On Mangiferin, the Coloring Matter of Mango (Mangifera indica Linn.). IV*[1]*. Isolation of 1,3,6,7-Tetrahydroxyxanthone and the Skeletal Structure of Mangiferin*[2]

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In Part III[1] of this series, it was shown that mangiferin is a substance agreeing with the composition $C_{19}H_{18}O_{11}$. For the structure of this pigment, Wiechowski[3] proposed a formula having a skeleton of 1,4,5,7-tetrahydroxyxanthone, but failed to offer any evidence supporting such an assumption. The fact that mangiferin changes into euxanthic acid in rabbit body[3] indicates that the pigment molecule possesses a xanthone skeleton or a structure that can easily resume the xanthone skeleton, and Gorter[4] proposed a structural formula favoring the latter. Although structural formulae were proposed, all attempts for the experimental evidence of skeletal structure of the pigment molecule have been unsuccessful.

Mangiferin resists hydrolysis with hydrochloric or sulfuric acid and the "genin" can not be isolated by such a method. Wiechowski[3] tried the application of hydroiodic acid but has not isolated any crystalline product. The writer succeeded in isolating a crystalline product by heating the pigment in phenol with hydriodic or hydrobromic acid.

This product was not found to be purifiable *per se* when it was acetylated, and the acetate was recrystallized from glacial acetic acid to colorless crystals, $C_{21}H_{16}O_{10}$, m.p. 200°C. Hydrolysis of this acetate with an acid afforded a product (I) as pale yellow crystalline powder or microneedle crystals, which colored markedly at 320°C, became brown at around 350°C and decomposed at 370–371°C. The analytical values agreed with $C_{13}H_8O_6$. This compound showed its phenolic property by coloring green with ferric chloride. The crude decomposition product melted with decomposition at 368°C before acetylation and its ultraviolet absorption spectrum agreed practically with that of I, recovered from the purified acetyl derivative; it is therefore certain that no cyclization or other changes in the molecular structure had taken place during acetylation.

Methylation of I with diazomethane gives a derivative (II), $C_{16}H_{14}O_6$, m.p. 218–219°C, which indicates the presence of a free phenolic hydroxyl by the positive ferric chloride reaction. Its acetylation gives crystals of m.p. 216°C, $C_{18}H_{16}O_7$, negative to the ferric chloride test, but its hydrolysis reverts it to II, m.p. 218–219°C. Application of diazomethane to II results in the total recovery of the unchanged II. It follows, therefore, that I contains a phenolic hydroxyl that is not methylated with diazomethane.

The melting points and the analytical

* *Mangiferine*, used to date, is hereafter corrected to *mangiferin* in accordance with international nomenclature.

1) Part III: S. Iseda, *J. Kumamoto Women's Univ.*, **9** (1957), in press. (in Japanese).

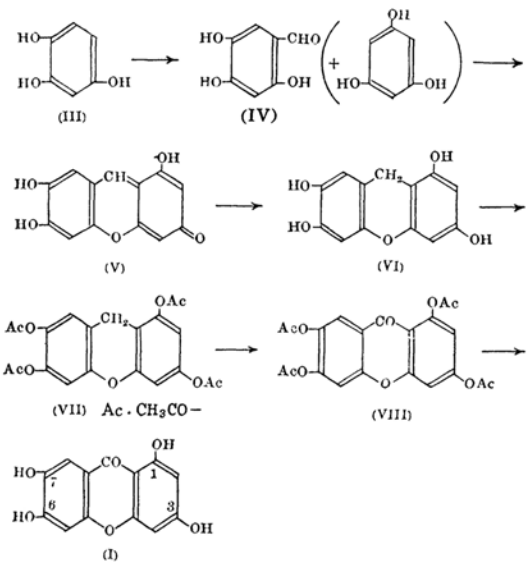
2) Presented at the Kyushu Local Meeting of the Chemical Society of Japan, Kumamoto, December 26, 1956.

3) W. Wiechowski, *Arch. exp. Pathol. Pharmakol.*, **97**, 462 (1923).

4) K. Gorter, *Bull. Jardin Bot. Buitenzorg*, (iii) **4**, 260 (1922).

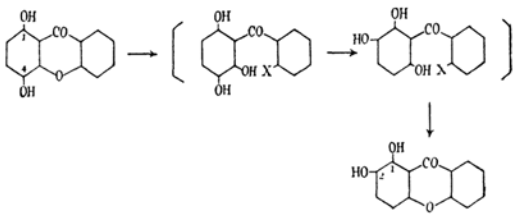
values of the acetyl derivative of I and those of II are respectively identical with the values[5] of 1,3,6,7-tetraacetoxyxanthone, m.p. 198°C, and 1-hydroxy-3,6,7-trimethoxyxanthone, m.p. 216°C. However, the melting point of 1,3,6,7-tetrahydroxyxanthone, thought to be identical with I, is given in the literature[5,6] as decomposing at 320°C. Through the courtesy of Prof. Shoji Shibata of University of Tokyo, a sample synthesized by Tanase[5] was made available. Mixed samples of the acetates and methyl ethers failed to show any depression of the melting point. The phenol also showed the same behavior when the synthetic product alone or in admixture with I was fused.

Since the Tanase sample was about 15 years old, a product was synthesized for comparison. Tanase reported two routes for the preparation of 1,3,6,7-tetrahydroxyxanthone. Hatsuda and Kuyama[6] reported the synthesis of 1,3,6,7-tetrahydroxyxanthone, m.p. 320°C(decomp.), by the preparation of diphenyl ether from 4-bromoveratrole and phloroglucinol dimethyl ether by the Ullmann reaction, according to Tanase's method, and then by deriving it to xanthone derivative by the application of oxalyl chloride. The writer carried out the synthesis by the second method (Scheme 1) and the product thereof was identical with the natural product, decomposing at 370°C. Since Tanase had observed that the products obtained by the two methods of synthesis are identical, the value of m.p. 320°C (decomp.) must indicate the initial browning temperature of the product.



Scheme 1

Heating of some kinds of flavone with hydroxyl group with hydriodic acid often causes rearrangement of the hydroxyl and this phenomenon is called the Wessely-Moser rearrangement[7]. This phenomenon has been observed in xanthones[8] and its mechanism is explained as follows (Scheme 2):



Scheme 2

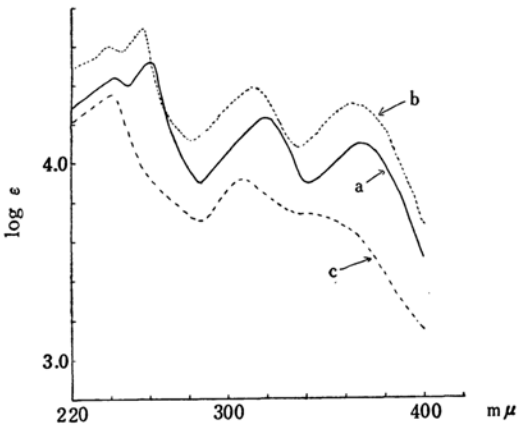
As will be clear from this mechanism, the rearrangement changes the hydroxyls in 1,4-positions to 1,2-positions (the reverse of this has not been observed). Therefore, the hydroxyl in 3-position is independent of this rearrangement and the rearrangement does not occur if there has been no hydroxyl in 1-position from the beginning. Considering such a mechanism, 1,3-dihydroxyxanthone would give a product with the same structure and 2,3-dihydroxyxanthone can not undergo such a rearrangement. Therefor, 1,3,6,7-tetrahydroxyxanthone can not be formed by rearrangement from other isomers. In other words, 1,3,6,7-tetrahydroxyxanthone must have been present in the pigment molecule from the beginning.

Next, considerations will be made on the possibility of a cyclization by the action of hydriodic acid where no xanthone ring was present in the pigment molecule itself. Since there is no possibility of a formation of a new benzene ring by the

5) Y. Tanase, *J. Pharm. Soc. Japan*, **61**, 341 (1941).
6) Y. Hatsuda and S. Kuyama, *J. Agr. Chem. Soc. Japan*, **29**, 14 (1955).
7) F. Wessely and G. H. Moser, *Monatsh.*, **56**, 97 (1930); F. Wessely and F. Kallab, ibid., **60**, 26 (1932); S. Hattori, *Acta Phytochim.*, **5**, 99,117,219 (1930); *J. Chem Soc. Japan*, **50**, 725 (1929); **51**, 570 (1930); Ber., **72**, 1914 (1939); R. C. Shah, C. R. Mehta and T. S. Wheeler, *J Chem. Soc.*, **1938**, 1555.
8) E. M. Philbin, J. Swirski and T. S. Wheeler, *Chem. & Ind.*, **1955**, 445.

action of hydriodic acid**, the point to be considered would be the γ-pyrone ring in the center. In such a case, there will be the diphenyl ether structure with the ketone portion severed and a benzophenone structure with the ether linkage severed. As the latter example, there is sulochrin[9], isolated from the mycelium of *Oospora sulphurea-ochracea*. If mango pigment belongs to the latter group, it must be a hexahydroxybenzophenone compound in order to cyclize into tetrahydroxyxanthone derivative. If such is the case, application of diazomethane must afford a tetramethyl ether, even if two hydroxyls will not be methylated due to a substituent and the chelation to the carbonyl group. However, the pigment only affords a dimethyl ether even when sufficient diazomethane reacted. Since there is no methoxyl group in the pigment itself and the color reaction denies*** the partial structure of *ortho*- and *para*-diphenols, the possibility of a benzophenone derivative for the pigment is excluded.

If the pigment is a diphenyl ether derivative, there must be one carboxyl radical *ortho* to ether linkage, but the infrared spectra*** of the pigment and its methyl derivative do not indicate the presence of a carboxyl or an ester-carbonyl group. It therefore follows that the pigment can not be a diphenyl ether derivative. The fact that the ultraviolet absorption spectrum (Fig. 1) of mangiferin is quite similar to that of 1,3,6,7-tetrahydroxyxanthone supports the presence of such a structure in the pigment molecule.



Wave length

Fig. 1. Ultraviolet absorption spectra of mangiferin (a: ——), 1,3,6,7-tetrahydroxyxanthone (b: ·····), and its tetraacetate (c: - - -).

The known tetrahydroxyxanthone compounds isolated to date from natural substances may be found in the 1,3,5,8-isomer obtained from swertianolin or its aglycone, swertianol, isolated from *Swertia japonica Makino*[10] and *S. tomentosa Makino*[11] of Gentianaceae, and the 1,3,5,6-isomer[12] obtained as the oxidation product of jacareubin, the pigment of the heartwood of *Calophyllum brasiliensis* Camb. of Guttiferae. The skeletal compound of swertinin and decussatin, the pigments of *Swertia decussata*, is said to be the 1,2,6,8-isomer from absorption spectra[13]. The presence of a 1,3,6,7-isomer in natural substances to be the first example and this would be also the first example of a xanthone derivative in plants belonging to Anacardiaceae.

The writer expresses his gratitude to Professor Ryozo Hirohata of the Kyushu University, for his kind guidance and encouragement throughout the course of this work, to Professor Shoji Shibata of the Pharmaceutical Institute, University of Tokyo, for kind supply of a valuable sample, to Professor Yaichiro Tanase of the Pharmaceutical Faculty, Tokushima University, for kind advice and the supply of literature, to Professor Eigai Sebe of the Kumamoto University Medical School for kind help in analysis, and to Dr. Keihei Ueno of the Dojin Pharmaceutical Laboratory for ultraviolet spectral measurements. The writer is also indebted to Mr. Eikichi Aoyagi of the Miike Dye Industries, Oomuta, for elementary analyses.

Experimental

Unless otherwise noted, all melting and decomposition points are corrected.

Action of Hydriodic Acid on Mangiferin.—To 30 g. of phenol, 5.0 g. of finely pulverized mangiferin was added and mixed with warming (remains practically insoluble). While cooling, 50 cc. of hydriodic acid (*d* 1.7) was added

** There is a report [A. Mustafa and M. I. Ali, *J. Org. Chem.*, **21**, 849 (1956)] on the formation of a tetrahydroxanthone by the new formation of a cyclohexene ring in diene synthesis.

9) H. Nishikawa, *Bull. Agr. Chem. Soc. Japan*, **12**, 47 (1936); **13**, 1 (1937). *Acta Phytochim.*, **11**, 167 (1939).

*** See the forthcoming report, Part V.

10) Y. Asahina, J. Asano and Y. Ueno, This Bulletin, **17**, 104 (1942); *J. Pharm. Soc. Japan*, **62**, 22 (1942).

11) T. Nakaoki and Y, Hida, *J. Pharm. Soc. Japan*, **63**, 554 (1943).

12) F. E. King, T. J. King and L. C. Manning, *J. Chem. Soc.*, **1953**, 3932.

13) S. R. Dalal, S. Sethna and R. C. Shah, *J. Indian Chem. Soc.*, **30**, 457, 463 (1953); R. C. Shah, A. B. Kulkarni and S. R. Dalal, *J. Sci. Ind. Research*, **13B**, 175 (1954).

gradually and the mixture was gently refluxed for 6 hours. When cooled, the two-layered mixture was poured into an aqueous solution of sodium bisulfite with agitation and the dark brown, resinous substance that separated was washed thoroughly with water. This resinous substance was boiled with four 500-cc. portions of water, the oily substance insoluble in hot water was collected by filtration, and the cooling of the filtrate afforded a pale brown precipitate. Yield, 2.5 g. of m.p. 368–370°C (decomp.). Ferric chloride reaction: Dusky greenish black.

Action of Hydrobromic Acid on Mangiferin.—Application of hydrobromic acid (constant boiling product) under exactly the same conditions as for hydriodic acid, or even without the addition of phenol, 1,3,6,7-tetrahydroxyxanthone was isolated, though in a smaller yield. In the case of an application of the acid alone, without phenol, the pigment dissolved in hydrobromic acid on heating the mixture in an oil bath and a black insoluble matter separated out immediately. After cooling, the insoluble matter was collected, the portion soluble in ethyl acetate was acetylated, and the product was recrystallized from glacial acetic acid to 1,3,6,7-tetra-acetoxyxanthone, m. p. 200°C. Yield, 15–20 % of the original pigment.

1,3,6,7-Tetra-acetoxyxanthone.—Two grams of the substance of m.p. 368–370°C, soluble in hot water, obtained by the application of hydriodic acid to the pigment, was acetylated by heating with acetic anhydride and a drop of conc. sulfuric acid for 4 hours. This was decomposed by the addition of water, the precipitate was collected, washed with water, and dried. Five recrystallizations from as small an amount as possible of glacial acetic acid, twice using activated carbon, afforded colorless, long prisms m.p. 199.5–200°C, Yield, 1.6 g. Ferric chloride reaction: Negative. It precipitates out as thick, rhombic plates from hot saturated acetone solution but forms fine needles when the acetone solution is allowed to evaporate or when recrystallized from ethanol, but no difference in the melting point was observed in either case.

Anal. Found: C, 58.97, 59.13; H, 4.17, 3.88. Calcd. for $C_{21}H_{16}O_{10}$: C, 58.88; H, 3.77 %.

No depression of the melting point was observed on admixture with the synthesized 1,3,6,7-tetra-acetoxyxanthone, m.p. 198°C. It can be obtained by direct acetylation of the resinous substance obtained on decomposition with hydriodic acid, without extraction with hot water, but the yield is poor.

1,3,6,7-Tetrahydroxyxanthone.—A mixture of 350 mg. of the tetra-acetate, m.p. 199.5–200°C, in 20 cc. of ethanol containing 5 cc. of conc. hydrochloric acid was refluxed for 5 hours, the solvent was evaporated under a reduced pressure, and water was added to the bright yellow residue. The pale yellow mass (290 mg.), obtained on washing this residue with water and drying, was dissolved in 4 cc. of ethanol, filtered, and brought to 10 cc. with hot water. On cooling this solution, pale brown needles or a crystalline powder was obtained, which became slightly brownish at around 320°C, became brown colored at around 350°C, and blackened with decomposition at 370–371°C. It is easily soluble in acetone and ethanol and sparingly in ether. Air-dried product gave analytical values almost corresponding to $C_{13}H_8O_6 \cdot H_2O$.

Anal. Found: C, 55.72; H, 4.01. Calcd. for $C_{13}H_8O_6 \cdot H_2O$: C, 56.12; H, 3.62 %.

Sample dried for 10 hours in vacuo at room temperature.

Anal. Found: C, 59.89; H, 3.20. Calcd. for $C_{13}H_8O_6$: C, 60.01; H, 3.20 %.

The melting point determination of the synthesized 1,3,6,7-tetrahydroxyxanthone[5] alone or in admixture with the foregoing product gave the same results as above.

1-Hydroxy-3, 6, 7-trimethoxyxanthone.—Ether solution of diazomethane made from 2.0 g. of nitrosomethylurea was added to a suspension of 0.1 g. of 1,3,6,7-tetrahydroxyxanthone in 20 cc. of ether, by which the phenol gradually disappeared with evolution of bubbles. After 5 hours, the residual diazomethane was decomposed by the addition of a few drops of glacial acetic acid, the solvent was evaporated, and the residue was dissolved in 30 cc. of 5 % potassium hydroxide solution. This solution was continuously extracted with ether for 10 hours, and the colorless ether extract was evaporated, leaving 90 mg. of a pale yellowish brown residue. Recrystallization from dilute ethanol afforded pale yellowish brown microneedles, m.p. 218–219°C. They dissolve in conc. sulfuric acid to form a bright yellow solution with green fluorescence. It colors green gradually with ferric chloride.

Anal. Found: C, 63.50; H, 4.72. Calcd. for $C_{16}H_{14}O_6$: C, 63.56; H, 4.67 %.

Mixed m.p. with the synthesized 1-hydroxy-3,6,7-trimethoxyxanthone, m.p. 216°C[5], was 218°C.

1-Acetoxy-3, 6, 7-trimethoxyxanthone.—Acetylation of 0.1g. of 1-hydroxy-3,6,7-trimethoxyxanthone with acetic anhydride and a drop of conc. sulfuric acid gave 0.1 g. of a crude product, which was treated with a small amount of acetone to remove a minute amount of soluble pale yellow substance. Recrystallization of the residue from ethanol afforded fine prisms, m.p. 216°C. Ferric chloride reaction, negative.

Anal. Found: C, 62.81; H, 4.75. Calcd. for $C_{18}H_{16}O_7$: C, 62.79; H, 4.68 %.

Mixed m.p. with 1-hydroxy-3,6,7-trimethoxyxanthone, m.p. 218–219°C, was 180–195°C. Admixture with mangiferin dimethyl ether acetate, m.p. 216–217°C, melted at 190–205°C. Hydrolysis with ethanolic hydrochloric acid gave 1-hydroxy-3,6,7-trimethoxyxanthone.

Synthesis of 2,4,5-Trihydroxybenzaldehyde.—The hydroxyhydroquinone triacetate, obtained by the acetylation of benzoquinone, was hydrolyzed with ethanolic sulfuric acid, concentrated under a reduced pressure, and the residue, to which a small amount of water was added, was extracted with ether, from which hydroxyhydroquinone (III) was obtained. Twenty grams of (III) was dissolved in 200°cc.

14) Ref. 5 and L. Gattermann and M. Köbner, *Ber.*, **32**, 278 (1899).

of ether; 30 g. of freshly prepared zinc cyanide was added, and dry hydrogen chloride gas was bubbled through this solution. Ether was then evaporated, 120 cc. of water was added to the residue, and the mixture was warmed for 5 minutes. After cooling, the mixture was allowed to stand in an ice chest over night and the crystals that precipitated out were removed by filtration. The mother liquor was extracted with ether, ether was evaporated, and the residue crystallized on standing, without recrystallization, to 2,4.5-trihydroxybenzaldehyde IV as pale brown, long prisms, m.p. 229–230°C (decomp.). Yield, 20 g.

Anal. Found: C, 54.45; H, 4.28. Calcd. for $C_7H_6O_4$: C, 54.42; H, 4.11 %.

Synthesis of 1,3,6,7-Tetrahydroxyxanthone.—A solution of 7.3 g. of IV and 7.5 g. of phloroglucinol dissolved in 50 cc. of glacial acetic acid was mixed with 20 cc. of conc. hydrochloric acid, heated on a sand bath for 30 minutes, allowed to stand over night, and the reddish solid thereby formed was collected by filtration. The solid was washed consecutively with water, sodium bicarbonate solution, and water, and dried, affording 10 g. of 1,6,7-trihydroxyfluorone V. Without purification, this was mixed with 500 cc. of ethanol, mixed with 4 g. of 5 % palladium-charcoal and shaken in a hydrogen stream by which 1 l. of hydrogen was absorbed in 1.5 hours, the solution becoming almost colorless. The catalyst was filtered off and the filtrate was evaporated completely to dryness under a reduced pressure, affording 7.3g. of crude 1,3,6,7-tetrahydroxyxanthene VI. This was directly acetylated and treated by the usual method. Recrystallization of the product from a large amount of ethanol gave 7.5 g. of 1,3,6,7-tetra-acetoxyxanthene (VII), m.p. 177°C.

To a solution of 7.5 g. of VII, dissolved in a mixture of 50 cc. of glacial acetic acid and 30 cc. of acetic anhydride, 2.4 g. of chromium trioxide was added in small portions with shaking. After completion of the addition, the mixture was heated in a water bath for 3 hours, a large amount of water was added to the mixture, and the insoluble matter was recrystallized four times from glacial acetic acid to 5.5 g. of 1,3,6,7-tetra-acetoxyxanthone VIII, as colorless needles, m.p. 200°C, showing no depression on admixture with the product isolated from the natural substance.

Anal. Found: C, 58.78; H, 3.85. Calcd. for $C_{21}H_{16}O_{10}$: C, 58.88; H, 3.77 %.

This tetra-acetate VIII was hydrolyzed with an acid and the product was recrystallized from 30 % ethanol. The pale yellowish brown, crystalline powder thereby obtained gradually colored from around 320°C, became markedly brown at around 350°C, and decomposed with blackening at 370–371°C, the behavior being identical with that of 1,3,6,7-tetrahydroxyxanthone from the natural source.

Anal. Found: C, 59.88; H, 3.22. Calcd. for $C_{13}H_8O_6$: C, 60.01; H, 3.10 %.

Ultraviolet Absorption Spectra.—Measurements were made with the Beckman Model DU spectrophotometer of mangiferin in 70 % ethanol and 1,3,6,7-tetrahydroxyxanthone and its tetra-acetate in methanol.

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