# *On Mangiferin, the Coloring Matter of Mango (Mangifera indica Linn.). V.*[1] *Identification of Sugar Component and the Structure of Mangiferin*[2]

By Syun ISEDA

(Received April 11, 1957)

From the results previously[1,3] reported, it has become clear that mangiferin agrees with the composition of $C_{19}H_{18}O_{11}$ and includes a 1, 3, 6, 7-tetrahydroxyxanthone skeleton ($C_{13}H_8O_6$). The difference in the molecular formula $C_6H_{10}O_5(+H_2O)$ coincides with a hexose group. Wiechowski[4] already proved the formation of levulinic acid by boiling the "mangin" with hydrochloric acid for a long time and indicated the possible presence of a sugar moiety, but failed to isolate the sugar. Gorter[5] proposed a structure containing a glucuronic acid group, but did not isolate it. The present writer also attempted the detection of sugars as described in the first paper of this series[6], but did not obtain any concrete result.

1) Part IV: This Bulletin, **30**, 625 (1957).
2) Paper presented at the Kyushu Local Meeting of the Chemical Society of Japan, in Fukuoka, November 24, 1956.
3) S. Iseda, *J. Kumamoto Women's Univ.*, **9**, 45 (1957).
4) W. Wiechowski, *Arch. exptl. Pathol. Pharmakol.*, **91**, 462 (1923).
5) K. Gorter, *Bull. Jardin Bot. Buitenzorg*, [III], **4**, 260 (1922).
6) S. Iseda and T. Asai, *J. Med. Assoc. Formosa*, **38**, 447 (1939).

Application of periodic acid to the pigment in water results in coloration of the solution to reddish brown, indicating the liberation of iodine, but the consumption of periodic acid varied between 1 and 3 moles and definite values were not obtained. Steam distillation of this aqueous reaction mixture failed to show the presence of formaldehyde in the distillate. Direct distillation of this reaction mixture showed, however, the presence of formaldehyde in the distillate, though in an amount about 1/20 of the calculated. It is known that glucose or glucosides does not form formaldehyde by periodate oxidation[7] and since there is a consumption of periodic acid without the virtual formation of formaldehyde, it seems certain that the $C_6H_{10}O_5$ residue bonded to the xanthone skeleton is not a hexitol ether type.

The sugar is not split off when the pigment is heated with sulfuric or hydrochloric acid under ordinary pressure or with water under high pressure, but when it is heated with 1% or 10% sulfuric acid under high pressure at 140°C, and the aqueous solution of the product is extracted with ether* after deionization, a spot appears on the paper chromatogram of the aqueous solution. This spot was identical with that of glucose. It does not appear, when the heating conditions, such as temperature and duration, are different from those indicated above. This experiment makes it certain that there is a glucose in the mangiferin molecule, but how and why that sugar is not split off by ordinary method is not clear.

Infrared absorption spectrum of mangiferin as a potassium bromide disk is shown in Fig. 1.

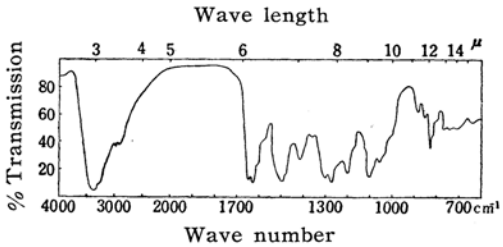



Fig. 1. Infrared spectra of mangiferin (KBr disk method).

The broad absorption around 3338 cm.$^{-1}$ indicates the presence of numerous hydroxyl groups, including those forming intramolecular hydrogen bond. The absorptions at 2932 and 2885 cm.$^{-1}$ indicate the presence of $CH_2$ in the molecule, but the amount seems to be small judging from its intensity. These absorptions, combined with those at 1490 and 1048 cm.$^{-1}$, suggest the presence of a primary alcoholic group in the molecule. Since there are no absorptions corresponding to $CH_3$ near 3000, 1450, and 1375cm.$^{-1}$, it can not be considered that the foregoing primary alcoholic group is due to the residual ethanol used for the purification of the pigment. There is no absorption in the region 1650 to 1750 cm.$^{-1}$ and the ester structure of Gorter[5] or the aldehyde structure of Wiechowski[4] can be excluded. The absorption at 1645 cm.$^{-1}$ is the carbonyl band of xanthone nucleus[8] and the sharp absorption at 828 cm.$^{-1}$ (821 cm.$^{-1}$ in Nujol) must be related to the xanthone structure, since it also appears in the spectrum of 1,3,6,7-tetrahydroxyxanthone (825 cm.$^{-1}$, Fig. 2).

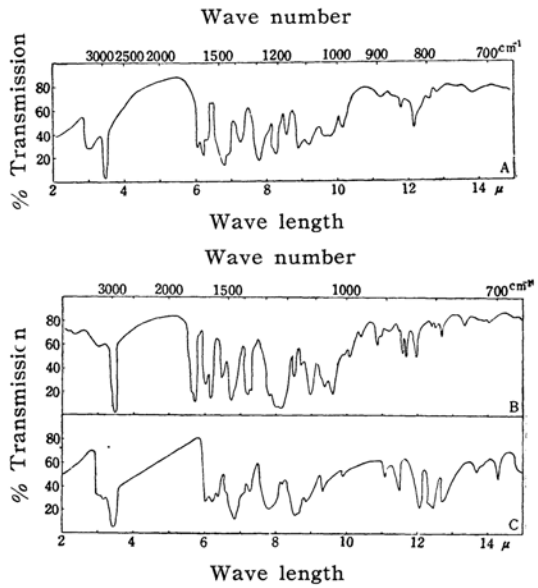



Fig. 2. Infrared spectra (in Nujol) of (A) mangiferin dimethyl ether, (B) mangiferin dimethyl ether acetate, (C) 1,3,6,7-tetrahydroxyxanthone.

According to Barker et al.[9] and Takahashi[10], α-D-glucopyranoside exhibits absorption at 844±8 cm.$^{-1}$ [9] or at 11.6—11.8 μ

7) P. Karrer and M. Pfaehler, *Helv. Chim. Acta*, **17**, 766 (1934).

* When this extraction with ether is omitted, a component that appears above (in ascending paper chromatography) the sugar spot and reduces its $R_f$, cannot be removed.

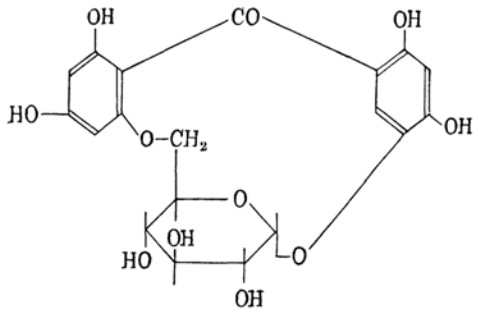
8) S. Kimoto [*J. Pharm. Soc. Japan*, **75**, 763 (1955)] gave 6.07μ (1645cm.$^{-1}$) for xanthone, but E. D. Bergmann and S. Pinchas [*J. chim. phys.*, **49**, 537 (1952); *Chem. Abstr.*, **47**, 3132 (1953)] gave 1658 cm.$^{-1}$. The following values (in Nujol) were obtained: 1,3,6,7-Tetrahydroxyxanthone (1640 cm.$^{-1}$), 1,3,6,7-tetra-acetoxyxanthone (1660 cm.$^{-1}$), 1-hydroxy-3,6,7-trimethoxyxanthone (1665 cm.$^{-1}$).

9) S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *J. Chem. Soc.*, **1954**, 171.

10) M. Takahashi, *J. Pharm. Soc. Japan*, **74**, 320 (1954); **75**, 237 (1955).

(862—848 cm.$^{-1}$)[10], irrespective of whether the compound is in the form of glycoside or its derivatives, such as methyl ether. Mangiferin exhibits an absorption at 844 cm.$^{-1}$ and its dimethyl ether and dimethyl ether acetate (Fig. 2) exhibit at 853 cm.$^{-1}$ (in Nujol), but such an absorption is not found in 1,3,6,7-tetrahydroxyxanthone. It follows that the absorption at 844 cm.$^{-1}$ in mangiferin must correspond to that of α-D-glucopyranoside.

The molecular structure shown in (I) may be proposed for mangiferin. Such a structure can be constructed on a molecular model, but this would hardly explain the similarity of the ultraviolet absorption spectrum between mangiferin and 1,3,6,7-tetrahydroxyxanthone, as shown in Part IV. Neither would it explain the formation of only a dimethyl ether with diazomethane. If two hydroxyls are present in the position *peri* to the carbonyl, one could easily be methylated[11].



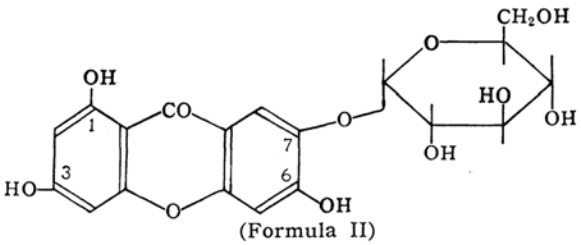
(Formula I)

From the foregoing results, it must be concluded that the sugar is bonded in α-glycosidic type to one of the four hydroxyls in 1,3,6,7-tetrahydroxyxanthone skeleton in mangiferin.

Attempted complete methylation of the pigment with dimethyl sulfate and anhydrous potassium carbonate in dry acetone, turned it into a dark brown syrup, which made it impossible to liberate the sugar portion by acid hydrolysis by the usual method. Assumption of the sugar bonding position was made through coloration reactions.

Mangiferin and its dimethyl ether are both positive to the Gibbs' reaction[12] and Liebermann's nitroso reaction[13], showing the presence of a free hydroxyl without a substituent in the *para*-position (i. e. in 1-position). The presence of a free *meta*-diphenol structure in the mangiferin molecule is known by Brauer's phosphomolybdic acid reaction[14], but this color reaction is negative in mangiferin dimethyl ether. Nagai and Hattori's color reaction[15] with chloropentammincobalt-(III) chloride, $[Co(NH_3)_5Cl]Cl_2$ is negative in both the pigment and its dimethyl ether, indicating the absence of an *ortho*- and *para*-diphenol structures in the molecule. Sevilla and DiMenza[16] have reported a color reaction characteristic of *ortho*-diphenol by ammonium metavanadate, but this was not found applicable in the case of 1,3,6,7-tetrahydroxyxanthone.

From the various experimental results described above, the sugar bonding position may be concluded to be in 6- or 7-position. From the biological fact that euxantic acid (1.7-dihydroxyxanthone 7-glucuronide) is excreted in the urine of rabbits and cattle fed with mangiferin, it may be certain that the sugar is bonded in 7- rather than 6-position. The following formula (II) is proposed as the structural formula for mangiferin.



(Formula II)

If mangiferin were to be represented by the foregoing formula (II), it would be necessary that the hydroxyls in 3- and 6-positions be removed to form euxanthic acid in an animal body. This point still requires further study.

## Experimental

All melting points are corrected.

**Reaction with Periodic Acid** — Mangiferin or its dimethyl ether was suspended in 5% sulfuric acid and powdered potassium periodate was added in small portions to it, by which the solution immediately turned reddish brown. This color faded with sodium thiosulfate and distillation yielded crystals of iodine. The amount of the periodate consumed by titration corresponded to 1.3—3.9 moles, failing to give any definite value. The insoluble portion obtained after the periodate oxidation remained a reddish brown amorphous

11) Y. Tanase, *J. Pharm. Soc. Japan*, **61**, 341 (1941).
12) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927); M. Tomita and S. Uyeo, *J. Pharm. Soc. Japan*, **61**, 449 (1941).
13) C. Liebermann, *Ber.*, **7**, 247, 1098 (1874).
14) K. Brauer, *Chem. Zeitg.*, **50**, 553 (1926).
15) W. Nagai and S. Hattori, *Acta Phytochim.*, **5**, 1 (1930); *J. Chem. Soc. Japan*, **51**, 162 (1930). Cf. ref. 11).
16) J. Sevilla and A. DiMenza, *Rev. asoc. biochim. argentina*, **14**, 17 (1947); *Chem. Abstr.*, **41**, 6172 (1947).

substance and it could not be induced to crystallize. Beilstein's halogen test: positive.

Aqueous solution of 1 g. of potassium periodate was added dropwise into a suspension of 300mg. of mangiferin in 30 cc. of 5 % sulfuric acid, with shaking, and the mixture was allowed to stand for 36 hours at room temperature (30°C) with occasional shaking. The clear supernatant was decanted and submitted to steam distillation. The addition of 1 cc. of 1 % ethanolic solution of dimedone to 30 cc. of the distillate failed to show the formation of a dimedone adduct. The reaction mixture obtained under the same conditions of oxidation, without separation of the supernatant, was treated with 100 cc. of water, and directly distilled in an oil bath to 50 cc. of the distillate (acid reaction). After removal of iodine crystals, 15 drops of 1 % ethanolic solution of dimedone was added. On standing the mixture over night, 12 mg. of colorless needle crystals were obtained. Recrystallization from ethanol afforded crystals melting at 188°C, undepressed on admixture with authentic sample of formaldimedone, m. p. 188°C.

**High-pressure Hydrolysis** — A mixture of 1.0 g. of mangiferin and 200 cc. of 10 % sulfuric acid was heated for 2 hours at 140°C in an autoclave. The black, insoluble matter that separated out was removed by filtration, the sulfat ion in the filtrate was quantitatively removed with barium hydroxide, and the filtrate concentrated to 50 cc. This solution was passed through the cation and anion exchange resin 'DIA' (product of Mitsubishi Kasei Co.) to effect complete deionization and further concentrated to 1 cc. of a pale yellow liquid.

This liquid was submitted to paper chromatography by the ascending method, using a mixture (4 : 2 : 1) of butanol : acetic acid: water as the developing solvent. A mixed solution of benzidine (1 g.), trichloroacetic acid (20 cc. of 40 %), glacial acetic acid (20 cc.), and 93 % methanol (160 cc.) was used as the coloring reagent.

In this case, the spot of pure glucose appeared at $R_f$ 0.23, while those of the first sample and the sample to which glucose had been added appeared at $R_f$ 0.20, accompanied by a somewhat broad, feathershaped region, above the spot, without any coloration.

This sample was diluted with about 10 volumes of water and extracted with ether for about 30 hours. The ether-soluble portion afforded a minute amount of yellowish green syrupy substance (which reduces the Fehling and ammoniacal silver nitrate solution). The aqueous solution left after ether extraction was again concentrated and submitted to paper chromatography by which the feathery region disappeared and the spots from pure glucose, the first sample, and the sample with added glucose all appeared at the same $R_f$ 0.24. The color of these spots was also of the same tone.

When 1 % sulfuric acid was used instead of 10 % one without alteration in other conditions, a spot also appeared at the same position.

Hydrolyses under the following conditions failed to indicate the presence of a sugar: heating with mere water at 120°C or 140°C for 2 or 4 hours, heating with 1 % or 10 % sulfuric acid at 120°C for 2 or 4 hours, or at 140°C for 4 hours.

**Gibbs' Reaction**[12] — To 1 cc. of saturated solution of mangiferin or its dimethyl ether in 70 % ethanol, a piece of sodium acetate crystal was added, followed by a few drops of freshly prepared ethanolic solution of 2,6-dichloroquinone chlorimide. Both gave bluish green (positive) coloration, while acetylmangiferin showed pale pinkish violet and 1,3,6,7-tetra-acetoxyxanthone a pale yellow color.

**Liebermann's Nitroso Reaction**[13] — A pale brown solution, obtained by dissolving sodium nitrite in well-chilled conc. sulfuric acid, was carefully superimposed on the conc. sulfuric acid solution of mangiferin or its dimethyl ether by which a reddish brown ring appeared at the zone of contact.

**Brauer's Reaction**[14] — To 1 cc. of saturated aqueous solution of the sample, 0.1 cc. of 10 % aqueous solution of phosphomolybdic acid was added (the color here obtained is designated as A). Then 10 % ammonia water was added carefully in drops (color here obtained is designated as B). Mangiferin showed pale yellowish brown in A and blue in B (positive to *meta* diphenol reaction), the blue color disappearing with excess of ammonia. 1,3,6,7-Tetrahydroxyxanthone showed blue color in A and bluish green in B (positive to *ortho*-diphenol reaction), while mangiferin dimethyl ether failed to show any coloration in either A or B.

**Color Reaction to Nagai-Hattori's Cobalt Reagent**[15] — To 2 cc. of saturated solution of the sample in 70 % ethanol, 5 drops of a saturated solution of the cobalt reagent** in ethanol was added. No color change was observed after 5 hours with mangiferin, its dimethyl ether, 1-hydroxy-3,6,7-trimethoxyxanthone, or in 1,3,6,7-tetra-acetoxyxanthone. When powdered mangiferin was floated on the ethanolic solution, the color changed to brown after 15 hours, although no change occured within 5 hours. In the case of 1,3,6,7-tetrahydroxyxanthone, the color changed to brown immediately, with turbidity.

**Sevilla-DiMenza's Reaction** — A few drops of 50 % sulfuric acid containing a small amount of ammonium metavanadate was added to 2 cc. of an aqueous solution containing 1—2 mg. of the sample, or a saturated aqueous solution in the case of mangiferin and xanthone compounds. Only pyrogallol out of the compounds listed below gave a positive reaction (wine red coloration) as described in the literature[16].

| Compound (position of OH) | Coloration |
|---|---|
| Resorcinol (1,3) | Slightly reddish yellow. |
| Hydroquinone (1,4) | ditto. |
| Pyrogallol (1,2,3) | Wine red—pale reddish brown. |
| Phloroglucinol (1,3,5) | Almost colorless. |

** $[Co(NH_3)_5Cl]Cl_2$

| | |
|---|---|
| Orcinol (1—$CH_3$; 3,5) | Dark gray—pale yellow with brown precipitate. |
| Mangiferin | Slightly reddish yellow, gradually turning pale, and finally pale yellowish brown. |
| 1,3,6,7-Tetrahydroxyxanthone | Pale dusky black with brown precipitate, gradually turning pale, with dark brown precipitate; finally the solution became pale yellowish brown. |

**Infrared Absorption Spectra**—Spectra were measured with the Perkin-Elmer Model 21 infrared spectrophotometer, by the potassium bromide disk method by Mr. Yusaku Ikegami of the Research Institute for Chemistry of Non-Aqueous Solution, Tohoku University, and by the Nujol method by Mr. Hirotaka Kozuma of the Technical Department, Shin Nippon Chisso Hiyro Co. Minamata.

The writer expresses his gratitude to those who supplied infrared data, to Assistant Professor Kazuyoshi Yagishita and Mr. Naoshi Toya of this laboratory for cooperation in part of the experiments, and to Mr. Chikashi Adachi, President of the Kumamoto Agricultural High School, for permission to use the autoclave.

*Department of Chemistry*
*Kumamoto Women's*
*University, Kumamoto*