was obtained. *Anal.* Calcd. for $C_{28}H_{30}O_8$: C, 68.01; H, 6.07; CH_3O, 50.20. Found : C, 68.33, 68.36, 68.15; H, 6.10, 5.90, 6.46; CH_3O, 49.23.

Alkaline Degradation of Ustilaginoidin A Hexamethyl Ether (Formation of Product B)——A suspension of ustilaginoidin A hexamethyl ether (600 mg.) in 25% NaOH (15 ml.) was refluxed under N_2 stream. During the reaction no volatile carbonyl compound was detected by Brady's reagent. After 2 hr. the brownish reaction mixture was acidified with HCl and the precipitates thus formed were dissolved in benzene to chromatograph over $CaHPO_4$ column.

The yellow band was eluted from the column and, the product was recrystallized from EtOH to give yellow leaflets, m.p. 234°(tentatively designated product B). Yield : 320 mg. IR : ν_{max} 1638 cm^{-1} (H-bonded C=O). It shows no fluorescence in the solution, and exhibits a blue coloration with $FeCl_3$. The product B was also obtained by the degradation of ustilaginoidin A hexamethyl ether using 3% ethanolic KOH. *Anal.* Calcd. for $C_{30}H_{30}O_{10}$: C, 65.45; H, 5.45; CH_3O, 33.82. Found : C, 65.77; H, 5.61; CH_3O, 32.92.

Product B Diacetate——Ac_2O was added to the solution of product B in pyridine under shaking. After 20 min., the mixture was poured into water, and the precipitates formed were dissolved in benzene to purify by chromatography on AcOH-pretreated alumina column. Recrystallization from MeOH-H_2O gave colorless leafits, m.p. 240°. *Anal.* Calcd. for $C_{34}H_{34}O_{12}$: C, 64.14; H, 5.70. Found : C, 64.22; H, 5.21.

Product B Dimethyl Ether——A mixture of product B (150 mg.), anhyd. K_2CO_3 (7.5 g.), Me_2SO_4 (4.5 ml.) and Me_2CO (15 ml.) was allowed to stand overnight. After 2 hr. refluxing, the reaction mixture was poured into water, and the precipitates thus obtained were dissolved in benzene to chromatograph. The product was eluted from a green fluorescent band on the $CaHPO_4$-column, and recrystallized from MeOH-H_2O to give slightly yellowish needles, m.p. 180~182°. Yield : 120 mg. IR : ν_{max} 1716 cm^{-1} (non-bonded C=O). It reacted with Brady's reagent, but failed to form crystalline product. *Anal.* Calcd. for $C_{32}H_{34}O_{10}$: C, 66.43; H, 5.92; CH_3O, 43.00. Found : C, 66.41, 66.33, 66.51, 66.37; H, 5.90, 5.88, 5.87, 5.85; CH_3O, 42.47.

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Summary

An orange red pigment, ustilaginoidin A, $C_{28}H_{18}O_{10}$, m.p. >300°, $[\alpha]_D$ −384°(dioxane), was isolated from false the smutted ball growing on rice spike. It has been shown that ustilaginoidin A possesses two hindered and four unhindered hydroxyls giving tetraacetate, tetrabenzoate, and hexamethyl ether. On alkaline degradation, ustilaginoidin A gave unstable product A and acetone. The product A afforded peracetate, $C_{36}H_{30}O_{16}$, and permethyl ether, $C_{28}H_{30}O_8$. Ustilaginoidin A hexamethyl ether yielded product B, $C_{30}H_{30}O_{10}$, on alkaline degradation, which gave diacetate and dimethyl ether. The ultraviolet spectral study has suggested a close structural correlation between the product A and 1,1′-binaphthalene or 1-phenylnaphthalene.

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