UDC 547.972 : 582.282.12

7. Kōno Kinoshita and Shoichi Nakajima : Studies on the Structure of Itaconitin. I.

(*Hoshi College of Pharmacy**)

One of the authors (Kinoshita) isolated a yellow crystalline substane melting at 169°, designated itaconitin, from the culture filtrate of *Aspergillus itaconicus*, from which a rich amount of itaconic acid and mannitol had formerly been obtained.[1] He also studied the culture condition of the mold for the production of itaconitin,[2] but the chemical nature of itaconitin has not yet been reported. In this paper, some experimental results which may contribute to the elucidation of the structure of itaconitin are described.

A yellow pigment, citrinin, of *Penicillium citrinum*[3] possesses the same melting point as itaconitin, but a depression of melting point occurs on mixed fusion with itaconitin.

Itaconitin is almost insoluble in water, soluble in ether, chloroform, and benzene, and easily soluble in acetone and ethanol. It dissolves readily in cold sodium hydrogen carbonate, and when warmed, vigorous effervescence occurs. In an alkaline solution, pH over 6.4, the yellow color of itaconitin completely disappears and on acidification, yellow crystals of itaconitin are recovered unchanged. It gives a strong red color when dissolved in concentrated sulfuric acid or in ammonia vapor.

Itaconitin and almost all of its derivatives are unstable in air and are sensitive to light. On standing for a month in ethanol solution under ordinary sunlight itaconitin turns into a transparent resin. The resinous substance is formed in a few minutes by addition of pyridine.

Itaconitin does not contain nitrogen, halogen, or methoxyls, and gives negative fuchsin, Legal, Ehrlich, and Mg-HCl reactions. Ferric chloride test in ethanol is negative for itaconitin, but, on addition of sodium hydrogen carbonate, it turns positive. The strong red color by the diazonium reaction with sulfanilic acid suggests the presence of a hydroxyl group, and, moreover, the fact that the itaconitin decolorizes bromine and potassium permanganate is attributable to its double bonds. Ferric hydroxamate and $KI-KIO_3$ tests are slightly positive.

Itaconitin was recrystallized from chloroform to orange prisms which readily released the crystal solvent giving an amorphous powder, while the yellow needles crystallized from ethanol did not take crystal solvent.

The ultraviolet spectra of itaconitin showed maximum absorption in methanol at 326 mμ ($\log \varepsilon$ 4.396) and in chloroform at 276 ($\log \varepsilon$ 4.306) and 388 mμ (4.200)(Fig. 1).

The molecular formula $C_{14}H_{14}O_5$ of itaconitin was identified through analytical values and molecular weights of itaconitin and its derivatives. Because of the formation of a monosemicarbazone and a mono-2,4-dinitrophenylhydrazone, itaconitin is likely to possess a ketone group in its molecule. The treatment of itaconitin with hydroxylamine, however, did not give an oxime but its addition product. On acetylation, itaconitin yielded yellow prisms of m.p. 136° with the molecular formula, $C_{16}H_{14}O_5$. This substance was hydrolyzed to produce orange rhombic crystals of m.p. 178°, whose molecular formula was $C_{14}H_{12}O_4$. When $C_{14}H_{12}O_4$ was treated with acetic anhydride,

---

* 2-chome, Ebara, Shinagawa-ku, Tokyo (木下広野, 仲嶋正一).

[1] K. Kinoshita : Acta Phytochim. (Japan), **5**, 276(1931).

[2] K. Kinoshita : Shigen Kagaku Kenkyusho Ihô, **17~18**, 78(1950).

[3] A. G. Hetherlington, H. Raistrick : Phil. Trans. Roy. Soc. London, Ser. B, **220**, 269(1931).

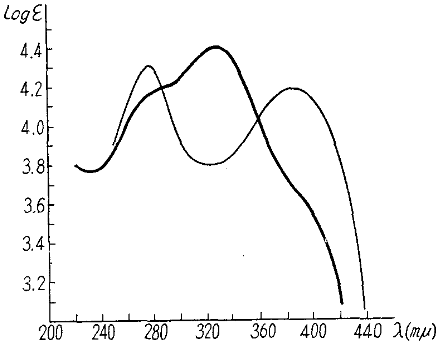

Fig. 1.
Ultraviolet Absorption Spectra of Itaconitin
—— in Methanol
------ in Chloroform

the yellow prisms of m.p. 136°, which did not lower on admixture with the abovementioned $C_{16}H_{14}O_5$, was obtained. Accordingly it is doubtless that $C_{16}H_{14}O_5$ and $C_{14}H_{12}O_4$ are monoacetylanhydroitaconitin and anhydroitaconitin, respectively.

$$C_{14}H_{14}O_5 \xrightarrow{Ac_2O} C_{16}H_{14}O_5 \underset{Ac_2O}{\overset{Na_2CO_3}{\rightleftharpoons}} C_{14}H_{12}O_4$$

Further verification of this assumption was made by measurement of the acetyl number of monoacetylanhydroitaconitin. Anhydroitaconitin did not produce a semicarbazone but an addition product with semicarbazide hydrochloride.

Itaconitin absorbed three moles of hydrogen on catalytic reduction to give a viscous oil, which was characterized as a 2,4-dinitrophenylhydrazone of yellow microrhombic crystals, m.p. 115°. The analytical value of the above substance suggested that hydrogenation caused no remarkable change in its molecule, exceptreduction of the double bonds. Moreover, the strong absorption band at 1592 cm^{-1} in the infrared spectrum of itaconitin disappeared on hydrogenation. It seems that the conjugated double bonds undoubtedly cause the yellow color of itaconitin. The characteristic instability of itaconitin may also be due to these double bonds occupying a larger portion of its molecule. Ferric hydroxamate reaction was remarkable in hexahydroitaconitin.

Through titration with alkali, using phenolphthalein as an indicator, itaconitin, anhydroitaconitin, and acetylanhydroitaconitin were shown to be di-, di-, and monobasic acids, respectively. However, the anhydroitaconitin is much decreased in apparent acidity compared with itaconitin, and is not easily soluble in cold sodium carbonate.

The authors wish to express their gratitude to Prof. S. Shibata of the University of Tokyo for giving helpful advices and all conveniences to carry out this work, to President S. Murata of this College for his kind encouragement, and to the analytical staff of the Japan Monopoly Corporation for the measurement of infrared spectra. Their deep thanks are also due to Prof. T. Kobayashi and Mr. T. Tabuchi of the Tokyo University of Education. The raw material used in this research was all kindly offered by them.

Experimental[4]

Itaconitin—The crude itaconitin was recrystallized three times from $CHCl_3$, then twice from EtOH to yellow needles, m.p. 168°. *Anal.* Calcd. for $C_{14}H_{14}O_5$: C, 64.11; H, 5.38; N, 0; mol. wt., 262. Found: C, 63.84, 63.71; H, 5.02, 5.11; N, 0; mol. wt. (cryoscopy in nitrobenzene), 240; neutral. equivalent (phenolphthalein), 117.3.

Itaconitin Monosemicarbazone—Itaconitin (130 mg.) in aq. EtOH was warmed on a water bath with semicarbazide hydrochloride (130 mg.) and crystalline AcONa (200 mg.) till nearly all EtOH was driven off. The crystalline powder that deposited after cooling was collected by filtration and

---

[4] All melting points are uncorrected.

recrystallized from MeOH-H_2O to orange microprisms (under microscope), m.p. 198°(decomp.). *Anal.* Calcd. for $C_{15}H_{17}O_5N_3$: C, 56.42; H, 5.37; N, 13.16. Found : C, 55.86; H, 5.67; N, 13.05.

This substance is soluble in sodium hydrogen carbonate under effervescence.

Itaconitin 2,4-Dinitrophenylhydrazone—Itaconitin (100 mg.), in a mixture of EtOH (0.4 cc.), conc. H_2SO_4 (0.4 cc.), and water (0.6 cc.), was warmed on a water bath with 2,4-dinitrophenylhydrazine reagent for several mins. The mixture was allowed to cool and the orange crystalline powder that deposited was collected and recrystallized from MeOH. Microcrystalline powder, m.p. 169°. *Anal.* Calcd. for $C_{20}H_{18}O_8N_4$: N, 12.67. Found : N, 12.52.

Hydroxylamine Adduct of Itaconitin—Water was added dropwise to itaconitin (100 mg.) in EtOH (3 cc.), until first trace of emulsification, and a drop of EtOH was further added to make the solution clear again. To this solution, $NH_2OH \cdot HCl$ (80 mg.) and crystalline AcONa (100 mg.) were added, and the mixture was boiled for 30 mins., when the color of the solution turned from red to yellow. After cool, the precipitated crystals were collected and recrystallized from H_2O-MeOH to yellow microprisms (under microscope), m.p. 210°(decomp.). *Anal.* Calcd. for $C_{14}H_{14}O_5 \cdot NH_2OH$: C, 56.94; H, 5.80; N, 4.74. Found : C, 56.78; H, 5.65; N, 4.69.

Treatment of Itaconitin with Acetic Anhydride—Itaconitin was suspended in 6 vol. of its weight of Ac_2O and the mixture was refluxed to complete solution. After 15 mins.' boiling, the reaction mixture was cooled and poured into water. After being washed repeatedly with water, acetylanhydroitaconitin crystallized from EtOH as yellow prisms, m.p. 136°, in a good yield. *Anal.* Calcd. for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93; $OCOCH_3$, 15.04; mol. wt. 286. Found : C, 66.91; H, 4.82; $OCOCH_3$, 15.39; mol. wt.(Rast), 311; neutral. equivalent (phenolphthalein), 255.

The use of AcONa or heating for longer time promotes resinification and causes a remarkable decrease in the yield.

Anhydroitaconitin—The above-obtained acetylanhydroitaconitin was warmed with 10% Na_2CO_3 solution(2 cc.) for 20 mins. to complete solution, which, after treatment with a small amount of activated charcoal, was acidified and precipitated crystals, m.p. 178.5°. This substance is insoluble in cold Na_2CO_3 solution. *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95; mol. wt. 244. Found : C, 68.89; H, 4.79; mol. wt.(Rast), 232; neutral. equivalent (phenolphthalein), 124.

Acetylation of Anhydroitaconitin—The procedure was essentially the same as that described for the treatment of itaconitin with Ac_2O. Yellow prisms (from EtOH), m.p. 136°, undepressed on admixture with the product obtained from itaconitin and Ac_2O. *Anal.* Calcd. for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found : C, 66.73; H, 4.74.

Semicarbazide Adduct of Anhydroitaconitin—The procedure as that for above-described itaconitin semicarbazone was followed. Recrystallization from MeOH-H_2O gave yellow microneedles (under microscope), m.p. 237°(decomp.). *Anal.* Calcd. for $C_{14}H_{12}O_4 \cdot NH_2CONHNH_2$: C, 56.42; H, 5.37; N, 13.16. Found : C, 56.81; H, 5.04; N, 12.87.

Hydrogenation of Itaconitin—i) A solution of 500 mg. of itaconitin in MeOH was catalytically hydrogenated over Pt-black at room temperature and 128.0 cc. (theoretical amount for 3 moles : 128.0 cc.) of hydrogen was absorbed in 2 hrs.

ii) A solution of 102 mg. of itaconitin in MeOH was hydrogenated catalytically over 10% Pd-C and 26.3 cc. (theoretical amount for 3 moles : 26.2 cc.) of hydrogen was absorbed in 0.5 hr. After hydrogenation, the solvent was driven off on a water bath to give a colorless viscous oil.

Hexahydroitaconitin 2,4-Dinitrophenylhydrazone—The same procedure as that for itaconitin 2,4-dinitrophenylhydrazone was followed. Recrystallized from MeOH to yellow rhombic crystals (under microscope), m.p. 115°. *Anal.* Calcd. for $C_{20}H_{24}O_8N_4$: C, 53.57; H, 5.39; N, 12.50. Found : C, 54.10; H, 5.35; N, 12.33.

Summary

Itaconitin, a yellow pigment from *Aspergillus itaconicus*, was preliminarily examined for the elucidation of its structural formula.

(Received September 12, 1957)