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10. Shoichi Nakajima, Koono Kinoshita,*1 and Shoji Shibata*2: Studies on the Structure of Itaconitin. IV.*3 The Reactions of Itaconitin and Anhydroitaconitin.*4

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It was reported in Part I[1] of this series that on catalytic hydrogenation with palladised charcoal, itaconitin, $C_{14}H_{14}O_5$, a yellow coloring matter of *Aspergillus itaconicus* KINOSHITA, absorbed three moles of hydrogen to afford hexahydroitaconitin $C_{14}H_{20}O_5$.

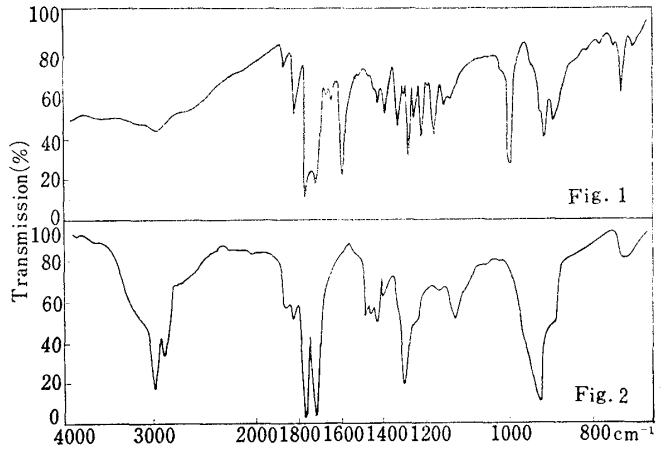

Fig. 1. Infrared Absorption Spectrum of Itaconitin (in KBr)

Fig. 2. Infrared Absorption Spectrum of Hexahydroitaconitin (in CCl_4)

A characteristic infrared absorption band of itaconitin at 1595 cm^{-1} (KBr) which indicated the presence of conjugated double bonds (Fig. 1) disappeared by the hydrogenation. Furthermore, the absence of absorption band in 1500~1600 cm^{-1} region of hexahydroitaconitin reveals that the hexahydro derivative as well as itaconitin itself possesses no aromatic system in its molecule (Fig. 2).

On treatment of itaconitin with *p*-nitrophenylhydrazine, a compound, $C_{20}H_{19}O_6N_3$, m.p. 203.5° (decomp.), was obtained. The mono-2, 4-dinitrophenylhydrazide of hexahydroitaconitin, which was described in Part I[1] as having m.p. 115°, was separated by the chromatography on dicalcium phosphate into two substances melting at 166° and 100°, respectively and both of which showed almost superimposable ultraviolet spectra.

It has already been reported[1] that on heating itaconitin with hydroxylamine on a steam-bath for thirty minutes, an addition product, $C_{14}H_{17}O_6N$, was yielded, while on heating for twenty minutes, a condensation product, $C_{14}H_{15}O_5N$, m.p. 231°, was produced.

Acetylanhydroitaconitin, $C_{16}H_{14}O_5$, was prepared from itaconitin either by the action of acetyl chloride and pyridine under ice-cooling or by boiling with acetic andydride.[1] The infrared absorption bands at 1212 and 1760 cm^{-1} (in KBr) of this compound showed that the product is a phenolic or an enolic acetate. Both itaconitin and anhydroitaconitin, $C_{14}H_{12}O_4$, gave rise to propionylanhydroitaconitin, $C_{17}H_{16}O_5$, m.p. 104°, on treatment with propionic anhydride. The ultraviolet spectrum of this propionyl derivative was superimposable on that of acetylanhydroitaconitin having maxima at 236 and 315 mμ with an inflexion at 370 mμ. The hydrolysis of this propionylanhydroitaconitin produced anhydroitaconitin, which possesses infrared absorption bands at 3420 and 3582

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*3 Part III. K. Kinoshita, S. Nakajima: This Bulletin, 8, 1051 (1960).
*4 Presented at Vth Japanese Symposium on the Chemistry of Natural Products (Oct. 1961). Proceedings, p. 19 (in Japanese).
1) Part I. K. Kinoshita, S. Nakajima: This Bulletin, 6, 31 (1958).

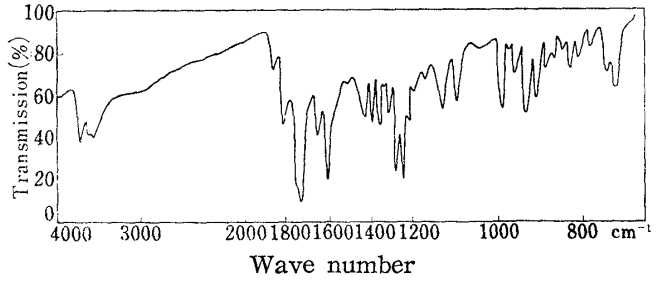


Fig. 3. Infrared Absorption Spectrum of Anhydroitaconitin (in KBr)

cm^{-1} due to the free hydroxyl group (Fig. 3).

On heating anhydroitaconitin with 2,4-dinitrophenylhydrazide, $C_{20}H_{16}O_7N_4$, m.p. 254° (decomp.), was afforded similarly as in the case of itaconitin. Acetylanhydroitaconitin and anhydroitaconitin reacted with *o*-phenylenediamine to afford, with loss of one mole of water, the condensation products $C_{22}H_{20}O_4N_2$, m.p. 210°, and $C_{20}H_{18}O_3N_2$, m.p. 222°, respectively.

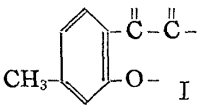
The presence of two C-methyl groups in anhydroitaconitin was revealed by the Kuhn-Roth oxidation. The nuclear magnetic resonance spectrum*[5] of this compound gave two singlet methyl signals at δ_{H_2O} 2.65 and 2.89 p.p.m., indicating that these methyls were attached to quaternary carbon atoms.

On oxidation of anhydroitaconitin with potassium permanganate, only oxalic acid was yielded, whereas treatment with conc. nitric acid by heating gave a phenolic nitro compound, yellow prisms of melting at 107°, which was proved to be identical with 2,4,6-trinitro-*m*-cresol (V) synthesized from thymol.[2]

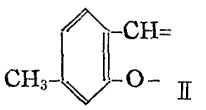
Ozonolysis of anhydroitaconitin using acetone, methyl formate or ethyl acetate as the solvent, and the subsequent treatment with hot water afforded carbon dioxide, acetaldehyde and a product, $C_8H_8O_2$, m.p. 60°. The latter compound was shown to be an aromatic chelated aldehyde by the chemical reactions and by the infrared absorption maxima at 1653, 2745, and 2855 cm^{-1}. On oxidation with ammoniac silver nitrate solution, this aldehyde was converted into the corresponding acid, $C_8H_8O_3$, which was identified to be 2,4-cresotic acid (VII).

The results of ozonolysis would suggest that anhydroitaconitin possesses the alternative partial structures (I) or (II) in its molecule. The partial structure (I) can be excluded, since the occurrence of phenylglyoxalic acid derivative has never been found in the process of ozonolysis, whereas 2,4-cresotaldehyde (VI) has been obtained to support the structure (II).

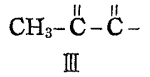
In addition, the formation of acetaldehyde would suggest the presence of the partial structures (III) or (IV) in its molecule. However, the foregoing nuclear magnetic resonance result supports that the methyl group is attached to tertiary carbon atom as shown in the partial structure (III).



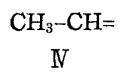
I



II



III



IV

When anhydroitaconitin was catalytically hydrogenated in acetone over 10% paradised charcoal, a dihydro derivative, $C_{14}H_{14}O_4$, m.p. 105°, was afforded, which gave three carbonyl bands at 1760, 1818, and 1859 cm^{-1} (in KBr) in the infrared spectrum, comparable to those bands of starting material (=anhydroitaconitin) at 1743, 1808, and 1849 cm^{-1} (in KBr) (Fig. 3). Added to this fact, the appearance of C-H stretching vibration bands at 2865 and 2925 cm^{-1}, due to a methylene group, in the infrared spectrum of dihydroanhydroitaconitin suggests that the hydrogenation occurred in the double bond remaining the carbonyl groups unaffected.

*[5] The NMR spectrum was measured in NaOD at 60 Mc.p.s. using a Varian Associates A-60 apparatus.

2) M. Yamazaki, T. Usui, S. Shibata : This Bulletin, **11**, 363 (1963).

Ozonization of dihydroanhydroitaconitin followed by the subsequent treatment with hot water gave no evidence of producing 2,4-cresotaldehyde, but a resinous product, which was then oxidized with potassium permanganate to yield succinic acid. Accordingly the hydrogenation of anhydroitaconitin seemed to take place at the double bond linked directly to the benzene nucleus.

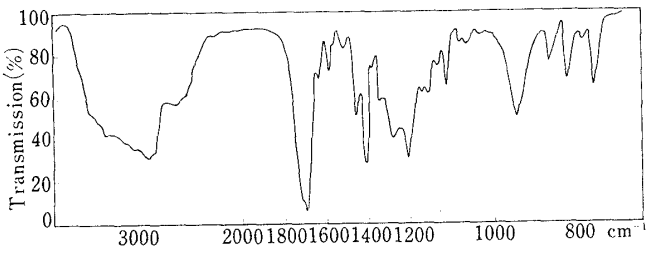


Fig. 4. Infrared Absorption Spectrum of $C_{14}H_{18}O_5$ Acid (in KBr)

The infrared absorption band of the dihydroanhydroitaconitin at 1679 cm^{-1} (in KBr) shows one double bond is retained intact against the above catalytic hydrogenation. This was verified by the catalytic hydrogenation of anhydroitaconitin in aquous

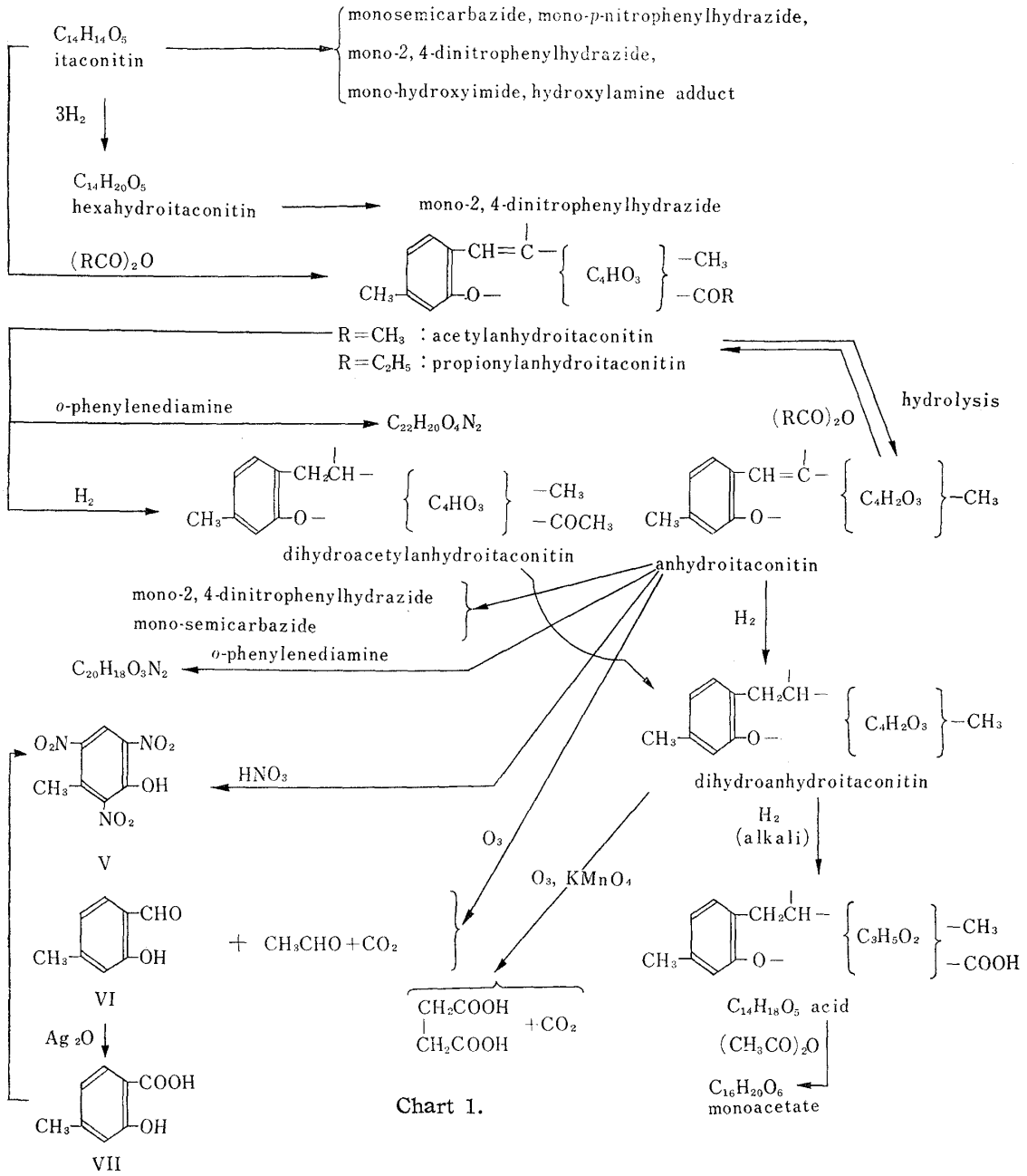


Chart 1.

alkaline solution, where two moles of hydrogen were absorbed very slowly during a period of twenty-four hours to give a water-soluble colorless product, m.p. 145°.

The infrared absorption bands of this compound at 1516, 1594, 1626 cm^{-1} (phenyl) and 941, 1200, 1420, 1695, 1715 cm^{-1} (COOH) (Fig. 4) revealed that it is an aromatic carboxylic acid. This compound was formulated as $C_{14}H_{18}O_5$ on the basis of the analytical figures of the corresponding monoacetate, $C_{16}H_{20}O_6$, m.p. 148°.

Thus the reactions of itaconitin and anhydroitaconitin mentioned above are formulated in chart 1.

Experimental*6

Itaconitin——Itaconitin was purified by chromatography on $CaHPO_4$ followed by crystallization from $CHCl_3$. IR ν_{max}^{KBr} cm^{-1} : 1388 (CH_3), 1595 (conj. double bond), 1709, 1749, 1809, 1855 (C=O) (Fig. 1). Neutral. equivalent (phenolphthalein) 95 (at hot).

Hexahydroitaconitin——Purification was made by distillation under a diminished pressure, otherwise it was decomposed. B.$p_{0.05}$ 175~177°. IR $\nu_{max}^{CCl_4}$ cm^{-1} : 1386 (CH_3), 1715, 1765, 1814, 1843 (C=O), 2870, 2940 (CH_2), 922, 1285, 1410 (Fig. 2). UV λ_{max}^{EtOH} mμ (log ε) : 210 (3.91), 250 (sh.) (3.50). *Anal.* Calcd. for $C_{14}H_{20}O_5$: C, 62.67; H, 7.51. Found : C, 62.63, 62.67; H, 7.50, 7.51; n_D^{20}, 1.4757.

On standing for 1 month in a refrigerator, it was unable to obtain in a crystalline form.

Itaconitin *p*-Nitrophenylhydrazide——To a solution of itaconitin (131 mg., 0.005 mole) in EtOH (3 ml.) was added *p*-nitrophenylhydrazine (76.5 mg., 0.005 mole) dissolved in AcOH–H_2O (1:2). On heating the red solution on a steam-bath for 5 min., red crystals were obtained, which were recrystallized from dil. AcOH to give red microneedles, m.p. 203.5° (decomp.). IR ν_{max}^{KBr} cm^{-1} : 1337 (NO_2), 1390 (CH_3), 1510, 1588, 1604 (phenyl, conj. double bond, and NO_2), 1696, 1715, 1764 (C=O). *Anal.* Calcd. for $C_{20}H_{19}O_6N_3$: C, 60.45; H, 4.82; N, 10.58. Found : C, 60.52, 60.23; H, 5.11, 4.90; N, 10.08, 10.27, 10.21.

Hexahydroitaconitin 2,4-Dinitrophenylhydrazide——The crude 2,4-dinitrophenylhydrazide prepared by the same procedure as described in Part 1,[1] was washed with a small amount of MeOH, dried and dissolved in $CHCl_3$–benzene (1:1). The solution was chromatographed on a $CaHPO_4$ column using the same solvent. From the bottom band was obtained yellow microcrystals (from MeOH), m.p. 100°. IR ν_{max}^{KBr} cm^{-1} : 1345 (NO_2), 1550, 1605, 1623 (phenyl and NO_2), 1711 (sh.), 1731, 1782 (C=O), 2860, 2932 (CH_2), 917, 1032, 1183, 1422. UV λ_{max}^{EtOH} mμ (log ε) : 222 (4.58), 237 (sh.) (4.36), 257 (4.17), 322 (4.30). *Anal.* Calcd. for $C_{20}H_{24}O_8N_4$: N, 12.50. Found : N, 12.20.

The second band gave yellow microcrystals (from MeOH), m.p. 166°. The IR spectrum of this compound was also superimposable with that of the above product, except that two absorption bands at 1032 and 1183 cm^{-1} were present in the former and lacked in the latter. UV λ_{max}^{EtOH} mμ (log ε) : 222 (4.67), 237 (sh.) (4.51), 255 (4.35), 325 (4.47). *Anal.* Calcd. for $C_{20}H_{24}O_8N_4$: N, 12.50. Found : N, 11.92. The 2,4-dinitrophenylhydrazine reagent unreacted was removed readily as it was very strongly adsorbed on the top of the column.

Treatment of Itaconitin with Hydroxylamine——A mixture of itaconitin, $NH_2OH \cdot HCl$ and AcONa in aq. EtOH was boiled for 20 min. The reaction product precipitated after cooling was recrystallized from MeOH to give yellow microneedles, m.p. 231°. *Anal.* Calcd. for $C_{14}H_{15}O_5N$: C, 60.64; H, 5.45; N, 5.05. Found : C, 60.87; H, 5.91; N, 4.93.

Treatment of Itaconitin with Acetyl Chloride and Pyridine (Acetylanhydroitaconitin)——Itaconitin was treated with AcCl and pyridine in the usual way, and the crude product obtained was chromatographed on $CaHPO_4$ column with benzene. From the bottom band acetylanhydroitaconitin[1] was eluted, while from the second yellow fluorescent band a resinous product was given. IR ν_{max}^{KBr} cm^{-1} : 978 (*trans* CH=CH), 1212, 1760 (phenolic $COCH_3$), 1394 (CH_3), 1504, 1615, 1640 (phenyl), 1808, 1850 (C=O). UV λ_{max}^{EtOH} mμ (log ε) : 236 (sh.) (4.27), 316 (4.44), 374 (sh.) (3.50).

Propionylanhydroitaconitin——A suspension of itaconitin (300 mg.) in propionic anhydride (2 ml.) was heated at 160° in an oil-bath for 15 min. After cooling, the resulting reddish yellow solution was poured into ice water and neutralized with $NaHCO_3$ to give crude brownish yellow crystals (320 mg.), which were recrystallized from petr. benzin–benzene to yellow long prisms, m.p. 104°. IR ν_{max}^{KBr} cm^{-1} : 968, 978 (*trans* CH=CH), 1391 (CH_3), 1504, 1570, 1615, 1640 (phenyl and double bond), 1760, 1813, 1855 (C=O). UV λ_{max}^{EtOH} mμ (log ε) : 236 (4.22), 315 (4.61), 370 (sh.) (4.07). *Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found : C, 68.18; H, 5.40.

Hydrolysis of Propionylanhydroitaconitin (Anhydroitaconitin)——A solution of the propionylanhydroitaconitin (300 mg.) obtained as above in 10% aq. Na_2CO_3 (5 ml.) and Me_2CO (8 ml.) was refluxed for 10 min.

*6 All melting points were uncorrected. Ultraviolet spectra were measured with Cary Model 11 recording spectrophotometer, and infrared spectra with Koken Model DS-301 spectrophotometer.

After removing Me_2CO, the reaction mixture was acidified while hot with conc. HCl, and heated on a steam-bath for further 10 min. The crude product was crystallized from $CHCl_3$ to orange prisms, m.p. 178.5°, undepressed on admixture with the anhydroitaconitin from acetylanhydroitaconitin.[1] IR ν_{max}^{KBr} cm^{-1}: 982 (*trans* CH=CH), 1388 (CH_3), 1508, 1603, 1625 (phenyl), 1743, 1808, 1849 (C=O), 3420, 3582 (OH) (Fig. 3). UV λ_{max}^{EtOH} mμ (log ε): 257 (3.95), 300 (4.10), 353 (4.22), 396 (4.23). Calcd. for 2 C-Me, 12.31. Found: C-Me, 8.99; neutral. equivalent (phenolphthalein), 205 (in cool),*7 128 (in hot).

Propionylation of Anhydroitaconitin (Propionylanhydroitaconitin)——The procedure was essentially the same as that described for the treatment of itaconitin with propionic anhydride. Yellow long prisms (from petr. benzin-benzene), m.p. 104°, undepressed on admixture with the product from itaconitin.

Anhydroitaconitin 2,4-Dinitrophenylhydrazide——A mixture of anhydroitaconitin (122 mg.), EtOH (3 ml.), conc. H_2SO_4 (0.4 ml.) and H_2O (0.6 ml.) was heated with 2,4-dinitrophenylhydrazine reagent (99 mg.) on a steam-bath for 5 min. The reaction mixture was allowed to cool, and the precipitate deposited was collected by filtration, washed with H_2O then with a small amount of EtOH. The crude product thus obtained was purified by chromatography on $CaHPO_4$ using dioxane-hexane (1:10) as the developing solvent. Orange microcrystals (from dioxane-H_2O) of melting at 254° (decomp.) was obtained. IR ν_{max}^{KBr} cm^{-1}: 985 (*trans* CH=CH), 1343 (NO_2), 1390 (CH_3), 1504, 1527, 1606, 1617 (phenyl, double bond, and NO_2), 1721, 1768 (C=O), 3370, 3485 (OH). *Anal.* Calcd. for $C_{20}H_{16}O_7N_4$: C, 56.60; H, 3.80; N, 13.20. Found: C, 56.64, 56.33; H, 3.94, 4.01; N, 12.69.

Condensation Reaction of Anhydroitaconitin with *o*-Phenylenediamine——A red solution of anhydroitaconitin (122 mg.) and *o*-phenylenediamine (70 mg.) dissolved in EtOH (5 ml.) was allowed to stand for two days in a dark place, when an almost pure condensation product (147 mg.) precipitated, which was washed with EtOH to give orange prisms, m.p. 222°. IR ν_{max}^{KBr} cm^{-1}: 1501, 1607, 1624 (phenyl and double bond), 1696, 1762 (C=O). *Anal.* Calcd. for $C_{20}H_{18}O_3N_2$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.63; H, 5.37; N, 7.90.

Condensation Reaction of Acetylanhydroitaconitin with *o*-Phenylenediamine——Acetylanhydroitaconitin (600 mg.) was treated with a solution of *o*-phenylenediamine (300 mg.) in MeOH (30 ml.), and the mixture was heated at 80° for 5 min. The yellow-orange needles formed was taken up on a filter and washed with a small amount of MeOH. M.p. 210°. IR ν_{max}^{KBr} cm^{-1}: 1378 (CH_3), 1504, 1607, 1630 (phenyl and conj. double bond), 1700, 1780 (C=O), 1396. *Anal.* Calcd. for $C_{22}H_{20}O_4N_2$: C, 70.20; H, 5.36; N, 7.44. Found: C, 69.82; H, 5.29; N, 7.43.

Oxidation of Anhydroitaconitin with Potassium Permanganate——Anhydroitaconitin was suspended in H_2O and neutralized with dil. aq. NaOH. Then to the resulting solution was added $KMnO_4$ in small portions until a purple color was kept. The mixture was then decolorized by heating on a steam-bath, and filtered while hot. The precipitate was washed with H_2O and the combined filtrate and washings were acidified with HCl and extracted with Et_2O. The yellowish sticky crystalline residue after removing the solvent was washed with Et_2O, and sublimed onto a cover-glass. The sublimate, colorless cubes, m.p. 177°, was confirmed as being oxalic acid by a mixed fusion.

Treatment of Anhydroitaconitin with Nitric Acid (2,4,6-Trinitro-*m*-cresol)——A suspension of anhydroitaconitin (1.5 g.) in conc. HNO_3 (d=1.325) was heated on a steam-bath for 3 hr. During the period, a brown tar floating on the surface dissolved to form a clear solution. After standing overnight, the yellow transparent solution was diluted with H_2O (60 ml.) and extracted thrice with each 50 ml. portions of benzene. The combined extract was washed with H_2O (20 ml.), dried over anhyd. Na_2SO_4, concentrated to a volume of 20 ml. and was passed through the column of $CaHPO_4$ (2×20 cm.). The residue obtained from the pale yellow band was crystallized from $CHCl_3$ to give thin yellow prisms (850 mg.) of melting at 107°. IR ν_{max}^{KBr} cm^{-1}: 928 (penta-substituted phenyl), 1353, 1540 (NO_2), 1378 (CH_3), 1510, 1606, 1634 (phenyl). *Anal.* Calcd. for $C_7H_5O_7N_3$: C, 34.58; H, 2.07; N, 17.28. Found: C, 34.49, 34.55; H, 2.02, 2.30; N, 17.75.

This substance was confirmed to be identical with the 2,4,6-trinitro-*m*-cresol prepared from thymol[2] by mixed fusion and comparison of IR spectra.

Ozonolysis of Anhydroitaconitin (2,4-Cresotaldehyde, Acetaldehyde and CO_2)——An orange solution of anhydroitaconitin (1 g.) in methyl formate (40 ml.) was cooled to 0° and treated with ozone for 4 hr., when the color of the solution changed to light yellow. After removal of the solvent *in vacuo*, the residue was mixed with 3% NaOH (30 ml.) and subjected to a steam-distillation under N_2 stream. To the distillate was added a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* HCl, and the orange crystalline precipitate was filtered up and extracted twice with each 20 ml. portions of boiling EtOH. The crude EtOH-soluble 2,4-dinitrophenylhydrazone thus obtained was passed through silica gel column using $CHCl_3$ as the solvent. The unreacted reagent was readily removed in this procedure, as it was absorbed very strongly on the top of the column. Recrystallization from EtOH gave orange plates (70 mg.), m.p. 161°,

*7 The observed value of the neutral. equivalent, 124, reported in Part I was determined by a back titration. It is noticeable that this value is almost corresponding to the observed value in hot.

which agreed with the authentic acetaldehyde 2,4-dinitrophenylhydrazone by mixed melting point determination and comparison of IR spectra.

The EtOH-insoluble part of 2,4-dinitrophenylhydrazone was dissolved in $CHCl_3$ and chromatographed on silica gel. The material eluted with the same solvent was crystallized from tetrahydrofuran to give orange plates (100 mg.), m.p. 258° (decomp.), which was confirmed to be the 2,4-dinitrophenylhydrazone of 2,4-cresotaldehyde. *Anal.* Calcd. for $C_{14}H_{12}O_5N_4$ (2,4-cresotaldehyde 2,4-dinitrophenylhydrazone): C, 53.16; H, 3.82; N, 17.72. Found: C, 53.25; H, 3.86; N, 18.17.

On boiling the residue of the steam-distillation after acidification with dil. H_2SO_4(1:1) (10 ml.), CO_2 gas was liberated which was trapped into 5% $Ba(OH)_2$ to yield $BaCO_3$ (187 mg.). The residue was then extracted with Et_2O to separate light yellow crystals (290 mg.) which were recrystallized from hexane to yield sublimable colorless long prisms, m.p. 60°, having a characteristic odor of salicylic aldehyde. IR ν_{max}^{KBr} cm^{-1}: 1388 (CH_3), 1508, 1578, 1625 (phenyl), 1653 (chelated aldehyde). *Anal.* Calcd. for $C_8H_8O_2$ (2,4-cresotaldehyde): C, 70.57; H, 5.92. Found: C, 70.68; H, 5.83.

A mixture of the above-obtained aldehyde, m.p. 60° (100 mg.), $AgNO_3$ (200 mg.) in NH_4OH (2 ml.) and NaOH (200 mg.) in H_2O (2 ml.) was heated for 15 min. on a boiling water bath. The hot reaction mixture was filtered, and the precipitate was washed with H_2O. The combined filtrate and washings were acidified with dil. HNO_3, and the precipitates were recrystallized from hexane-$CHCl_3$ to give colorless prisms, m.p. 175°. *Anal.* Calcd. for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 62.90; H, 5.20.

This substance was proved to be identical with the 2,4-cresotic acid synthesized from *m*-cresol by the Kolbe-Schmidt reaction, by mixed fusion and comparison of IR spectra.

Catalytic Hydrogenation of Anhydroitaconitin (Dihydroanhydroitaconitin)——A solution of anhydroitaconitin (3.23 g.) in Me_2CO (100 ml.) was catalytically hydrogenated over 10% Pd-C at room temperature, and 362 ml. (theoretical amount for 1 mole: 297 ml.) of hydrogen was absorbed in 10 min. After filtration and evaporation, a light yellow oil was yielded which solidified on cooling. The product was dissolved in $CHCl_3$ and subjected to column chromatography on $CaHPO_4$. The yellow band eluted gave a crude product (2.8 g.) of dihydroanhydroitaconitin that was purified by recrystallization twice from benzene-hexane and thrice from benzene to give colorless plates, m.p. 105°. This substance decolorizes Br_2 or $KMnO_4$, gives positive Tollens, and diazonium reaction, but negative $FeCl_3$ reaction. IR ν_{max}^{KBr} cm^{-1}: 1386 (CH_3), 1522, 1591, 1615 (phenyl), 1679 (double bond), 1760, 1818, 1859 (C=O), 2865, 2925 (CH_2), 3530 (OH). *Anal.* Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.12; H, 5.73; neutral. equivalent (phenolphthalein), 205 (at cool), 129 (at hot).

From the washings of $CaHPO_4$ column by Me_2CO was obtained a brown crystalline mass (170 mg.) that was not fully investigated.

Treatment of 2,4-Cresotic Acid with Nitric Acid (2,4,6-Trinitro-*m*-cresol)——2,4-Cresotic acid (0.5 g.) was reacted with HNO_3 (d=1.325) (15 ml.) by heating in a boiling water bath for 3 hr. After cooling, the reaction mixture was treated with H_2O (150 ml.) and allowed to stand overnight in a refrigerator. Almost pure pale yellow needles, m.p. 108°, separated was identified with 2,4,6-trinitro-*m*-cresol prepared from anhydroitaconitin or thymol.

Succinic Acid from Dihydroandrohydroitaconitin——A stream of ozone was introduced into a solution of dihydroanhydroitaconitin (1.2 g.) in AcOEt (20 ml.) on cooling at 0°. A brownish yellow resinous substance obtained after removal of the solvent *in vacuo*, was subjected to a steam-distillation to remove volatile substance. To the brownish resinous residue was added a sufficient amount of powdered $KMnO_4$, when the reaction proceeded with evolution of heat liberating CO_2. After the effervescence had ceased, the reaction mixture was filtered, and the filtrate was made acidic with HCl. The brownish resin separated out was removed, and the filtrate was extracted 3 times with each 200 ml. of Et_2O. The crude product (28 mg.) that remained after removal of the solvent was purified by recrystallization from H_2O giving colorless prisms, m.p. 185°, which agreed with the authentic sample of succinic acid in mixed melting point determination and comparison of IR spectra.

Catalytic Hydrogenation of Acetylanhydroitaconitin (Dihydroacetylanhydroitaconitin)——A solution of acetylanhydroitaconitin (200 mg.) in Me_2CO (50 ml.) was catalytically hydrogenated over 10% Pd-C at room temperature, and 20.4 ml. (theoretical amount for 1 mole: 15.6 ml.) of hydrogen was absorbed. After treating in the usual manner, the oily residue was dissolved in a small amount of MeOH. On standing at room temperature for 2 days, large cubic crystals involving MeOH as the crystal solvent, m.p. 85° (moistened at 67°) were separated. IR ν_{max}^{KBr} cm^{-1}: 1375 (CH_3), 1510, 1584, 1628 (phenyl), 1681 (double bond), 1758, 1819, 1862 (C=O), 2855, 2927 (CH_2), 1221. *Anal.* Calcd. for $C_{16}H_{16}O_5 \cdot CH_3OH$: C, 63.74; H, 6.29. Found: C, 64.39; H, 5.69.

Hydrolysis of Dihydroacetylanhydroitaconitin (Dihydroanhydroitaconitin)——A mixture of dihydroacetylanhydroitaconitin (500 mg.), Me_2CO (1 ml.) and 10% Na_2CO_3 (1 ml.) was refluxed for 1 hr. After removal of Me_2CO, the reaction mixture was acidified with HCl to yield colorless plates, m.p. 105°, which agreed with the dihydoanhydroitaconitin prepared from anhydroitaconitin.

Catalytic Hydrogenation of Anhydroitaconitin in Alkali ($C_{14}H_{18}O_5$ Acid)——A solution of anhydroitaconitin (635 mg.) was hydrogenated in 0.5% NaOH (60 ml.) over 10% Pd-C catalyst. Three moles (117 ml.) of hydrogen was quantitatively absorbed during a period of 24 hr. The catalyst was removed by filtration,

washed with H_2O and the filtrate combined with washings was acidified with HCl. Extraction with 5 portions of 200 ml. each of Et_2O, gave a crude reduction product (460 mg.) that was washed with $CHCl_3$, crystallized from $CHCl_3$–Me_2CO to give colorless leaflets (under microscope), m.p. 145°. This substance is insoluble in petr. benzin or $CHCl_3$ and gives a red coloration with $FeCl_3$ in EtOH. When it was heated rapidly up to 120°, it decomposed with effervescence, but when the temperature was raised slowly, it did not melt below 145°. IR ν_{max}^{KBr} cm^{-1}: 941, 1200, 1420, 1695, 1715 (COOH), 1380 (CH_3), 1516, 1594, 1626 (phenyl). Neutral. equivalent; 143.

Acetylation of $C_{14}H_{18}O_5$ Acid——The above-obtained $C_{14}H_{18}O_5$ acid (500 mg.) was boiled with Ac_2O (4 ml.) for 1 hr. Then the solution was evaporated *in vacuo* to remove Ac_2O, and the resinous substance remained washed with ligroin, dissolved in Et_2O, decolorized with active charcoal, and then crystallized by adding petr. benzin. A sample for analysis was prepared by further recrystallization from H_2O. Colorless needles, m.p. 148°. IR ν_{max}^{KBr} cm^{-1}: 1225, 1760 (phenolic or enolic $COCH_3$), 1373 (CH_3), 1506, 1584, 1623 (phenyl), 1695, 1711 (COOH). *Anal.* Calcd. for $C_{16}H_{20}O_6$: C, 62.32; H, 6.54; Acetyl (monoacetate), 13.96. Found: C, 62.34; H, 6.56; Acetyl, 14.29; neutral. equivalent (phenolphthalein), 162.

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Summary

The ozonolysis of anhydroitaconitin which was prepared from itaconitin by the action of acetic or propionic anhydride followed by hydrolysis, yielded 2,4-cresotaldehyde liberating acetaldehyde and carbon dioxide. Treatment of the same substance with nitric acid afforded 2,4,6-trinitro-*m*-cresol. These and other experimental results showed that the anhydroitaconitin was an aromatic compound having a *m*-cresyl grouping with an adjacent ethylenic linkage. The catalytic hydrogenation of anhydroitaconitin in acetone gave dihydroanhydroitaconitin, whereas in alkali gave a carboxylic acid, $C_{14}H_{18}O_5$.

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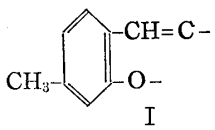
11. Shoichi Nakajima: Studies on the Structure of Itaconitin. V.*1 On the Alkaline Hydrogenation Product of Anhydroitaconitin.*2

(*Hoshi College of Pharmacy**3)

On the basis of the experimental results reported in the preceding paper*1 anhydroitaconitin,[1] a dehydration product of itaconitin, was formulated partially as I.



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By the present study, it was found that ozonolysis of acetylanhydroitaconitin*1,1) yielded 2,4-cresotaldehyde. This appears to suggest that the O-functional group directly attached to the benzene nucleus in

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*1 Part Ⅳ. S. Nakajima, K. Kinoshita, S. Shibata: This Bulletin, **13**, 58 (1965).

*2 Presented at Ⅷth Japanese Symposium on the Chemistry of Natural Products (Oct. 1963). Proceedings, p. 144 (in Japanese).

*3 2-Chome, Ebara, Shinagawa-ku, Tokyo (仲嶋正一).

1) Part I. K. Kinoshita, S. Nakajima: *Ibid.*, **6**, 31 (1958).