
(Hoshi College of Pharmacy)

One of the authors (Kinoshita) isolated a yellow crystalline substance 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, Ehlich, 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 diazoon 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\(_4\) 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 m\(_\mu\) (log \(\varepsilon\) 4.396) and in chloroform at 276 (log \(\varepsilon\) 4.306) and 388 m\(_\mu\) (4.200)(Fig. 1).

The molecular formula C\(_{14}\)H\(_{16}\)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 oxide but its addition product. On acetylation, itaconitin yielded yellow prisms of m.p. 136° with the molecular formula, C\(_{16}\)H\(_{18}\)O\(_4\). This substance was hydrolyzed to produce orange rhombic crystals of m.p. 178°, whose molecular formula was C\(_{14}\)H\(_{14}\)O\(_5\). When C\(_{14}\)H\(_{14}\)O\(_4\) was treated with acetic anhydride,

\(^*\) 2-chome, Ebara, Shinagawa-ku, Tokyo (本下広野, 伸飽正一).
1) K. Kinoshita: Acta Phytochim. (Japan), 5, 276(1931).
the yellow prisms of m.p. 136°, which did not lower on admixture with the above-mentioned C_{10}H_{14}O_{5}, was obtained. Accordingly it is doubtless that C_{10}H_{14}O_{5} and C_{14}H_{12}O_{4} are monoacetylanhydritoitaconitin and anhydroitaconitin, respectively.

\[
\begin{align*}
\text{C}_{14}\text{H}_{12}\text{O}_{4} & \xrightarrow{\text{Ac}_2\text{O}} \text{C}_{10}\text{H}_{14}\text{O}_{5} & \xrightarrow{\text{Na}_2\text{CO}_3} \text{C}_{10}\text{H}_{14}\text{O}_{5} & \xrightarrow{\text{Ac}_2\text{O}} \text{C}_{14}\text{H}_{12}\text{O}_{4}
\end{align*}
\]

Further verification of this assumption was made by measurement of the acetyl number of monoacetylanhydroitaconitin. Anhydritoitaconitin 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 microcrystalline crystals, m.p. 115°. The analytical value of the above substance suggested that hydrogenation caused no remarkable change in its molecule, except reduction 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 hexahydritoitaconitin.

Through titration with alkali, using phenolphthalein as an indicator, itaconitin, anhydritoitaconitin, and acetylanhydritoitaconitin were shown to be di-, di-, and mono-basic acids, respectively. However, the anhydritoitaconitin 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 advice 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**

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_{12}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. (cryoscopic in nitrobenzene), 240; neutral equivalient (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–H₂O to orange microprisms (under microscope), m.p. 198°(decomp.). Anal. Calcd. for C₁₅H₁₂O₅N₃: 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. H₂SO₄ (0.4 cc.), and water (0.6 cc.), was warmed on a water bath with 2,4-dinitrophenylhydrazone 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 C₁₄H₁₀O₅·N₂H₂O : 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, NH₂OH·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 H₂O–MeOH to yellow microprisms (under microscope), m.p. 210°(decomp.). Anal. Calcd. for C₁₄H₁₀O₅·N₂H₂O : 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 Ac₂O 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, acetyl-anhydroitaconitin crystallized from EtOH as yellow prisms, m.p. 136°, in a good yield. Anal. Calcd. for C₁₅H₂₀O₅ : C, 67.12; H, 4.93; OCOCH₃, 15.04; mol. wt. 286. Found : C, 66.81; H, 4.82; OCOCH₃, 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 acetyl-anhydroitaconitin was warmed with 10% Na₂CO₃ 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 Na₂CO₃ solution. Anal. Calcd. for C₁₅H₁₀O₅ : C, 68.84; H, 4.95; mol. wt. 244. Found : C, 68.98; H, 4.79; mol. wt.(Rast), 222; neutral. equivalent (phenolphthalein), 124.

Acetylation of Anhydroitaconitin—The procedure was essentially the same as that described for the treatment of itaconitin with Ac₂O. Yellow prisms (from EtOH), m.p. 136°, undepressed on admixture with the product obtained from itaconitin and Ac₂O. Anal. Calcd. for C₁₆H₂₁O₅ : 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–H₂O gave yellow microneedles (under microscope), m.p. 237°(decomp.). Anal. Calcd. for C₁₅H₁₉O₅·NH₂CONHNH₂ : 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.

Hexaehydroitaconitin 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 C₁₉H₁₆O₅N₄ : C, 53.57; H, 5.39; N, 12.50. Found : C, 54.10; H, 5.38; N, 12.93.

Summary

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

(Received September 12, 1957)