[Chem. Pharm. Bull. 28(8)2482—2486(1980)]

Biosynthesis of Mangiferin in *Anemarrhena asphodeloides* Bunge. II.[1] *C*-Glucosylation of Mangiferin[2]

Masao Fujita and Takao Inoue

Hoshi College of Pharmacy[3]

(Received March 31, 1980)

A benzophenone, maclurin-1,3,5-$^{14}C_3$ (**3**), was efficiently incorporated into *C*-glucosylxanthones [mangiferin (**1**) and isomangiferin (**2**)] of *Anemarrhena asphodeloides* without randomization, but the labelled aglycone of **1** and **2** (1,3,6,7-tetrahydroxyxanthone-2,4,9a-$^{14}C_3$) (**4**) was essentially not incorporated. Furthermore, the incorporation of phenylalanine-3-^{14}C into **1** and **2** was clearly suppressed by the addition of non-labelled maclurin (**3**) to the precursor solution.

These results indicate that *C*-glucosylation of **1** and **2** occurs at the stage of maclurin (**3**), prior to the formation of the xanthone nucleus, and that **1** and **2** may be biosynthesized *via* 3-*C*-glucosylmaclurin (**6**). A biosynthetic route is proposed for mangiferin (**1**) and related *C*-glucosylxanthones.

Keywords——*Anemarrhena asphodeloides* Bunge; biosynthesis; *C*-glucosylation of xanthone; mangiferin; isomangiferin; maclurin-1,3,5-$^{14}C_3$; 1,3,6,7-tetrahydroxyxanthone-2,4,9a-$^{14}C_3$; phloroglucinol-2,4,6-$^{14}C_3$

In the previous paper, we proposed that the aglycone of the *C*-glucosylxanthones mangiferin (**1**) and isomangiferin (**2**) is biosynthesized *via* a benzophenone, maclurin (**3**), derived from *p*-coumarate and two malonates in *Anemarrhena asphodeloides* Bunge (Liliaceae).[1]

Regarding the *C*-glucosylation of flavonoids, Wallace and Grisebach[4] reported that *C*-glucosylation of flavones occurs at the flavanone level in *Spirodela* and *Lemna* species. In addition, we studied the biosynthesis of an isoflavone *C*-glucoside, puerarin, in *Pueraria* root and reported that *C*-glucosylation of the isoflavone probably takes place at the chalcone stage.[5]

Since mangiferin (**1**) co-occurs with flavone *C*-glucosides in several families of plants,[6] it is interest to study whether its *C*-glucosylation occurs at the stage of benzophenone or xanthone. Bhatia and Seshadri[7] synthesized mangiferin (**1**) from the aglycone (1,3,6,7-tetrahydroxyxanthone) (**4**) and α-acetobromoglucose on the basis of the consideration that *C*-glucosylation might occur at the aglycone (**4**) stage. Aritomi and Kawasaki[8] first isolated an isomer of **1**, isomangiferin (**2**), along with **1** from *A. asphodeloides*, suggesting in view of the co-occurrence of **1** and **2** that these isomers may be formed *via* *C*-glucosylation of maclurin (2,3′,4,4′,6-pentahydroxybenzophenone) (**3**).

We have studied the *C*-glucosylation of **1** and **2** in *A. asphodeloides*, and now propose that their *C*-glucosylation occurs at the stage of a benzophenone, maclurin (**3**).

1) Part I: M. Fujita and T. Inoue, *Chem. Pharm. Bull.*, **28**, 2476 (1980).
2) A part of this study was reported briefly in *Tetrahedron Lett.*, **1977**, 4503 by M. Fujita and T. Inoue.
3) Location: *Ebara 2-4-41, Shinagawa-ku, Tokyo, 142, Japan.*
4) J.W. Wallace, T.J. Mabry, and R.E. Alston, *Phytochemistry*, **8**, 93 (1969); J.W. Wallace and H. Grisebach, *Biochim. Biophys. Acta*, **304**, 837 (1973).
5) T. Inoue and M. Fujita, *Chem. Pharm. Bull.*, **25**, 3226 (1977).
6) E.C. Bate-Smith, *Mem. Soc. Bot. Fr.*, **1965**, 16; T. Swain, *ibid.*, **1965**, 184; E.C. Bate-Smith and T. Swain, *Lloydia*, **28**, 313 (1965); T. Tomimori and M. Komatsu, *Yakugaku Zasshi*, **89**, 1276 (1969); I. Carpenter, H.D. Locksley and F. Sheinmann, *Phytochemistry*, **8**, 2013 (1969); K. Hostettmann and H. Wagner, *ibid.*, **16**, 821 (1977); C.A. Williams, *ibid.*, **18**, 803 (1979).
7) V.K. Bhatia and T.R. Seshadri, *Tetrahedron Lett.*, **1968**, 1741.
8) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.*, **18**, 2327 (1970).

Results and Discussion

Labelled compounds were prepared as follows. 1,3,6,7-Tetrahydroxyxanthone-2,4,9a-$^{14}C_3$ (**4**) was obtained by demethylation of 1,3,7-trihydroxy-6-methoxyxanthone-^{14}C[9] which was synthesized from 2,5-dihydroxy-4-methoxybenzoic acid and phloroglucinol-2,4,6-$^{14}C_3$ (**5**). Maclurin-1,3,5-$^{14}C_3$ (**3**) was synthesized from **5** and protocatechunitrile by means of the Hoesh reaction,[10] and **5** was prepared from diethylmalonate-2-^{14}C.[11]

In order to study the stage of *C*-glucosylation in **1** and **2**, two series of experiments were performed. Firstly, ^{14}C-labelled aglycone (**4**) and maclurin (**3**) were fed to the intact plants (cotton wick) or the aerial parts of *Anemarrhena asphodeloides*, which contains mangiferin (**1**) and isomangiferin (**2**) in the whole plant.[8,12] The above labelled compounds were administered as sodium salts in aqueous solution. After feeding for 50 and 70 hr, the radioactive mangiferin (**1**) and isomangiferin (**2**) were isolated from the plant materials. In some experiments, radioactive **1** and **2** were degraded to the aglycone (**4**), followed by acetylation and purification as the tetraacetate.

Table I. Incorporations of Labelled Aglycone (**4**) and Maclurin (**3**) into Mangiferin and Isomangiferin

Expt.	Precursors (Sp. act.: amount fed)		*C*-Glucosyl-xanthones	Yield (mg)	Incorporation (%)	Sp. act. (dpm/mM) *C*-Glucosyl-xanthones	Sp. act. (dpm/mM) Aglycone[a] (tetraacetate)
1.	1,3,6,7-Tetrahydroxy-xanthone-2,4,9a-$^{14}C_3$ (**4**) (8.51×10^7 dpm/mM, 5 mg)	(a)[b]	Mangiferin	66	0.04	3.73×10^3	1.41×10^3(37.8)
			Isomangiferin	8.9	0.002	1.25×10^3	
		(b)[c]	Mangiferin	238	0.04	1.27×10^3	
2.	Maclurin-1,3,5-$^{14}C_3$ (**3**) (3.05×10^7 dpm/mM, 5 mg)	(a)[b]	Mangiferin	41	0.38	2.28×10^4	2.23×10^4(97.9)
			Isomangiferin	6.2	0.03	1.10×10^4	1.06×10^4(96.4)
		(b)[c]	Mangiferin	180	0.47	6.46×10^3	

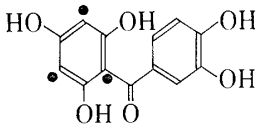
a) Figures in parentheses show % ratios to the specific activity of the *C*-glucosylxanthones.
b) Wick-feed to whole plants (feeding period: 70 hr)
c) Absorption into excised leaves (feeding period: 50 hr)

As shown in Table I, the incorporation of the labelled aglycone (**4**) into **1** and **2** was very poor, and about 60% of the label in **1** was present in the sugar moiety (Expt. 1). In contrast, maclurin-^{14}C (**3**) was much more effectively incorporated into **1** and **2** than the aglycone-^{14}C (**4**), and the radioactivity in **1** and **2** was largely localized in the xanthone moiety (Expt. 2). Furthermore, the aglycone (**4**) of **1** obtained after feeding maclurin-^{14}C (**3**) was degraded to phloroglucinol by potash fusion. The label in the aglycone (**4**) was largely present in the phloroglucinol moiety, as shown in Chart 1, and these results showed that **3** was incorporated into **1** without randomization.

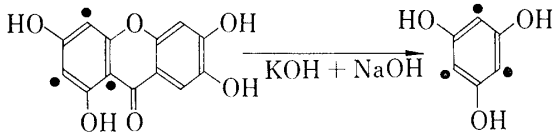
Secondly, three parallel experiments were performed in which (a) phenylalanine-3-^{14}C alone, (b) a mixture of phenylalanine-3-^{14}C and inactive aglycone (**4**) (10 mg), or (c) a mixture of phenylalanine-3-^{14}C and inactive maclurin (**3**) (10 mg) was fed to the aerial parts of the plants under the same conditions. As shown in Table II, in case (c) only, the incorporation of ^{14}C into **1** and **2** was clearly suppressed to about 30% of that in case (a).

These results show that mangiferin (**1**) and isomangiferin (**2**) were biosynthesized *via* a benzophenone, maclurin (**3**), that *C*-glucosylation occurs at the stage of **3** prior to the forma-

9) P. Yates and G.H. Stout, *J. Am. Chem. Soc.*, **80**, 1791 (1958).
10) K. Hoesh and T. Zarzecki, *Chem. Ber.*, **50**, 462 (1917).
11) L. Patschke, W. Barz, and H. Grisebach, *Z. Naturforsch.*, **19b**, 1110 (1964).
12) N. Morita, M. Shimizu, and M. Fukuta, *Yakugaku Zasshi*, **85**, 374 (1965).



3



4

Sp. act.: 2.23×10^4 dpm/mM (100%) Sp. act.: 2.18×10^4 dpm/mM (97.8%)

Chart 1. Degradation of the Aglycone (**4**) of Mangiferin from *A. asphodeloides* after Feeding Maclurin-1,3,5-$^{14}C_3$ (**3**)

Table II. Incorporation of Phenylalanine-3-^{14}C into Mangiferin and Isomangiferin

Expt.	Precursors[a]	Mangiferin				Isomangiferin			
		Yield (mg)	Sp. act. (dpm/mM)	Incorporation (%)	Ratio[c] (%)	Yield (mg)	Sp. act. (dpm/mM)	Incorporation (%)	Ratio[c] (%)
3. (a)	Phenylalanine-3-^{14}C	231	1.30×10^6	3.20	100	36	8.02×10^5	0.31	100
(b)	Phenylalanine-3-^{14}C +1,3,6,7-tetrahydroxyxanthone (**4**)[b]	238	1.16×10^6	2.94	91.9	40	6.32×10^5	0.27	87.1
(c)	Phenylalanine-3-^{14}C +maclurin (**3**)[b]	193	4.86×10^5	1.00	31.3	32	1.59×10^5	0.05	16.1

Feeding period: 50 hr.
a) Phenylalanine-3-^{14}C fed: 10 μCi each.
b) Inactive compounds fed: 10 mg each.
c) Ratio to incorporation of phenylalanine-3-^{14}C.

tion of the xanthone nucleus, and that 3-*C*-glucosylmaclurin (**6**) is a possible intermediate in the biosynthesis of **1** and **2**.

When **6** is converted to *C*-glucosylxanthone by ring closure, four isomeric *C*-glucosylxanthones could be formed, as pointed out by Aritomi and Kawasaki.[8] Two of them are mangiferin (**1**) and isomangiferin (**2**), and the others are 1,3,5,6-tetrahydroxyxanthone-2-*C*-glucoside (**7**) and -4-*C*-glucoside (**8**). The co-ocurrence of **1** and **2** in several species[13] and that of **1**, its 3-methyl ether (homomangiferin)(**9**) and **2** in *Mangifera indica* have been reported.[14] Mangiferin (**1**) was found along with **7** in *Canscora decussata* by Ghosal and Chaudhuri,[15] while Arisawa *et al.*[16] reported the isolation of **1** and the 5-methyl ether of **7** (irisxanthone) (**10**) from *Iris florentina*. Recently Ghosal *et al.*[17] isolated the trimethyl ether of **7** along

13) D.M. Smith and J.B. Harbone, *Phytochemistry*, **10**, 2117 (1971); V.I. Glyzin, A.I. Ban'Kovskii, M.G. Pimenov, and K.I. Boryaev, *Khim. Prir. Soedin.*, **9**, 434 (1973) [*C.A.*, **79**, 79119 (1973)]; B.A. Bohm, *Phytochemistry*, **14**, 287 (1975); M. Arisawa, H. Kizu, and N. Morita, *Chem. Pharm. Bull.*, **24**, 1609 (1976).
14) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.*, **18**, 2224 (1970); N.A.M. Saleh and M.A.I. El-Ansori, *Planta Medica*, **28**, 124 (1975).
15) R.K. Chaudhuri and S. Ghosal, *Phytochemistry*, **10**, 2425 (1971); S. Ghosal and R.K. Chaudhuri, *ibid.*, **12**, 2035 (1973).
16) M. Arisawa, N. Morita, Y. Kondo, and T. Takemoto, *Chem. Pharm. Bull.*, **21**, 2562 (1973).
17) S. Ghosal, D.K. Jaiswal, and K. Biswas, *Phytochemistry*, **17**, 2119 (1978).

with **9** instead of **1** from *Hoppea dichotoma*. Although **8** has not been recorded in the literature, it is interesting that the other *C*-glucosylxanthones (**2**, **7** and **10**) do co-occur with **1**. These findings strongly suggest that 3-*C*-glucosylmaclurin (**6**) is a key intermediate in the biosynthesis of the above *C*-glucosylxanthones.

A biosynthetic route for mangiferin (**1**) and related *C*-glucosylxanthones is proposed in Chart 2.

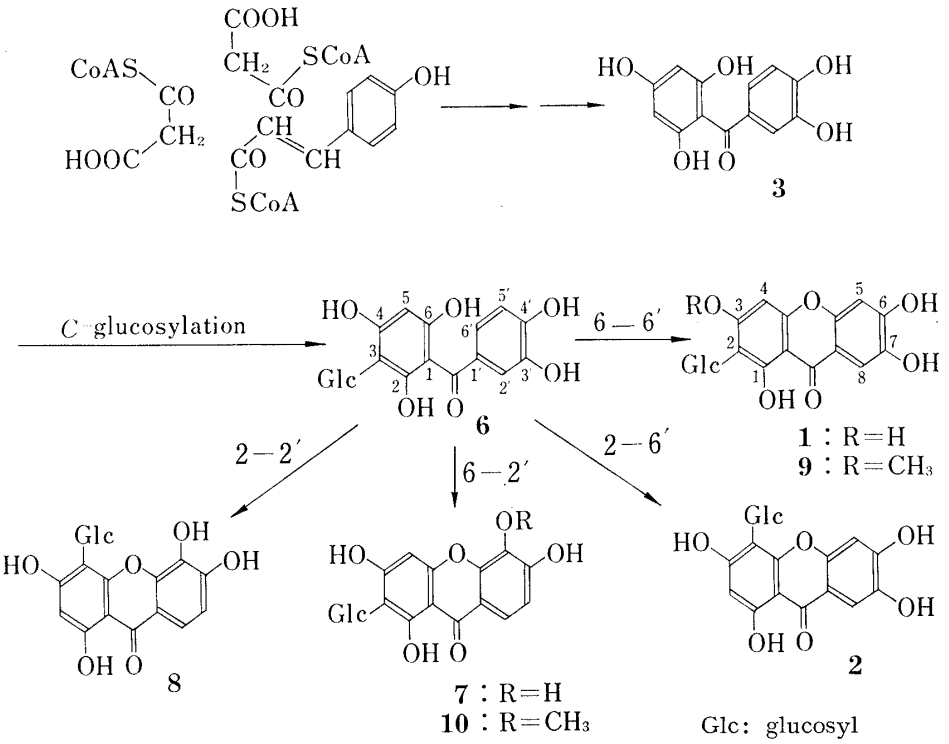


Chart 2. Probable Biosynthetic Route to Mangiferin and Related *C*-Glucosylxanthones

Experimental

Malonic acid-2-^{14}C was purchased from the Radiochemical Centre, Amersham and diethyl malonate-2-^{14}C from New England Nuclear Corp. All radioactive samples were counted with an Aloka LSC-602 liquid scintillation counter in a POP-POPOP-naphthalene-dioxane scintillator solution. The purity of radioactive compounds synthesized was examined by radio-chromatography (Aloka JTC-201 scanner).

Phloroglucinol-2,4,6-$^{14}C_3$ (5)[11]——Diethyl malonate-2-^{14}C (1 mCi, 35 mg) and diethyl malonate (4 g) were added to EtOH (8 ml) containing Na (0.3 g), and the mixture was heated at 135° for 4 hr. After cooling, the reaction mixture was diluted with H_2O (8 ml) and washed with ether (3×40 ml). The aqueous layer was acidified with 30% H_2SO_4 and extracted with ether (5×40 ml). The ethereal layer was washed with H_2O, dried over anhyd. $MgSO_4$ and concentrated to give crude diethyl 2,4,6-trihydroxyisophthalate-1,3,5-$^{14}C_3$ (0.78 g) as a pale brown solid. The radioactive crude ester was diluted with carrier (0.6 g) and recrystallized from EtOH to give colorless needles (864 mg), mp 103—104°. A solution of the ester in 60% KOH (5.1 ml) was heated for 2.5 hr at 100° under a stream of nitrogen, acidified with dil. HCl and extracted with ether (10×30 ml). Concentration of the ether layer afforded crude phloroglucinol-2,4,6-$^{14}C_3$ (**5**), which was purified by silica gel chromatography using C_6H_6–EtOAc (1:1) as an eluent, followed by sublimation at 150—180° *in vacuo* to give a white powder (248 mg), mp 213—215°. Sp. act. 8.80×10^7 dpm/mM.

1,3,6,7-Tetrahydroxyxanthone-2,4,9a-$^{14}C_3$ (4)——A mixture of **5** (85 mg), 2,5-dihydroxy-4-methoxybenzoic acid (87 mg), fused $ZnCl_2$ (0.5 g) and $POCl_3$ (1.5 ml) was heated at 70° for 2 hr and poured into ice water (40 ml). After a day, the resulting precipitates were collected, washed with H_2O, and then suspended in 5% $NaHCO_3$ (20 ml). The suspension was centrifuged, then the separated precipitates were dissolved in MeOH (30 ml), and the solution was filtered. The filtrate was chromatographed on silica gel and elution with AcOEt gave 1,3,7-trihydroxy-6-methoxyxanthone-2,4,9a-$^{14}C_3$ (48 mg) as a pale yellow powder. A mixture of the above radioactive xanthone (48 mg) and HI (d=1.7, 16 ml) was refluxed for 2.5 hr and poured

into satur. $NaHSO_3$ solution. The resulting precipitates were collected and washed with H_2O, followed by recrystallization from aq. EtOH to give yellow crystals (32 mg), mp 307—308°. Sp. act. 8.51×10^7 dpm/mM.

Maclurin-1,3,5-$^{14}C_3$ (3)[10]——Na-dry ether (6 ml) was added to a mixture of **5** (80 mg), anhyd. phloroglucinol (160 mg), protocatechunitrile (300 mg) and fused $ZnCl_2$ (1 g), and the mixture was saturated with dry HCl. After 30 min, dry ether (3 ml) was added to the mixture, and the whole was heated at 60° for 3 hr under a stream of HCl. After standing overnight, the reaction mixture was diluted with H_2O (5 ml) and washed with ether (3×20 ml). The aqueous layer was gently warmed to remove ether, neutralized with dil. NH_4OH and refluxed for 2 hr. After cooling, the aqueous solution was acidified with a few drops of 10% HCl and extracted with ether (5×20 ml). The ether layer was washed with H_2O, dried over anhyd. Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography using $CHCl_3$ and ether successively as eluents. The elution with ether afforded crude crystals, which were recrystallized from H_2O to give yellow crystals (21.4 mg), mp 199—200°. Sp. act. 3.05×10^7 dpm/mM.

Feeding Experiments——Labelled compounds were dissolved in a minimum amount of 0.1 N NaOH, diluted with an adequate amount of H_2O and administered to *A. asphodeloides* in the flowering period. In Expts. 1a and 2a the precursor solution was fed to the intact plants from the rhizomes by the cotton wick method. The solution was almost completely absorbed by the plants within 8—10 hr and subsequently small amounts of H_2O were frequently supplied in order to ensure that all the remaining precursor was absorbed. In the other feeding experiments (1b, 2b and 3), the precursor was administered to the aerial parts of the plants essentially as described previously.[1] After feeding for 50 and 70 hr, the plants were cut into small pieces and dried at 60°.

Isolation and Degradation of Mangiferin (1) and Isomangiferin (2)——Isolation of **1** and **2** from the plant materials, and their degradation with HI to the aglycone (**4**), as well as that of **4** by alkali fusion to phloroglucinol, were performed according to the reported procedures.[1] In the previous paper, alkali fusion was carried out with mangiferin (**1**), but in this case it was performed with the aglycone (**4**) in order to increase the yield of phloroglucinol. Radioactive **4** (18 mg) was diluted with about twice the amount of carrier and fused in a mixture of KOH and NaOH at 290—300° for 1 hr to yield phloroglucinol (4.1 mg).

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.