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BIOSYNTHESIS OF MANGIFERIN IN *ANEMARRHENA ASPHODELOIDES*: INTACT INCORPORATION OF C_6—C_3 PRECURSOR INTO XANTHONE

Masao Fujita and Takao Inoue*
Hoshi College of Pharmacy
Ebara 2-4-41, Shinagawa-ku, Tokyo, Japan

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Mangiferin (1) is a C-glycosylxanthone which is widely distributed in several families.[1]

It has been suggested that naturally occurring xanthones are biosynthesized *via* benzophenone-like intermediate derived wholly from polyketide in fungi, and from shikimate—polyketide in higher plants.[2]

Regarding the biosynthesis of xanthones in *Gentiana lutea*, Floss and Rettig[3] demonstrated that the phloroglucinol ring of gentisin (gentisein 7-methyl ether) is derived from acetate, and later, Gupta and Lewis[4] have reported that gentisein (1,3,7-trihydroxyxanthone) and related xanthones are biosynthesized by oxidative coupling of a benzophenone, derived from three malonates and an intermediate (C_6—C_1) formed by loss of two carbon fragments from phenylalanine.

In connection with our studies on C-glycosylation of flavonoid[5] the biosynthesis of mangiferin (1) has been studied using the aerial parts of *Anemarrhena asphodeloides* Bunge (Liliaceae) which contains 1.[6] We now wish to propose the biosynthetic route of 1 based on intact incorporation of a C_6—C_3 unit from shikimate as shown in Scheme.

Various ^{14}C-labelled compounds were fed to the plants (excised aerial parts) and radioactive mangiferin (1) isolated from the materials was degraded with hydroiodic acid to the aglycone (1,3,6,7-tetrahydroxyxanthone) (2), followed by acetylation[7] (Table 1). Phenylalanine-1-^{14}C, -2-^{14}C, -3-^{14}C and *p*-coumaric acid-2-^{14}C were efficiently incorporated into 1 and in all the cases the radioactivity was localized in the xanthone moiety. In contrast, *p*-hydroxybenzoic acid- and protocatechuic acid-[carboxyl-^{14}C] showed much lower incorporation in comparison with the above C_6—C_3 precursors and also about 30 % of the label was distributed in the xanthone moiety and the remains in the sugar moiety. These findings suggest that the C_6—C_3 compounds, and not the C_6—C_1 ones, could be direct precursors for the biosynthesis of 1. If phenylalanine would be utilized in the formation of the aglycone (2) without loss of two carbon fragments, C-1 and C-2 carbons of phenylalanine must be incorporated into the phloroglucinol ring of 2. Thus mangiferin (1) obtained by feeding with labelled phenylalanine was degraded to phloroglucinol with potash fusion. The results showed that the radioactivity was almost exclusively present in the phloroglucinol ring of 1 when phenylalanine-1-^{14}C or -2-^{14}C was fed.

In order to obtain conclusive evidence for the participation of a C_6—C_3 unit, a mixture of *p*-coumaric acid-[ring-3,5-T_2] and -2-^{14}C was fed to the plants and the T/^{14}C ratio of mangiferin (1) isolated was compared with that of the precursor fed (Table 2). Considering loss (1/2) of T on arylhydroxylation in the biosynthesis, the results revealed that the doubly labelled *p*-coumaric

Table 1. Incorporation of ^{14}C-labelled precursors into mangiferin and radioactivity of degradation products

Precursors	Incorp. (%)	Spec. act. (dmp/mM) Mangiferin	Aglycone (2)* (tetraacetate)	Phloroglucinol*
1. Phenylalanine				
-1-^{14}C (12.5 μCi)	1.15	2.74×10^5	2.67×10^5 (97.4)	2.65×10^5 (96.7)
-2-^{14}C (12.5 μCi)	1.22	2.84×10^5	2.81×10^5 (98.9)	2.77×10^5 (97.5)
-3-^{14}C (12.5 μCi)	1.47	3.57×10^5	3.55×10^5 (99.4)	8.92×10^3 (2.5)
2. *p*-Coumaric acid				
-2-^{14}C (3.26 μCi)	1.19	1.13×10^5	1.14×10^5 (100.9)	
3. *p*-Hydroxybenzoic acid				
-[carboxyl-^{14}C] (100 μCi)	0.02	5.64×10^4	1.51×10^4 (26.8)	
4. Protocatechuic acid				
-[carboxyl-^{14}C] (1.78 μCi)	0.04	2.20×10^3	7.28×10^2 (33.1)	

Feeding period : 1. 15 hours, 2 — 4. 50 hours.

* Figures in parentheses show % ratios to specific activity of mangiferin.

Table 2. Ratio of T and ^{14}C activities in the precursor and mangiferin after feeding T- and ^{14}C-labelled *p*-coumaric acid

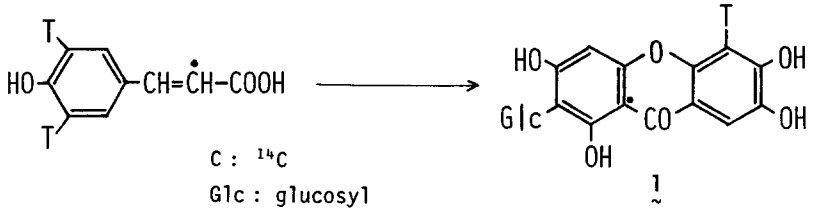
	T/^{14}C*	Spec. act. (dpm/mM) T	^{14}C	Incorp. (%) T	^{14}C	T/^{14}C†
5. (a)	1.68	7.39×10^4	9.57×10^4	0.52	1.11	1.54
(b)	1.65	7.66×10^4	1.03×10^5	0.52	1.16	1.49

Precursor : a mixture of *p*-coumaric acid-[ring-3,5-T_2] (6.56×10^8 dpm/mM) + *p*-coumaric acid-2-^{14}C (3.96×10^8 dpm/mM), 3 mg each.

Feeding period : (a) 50 hours, (b) 70 hours.

* Ratio in the precursor fed.

† Ratio in mangiferin ; this ratio is corrected for loss of T on aryl-hydroxylation in biosynthesis.



C : ^{14}C

Glc : glucosyl

1

acid was incorporated into 1 without almost change of the T/^{14}C ratio.

All the feeding experiments indicate that the aglycone (2) can be biosynthesized by the cyclization of an intermediate derived from *p*-coumarate and two malonates (Scheme). This conclusion presents the occurrence of a new route for xanthone biosynthesis which is different from that of the xanthones in *Gentiana lutea*.

Bhatia and Seshadri[8] synthesized mangiferin (1) from the aglycone (2) and α-acetobromoglucose on the basis of the consideration that C-glucosylation might occur at the stage of 2. Aritomi and Kawasaki[9] first isolated a isomer of 1, isomangiferin (3), along with 1 from the aerial parts of *Anemarrhena asphodeloides*, suggesting from the co-occurrence of 1 and 3 that these isomers seem to be formed *via* C-glucosylation of maclurin (2,3',4,4',6-pentahydroxybenzophenone) (4).

In confirmation of the above suggestion, ^{14}C-labelled aglycone (1,3,6,7-tetrahydroxyxanthone-2,4,9a-$^{14}C_3$) and maclurin-1,3,5-$^{14}C_3$ were fed to the plants (Table 3). The incorporation of maclurin-^{14}C into 1 was found to be much higher than that of the aglycone-^{14}C. In addition to these data, three parallel experiments were performed in which (a) phenylalanine-3-^{14}C alone, (b) a mixture of phenylalanine-3-^{14}C and inactive aglycone (2), or (c) a mixture of phenylalanine-3-^{14}C and inactive maclurin (4) was fed to the plants under the same conditions. Only in the case of (c) the incorporation of ^{14}C was obviously suppressed to about 30 % than in the case of (a). These results show that C-glucosylation occurs at the stage of maclurin (4) prior to the formation of xanthone nucleus and that mangiferin (1) would be biosynthesized *via* 3-C-glucosylmaclurin (5) as a possible intermediate.

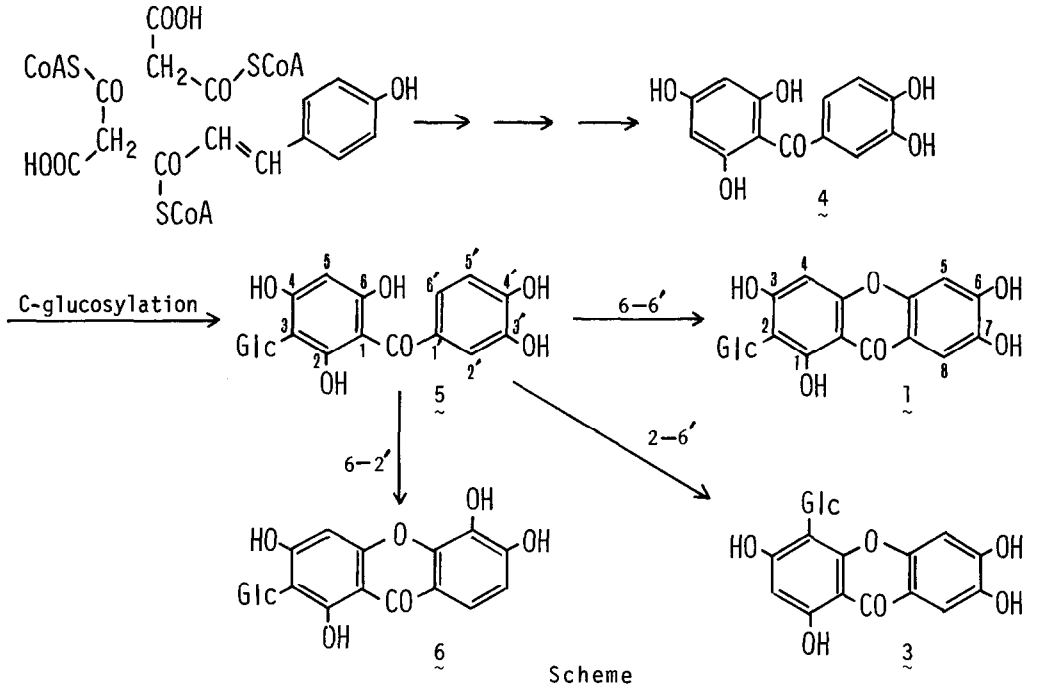
When 5 is converted to C-glucosylxanthone by ring closure, four isomeric C-glucosylxanthones could be formed, as suggested by Aritomi and Kawasaki.[9] Two of them are mangiferin (1) and isomangiferin (3), and two others are 1,3,5,6-tetrahydroxyxanthone-2-C-glucoside (6) and -4-C-glucoside (7). Recently 6 and its 5-methyl ether (irisxanthone) (8) were isolated from *Canscora decussata*[10] and *Iris florentina*[11] along with 1 respectively, although 7 has not been recorded in the literature. The co-occurrence of 1, its 3-methyl ether (homomangiferin)[7] and 3 in *Mangifera*

Table 3. Incorporation of ^{14}C-labelled aglycone (2), maclurin and phenylalanine into mangiferin

	Precursors	Spec. act. (dpm/mM)	Incorp. (%)	Ratio (%)
6.	1,3,6,7-Tetrahydroxyxanthone (2) -2,4,9a-$^{14}C_3$ (0.74 μCi)	1.27×10^3	0.04	—
7.	Maclurin-1,3,5-$^{14}C_3$ (0.26 μCi)	6.46×10^3	0.47	—
8. (a)	Phenylalanine-3-^{14}C (10 μCi)	1.30×10^6	3.20	100
(b)	Phenylalanine-3-^{14}C (10 μCi) + 1,3,6,7-tetrahydroxyxanthone (2)*	1.16×10^6	2.94	91.9
(c)	Phenylalanine-3-^{14}C (10 μCi) + maclurin (4)*	4.86×10^5	1.00	31.3

Feeding period : 50 hours. * Inactive compounds (2 and 4) added : 10 mg each.

indica,[12] and that of 1 and 3 in several species[13] have been reported. In particular, it should be noted that each of 3, 6 and 8 co-occurs with 1. These facts strongly support that 3-C-glucosylmaclurin (5) would be a key intermediate for the biosynthesis of the above C-glucosylxanthones. Probable biosynthetic route of mangiferin (1) and related C-glucosylxanthones is shown in Scheme.



Scheme

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