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Inhibitory Effects of Flavonoids on Animal Fatty Acid Synthase

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The inhibitory effects of 15 flavonoids on animal fatty acid synthase (FAS, EC 2.3.1.85) were investigated, and 9 of them were found to inhibit FAS with IC_{50} (the inhibitor concentration inhibiting 50% of the activity of FAS) values ranging from 2 to 112 μM. A structure-activity relationship study showed that the flavonoids containing two hydroxyl groups in the B ring and 5,7-hydroxyl groups in the A ring in combination with a C-2, 3 double bond were the most inhibitory. Morin (IC_{50} = 2.33 ± 0.09 μM) was further investigated kinetically to detail the inhibitory mechanism. The results showed that morin inhibited the overall reaction of FAS competitively with Ac-CoA, noncompetitively with Mal-CoA and in a mixed manner with NADPH. The study indicated that morin bound reversibly to the β-ketoacyl synthase domain of FAS to inhibit the elongation of the saturated acyl groups in fatty acids synthesis.

Key words: FAS, flavonoid, inhibition, inhibitor, morin.

Abbreviations: Ac-CoA, acetyl-CoA; AcAc-CoA, acetoacetyl-CoA; ACP, acyl carrier protein; EGCG, epigallocatechin gallate; FAS, fatty acid synthase; IC50, the inhibitor concentration required to inhibit the enzyme activity to 50% of that observed in the absence of the inhibitor; Mal-CoA, malonyl-CoA.

Animal FAS is an important enzyme participating in energy metabolism *in vivo*, and is related to various human diseases. In connection with its novel actions, recent studies showed that FAS inhibitors can reduce food intake and body weight (*1*) and also have significant antitumor activity (*2*). However, only a few FAS inhibitors with clinical potential, such as cerulenin, C75 (a synthetic compound) and epigallocatechin gallate (EGCG), have been reported (*2–4*).

Animal FAS includes two identical multifunctional polypeptide chains, each containing six discrete functional domains with enzymatic activity. FAS synthesizes fatty acid, mainly palmitate, *de novo* from the substrates acetyl-CoA (Ac-CoA), malonyl-CoA (Mal-CoA), and NADPH through its six active sites, *i.e.* acetyl/malonyl transferase, β-ketoacyl synthase, β-ketoacyl reductase, β-hydroxyacyl dehydratase, enoyl reductase and thioesterase (*5*, *6*).

$$CH_3COS\text{-}CoA + 7HOOCCH_2COS\text{-}CoA + 14NADPH + 14H^+ \rightarrow CH_3CH_2(CH_2CH_2)_6CH_2COOH + 14NADP^+ + 6H_2O + 8CoA\text{-}SH + 7CO_2$$

A malonyl group derived from Mal-CoA is condensed with an acetyl group from Ac-CoA. The resultant β-ketoacyl derivative is reduced in three consecutive steps, β-ketoacyl reduction, β-hydroxyacyl dehydration and enoyl reduction, to the saturated acyl derivative, which then acts as a primer for further elongation and reduction cycles to yield ultimately a palmitoyl derivative. The latter is hydrolyzed by thioesterase to free palmitate (*5–7*).

Flavonoids with different chemical structures are widely distributed in plant foods, vegetables, fruits and beverages (tea and red wine) and, therefore, are ingested regularly with the human diet. A large number of biological actions of flavonoids have been described, which overall are believed to be beneficial for health (*8–10*). In the present work, we found some flavonoids that can inhibit animal FAS, which could make flavonoids a potential candidate in the treatment of obesity. The study also provided explanations for the antitumor effects of some flavonoids (*11*). The structure-activity relationship of flavonoids and the detailed kinetic mechanism of the inhibition of FAS by flavonoids were elucidated.

MATERIALS AND METHODS

Materials—Ac-CoA, Mal-CoA, NADPH, acetoacetyl-CoA (AcAc-CoA), morin, luteolin, fisetin, baicalein, flavone, flavonol, rutin, hesperetin, (±)-epicatechin, (–)-epigallocachin, galangin, quercetin and kaempferol were purchased from Sigma-Aldrich. Myricetin and (±)-taxifolin were generous gifts from Procter & Gamble Company. All other reagents were local products with purity of analytical grade. The flavonoids were used as received for comparison.

Preparation of FASs and Substrates—Duck FAS were used. The preparation, storage and use of FAS were performed as described previously (*12*). Briefly, the FAS preparation was homogeneous on polyacrylamide gel electrophoresis in the presence and absence of SDS. The enzyme and substrate concentrations were determined by spectrophotometry with the following extinction coefficients: FAS, 4.83×10^5 M^{-1} cm^{-1} at 279 nm; Ac-CoA, 1.54×10^4 M^{-1} cm^{-1} at 259 nm, pH 7.0; Mal-CoA, 1.46×10^4 M^{-1} cm^{-1} at 260 nm, pH 6.0; NADPH, 6.02×10^3 M^{-1} cm^{-1} at 340 nm and 1.59×10^4 M^{-1} cm^{-1} at 259 nm, pH 9.0.

Assay of FAS Activity—The overall FAS activity was determined using an Amersham Pharmacia Ultrospec 4300 pro UV-Vis spectrophotometer at 37°C by following

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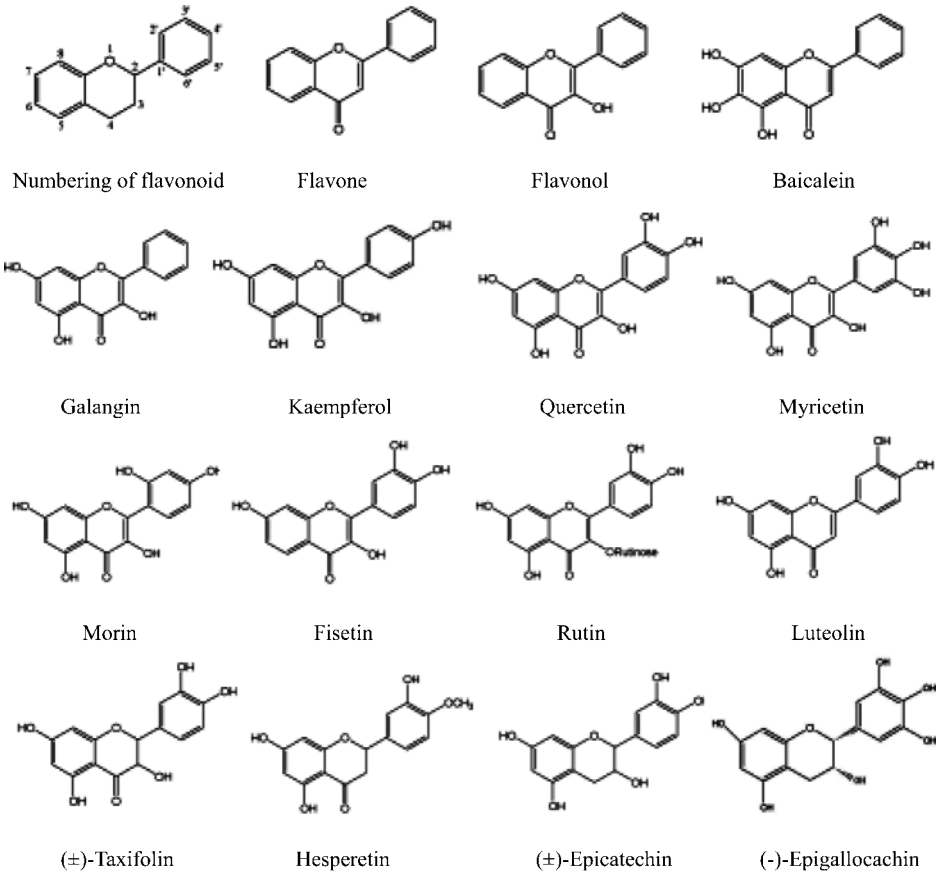


Fig. 1. **Structure of the flavonoids used in this study.**

the decrease of NADPH at 340 nm. The reaction mixture contained 100 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 1 mM dithiolthreitol, 3 μM Ac-CoA, 10 μM Mal-CoA, 35 μM NADPH, and 10 μg of FAS in a total volume of 2.0 ml (*12*).

The reaction using AcAc-CoA as primer was determined by the same method except that 3 μM Ac-CoA was substituted by 20 μM AcAc-CoA.

The β-ketoacyl reduction and enoyl reduction activities were determined at 37°C by measuring the change of absorption at 340 nm. The ketoacyl reduction reaction mixture (2 ml) contained 40 mM ethyl acetoacetate, 35 μM NADPH, 1 mM EDTA, 1 mM dithiolthreitol and 10 μg of FAS in 100 mM phosphate buffer, pH 7.0. The enoyl reduction reaction mixture (2 ml) contained 40 mM ethyl crotonate, 35 μM NADPH, 1 mM EDTA, 1 mM dithiolthreitol and 50 μg of FAS in 10 mM phosphate buffer, pH 6.3 (*13–15*).

For the reduction study of AcAc-CoA, the standard incubation mixture contained, in a total volume of 2 ml, 0.1 M potassium phosphate buffer, pH 7.6, 1 mM dithiolthreitol, 20 μM AcAc-CoA, 35 μM NADPH, and 50 μg of FAS. AcAc-CoA reduction includes four reactions, transacylation, β-ketoacyl reduction, dehydration and enoyl reduction, which are catalyzed by four component enzymes and ACP in fatty acid synthase (*16*, *17*). After temperature equilibration at 37°C for 10 min, the reaction was initiated by the addition of FAS and the initial velocity was determined from the decrease in absorbance at 340 nm (*17*).

Inhibition Studies of Flavonoids—Inhibition by flavonoids was investigated by adding the inhibitors to the reaction system before the addition of FAS to initiate the reaction. The flavonoids were dissolved in dimethyl sulfoxide (DMSO) and added to the reaction mixtures described above. The final concentration of DMSO was under 0.5% (v/v), to avoid the interference with FAS activity. The extent of inhibition by the addition of inhibitors was measured by reference to the half inhibition concentration (IC_{50}).

Determination of the Descriptor of Flavonoids—The partition coefficients (log*P*) of flavonoids were predicted by ACD/ChemSketch software.

RESULTS

Structure–Activity Relationship of Flavonoids against Animal FAS—Inhibitory effects of various concentrations of flavnoids (Fig. 1) on the overall reaction of animal FAS were examined *in vitro*. As a result, nine chemically related flavonoids (five flavonol derivatives, two flavone derivatives and two flavanone derivatives) were found to inhibit FAS. Morin showed the most potent concentration-dependent inhibition of FAS among the flavonoids investigated. Figure 2 show that only 2.33 μM morin inhibited 50% of the overall reaction of FAS. The order of the inhibitory potencies was morin > (≈) luteolin > quercetin > kaempferol > fisetin > myricetin > (±)-taxifolin > hesperetin > baicalein. Tabel 1 lists their IC_{50} values.

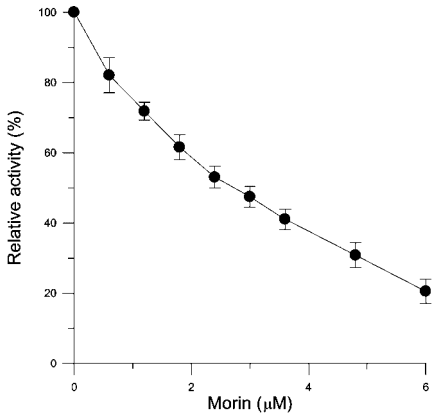


Fig. 2. **Effects of morin on FAS activity.** The overall reaction of FAS was measured in the presence of various concentrations of morin. Each datum is the mean from three experiments.

To evaluate the structural requirements for FAS inhibition, a structure-activity study of a series of compounds including some inactive flavonoids was conducted.

Morin, luteolin, quercetin, kaempferol, fisetin, myricetin, and baicalein are derived from the flavone skeleton and differ in the position of hydroxyl group and the degree of hydroxylation, which could influence their inhibitory activity against FAS. It was interesting to compare the activities of galangin (IC_{50} > 100 μM), kaempferol (IC_{50} = 10.38 ± 0.67 μM), quercetin (IC_{50} = 4.29 ± 0.34 μM), morin (IC_{50} = 2.33 μM) and myricetin (IC_{50} = 27.18 ± 0.24 μM), whose structural differences lie in the number of hydroxyl groups in the B ring, varying from none to three. Galangin, which lacks a hydroxyl group in the B ring, showed little activity, while the other flavonoids with different degrees of hydroxylation in the B ring inhibited FAS potently, morin and quercetin with two hydroxyl groups in the B ring being the most potent. Thus, the hydroxyl groups in the B ring of the flavonoids are very important for the inhibition of FAS. On the contrary, the presence of the hydroxyl group in the C ring (3-hydroxyl group) or 5-hydroxyl group in the A ring was not indispensable to activity. For example, luteolin (IC_{50} = 2.52 ± 0.10 μM), which had the same structure as quercetin but lacked the 3-hydroxyl group, showed activity similar to quercetin, and fisetin (IC_{50} = 18.78 ± 0.49 μM), which lacks the 5-hydroxyl group in the quercetin skeleton, was also a potent inhibitor of FAS, though less active than quercetin.

The flavanone (dihydroflavone) derivatives, (±)-taxifolin (IC_{50} = 41.16 ± 0.59 μM) and hesperetin (IC_{50} = 68.86 ± 4.49 μM), also had inhibitory activity against FAS but were less inhibitory than the active flavone or flavonol derivatives other than baicalein. The IC_{50} value of (±)-taxifolin, which had the identical structure to quercetin but lacked the 2, 3 double bond, was much higher than that of quercetin. This showed that the 2, 3 double bond contributed to activity. Compared with the flavanone derivatives, the flavanol derivatives, (±)-epicatechin and (–)-epigallocachin, had lower FAS inhibitory activity, which indicated that the absence of the 4-carbonyl group was disadvantageous to FAS inhibitory activity of the flavonoids.

Kinetic Mechanism of inhibition of Animal FAS by Flavonoids—Morin was used as a representative flavonoid in a study to elucidate the mechanism of the reversible inhibition of the overall reaction of FAS by flavonoids. Possible interference by morin at each substrate-binding site was examined by holding the concentration of morin at a constant value, and measuring the effect of increasing one substrate concentration (the other substrate concentrations were fixed) on the initial reaction rate. Double-reciprocal plots of the results (Fig. 3) yielded three families of straight lines, which had different intersections, for three substrates of FAS. These lines for Ac-CoA substrate (Fig. 3A) have a common intercept on the Y-axis, indicating that morin inhibited FAS competitively with Ac-CoA. The dissociation constant for inhibitor binding, K_i of 3.57 μM, was obtained from the secondary plot of the slopes of these lines versus morin concentra-

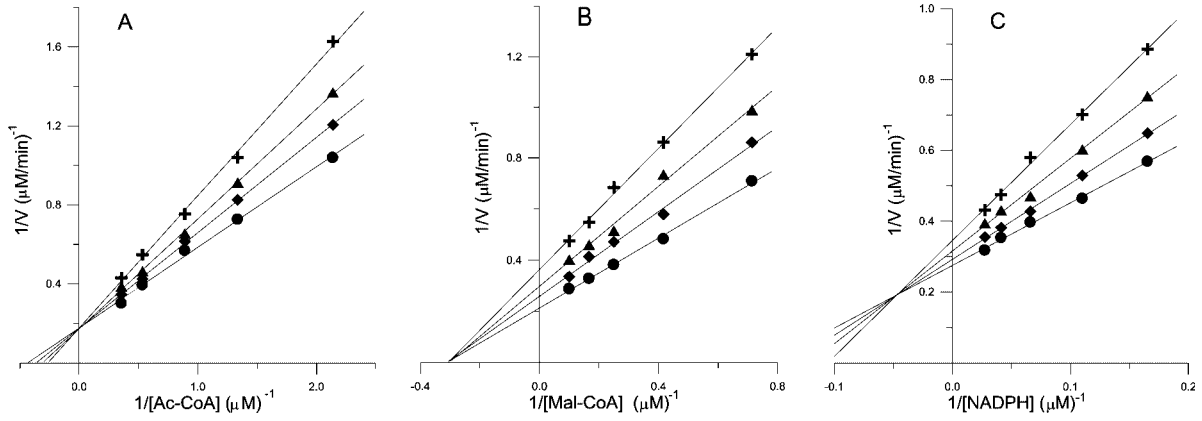


Fig. 3. **Lineweaver-Burk plot for inhibition of FAS by morin.** The overall reaction of FAS was measured. (A) Concentrations of Mal-CoA and NADPH were fixed at 10 μM and 35 μM respectively. Ac-CoA was the variable substrate. The concentrations of morin were: 0 μM (circles), 0.6 μM (diamonds), 1.2 μM (triangles), and 1.8 μM (crosses). (B) Concentrations of Ac-CoA and NADPH were fixed at 3 μM and 35 μM respectively. Mal-CoA was the variable substrate. The concentrations of morin were: 0 μM (circles), 0.6 μM (diamonds), 1.2 μM (triangles), and 1.8 μM (crosses). (C) Concentrations of Ac-CoA and Mal-CoA were fixed at 3 μM and 10 μM respectively. NADPH was the variable substrate. The concentrations of morin were: 0 μM (circles), 0.6 μM (diamonds), 1.2 μM(triangles), and 1.8 μM (crosses).

Table 1. **Inhibitory effects of flavonoids on FAS.**

Flavonoid	Class	IC_{50}[a] (μM)	log*P*
Morin	Flavonol	2.33 ± 0.09	1.61 ± 0.70
Luteolin	Flavone	2.52 ± 0.10	2.40 ± 0.65
Quercetin	Flavonol	4.29 ± 0.34	2.07 ± 0.72
Kaempferol	Flavonol	10.38 ± 0.67	2.05 ± 0.60
Fisetin	Flavonol	18.78 ± 0.49	2.52 ± 0.62
Myricetin	Flavonol	27.18 ± 0.24	2.11 ± 0.74
Baicalein	Flavone	111.69 ± 2.29	3.31 ± 0.62
Galangin[b]	Flavonol	>100	2.83 ± 0.59
Flavone	Flavone	n.i.[c]	3.56 ± 0.43
Flavonol	Flavonol	n.i.	3.76 ± 0.43
Rutin	Flavonol	n.i.	1.22 ± 1.01
(±)-Taxifolin	Flavanone	41.16 ± 0.59	1.82 ± 0.41
Hesperetin	Flavanone	68.86 ± 4.49	2.90 ± 0.39
(±)-Epicatechin	Flavanol	n.i.	0.45 ± 0.38
(–)-Epigallocachin	Flavanol	n.i.	–0.14 ± 0.39

[a]The IC_{50} values are the means ± SD for three experiments. [b]Galangin is poorly soluble in reaction mixture. About 20% of FAS overall reaction was inhibited in the presence of 100 μM galangin. [c]n.i is no inhibition(IC_{50} > 1 mM).

tion. The lines for Mal-CoA (Fig. 3B) intersect on the X-axis, from which it is concluded that morin is a typically noncompetitive inhibitor of FAS against Mal-CoA. The dissociation constant for inhibitor binding was calculated from the secondary plot of slopes versus morin concentration (K_i of 2.46 μM) or from the secondary plot of intercepts versus morin concentrations (K_i' of 2.50 μM). However, the lines for NADPH (Fig. 3C) have a point of intersection in the second quadrant, indicating that the inhibition was of the mixed competitive and noncompetitive type. The dissociation constant for morin binding with free enzyme (E), K_i of 2.05 μM, was obtained from the secondary plot of the slopes of these lines versus morin concentrations, while the dissociation constant for morin binding with enzyme-substrate complex (ES), K_i' of 6.95 μM, was obtained from the secondary plot of the intercepts versus morin concentrations.

To determine whether the flavonoids had the same inhibitory mode, the other two flavonoids, luteolin (flavone derivative) and taxifolin (flavanone derivative), were examined for the types and constants of inhibition. The results (Table 2) revealed that the flavonoids inhibiting FAS activity exhibited the same kinetic inhibitory mechanism. They compete with Ac-CoA for the same active site of FAS, which is different from the binding site of Mal-CoA or NADPH, but NADPH influenced the rate of binding of the flavonoids to FAS, in contrast to Mal-CoA.

Inhibition of Some Partial Reactions of Animal FAS—Some partial reactions of FAS were measured in the presence of morin. It was found that the β-ketoacyl and enoyl reduction were not influenced by morin (Fig. 4). At the same inhibitor concentration of 6.0 μM, 80% of the overall reaction of FAS was inhibited, but only 3.9% of the β-ketoacyl reduction and 3.6% of the enoyl reduction of FAS were lost. The results showed that morin did not inhibit the β-ketoacyl reductase and enoyl reductase domains of FAS.

Table 2. **Inhibition types and inhibition constants of FAS for every substrate by flavonoids.**

Substrate	Inhibitor	Inhibition type	Inhibition constant (μM)	
			K_i	K_i'
Ac-CoA	Morin	Competitive	3.57	–
	Luteolin	Competitive	1.20	–
	Taxifolin	Competitive	20.53	–
Mal-CoA	Morin	Noncompetitive	2.46	2.50
	Luteolin	Noncompetitive	9.80	10.01
	Taxifolin	Noncompetitive	94.96	94.27
NADPH	Morin	Mixed[a]	2.05	6.95
	Luteolin	Mixed[a]	4.15	10.75
	Taxifolin	Mixed[a]	37.96	68.53

[a]Mixed competitive and noncompetitive inhibition.

Given that NADPH substrate was present in the reaction mixture, FAS could reduce AcAc-CoA. The reduction entailed four component enzymes, acyl-transferase, β-ketoacyl reductase, β-hydroxyacyl dehydratase, and enoyl reductase, and ACP in FAS (*17*). Interestingly, flavonoids did not inhibit the AcAc-CoA reduction. In the presence of 6.0 μM morin in the reaction mixture, only 20% activity of the overall reaction of FAS remained, whereas 96% of the AcAc-CoA reaction remained (Fig. 4). This is evidence that morin did not target the four component enzymes involved in the AcAc-CoA reaction.

When AcAc-CoA was used as primer instead of Ac-CoA in fatty acid synthesis, the reaction activity of FAS, which required all six component enzymes and ACP (*17*), was potently inhibited by morin. The IC_{50} value of 3.62 ± 0.15 μM was kinetically comparable to that, 2.33 ± 0.09 μM, for the overall reaction of FAS using Ac-CoA as primer (Fig. 5). In order to explore further the effect of morin on the reaction using AcAc-CoA as primer in fatty acid synthesis, the possibility of competitive binding of morin to the site of AcAc-CoA binding was examined with fixed concentrations of Mal-CoA and NADPH. A double-reciprocal plot of the results showed that morin inhibited FAS noncompetitively with AcAc-CoA, with K_i' of 6.04 μM (Fig. 6).

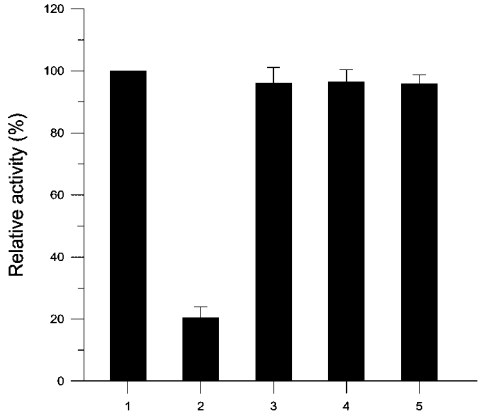


Fig. 4. **Inhibition of the overall reaction and some partial reactions of FAS by morin.** The concentration of morin in the reaction mixtures was 6.0 μM. Data were derived from three experiments. (1) is the control, (2) is the overall reaction, (3) is the β-ketoacyl reduction, (4) is the enoyl reduction, and (5) is the AcAc-CoA reduction.

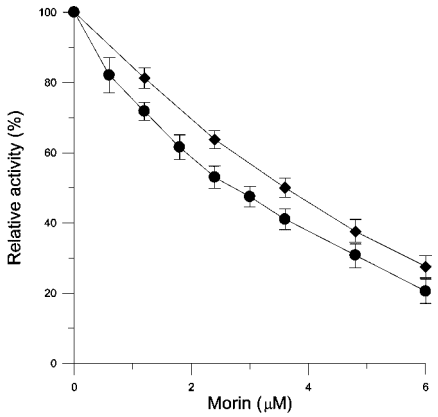


Fig. 5. **Effects of morin on FAS activity.** The inhibition of FAS in the presence of various concentrations of morin was measured. Inhibition of the overall reaction (circles); inhibition of the reaction using AcAc-CoA as primer (diamonds). Data are the means values from three experiments.

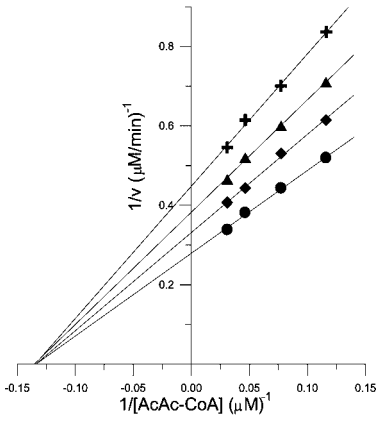


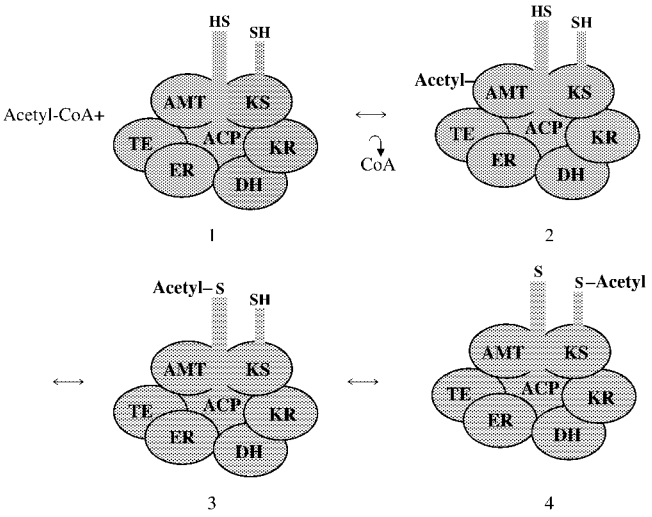
Fig. 6. **Lineweaver-Burk plot for inhibition of the reaction using AcAc-CoA as primer by morin.** Concentrations of Mal-CoA and NADPH were fixed at 10 μM and 35 μM respectively, and AcAc-CoA was the variable substrate. The concentrations of morin were: 0 μM (circles), 1.2 μM (diamonds), 2.4 μM (triangles), and 3.6 μM (crosses)

DISCUSSION

In a preliminary screening, we have found that nine flavonoids inhibit animal FAS. Compared with some reported previously FAS inhibitors, C75 (*2*) inhibits FAS by irreversibly binding to it, while EGCG (*4*) and cerulenin (*3*) can inhibit FAS reversibly like flavonoids. The IC_{50} values of most active flavonoids (2.33 μM for the most potent, morin) are lower than that of EGCG (52 μM) or cerulenin (54 μM). This shows that the flavonoids are very potent inhibitors of FAS and deserve to be further explored.

The log*P* values in Table 1 indicate that the FAS-inhibitory activity of flavonoids was restrained by their lipophilic nature. The log*P* values of the active flavonoids mainly distributed between 1.6 and 2.6. If the values were outside this range, the inhibitory activity decreased sharply (hesperetin, galangin and baicalein) or disappeared [flavone, flavonol, rutin, (±)-epicatechin and (–)-epigallocachin]. The lipophilic nature depended on the molecular structure, and so the differences in molecular structures accounted for the different activities of flavonoids. Based on the structure-activity relationship of flavonoids, the flavone derivatives with two hydroxyl groups in the B ring and 5,7-hydroxyl groups in the A ring (morin, quercetin and luteolin) presented the optimal pattern for inhibitors of FAS.

Animal FAS has six component enzymes (*5*, *6*), and if any one of these is inhibited, the overall FAS reaction will not proceed. It was found that morin was highly related to the acetyl group binding site. Morin inhibited FAS competitively with Ac-CoA and noncompetitively with Mal-CoA, which suggested that morin binds competitively to the binding site of acetyl group but does not influence the binding of malonyl group to FAS. There are two possible explanations for the observed results. One possibility is that morin binds reversibly to acetyl transferase, on the basis that Ac-CoA is the direct substrate of acetyl transferase. However, FAS employs the same acyl-transferase (also called acetyl/malonyl transferase) for loading both substrates, Ac-CoA and Mal-CoA (*18*); moreover, acyl-transferase has an identical active site for the two substrates (*19–22*). If morin binds to the acyl-transferase, morin should also inhibit FAS competitively with Mal-CoA, as it does with Ac-CoA, but the noncompetitive experimental result was not consistent with this speculation. Another alternative is that morin acts on the saturated acyl (including acetyl) binding site of the β-ketoacyl synthase domain of FAS. In order to initiate the fatty acid synthesis, the acetyl moiety from Ac-CoA must bind to active cysteine-SH of β-ketoacyl synthase (*19*, *23*) in the process of loading of substrates onto FAS (Scheme 1), while the malonyl moiety binds only to the phosphopantetheine-SH of ACP (*19*, *24*). If the inhibitor binds reversibly to the saturated acyl binding site of the β-ketoacyl synthase, it prevents transfer of the acetyl group to the β-ketoacyl synthase, as a result, it is able to inhibit through feedback Ac-CoA binding to the acyl-transferase. Conversely, when Ac-CoA binds to FAS, the acetyl group



Scheme 1. **The process of loading Ac-CoA substrate onto FAS.** AMT: acyl-transferase; KS: β-ketoacyl synthase; KR: β-ketoacyl reductase; DH: β-hydroxyacyl dehydratase; ER: enoy reductase; TE: thioesterase.

will transfer quickly to the saturated acyl binding site of the β-ketoacyl synthase, which removes the inhibition of FAS by flavonoids. Therefore, there is a competitive relationship between active flavonoids and Ac-CoA substrate of FAS. Since the malonyl group binds only to ACP-FAS, active flavonoids leave Mal-CoA unaffected. The experimental results were consistent with such a hypothesis

Based on the above observations, the inhibition of FAS by active flavonoids was considered to be closely related to the acetyl binding site. Interestingly, when AcAc-CoA was used as primer instead of Ac-CoA in fatty acid synthesis, FAS activity was still potently inhibited by morin. However, morin was a noncompetitive inhibitor of FAS against AcAc-CoA. This suggested that like Mal-CoA, the binding of AcAc-CoA to FAS, was independent of morin. In fact, the results confirm further the hypothesis that morin binds to the saturated acyl binding site of the β-ketoacyl synthase domain. The acetoacetyl group from AcAc-CoA was transferred by acyl-transferase from CoA to the phosphopantetheine-SH of ACP, and then became indistinguishable from the acetoacetyl group formed on FAS by the condensation of acetyl and malonyl groups during the synthesis of fatty acids (*15*), *i.e.*, acetoacetyl moieties were only transferred to ACP not to β-ketoacyl synthase of FAS, and then the fatty acid synthesis was initiated. The loading pathway of AcAc-CoA was the same as that of Mal-CoA. Thereby morin did not influence the binding of AcAc-CoA to FAS.

The inhibition of some partial reactions involved in the fatty acid synthesis by morin further confirmed this conclusion. The reduction of β- ketoacyl and enoyl, which entailed only β- ketoacyl reductase and enoyl reductase, respectively, were not inhibited by morin. The most convincing evidence come from the AcAc-CoA reduction. The reduction involved four component enzymes, acyl-transferase, β-ketoacyl reductase, β-hydroxyacyl dehydratase and enoyl reductase, and skipped the β-ketoacyl synthase and thioesterase, compared with the reaction using AcAc-CoA as primer in fatty acid synthesis (*17*). However, morin potently inhibited the reaction using AcAc-CoA as primer but did not affected the AcAc-CoA reduction. In the AcAc-CoA reduction, the acetoacetyl group was reduced to a butyryl group, then released from FAS by transferring to free CoA. Another pathway for the reaction uses AcAc-CoA as primer in the presence of Mal-CoA: the butyryl group formed from AcAc-CoA reduction in the first cycle could undergo chain elongation by the following six cycles involving β-ketoacyl synthase. It is noteworthy that when the β-ketoacyl synthase in the reaction using AcAc-CoA as primer was inhibited, FAS still had AcAc-CoA reduction activity, but since the V_{max} of the AcAc-CoA reduction was much lower than that of the reaction using AcAc-CoA as primer, the flavonoid inhibitors showed inhibitory activity to the reaction using AcAc-CoA as primer. Combined with the above analyses, the conclusion was drawn that morin binds reversibly to the acyl binding site of β-ketoacyl synthase domain of FAS to prevent the transfer of saturated acyl groups (including the acetyl group) from phosphopantetheine-SH of ACP to the active cysteine-SH of the β-ketoacyl synthase of FAS, while this transfer is indispensable to the elongation of saturated acyl derivatives in fatty acid synthesis (*6*, *18*).

We evaluated the structural requirements of flavonoids for the inhibition of animal FAS, and elucidated the inhibitory mechanism, which will be beneficial for further discovery or synthesis of flavonoids inhibitors of FAS. It may contribute to the discovery of new drugs for the treatment of obesity and cancer.

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REFERENCES

1. Loftus, T.M., Jaworsky, D.E., Frehywot, G.L., Townsend, C.A., Ronnett, G.V., Lane, D.M., and Kuhajda, F.P. (2000) Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* **288**, 2299–2300
2. Kuhajda, F.P., Pizer, E.S., Li, J.N., Mani, N.S., Frehywot, G.L., and Townsend, C.A. (2000) Synthesis and antitumor activity of an inhibitor of fatty acid synthase. *Proc. Natl Acad. Sci. USA* **97**, 3450–3454
3. Vance, D., Goldberg, I., Mitsuhashi, O., and Bloch, K. (1972) Inhibition of fatty acid synthetases by the antibiotic cerulenin. *Biochem. Biophys. Res. Commun.* **48**, 649–656
4. Wang, X. and Tian, W. (2001) Green tea epigallocatechin gallate: a natural inhibitor of fatty-acid synthase. *Biochem. Biophys. Res. Commun.* **288**, 1200–1206
5. Wakil, S. (1989) J. Fatty acid synthase, a proficient multifunctional enzyme. *Biochemistry* **28**, 4523–4530
6. Smith, S. (1994) The animal fatty acid synthase: one gene, one polypeptide, seven enzymes. *FASEB J.* **8**, 1248–1259
7. Rock, C.O., Goelz, S.E., and Cronan, J.E. (1981) Phospholipid synthesis in *Escherichia coli*. Characteristics of fatty acid transfer from acyl-acyl carrier protein to sn-glycerol 3-phosphate. *J. Biol. Chem.* **256**, 736–742
8. Rice-Evans, C.A. and Packer, L. (1997) *Flavonoids in Health and Disease*, Marcel Dekker, New York
9. Middleton, J.E., Kandaswami, C., and Theoharides, T.C. (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **52**, 673–751
10. Virgili, F., Scaccini, C., Hoppe, P.P., Krämer, K., and Packer, L. (2001) Plant phenols and cardiovascylar disease: antioxidants and cell modulators in *Nutraceuticals in Health and Disease Prevention* (Krämer, K., Hoppe, P.P., and Packer, L., eds.) pp. 187–215, Marcel Dekker, New York
11. Heo, M.Y., Sohn, S.J., and Au, W.W. (2001) Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. *Mutat. Res.* **488**, 135–150
12. Tian, W.X., Hsu, R.Y., and Wang, Y.S. (1985) Studies on the reactivity of the essential sulfhydryl groups as a conformational probe for the fatty acid synthetase of chicken liver. Inactivation by 5, 5′-dithiobis-(2-nitrobenzoic acid) and intersubunit cross-linking of the inactivated enzyme. *J. Biol. Chem.* **260**, 11375–11387
13. Tian, W.X., Liang, Q., Qu, B.H., and Xiao, J. (1992) The inhibition of carboxylates to fatty acid synthase of duck. *Acta Biophys. Sin. (China)* **8**, 22–28
14. Tian, W.X. and Cai, H. (1994) The denaturation and inactivation of fatty acid synthase from duck liver in guanidine hydrochloride. *Acta Biophys. Sin. (China)* **10**, 6–12
15. Kumar, S., Dorsey, J.A., Muesing, R.A., and Porter, J.W. (1970) Comparative studies of the pigeon liver fatty acid synthetase complex and its subunits. Kinetics of partial reactions and the number of binding sites for acetyl and malonyl groups. *J. Biol. Chem.* **245**, 4732–4744
16. Wu, B.N., Park, Y.D., Tian, W.X., and Zhou, H.M. (2001) Unfolding and inactivation of fatty acid synthase from chicken

liver during urea denaturation. *Biochim. Biophys. Acta* **1549**, 112–121
17. Dodds, P.F., Guzman, M.G., Chalberg, S.C., Anderson, G.J., and Kumar, S. (1981) Acetoacetyl-CoA reductase activity of lactating bovine mammary fatty acid synthase. *J. Biol. Chem.* **256**, 6282–6290
18. Smith, S., Witkowski, A., and Joshi, A.K. (2003) Structural and functional organization of the animal fatty acid synthase. *Prog. Lipid Res.* **42**, 289–317
19. Joshi, V.C., Plate, C.A., and Wakil, S.J. (1970) Studies on the mechanism of fatty acid synthesis. 23. The acyl binding sites of the pigeon liver fatty acid synthetase. *J. Biol. Chem.* **245**, 2857–2867
20. Plate, C.A., Joshi, V.C., and Wakil, S.J. (1970) Studies on the mechanism of fatty acid synthesis. XXIV. The acetyl- and malonyltransacylase activities of pigeon liver fatty acid synthetase. *J. Biol. Chem.* **245**, 2868–2875
21. McCarthy, A.D. and Hardie, D.G. (1983) The multifunctional polypeptide chains of rabbit-mammary fatty-acid synthase. Stoichiometry of active sites and active-site mapping using limited proteolysis. *Eur. J. Biochem.* **130**, 185–193
22. Mikkelsen, J., Hojrup, P., Rasmussen, M.M., Roepstorff, P., and Knudsen, J. (1985) Amino acid sequence around the active-site serine residue in the acyltransferase domain of goat mammary fatty acid synthetase. *Biochem. J.* **227**, 21–27
23. Mikkelsen, J., Smith, S., Stern, A., and Knudsen, J. (1985) Stoichiometry of substrate binding to rat liver fatty acid synthetase. *Biochem. J.* **230**, 435–440
24. Phillips, G.T., Nixon, J.E., Abramovitz, A.S., and Porter, J. (1970) W. Identification of the sites of binding of acetyl and malonyl groups to the pigeon liver fatty acid synthetase complex. *Arch. Biochem. Biophys.* **138**, 357–371
25. Dinya, Z. and Hetenyi, E. (1975) Relationship between the electron structure and biological action of some flavonoids in *Flavonoid Chemistry and Biochemistry* (Farkas, L., Gabor, M., and Kallay, F., eds.) pp. 240–256, Akadimiai Kiado, Budapest
26. Parmar, N.S. and Ghosh, M.N. (1980) Current trends in flavonoid research. *Ind. J. Pharm.* **12**, 213–228
27. Imamura, Y., Migita, T., Uriu, Y., Otagiri, M., and Okawara, T. (2000) Inhibitory effects of flavonoids on rabbit heart carbonyl reductase. *J. Biochem.* **127**, 653–658