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Inhibition of Intestinal α-Glucosidases and Sugar Absorption by Flavones

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Forty flavones have been tested for inhibitory activity toward rat small intestinal sucrase and maltase with the object of developing a new type of sugar absorption inhibitor for the treatment of diabetes and obesity. It was suggested that the greater the number of hydroxyl groups, the higher the inhibitory activity. The IC_{50} values of the two most potent flavones, 3′,4′,5,6,7-pentahydroxyflavone (**24**, PHF) and 3′,4′,5,6,7-pentahydroxy-3-methoxyflavone (**27**, PHMF), for sucrase were 13 and 28 μM, and those for maltase were 29 and 31 μM. The types of inhibition by PHF and PHMF were both uncompetitive for sucrase and non-competitive for maltase. PHF at 25 mg/kg significantly inhibited the intestinal absorption of sucrose, but not maltose, in rats. These results indicate that PHF (**24**) and more potent flavones, if they can be found, may be useful clinically.

Keywords——flavonoid; flavone; sucrase; maltase; intestinal α-glucosidase; inhibition of sugar absorption; inhibitor

The control of food intake by modulating absorptive processes is considered to be useful especially for patients suffering from diabetes or obesity. One method by which the postprandial increments in blood glucose concentration can be decreased is the use of undigestible dietary fiber.[1] Recently more direct chemical methods to achieve this effect have been introduced with the development of inhibitors of intestinal α-glucosidase (*e.g.* acarbose[2] and *p*-alkanoyl toluenes[3]) and of α-amylase (*e.g.* cycloleucine).[4] In particular, acarbose, a microbial pseudosaccharide, has been shown to reduce effectively the intestinal absorption of sugar in man.[5]

Flavonoids, which are among the most ubiquitously distributed compounds in the plant kingdom, have been shown to possess a variety of biochemical and pharmacological activities[6] including inhibitory activity toward yeast α-glucosidase.[7] On the other hand, it was suggested that the lack of success of a flavonoid treatment may be ascribed to the failure of the compound to reach its target, if the target is not in the gastrointestinal tract.[6b,8]

All these facts prompted us to assess the inhibitory activity of flavonoids against small intestinal α-glucosidases in an attempt to develop a new type of sugar absorption inhibitor.

In this paper, we describe the results of a screening test of 40 flavones for activity as inhibitors of rat small intestinal α-glucosidases, the properties of the inhibitions by the two most potent flavones, and the inhibitory effect of the most potent flavone, 3′,4′,5,6,7-pentahydroxyflavone (**24**, PHF), on the intestinal absorption of sugar.

Materials and Methods

Chemicals——Isomaltose was purchased from Wako (Osaka, Japan). Soluble starch and glucose dehydrogenase were from Kanto (Tokyo, Japan). Human pancreatic and salivary α-amylases were prepared from pancreatic tissues and saliva, respectively, as described previously.[9] A kit for blood sugar test (GOD-Perid-Test) was from Boehringer (Mannheim, Germany). Amylochrome (a kit for assaying α-amylase activity) was from Roche (Basel, Switzerland). Flavones were isolated from various plants or chemically synthesized as reported elsewhere.[10]

Preparation of Membrane Vesicles from Small Intestinal Brush Border——Brush border membrane vesicles were prepared from the rat small intestine frozen at −20 °C as described by Kessler *et al.*[11] The prepared vesicles were suspended in 0.02 M maleate buffer (pH 6.0) and stored at 4 °C.

Assays of Small Intestinal α-Glucosidases——Brush border membrane vesicles were used as the preparation of small intestinal α-glucosidases such as sucrase, maltase, isomaltase, trehalase, and glucoamylase. Reactions for enzyme assays were performed as illustrated in Chart 1. After incubation at 37 °C for 15 min, optical densities of

	reaction mixture			
	A	B	C	D
suspension of membrane vesicles	50 μl	50 μl	50 μl	50 μl
0.2 M maleate buffer (pH 6.0)	50 μl	50 μl	50 μl	50 μl
substrate solution	50 μl	—	50 μl	—
0.2 mM flavone	—	—	50 μl	50 μl
H_2O	50 μl	100 μl	—	50 μl
incubation at 37 °C for 5 min				
0.3 M Tris–HCl (pH 7.6)/2.2 mM NAD/ 100 U/l mutarotase	1 ml	1 ml	1 ml	1 ml
500 kU/l glucose dehydrogenase	10 μl	10 μl	10 μl	10 μl
incubation at 37 °C for 15 min				
measurement of absorbance at 340 nm	OD_A	OD_B	OD_C	OD_D

Chart 1. Reaction Procedure for the Assay of Intestinal α-Glucosidases

reaction mixtures were measured at 340 nm against a water blank. By using the optical densities, OD_A, OD_B, OD_C, and OD_D, corresponding to reaction mixtures, A, B, C, and D, the percent inhibition of enzyme activities was calculated as follows.

$$\text{percent inhibition} = \frac{OD_A - OD_B - (OD_C - OD_D)}{OD_A - OD_B} \times 100$$

Brush border membrane vesicles were adequately diluted with 0.02 M maleate buffer (pH 6.0) for each enzyme activity. Substrate solutions for sucrase, maltase, isomaltase, trehalase, and glucoamylase were 400 mM sucrose, 40 mM maltose, 100 mM isomaltose, 100 mM trehalose, and 2% soluble starch, respectively. Flavones were dissolved in dimethylsulfoxide to give a concentration of 5 mM and then diluted to 0.2 mM with water. Tris (0.3 M) added at 5 min after the start of incubation acts as a strong inhibitor of small intestinal α-glucosidases and completely stops the hydrolysis of sugar by the enzymes.

Assay of α-Amylases——Activities of human pancreatic and salivary α-amylases were assayed with Amylochrome (α-amylase assay kit) according to the instructions of the manufacturer. In order to determine the inhibitory activities of flavones, each compound was dissolved in dimethylsulfoxide and added, in a volume of 0.01 ml, to the reaction mixture. The same amount (0.01 ml) of vehicle did not affect the α-amylase activity.

Effect of Flavone on the Intestinal Absorption of Sugar——Male Sprague Dawley rats (170—200 g) were fasted for 26 h (from 10 a.m. to 12 a.m. of the next day) before use. Each of the animal groups (test group, vehicle-control group, and sugar-control group) consisted of 5 rats. A test flavone, PHF (**24**), was dissolved in a solution containing 0.1 N NaOH and 2% Na_2CO_3 and then neutralized with 0.5 N HCl. After adding sugar (sucrose or maltose) and water to the solution, the concentrations of PHF and sugar were brought to 2.17 mg/ml and 0.13 g/ml, respectively. A solution containing PHF and sugar was given to the test group by gavage. Dose of PHF and sugar were 25 mg/kg and 1.5 g/kg, respectively. Vehicle-controls received vehicle only. Sugar-controls received sugar solutions (0.13 g/ml) at a dose of 1.5 g sugar/kg. Blood glucose concentrations were determined before and at 0.5, 1, 1.5 and 2 h after dosing. Blood samples (10 μl each) were collected by slightly cutting the tail vein with a razor and deproteinized by the method of Somogyi.[12] The supernatants were subjected to glucose determination with the GOD-Perid-Test.

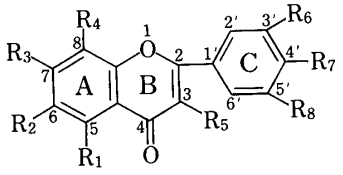
Determination of IC_{50} Values——The IC_{50} value (50% inhibitory concentration) was determined graphically from a plot of percent inhibition *vs.* log of flavone concentration (40, 120, 200, and 300 μM).

Results and Discussion

Inhibitory Activities of Flavones toward Sucrase and Maltase of Small Intestinal Brush Border Membrane Vesicles

The inhibitory activities toward small intestinal sucrase and maltase of flavones (**1**—**29**) substituted with hydroxyl and/or methoxyl groups are shown in Table I, in which flavones are arranged primarily according to the number of hydroxyl groups. Compounds **1**—**6**, **7**—**16**, **17**—**23**, and **24**—**29** have 2, 3, 4, and 5 or 6 hydroxyl groups, respectively. Table II illustrates the inhibitory activities of flavones (**30**—**40**) with substituents other than hydroxyl and methoxyl groups. At first glance, the group of flavones (**24**—**29**) possessing 5 or 6 hydroxyl groups is more potent than other groups of flavones. The most potent flavones were PHF (**24**) and 3′,4′,5,6,7-pentahydroxy-3-methoxyflavone (**27**, PHMF). The free hydroxyl groups at positions 5, 6, and 7 may be essential for the inhibitory activity. Methoxylation at position 8 is

TABLE I. Inhibitory Activities of Flavones with Hydroxyl and Methoxyl Groups toward Sucrase and Maltase of the Small Intestine



Compd. No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Inhibition (%) at 50 μM[a] Sucrase	Maltase
1	OH	OH	OMe	OMe	H	H	H	H	<5	<5
2[b]	OMe	H	OH	OH	H	H	OMe	H	<5	9
3	OH	OMe	OH	OMe	H	H	OMe	H	<5	10
4	OH	OMe	OMe	H	H	OMe	OH	H	<5	<5
5	OMe	OH	OMe	OMe	H	OMe	OH	H	18	18
6	OMe	OMe	OMe	OMe	H	OH	OH	H	15	13
7	OH	OH	OH	OMe	H	H	H	H	25	8
8	OH	H	OH	OH	H	H	OMe	H	<5	5
9	OH	OH	OMe	OMe	H	H	OH	H	9	9
10	OH	OMe	OH	OMe	H	H	OH	H	<5	<5
11	OH	OMe	OMe	H	H	OH	OH	H	5	<5
12[b]	OMe	H	OH	OH	H	OMe	OH	H	11	15
13	OH	OH	OMe	OMe	H	OMe	OH	H	14	9
14	OH	OMe	OMe	H	OMe	OH	OH	H	8	35
15	OMe	OH	OMe	OMe	H	OH	OH	H	15	18
16	OMe	H	OH	OMe	OMe	OH	OH	H	<5	18
17	OH	H	OH	OH	H	H	OH	H	<5	14
18	OH	OH	OH	OMe	H	H	OH	H	20	21
19	OH	OH	OMe	H	H	OH	OH	H	<5	7
20	OH	H	OH	OMe	H	OH	OH	H	35	21
21	OH	OH	OH	OMe	H	OMe	OH	H	23	19
22	OH	OMe	OH	H	OMe	OH	OH	H	9	24
23	OH	H	OH	OMe	OMe	OH	OH	H	16	24
24 (PHF)	OH	OH	OH	H	H	OH	OH	H	82	60
25	OH	H	OH	OH	H	OH	OH	H	22	21
26	OH	OH	OH	OMe	H	OH	OH	H	33	25
27 (PHMF)	OH	OH	OH	H	OMe	OH	OH	H	64	62
28	OH	H	OH	OH	H	OH	OH	OH	32	42
29[c]	OH	H	OH	H	OH	OH	OH	OH	28	37

a) Assays were carried out as described in Materials and Methods. Each value is the mean of two determinations. *b*) The methods for synthesizing these compounds will be reported elsewhere. *c*) Myricetin.

TABLE II. Inhibitory Activities of Flavones with Substituents other than Hydroxyl and Methoxyl Groups toward Sucrase and Maltase of the Small Intestine

Compd. No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Inhibition (%) at 50 μM[a] Sucrase	Maltase
30[b]	OMe	OMe	OMe	OMe	H	$OCOC_3H_7$	$OCOC_3H_7$	H	8	6
31	OAc	OAc	OAc	OAc	H	OAc	OAc	H	16	16
32[b]	OMe	OMe	OMe	OMe	H	OH	OPO_3Na_2	H	22	15
33[b]	OMe	$OC_{10}H_{21}$	OMe	OMe	H	OH	OH	H	<5	14
34	OH	OMe	$OC_{12}H_{25}$	H	H	OH	OH	H	<5	21
35	OH	H	$OC_{12}H_{25}$	OMe	H	OH	OH	H	6	9
36	OH	OC_5H_{11}	OMe	H	H	OH	OH	H	<5	18
37	OH	H	OMe	$OC_{12}H_{25}$	H	OH	OH	H	<5	<5
38	OH	$OC_{12}H_{25}$	OMe	H	H	OH	OH	H	<5	<5
39	OH	$OC_{18}H_{37}$	OMe	H	H	OH	OH	H	5	<5
40[c]	OH	H	OH	H	$OC_6H_{11}O_4$[d]	OH	OH	H	<5	17

a) Assays were carried out as described in Materials and Methods. Each value is the mean of two determinations. *b*) The methods for synthesizing these compounds will be reported elsewhere. *c*) Quercitrin. *d*) L-Rhamnosyl.

TABLE III. Inhibitory Activities of PHF and PHMF toward Small Intestinal α-Glucosidases

Compd.	Inhibition (%) at 50 μM[a] Sucrase	Maltase	Isomaltase	Trehalase	Glucoamylase
PHF[b]	82	60	32	22	49
PHMF[c]	64	62	18	20	51

a) Assays were carried out as described in Materials and Methods. Each value is the mean of two determinations. *b*) 3′,4′,5,6,7-Pentahydroxyflavone (**24**). *c*) 3′,4′,5,6,7-Pentahydroxy-3-methoxyflavone (**27**).

TABLE IV. IC_{50} Values of Flavones toward Small Intestinal α-Glucosidases and Types of Inhibitions

Compd.	IC_{50} (μM)[a] Sucrase	Maltase	Type of inhibition Sucrase	Maltase
PHF[b]	13	29	Uncompetitive	Non-competitive
PHMF[c]	28	31	Uncompetitive	Non-competitive

a) Values were determined as described in Materials and Methods. *b*) 3′,4′,5,6,7-Pentahydroxyflavone (**24**). *c*) 3′,4′,5,6,7-Pentahydroxy-3-methoxyflavone (**27**).

detrimental to the activity, in that **26** is less potent than **24**. The hydroxylation and/or methoxylation of ring C at positions 3′ and 4′ may be without appreciable effect, since the inhibitory activities of **7**, **18**, **21**, and **26** are at similar levels.

Properties of the Inhibitions of Small Intestinal α-Glucosidases by PHF (24) and PHMF (27)

The inhibitory activities of PHF (**24**) and PHMF (**27**) toward small intestinal isomaltase,

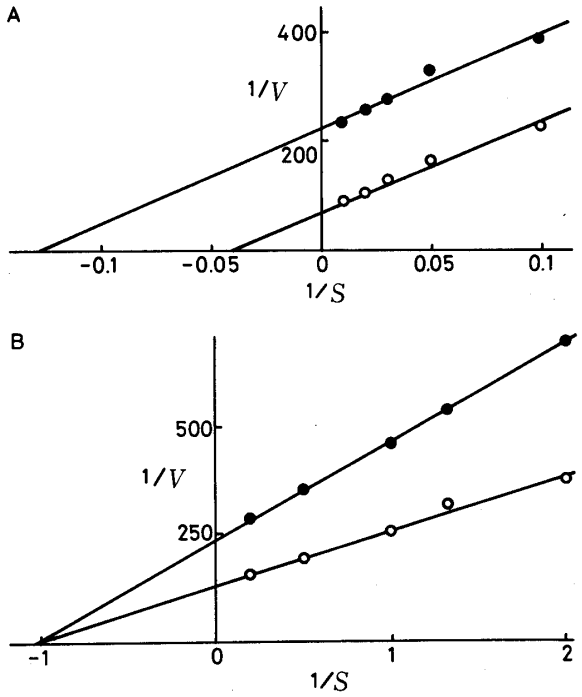


Fig. 1. Lineweaver–Burk Plots for Inhibition of Small Intestinal Sucrase and Maltase by PHF

—○—, control; —●—, in the presence of PHF (**24**) at 15 μM for sucrase (A) and at 30 μM for maltase (B). The enzyme assays were carried out in the presence and absence of PHF as described in Materials and Methods except that the substrate concentration was varied. The velocity (V) and the substrate concentration (S) are expressed as μmol sugar hydrolyzed/min and mM, respectively.

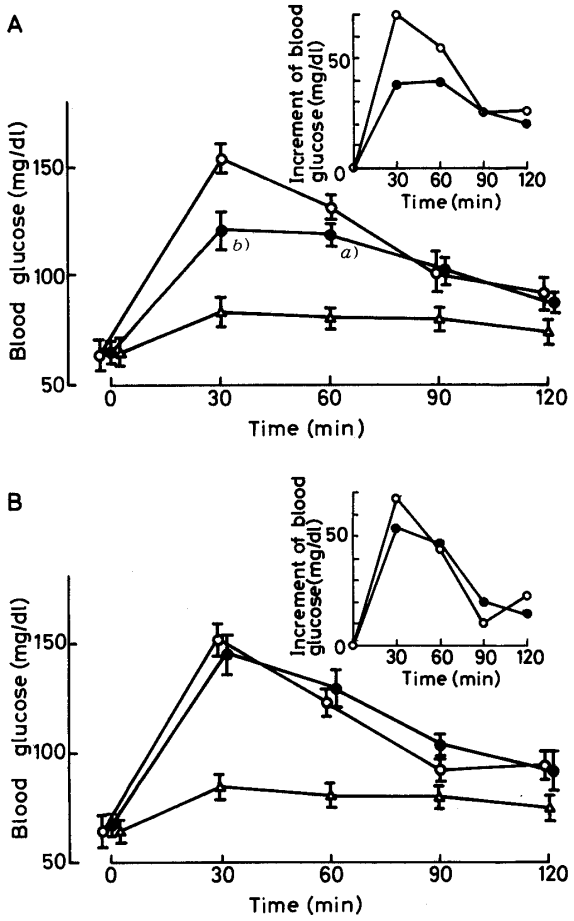


Fig. 2. Effect of PHF on the Intestinal Absorption of Sugar

PHF (**24**) and sugar (sucrose or maltose) were orally given at doses of 25 mg/kg and 1.5 g/kg, respectively, in the test group. Blood glucose concentrations were followed for 2 h after loading sugar. Further details of the experiments are given in Materials and Methods. Values and vertical bars represent means ± S.D. for five experiments. A, sucrose loading; B, maltose loading. —●—, test group; —○—, sugar-control group; —△—, vehicle-control group. Insets show the increment of blood glucose concentration as a function of time, calculated according to the following equation.

$$\begin{aligned}&\text{increment of blood glucose concentration in the test group}\\&=[\text{Test}]_t-[\text{Test}]_0-([\text{Vehicle}]_t-[\text{Vehicle}]_0)\end{aligned}$$

where $[\text{Test}]_0$ and $[\text{Test}]_t$ are blood glucose concentrations determined before and t min after dosing in the test group, and $[\text{Vehicle}]_0$ and $[\text{Vehicle}]_t$ are those in the vehicle-control group. The increment of blood glucose concentration in the sugar-control group was similarly calculated.

a) $p<0.05$ as compared to the sugar-control. *b*) $p<0.01$ as compared to the sugar-control.

trehalase, and glucoamylase, along with those toward sucrase and maltase, are listed in Table III. Both PHF (**24**) and PHMF (**27**) at 50 μM clearly inhibited small intestinal α-glucosidases tested. Isomaltase and trehalase, however, were less susceptible to inhibition by the flavones than the other three α-glucosidases. Human pancreatic and salivary α-amylases were not

inhibited at all by PHF (**24**) and PHMF (**27**) at 0.5 mM (data not shown).

The IC_{50} values of PHF (**24**) and PHMF (**27**) toward sucrase and maltase as well as the types of inhibitions are summarized in Table IV. The types of inhibitions of sucrase and maltase by PHF (**24**) were found to be uncompetitive and non-competitive, respectively, by means of Lineweaver–Burk plots (Fig. 1). Similar plots were also obtained with PHMF (**27**) (data not shown). Uncompetitive and non-competitive inhibitors appear to be preferable to competitive inhibitors in therapeutic use for the present purpose, since the binding of the former inhibitors, but not the latter ones, to enzymes is unaffected or less affected by substrate concentration.

Effect of PHF on the Intestinal Absorption of Sugar

We investigated the inhibitory effect of PHF (**24**), the most potent compound among the 40 flavones tested, on the intestinal absorption of sugar (sucrose or maltose) in rats. As judged from the protective effect of PHF (**24**) against the increase of blood glucose concentration after loading sugar, the compound at 25 mg/kg was significantly effective in inhibiting the intestinal absorption of sucrose, but not that of maltose (Fig. 2). PHF (**24**) at 12.5 mg/dl, however, little affected the intestinal absorption of sucrose or maltose (data not shown). Negligible inhibition of maltose absorption, as distinct from sucrose absorption, by PHF (**24**) (25 mg/kg) may be a reflection of the fact that PHF (**24**) is twice as potent against small intestinal sucrase than against maltase, as shown in Table IV. An alternative explanation is that such α-glucosidases as sucrase, isomaltase, and glucoamylase can also hydrolyze maltose[13] and thus effective inhibition of maltose hydrolysis is not easy.

Acarbose at 4 μM inhibited rat small intestinal sucrase and maltase by 60 and 58%, respectively (unpublished data). Comparing these values with the present data on PHF (**24**), this flavone is at least one order of magnitude less potent than acarbose. Flavones, however, occur widely in the plant kingdom, chemical syntheses of flavones are relatively easy, and the toxicity of flavones is very low in animals.[6b] The present results, together with these features, suggest that there may be other flavones with more potent inhibitory activity toward small intestinal α-glucosidases and that PHF (**24**) and more potent flavones may be useful clinically for treating diabetes and obesity.

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References

1) D. J. A. Jenkins, T. M. S. Wolever, A. R. Leeds, M. A. Gassull, P. Haisman, J. Dilawari, D. V. Goff, G. L. Metz, and K. G. M. M. Alberti, *Br. Med. J.*, **1**, 1392 (1978); K. Doi, M. Matsuura, A. Kawara, S. Baba, and K. Nishikawa, *J. Japan Diabetes Soc.*, **25**, 197 (1982).
2) W. F. Caspary and S. Graf, *Res. Exp. Med.*, **175**, 1 (1979); E. Truscheit, W. Frommer, B. Junge, L. Müller, D. D. Schmidt, and W. Wingender, *Angew. Chem. Int. Ed. Engl.*, **20**, 744 (1981).
3) R. S. Ho and C. G. Aranda, *Arch. Int. Pharmacodyn. Ther.*, **237**, 98 (1979).
4) C. G. Aranda, R. S. Ho, and W. R. Sterling, *Proc. Soc. Exp. Biol. Med.*, **162**, 401 (1979).
5) G. Sachse and B. Willms, *Diabetologia*, **17**, 287 (1979); D. J. A. Jenkins, R. H. Taylor, D. V. Goff, H. Fielden, J. J. Misiewicz, D. L. Sarson, S. R. Bloom, and K. G. M. M. Alberti, *Diabetes*, **30**, 951 (1981); J. Radziuk, F. Kemmer, T. Morishima, P. Berchtold, and M. Vranic, *ibid.*, **33**, 207 (1984).
6) *a*) J. Okuda, I. Miwa, K. Inagaki, T. Horie, and M. Nakayama, *Biochem. Pharmacol.*, **31**, 3807 (1982); *b*) B. Havsteen, *ibid.*, **32**, 1141 (1983); *c*) R. L. Mower, R. Landolfi, and M. Steiner, *ibid.*, **33**, 357 (1984).
7) M. Iio, A. Yoshioka, Y. Imayoshi, C. Koriyama, and A. Moriyama, *Agric. Biol. Chem.*, **48**, 1559 (1984).
8) S. D. Varma and J. H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976); J. Metzner, H. Bekemeier, E.-M. Schnedewind, and U. Wenzel, *Pharmazie*, **34**, 185 (1979).
9) J. J. Marshall and I. Miwa, *Biochim. Biophys. Acta*, **661**, 142 (1981).
10) T. Horie, M. Masumura, K. Kase, K. Fukui, and M. Nakayama, *Nippon Kagaku Kaishi*, **1974**, 2400; T. Horie,

ibid., **1978**, 748; T. Horie, H. Kourai, M. Nakayama, M. Tsukayama, and M. Masumura, *ibid.*, **1980**, 1397; M. Nakayama, I. Mori, and T. Horie, *ibid.*, **1983**, 161; T. Horie, M. Masumura, and S. Okumura, *Nippon Kagaku Zasshi*, **83**, 465 (1962); K. Fukui, M. Nakayama, T. Matsui, M. Masumura, and T. Horie, *ibid.*, **90**, 1270 (1969); M. Nakayama, K. Fukui, T. Horie, Y. Shimizu, and M. Masumura, *ibid.*, **91**, 1174 (1970); K. Fukui, M. Nakayama, and T. Horie, *Bull. Chem. Soc. Jpn.*, **42**, 1649 (1969); T. Horie, H. Kourai, and N. Fujita, *ibid.*, **56**, 3773 (1983); T. Horie, H. Kourai, M. Tsukayama, M. Masumura, and M. Nakayama, *Yakugaku Zasshi*, **105**, 232 (1985); L. Farkas and M. Nógrádi, *Tetrahedron Lett.*, **1966**, 3759; A. Ulubelen, K. M. Kerr, and T. J. Mabry, *Phytochemistry*, **19**, 1761 (1980); T. Horie, H. Kourai, M. Tsukayama, T. Yokoyama, T. Yoshimoto, S. Yamamoto, and S. Kohno, Abstracts of Papers, The 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April 1985, p. 546.

11) M. Kessler, O. Acuto, C. Storelli, H. Murer, M. Müller, and G. Semenza, *Biochim. Biophys. Acta*, **506**, 136 (1978).

12) M. Somogyi, *J. Biol. Chem.*, **160**, 69 (1945).

13) G. Semenza, "Carbohydrate Metabolism and Its Disorders," Vol. 3, ed. by P. J. Randle, D. F. Steiner, and W. J. Whelan, Academic Press, London, 1981, pp. 425—479.