# Occurrence of Mangiferin in *Hiptage madablota* Geartn.

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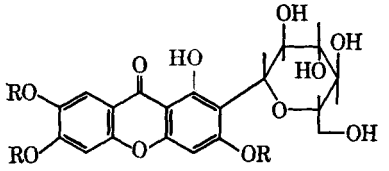
**A bright yellow, crystalline compound, m.p. 271–274°, isolated from the root-bark of *Hiptage madablota* Geartn. has been identified as mangiferin, 1,3,6,7-tetrahydroxy-2-C-β-D-glucosylxanthone, by direct comparison of its properties and those of its derived 3,6,7-trimethyl ether with those of authentic samples. Mangiferin was assayed in three biological test systems and the results are given.**

In the course of studies on the structures of the endecaphyllins, a group of glucose polyesters of 3-nitropropanoic acid (1), the authors' attention was naturally directed to the report of Gorter (2) concerning hiptagin, a nitrogenous glucoside which was isolated from *Hiptage madablota* Geartn. (family *Malpighiacae*), since available evidence strongly suggested that hiptagin was very closely related to the endecaphyllins. Indeed, it was found that hiptagin (2) and endecaphyllin X (1) are identical and have the structure 1,2,4,6-tetra-*O*-(3-nitropropanoyl)-β-D-glucopyranoside (3). The authors deal here with the isolation of a new constituent of *H. madablota* and its identification as mangiferin (I).




I, R = H
II, R = $CH_3$

A granular solid which precipitated during acetone extraction of defatted root bark was collected and recrystallized several times from 50% aqueous alcohol to provide a bright yellow substance with melting point 271–274° dec. The spectroscopic properties of this substance pointed to the presence of a hydroxyxanthone moiety (4, 5) and quickly led to the supposition that the compound was mangiferin (I) on the basis of correspondence with published infrared (6), ultraviolet (4, 6, 7), and NMR (8) data in addition to agreement with the literature values for its melting point (9) and optical rotation (10). Furthermore, diazomethylation provided a derivative whose properties accorded with those of the known (7) trimethyl ether, II. Finally, the identification of the materials used was confirmed by direct comparison with authentic samples of I and II generously provided by Haynes (8, 11).

Although mangiferin has been long known as a constituent of the mango tree (*Mangifera indica* L., family *Anacardiaceae*), its structure has only recently been established (8, 12), thus permitting its inclusion in the rather unusual class of natural products, the *C*-glycosyl compounds. Reviews of earlier chemical studies have appeared (11). In addition to *M. indica*, and now *H. madablota*, the following species are reported to contain mangiferin: *Salacia prinoides* L. (*Hippocrateaceae*) (13), *Hedysarum obscurum* L. (*Papilionaceae*) (14), *Belamcanda chinensis* Adans (*Iridaceae*) (15), various *Iris* spp. belonging to sections Pogonoris, Apogon, and Pardanthopsis (*Iridacea* (15), *Athyrium mesosorum* Roth (*Polypodeaceae*) (16), *Apholoia madagascariensis* Clos (*Flacourtiaceae*) (17), *Cuscuta reflexa* Tourn. (*Convolvulaceae*) (17), *Smilax glycyphylla* Tourn. (*Liliacea* (10), *Madhuca utilis* Ridley (*Sapotaceae*) (7), and *Anemarrhenae rhizoma* B. (*Liliaceae*) (18).

The authors are aware of only two reports on biological properties of mangiferin. One of these describes increased diuresis in the adrenalectomized rat (19) while the other deals with the cardiotonic effects on the isolated frog heart (20). The cytotoxic effect of mangiferin on Sarcoma 180 cells grown in stationary cell culture has now been studied and the compound was found to be inactive. An $ID_{50}$ value of 440 mcg./ml. was determined which is well outside the range (6 mcg./ml. or below) of potential interest. A number of more simply substituted xanthones have been subjected to this bioassay with a few showing significant activity. These data will be discussed in detail in a subsequent publication (21).

Results from two additional assays using cell-free systems from *Escherichia coli* have been kindly provided by Dr. C. Coutsogeorgopoulos at the Roswell Park Memorial Institute. Mangiferin ($10^{-4}$ *M*) displayed low inhibitory activity (15–20%) on the polyuridylic acid-stimulated synthesis of polyphenylalanine (22) in the presence of ribosomes washed with ammonium chloride (23). In the second system, the reaction between puromycin and polylysyl-ribonucleic acid (23) was inhibited by mangiferin ($10^{-4}$ *M*) to the extent of 35–40% whereas chloramphenicol showed 50–60% inhibition.

## EXPERIMENTAL

**Mangiferin**—Finely ground, air-dried root bark (200 Gm.) of *H. madablota* was defatted with petroleum ether (b.p. 60–80°, 300 ml.) in a Soxhlet extractor for 30 hr. It was observed that after about 12 hr./extraction with acetone (300 ml.), a granular solid began to deposit. At the end of the extraction (48 hr.) the solid was collected on a filter and washed with acetone, yield 0.025%. Recrystallization from 50% aqueous ethanol afforded yellow crystals, m. p. 271–274° dec. Literature values, 271° (9), 268° (7), 269° (8); $\lambda_{max.}^{KBr}$ 3450–3100, 1650, 1625, 1600, 1570, 1500, 1260, 1100, 870 $cm.^{-1}$ $\lambda_{max}^{95\%\ ethanol}$ 244.5, 260.5, 321.0, 375.5 mμ, log ε 4.03, 4.13, 3.43, 3.41; $\lambda_{max.}^{95\%\ ethanol}$, NaOH 245, 261, 400 mμ, log ε 4.05, 4.10, 4.12. These spectra were identical with those of an authentic sample.

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**Mangiferin Trimethyl Ether**—A suspension of mangiferin (30 mg.) in 2 ml. methanol and 8 ml. ether was treated with an excess of ethereal diazomethane following the procedure of Hawthorne and co-workers (7). After twice being precipitated from aqueous acetone, the product had m.p. 298–299° and provided infrared and ultraviolet spectra identical with those of an authentic sample. Literature m.p. 298–299° (7).

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***Keyphrases***

*Hiptage madablota*
Mangiferin—isolation, identification
Biological activity—mangiferin
IR spectrophotometry—structure
UV spectrophotometry—structure


# Deficiency of Vitamin $B_{12}$ in *Chlorella*

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**Repeated systematic attempts to detect vitamin $B_{12}$ in two species of *Chlorella* at different times during the culture growth period failed to reveal consistent or significant evidence of $B_{12}$ or $B_{12}$-like activity. The results are discussed in terms of other reports in the literature.**

As PART of a continuing investigation of the vitamin content of two *Chlorella* species (1–4), extracts of *C. vulgaris* and of *C. pyrenoidosa* have been assayed for vitamin $B_{12}$ (cyanocobalamin and related factors). This seemed of particular interest in view of the conflicting reports in the literature concerning occurrence of the vitamin in chlorophyllous plants—especially the algae and particularly the Chlorococcales.

In an important review of vitamin $B_{12}$ in the metabolism of microorganisms, Ford and Hutner (5) state "$B_{12}$ . . . is not present in green plants and yeast; it is synthesized by bacteria and probably by blue-green, brown, and red algae, but not by green algae." Others have suggested that $B_{12}$ found associated with seaweeds (whether red, brown, or green) can be attributed to bacteria living epiphytically on the algae (6, 7) or to bacterial contaminants in the distilled water used in preparing specimens for assay (8).

Referring specifically to *Chlorella* (species unspecified), Hutner (9) commented on the lack of $B_{12}$ in the organism while, in contrast, Hashimoto reported significant levels of the vitamin (av. 61 $\mu$ mcg./mg. dry wt.) in "pure cultured" *Chlorella ellipsoidea* and he considered this a sufficiently high level to warrant further study (10).

## METHODS

*Chlorella vulgaris* and *C. pyrenoidosa* were cultured aseptically by both the column and the round-bottom flask techniques described previously (11): light intensity in the former was 600 ftc. and in the latter 1250 ftc. Column cultures were harvested at different times from Day 5 to Day 21; flask cultures were harvested at intervals from 3 weeks to 10 weeks postinoculation.

Assays were performed using *Euglena gracilis* (ATCC 12716) and *Ochromonas malhamensis* (ATCC 11532) in the appropriate Difco media. Acid-cleaned glassware and fresh glass-redistilled

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