Summary

The structural formula of anhydroitaconitin was established as 2-methyl-3-(*trans*-2-hydroxy-4-methylstyryl)maleic anhydride. The structures of the reaction products of anhydroitaconitin with diazomethane, *o*-phenylenediamine or various ketonic reagents, and acetylanhydroitaconitin, propionylanhydroitaconitin, dihydroanhydroitaconitin, dihydroacetylanhydroitaconitin and all other derivatives hitherto prepared were determined. The ultraviolet, infrared and nuclear magnetic resonance data were provided to support these formulae.

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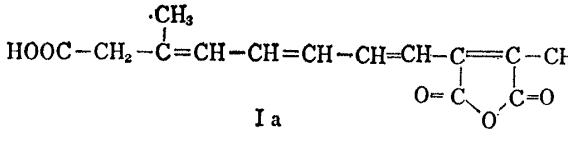
13. Shoichi Nakajima : Studies on the Structure of Itaconitin. VII.*1 The Structures of Itaconitin and Its Derivatives.*2

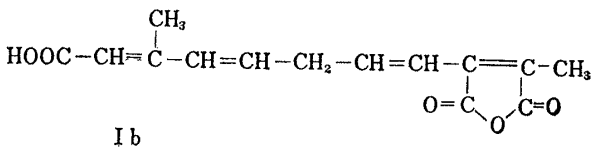
(*Hoshi College of Pharmacy**3)

Anhydroitaconitin, the dehydro-product of itaconitin, has been established to possess the structural formula (Ⅲ) as mentioned in Part Ⅵ*1 of this series of works. The present paper deals with the determination of the structures of itaconitin and its derivatives.

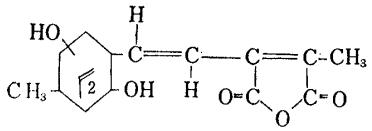
As mentioned in Part Ⅳ[1] of this series, itaconitin which is originally a non-aromatic substance is converted into an aromatic compound, acetylanhydroitaconitin (Ⅱ), on acetylation process, and the latter gives Ⅲ by deacetylation. The easy formation of benzene ring in Ⅱ, or Ⅲ, from itaconitin can only be deduced when one of the structures (Ia), (Ib), and (Ic) is adopted for itaconitin.



Ia



Ib



Ic

Of these formulae, Ic must have an asymmetric carbon atom in the six-membered ring of its molecule. However, itaconitin was found to have no optical activity. Moreover all the attempts for the acetylation without accompanying dehydration and

*1 Part Ⅵ. S. Nakajima : This Bulletin, **13**, 69 (1965).
*2 This work was presented at the monthly meeting of the Kanto Branch of Pharmaceutical Society of Japan on Feb., 1964.
*3 2-Chome, Ebara, Shinagawa-ku, Tokyo (仲嶋正一).
1) Part Ⅳ. S. Nakajima, K. Kinoshita, S. Shibata : *Ibid.*, **13**, 58 (1965).

aromatization by dehydrogenation which might be accessible by the formula (Ic) were failed.

It has already been reported that itaconitin or hexahydroitaconitin reacts with the ketonic reagents forming semicarbazide,[2] *p*-nitrophenylhydrazide,[1] 2,4-dinitrophenylhydrazide,[2] hydroxyimide[1] of itaconitin, or 2,4-dinitrophenylhydrazide of hexahydroitaconitin.[1] The chemical structures of these derivatives can readily be postulated by the analogy of the corresponding products of anhydroitaconitin and its derivatives.*[1] Thus the two bands appearing in the infrared spectra of these products, 1732 and 1780 cm^{-1} of itaconitin semicarbazide (KBr), 1732 and 1780 cm^{-1} of itaconitin 2,4-dinitrophenylhydrazide ($CHCl_3$), 1715 and 1764 cm^{-1} of itaconitin *p*-nitrophenylhydrazide (KBr) (Fig. 1), 1731 and 1782 cm^{-1} of hexahydroitaconitin 2,4-dinitrophenylhydrazide (KBr) are explicable by the α,β-unsaturated five-membered cyclic imide structure. Furthermore, it should be noted that these compounds have another strong infrared absorption bands in their carbonyl region; for an example, 1696 cm^{-1} of itaconitin *p*-nitrophenylhydrazide (Fig. 1). As these compounds were formed by treatment with ketonic reagents, this carbonyl band should be based on a non-ketonic function, and it could be assigned to a carboxylic carbonyl group by the following evidences.

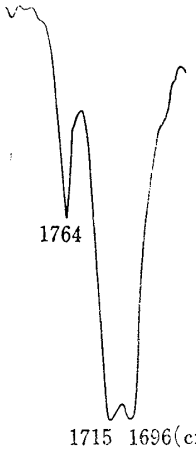


Fig. 1. Infrared Spectrum of Itaconitin *p*-Nitrophenylhydrazide (KBr)

In the infrared spectrum of itaconitin or hexahydroitaconitin (Figs., 1 and 2 in Part IV[1]), an absorption band near 1700 cm^{-1} (in KBr) is observed, which shifts to 1738 cm^{-1} in dioxane solution. This shift of band is usually explained by the splitting of the intermolecular bonding of carboxylic acid by the association with dioxane.

The presence of a carboxyl group in itaconitin, which favors the proposed formulae (Ia) and (Ib) excluding the possibility of Ic, was also proved by the observation of the infrared spectrum of hexahydroitaconitin ditoluide, m.p. 124°. The infrared spectrum (Fig. 2) of this compound showed absorption bands at 1520 and 1653 cm^{-1} (KBr) which shifted to 1515 and 1690 cm^{-1} in chloroform solution indicating the presence of the secondary amide, associated intermolecularly in KBr and nonassociated in the chloroform solution. Thus the formation of *p*-toluide indicated the carboxyl grouping in the original hexahydroitaconitin molecule. The absorption bands at 1702 and 1750 cm^{-1} assigned to α,β-unsaturated cyclic imide are also observed in the infrared spectrum (Fig. 2) of hexahydroitaconitin ditoluide.

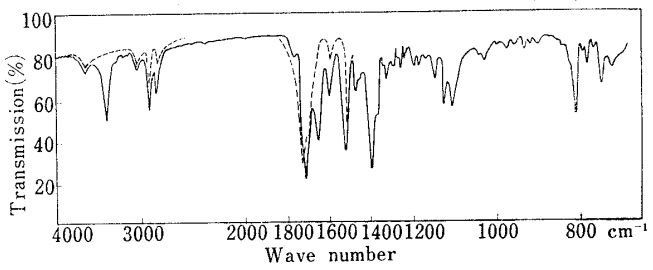


Fig. 2. Infrared Absorption Spectrum of Hexahydroitaconitin Ditoluide
——— in KBr ------ in $CHCl_3$

On oxidation of hexahydroitaconitin with potassium permanganate, liquid nonvolatile acids were produced liberating carbon dioxide and acetic acid. The nonvolatile acid mixture was purified by the method reported in Part II[3] and III[4] to obtain a phenylazoanilide, m.p. 218°, $C_{34}H_{36}O_2N_6$ (XIV) whose ultraviolet absorption curve showed two maxima at 237 and 349 mμ

2) Part I. K. Kinoshita, S. Nakajima: This Bulletin, **6**, 31 (1958).
3) Part II. K. Kinoshita, S. Nakajima: This Bulletin, **8**, 56 (1960).
4) Part III. *Idem*: *Idid.*, 8, 1051 (1960).

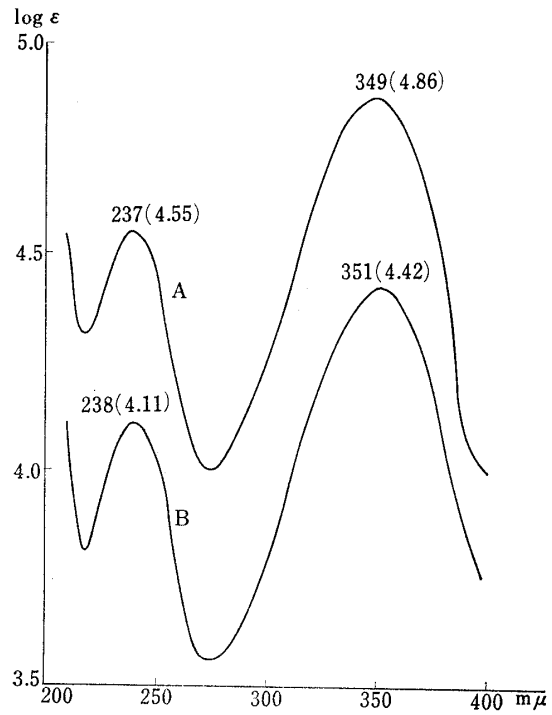


Fig. 3. Ultraviolet Spectra of *p*-Aminoazobenzene Derivatives of Hexahydroitaconitin Oxidation Product (A) and Pelargonic Acid (B)

alike that of common fatty acids derivatives. A comparison was made with pelargonic acid derivative in Fig. 3.

The degradation of hexahydroitaconitin with ozone afforded an α-ketonic acid corresponding to XII, which was treated with hydrogen peroxide and alkali to give a liquid (XIII) by decarboxylation, whose ethyl ester, $C_{14}H_{26}O_4$, boiling between 295° and 310° was

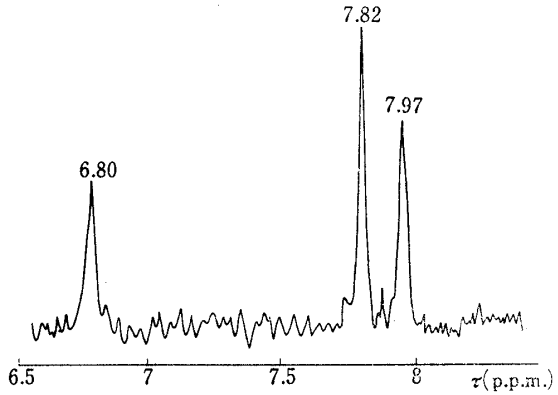


Fig. 4. Nuclear Magnetic Resonance of Itaconitin, measured in Deuterochloroform at 60 Mc.p.s.

identified with the synthetic diethyl-2-methyl-nonanedioate (XV)[5] by comparison of their infrared spectra. The identification was also made by comparing their relative retention times in the gas chromatography.

In view of the above evidences, the structural formula of hexahydroitaconitin can be represented by IX. Accordingly, itaconitin would be formulated as Ia or Ib. The infrared absorption band of itaconitin at 1709 cm^{-1} (Fig. 1 in Part IV[1]) due to the formula (Ib), as the carboxyl group in Ib should be conjugated with a double bond. Furthermore, the nuclear magnetic resonance spectrum of itaconitin (Fig. 4)*4 gave a singlet signal at 6.80 τ with the magnitude of two protons to show the presence of a methylene group adjacent to a quaternary carbon. This supported the formula (Ia) in excluding Ib.

The structure of itaconitin has thus finally been established to be represented by the formula (Ia), and the series of the reactions and derivations mentioned in this series of papers are formulated in Chart 1 :

The various derivatives of itaconitin which are formed by the action of ketonic reagents would be formulated as IV, V, VI, VII, and the hydroxylamine adduct[2] of itaconitin presumably as VIII, 2,4-dinitrophenylhydrazide and *p*-toluide of hexahydroitaconitin as X and XI, respectively.

The cyclization of Ia to II which takes place on treatment with acetic anhydride in good yield is one of the typical examples of a novel aromatization reaction of 3,5-dienoic acids, that has very recently been reported by Chiusoli and Agnés.[6] According to their description, no difference was noted in the reactions of *trans*- and *cis*-acids.

*4 NMR spectrum was measured in $CDCl_3$ at 60 Mc.p.s. using a Varian Associates A-60 apparatus and tetramethylsilane as an internal reference.

5) P. C. Freer, W. H. Perkin, jun. : J. Chem. Soc., **53**, 218 (1888).

6) G. P. Chiusoli, G. Agnés : Z. Naturforsch.,, **17B**, 852 (1962); Proc. Chem. Soc., **1963**, 310.

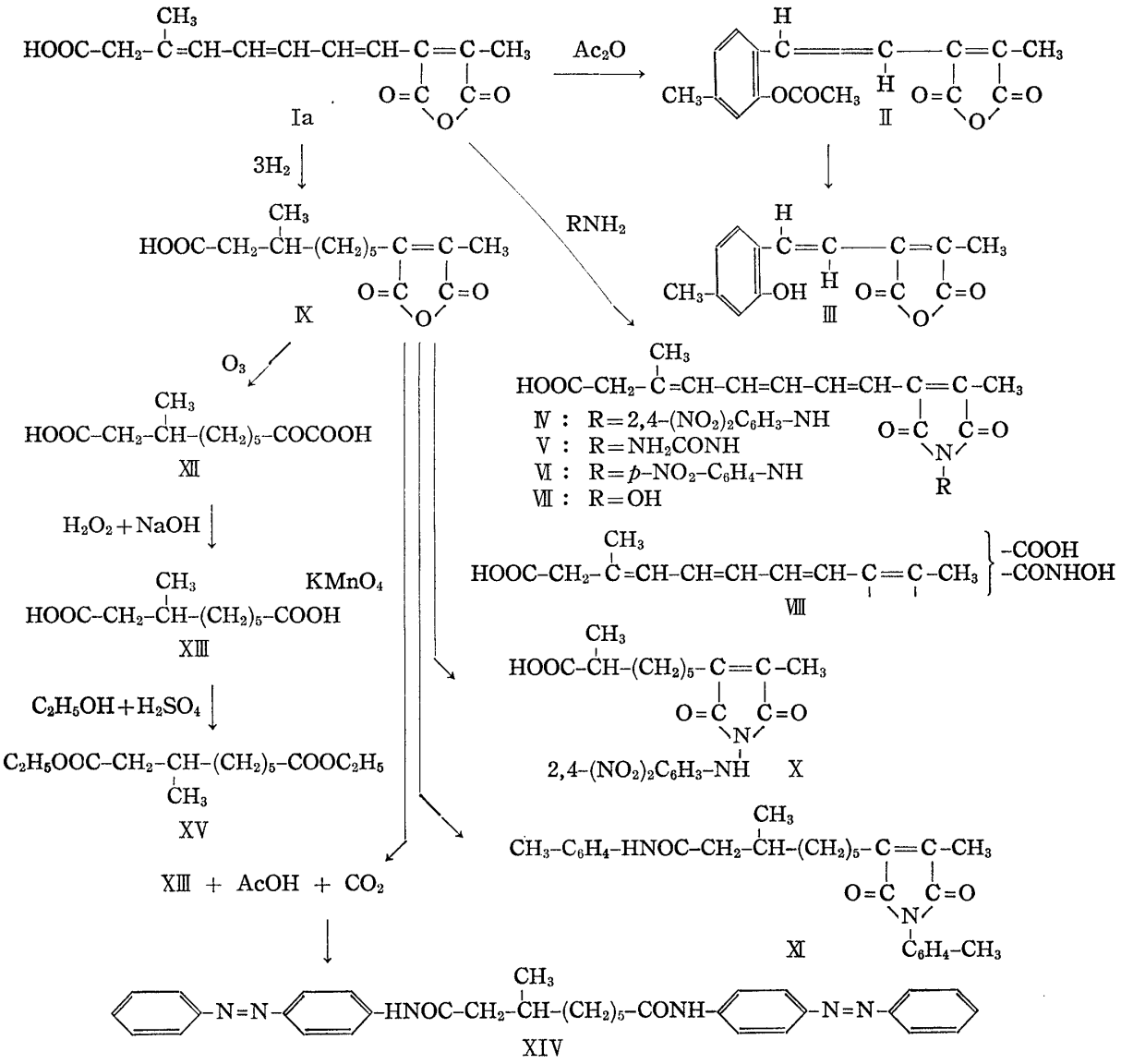


Chart 1.

Thus the configuration of the olefinic linkage of itaconitin has not been established by this cyclization reaction.

Experimental*5

Hexahydroitaconitin Ditoluide——a) Hexahydroitaconitin was boiled with 5 times of its weight of *p*-toluidine for 2 hr. After cooling, the brown mass was washed with dil. HCl, H_2O and dried. Recrystallization twice from MeOH–petr. benzin and once from MeOH gave colorless needles, m.p. 124°. IR ν_{max}^{KBr} cm^{-1} : 1520, 1653, 3325 (associated CONHR), 1600 (phenyl), 1711 (α,β-unsatd. 5-membered imide), 2855, 2925 (CH_2). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1515, 1690 (nonassociated CONHR), 1600 (phenyl), 1702, 1750 (α,β-unsatd. 5-membered imide), 2859, 2931 (CH_2). *Anal.* Calcd. for $C_{28}H_{34}O_3N_2$: C, 75.30; H, 7.67; N, 6.27. Found : C, 74.74; H, 7.67; N, 6.26; neutral. equivalent (phenolphthalein), 0.

b) Hexahydroitaconitin (100 mg.) was boiled with $SOCl_2$ for 2 hr. After removal of excess $SOCl_2$ by distillation, the residue was dissolved in benzene (10 ml.) and was treated with *p*-toluidine (700 mg.) in benzene (10 ml.). The brown crystalline mass obtained by evaporation of the solvent was recrystallized from a mixture of benzin–benzene to give colorless needles, m.p. 124°, undepressed on admixture with the product of a).

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

Oxidation of Hexahydroitaconitin with Potassium Permanganate (Formation of $C_{10}H_{18}O_4$ Acid, AcOH and CO_2)——To a suspension of hexahydroitaconitin (3.3 g.) in H_2O (50 ml.) was added pulverized $KMnO_4$ corresponding to 6 atom oxygen (9.1 g.) during a period of 20 min. with heating in water bath. After warming for further 5 min., the decolorized reaction mixture was filtered and the residue on filter was washed well with H_2O (600 ml.). The combined filtrate and the washings were concentrated *in vacuo* into ca. 50 ml., acidified with dil. H_2SO_4, then submitted to a steam-distillation. The distillate (250 ml.) was neutralized with 1/15*N* NaOH and concentrated under a diminished pressure to give a white powder (1.2 g.). The main part of this substance was proved to be Na salt of acetic acid by paper chromatography with BuOH saturated with 1.5*N* NH_4OH as solvent and B. C. G. as the developing reagent (Rf 0.14). To a water solution (20 ml.) of this substance (600 mg.) was added 2-bromo-4′-phenylacetophenone (1.0 g.) and EtOH, and the total mixture was refluxed for 4 hr. The crystalline residue yielded by evaporation of the solvent was purified by chromatography on alumina followed by recrystallization from EtOH giving colorless leaflets, m.p. 111°, undepressed on admixture with the synthetic sample of *p*-phenylphenacyl acetate.

The steam-distillation residue was extracted with Et_2O, which gave a sticky yellow liquid (1.3 g.) considered to be a fatty acid mixture by an examination of its IR spectrum. The acids mixture (650 mg.) was treated with $SOCl_2$ by boiling for 2 hr. After removing the excess $SOCl_2$ by distillation, the residue was treated with a solution of *p*-aminoazobenzene (1.0 g.) in benzene (20 ml.). The red precipitate deposited was dissolved again by boiling for 2 hr. After evaporation of the solvent, the product was immersed in conc. NH_4OH for 5 min. to make the HCl salt of excess *p*-aminoazobenzene free, and washed with H_2O, dried and treated with $CHCl_3$ (100 ml.). The $CHCl_3$-soluble part (835 mg.) was passed through alumina column (2.5×30 cm.) with the same solvent. The first band eluted was the unreacted reagent, and the residue from the second band which was sparingly soluble in $CHCl_3$ was combined with the above $CHCl_3$-insoluble portion (180 mg.), dissolved in pyridine-benzene (1:9) and was purified by passing twice through the alumina column with the same solvent. After repeated recrystallization from pyridine-H_2O, orange crystalline powder (60 mg.), m.p. 216~218°, was obtained. IR ν_{max}^{KBr} cm^{-1} : 1304, 1530, 1661, 3285 (CONHR), 1376 (CH_3), 1510, 1603 (phenyl), 2845, 2915 (CH_2). UV λ_{max}^{EtOH} mμ (log ε) : 237 (4.55), 349 (4.86). *Anal.* Calcd. for $C_{34}H_{36}O_2N_6$: C, 72.83; H, 6.47; N, 14.99. Found : C, 72.48; H, 6.54; N, 14.90.

Ozonolysis of Hexahydroitaconitin (2-Methylnonanedioic Acid)——A solution of the hexahydroitaconitin, which was prepared by a catalytic hydrogenation of itaconitin (5 g.) in Me_2CO (120 ml.) over 10% Pd-C, was subjected to ozonization under ice-cooling for 1.5 hr. The crystalline ozonide remained after removal of the solvent was treated with H_2O and boiled for 10 min. Then the reaction mixture was treated with 30% H_2O_2 (5 ml.) and 5% NaOH (10 ml.) and allowed to stand for 1 hr. at room temperature for decarboxylation. The resulting yellow clear solution was acidified with HCl, extracted with benzene (120 ml.), and the extract was dried over Na_2SO_4 and evaporated to dryness. The yellow sticky oily residue was esterified by boiling with EtOH (20 ml.) and conc. H_2SO_4 for 1.5 hr. After standing overnight, EtOH was distilled off, and the residue was washed with H_2O, aq. Na_2CO_3 solution and H_2O successively, and dried over Na_2SO_4. On evaporation a colorless sticky liquid (1.3 g.), b.p. 295~310° was obtained. IR $\nu_{max}^{cycloh\ xane}$ cm^{-1} : 1376 (CH_3), 1732, 1761 (ester CO), 2848, 2919 (CH_2). *Anal.* Calcd. for $C_{14}H_{26}O_4$: C, 65.08; H, 10.15. Found : C, 65.09; H, 10.15.

This substance was confirmed to be identical with the following synthetic diethylnonanedioate by comparison of IR spectra and their relative retention times in the gas chromatography*[6] of both substances.

Diethyl 2-Methylnonanedioate——2-Methylnonanedioic acid was prepared starting with ethyl 3-bromopropionate and ethyl acetoacetate, through the following intermediates : ethyl 2-acetylglutarate,[9] 5-oxohexanoic acid,[10] ethyl 5-oxohexanoate,[11] 1,5-dibromohexane,[13] tetraethyl 2-methylheptanetetracarboxylate,[5] and 2-methyl-1,1,7,7-heptanetetracarboxylic acid.[5] The final acid was then esterified by the method described above yielding a colorless liquid, b.p. 290~305°. *Anal.* Calcd. for $C_{14}H_{26}O_4$: C, 65.08; H, 10.14. Found : C, 64.93; H, 10.01.

This series of work was carried out under the guidance of Prof. S. Shibata, University of Tokyo, to whom the author wishes to express his gratitude. He is also indebted to Prof. Dr. K. Kinoshita of this college, who gave him this theme and constant encouragement, to Dr. U. Sankawa of the University of Tokyo for valuable discussions, to Mr. Murata, the president of this college for encouragement. The NMR spectra were measured by the staff of the Mitsubishi Kasei Co., Ltd. IR and UV spectral measurements and microanalyses were carried out by the members of the microanalytical laboratories of the

*[6] The conditions carried out and data obtained were as follows : Apparatus, Hitachi K. G. L.-2B; Column, 10% polyethyleneglycol on Diasolid M; Gas, He; Temperature, 200°; Flow rate 30 ml./min.; Rt., 2.2 min.

9) W. O. Emery : Ber., **24**, 282 (1891).
10) W. H. Bentley, W. H. Perkin, jun. : J. Chem. Soc., **69**, 1511 (1896).
11) D. Vorländer : Ann., **294**, 270 (1897).
12) E. V. Rudloff : Can. J. Chem., **36**, 486 (1958).
13) W. H. Perkin, jun. : J. Chem. Soc,. **51**, 722 (1887).

Faculty of the Pharmaceutical Sciences of the University of Tokyo and of the Hoshi College of Pharmacy. The author is grateful to them. This work was partly supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education, to which the author is also indebted.

Summary

It was found that itaconitin, a yellow coloring matter of a mould *Aspergillus itaconicus* KINOSHITA, possesses a 3,5-dienoic acid system which is converted into an aromatic ring by the action of acetic anhydride to give acetylanhydroitaconitin. Oxidation of hexahydroitaconitin with potassium permanganate, as well as the ozonolysis of the same substance yielded 2-methylnonanedioic acid. From these and other experimental data including nuclear magnetic resonance spectra, the structural formula of itaconitin and all its derivatives hitherto prepared were conclusively established.

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14. Toshio Nambara and Motohiko Katō : Analytical Chemical Studies on Steroids. IV*1. The Zimmermann Reaction of 16- and 17-Oxosteroids.*2

(*Faculty of Pharmaceutical Sciences, University of Tokyo**3)

The Zimmermann reaction has been widely used in the field of biochemistry as well as organic chemistry for the detection and determination of the compounds having active methylene group, particularly, of the oxosteroids. Since the reaction was discovered by Zimmermann[4] in 1935, several workers have investigated its specificity and applicability.[5~8] With regards to the reaction mechanism, it has been accepted that *m*-dinitrobenzene condenses with the active methylene group to yield a Meisenheimer-type compound (IIa)[9~12] as shown in Chart 1. This mechanism had not apparently been

*1 The previous papers, "Colorimetric determination of cholestan-3α-ol in the presence of cholestan-3β-ol and cholesterol,"[1] "Reactions of the 16-double bond of 5α,14β-androst-16-ene,"[2] and "Chemistry of 3β,12-dihydroxy-5α-androstan-17-ones"[3] were entitled as Part I, II and III of this series, respectively.

*2 A part of this paper was presented at the 84th Annual Meeting of Pharmaceutical Society of Japan in Tokyo (April, 1964).

*3 Motofuji-cho, Bunkyo-ku, Tokyo (南原利夫, 加藤元彦).

1) T. Nambara, R. Imai, S. Sakurai : Yakugaku Zasshi, **84**, 680 (1964).
2) T. Nambara, K. Hirai : This Bulletin, **12**, 836 (1964).
3) T. Nambara : *Ibid.*, **12**, 1253 (1964).
4) W. Zimmermann : Z. physiol. Chem., Hoppe-Seyler's, **233**, 257 (1935); **245**, 47 (1937).
5) K. Kaziro, T. Shimada : *Ibid.*, **249**, 220 (1937).
6) H. W. Marlow : J. Biol. Chem., **183**, 167 (1950).
7) H. Wilson : Arch. Biochem. Biophys., **52**, 217 (1954).
8) A. B. Roy : Austral. J. exp. Biol., **40**, 415 (1962).
9) J. Meisenheimer : Ann., **323**, 205 (1902).
10) T. Canbäck : Farm. Revy., **48**, 153 (1949) (C. A., **43**, 4650 (1949)).
11) M. Kimura : Yakugaku Zasshi, **73**, 1219 (1953).
12) M. Akatsuka : *Ibid.*, **80**, 389 (1960).