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Food Chemistry 101 (2007) 357–364
Food Chemistry
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# Development of lipase inhibitors from various derivatives of monascus pigment produced by *Monascus* fermentation

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Received 22 September 2005; accepted 29 November 2005

**Abstract**

Derivatives of monascus pigment having high lipase-inhibitory activities were developed and characterized. Various derivatives of monascus pigment were produced during *Monascus* fermentation with added L-, D-amino acids. From them, compounds having high inhibitory activities against a porcine pancreatic lipase were selected. Monascus pigments produced with aromatic and non-polar aliphatic L-, D-amino acids revealed strong inhibitory activities against the lipase. L-Trp and D-Tyr derivatives showed especially low $IC_{50}$ values of 61.2 and 103 μM, respectively. Further structure modifications of pigment derivatives were made with amino acid esters and dipeptides for enhanced inhibitory activity. L-Leu-OEt and L-Tyr-OEt derivatives made via further structure modifications showed high lipase-inhibitory activities with $IC_{50}$ values of 12.2 and 13.8 μM, respectively. The L-Leu-OEt derivative exhibited some specificity against porcine pancreatic lipase but not exhibit high activities against other digestive enzymes. Some compounds were developed from modification of monascus pigment followed by lipase-inhibitory activity tests.


*Keywords:* *Monascus* species; Pigment; Lipase inhibitor; Fermentation

## 1. Introduction

Obesity is a serious problem around the world, especially in developed countries as a key factor in diabetes, hypertension, cardiovascular diseases, and cancers of small intestine, uterus and breast (Balsiger, Murr, Poggio, & Sarr, 2000). Moderate exercise can be effective for control and therapy of obesity, even though it is not useful for everybody. In many cases, treatment of obesity depends on drugs and foodstuffs (Clapham, Arch, & Tadayyon, 2001; Pittler & Ernst, 2004). Anti-obesity drugs can have various functions, including reduction of food intake, alteration of metabolism, and increase of thermogenesis (Bray, 2000). Lipases are important in fat digestion for hydrolysis of fat to glycerol and free fatty acids (Mukherjee, 2003), which can be used for energy production or stored in adipose tissues (Mayes, 1983). Therefore, if the lipases in human body inhibited, fat absorption or obesity would be controlled. Tetrahydrolipstatin, a commercial anti-obesity drug, is a good example of lipase inhibitor (Borgström, 1988; Hadváry, Sidler, Meister, Vetter, & Wolfer, 1991).

Monascus pigments have been used for many years as a natural food colorant and a health food in East Asia (Ma et al., 2000). There are some reports that monacolin, a secondary metabolite produced from fermentation of *Monascus* species, can lower serum cholesterol and triglyceride levels in animals and humans (Endo, 1979; Jůzlová, Martínková, & Křen, 1996). There are a few reports for anti-microbial and immunosuppressive activities of monascus pigments (Martínková, Jůzlová, & Veselý, 1995; Martínková et al., 1999). However, additional biological activities of the pigments have rarely been reported. Monascus pigments consist of six major

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doi:10.1016/j.foodchem.2005.11.055

compounds including the yellows of monascin and ankaflavin, the oranges of monascorubrin and rubropunctatin, and the reds of monascorubramine and rubropunctamine (Birch et al., 1962; Fowell, Robertson, & Whelly, 1956; Hadfield, Holker, & Stanway, 1967; Hiroi, Shima, Isobe, & Kimura, 1975; Kurono, Nakanishi, Shindo, & Tada, 1963; Manchand, Whelly, & Chen, 1973). There is a report (Moll & Farr, 1976) that the orange compounds can be transformed in ethanol to red pigments in the presence of nitrogenous compounds when the oxygen moiety of orange pigment is replaced by a nitrogen moiety of added compounds. Lin, Yakushijin, Büchi, and Demain (1992) postulated a reaction between the orange pigment and compounds containing a primary amino group. Such a reaction would occur with strong aminophiles and amino acids via a Schiff base formation and dehydration (Martínková et al., 1999).

Previously, our group reported production of various amino acid derivatives of monascus pigments via fermentation and their color characteristics (Jung, Kim, Kim, & Shin, 2003). In this study, monascus derivatives having high lipase-inhibitory activities were screened using in vitro tests.

## 2. Materials and methods

### 2.1. Materials

All enzymes were purchased from Sigma–Aldrich Co. All chemicals were purchased from Sigma–Aldrich Co., Duksan Co., and Difco Co.

### 2.2. Microorganism and media

*Monascus* sp. J101 was used to produce amino acid derivatives of monascus pigments (Ju, Nam, Yoon, & Shin, 1994). Strain preservation and spore formation were perfomed on Hiroi agar medium consisting of 10% sucrose, 0.5% casamino acid, 0.3% yeast extract, 0.2% $NaNO_3$, 0.1% $KH_2PO_4$, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.001%$FeSO_4 \cdot 7H_2O$, and 2% agar powder in distilled water (w/v) (Hiroi, Shima, Suzuki, Tsukioka, & Ogasawara, 1979). Seed cultivations were performed with Mizutani medium consisting of 5% glucose, 2% bacto-peptone, 0.8% $KH_2PO_4$, 0.2% $CH_3COOH$, 0.1% NaCl, and 0.05% $MgSO_4 \cdot 7H_2O$ in distilled water (w/v) (Ju et al., 1994). The main culture medium for production of monascus pigment derivatives consisted of 5% glucose, 0.3% $NH_4NO_3$, 0.1% $KH_2PO_4$, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, and 0.001% $FeSO_4 \cdot 7H_2O$ in distilled water (w/v) (Jung et al., 2003). For production of monascus pigment darivatives, L-, D-amino acids, L-amino acid esters, or dipeptides were added at 0.7% to main culture broths at 48 h of cultivation. Media pH was adjusted to 6.6 prior to sterilization.

### 2.3. Cultivations

*Monascus* sp. J101 was grown on a Hiroi agar slant for 168 h at 30 °C for preparation of spore suspensions. After incubation, spores were scraped off by spatula and the spore concentration was adjusted to $1 \times 10^5$ CFU $ml^{-1}$. For seed cultures, spore suspensions were inoculated into 500 ml flasks containing 75 ml of Mizutani medium, followed by 48 h of cultivation at 30 °C. For main cultures, a 7% (v/v) seed culture was inoculated into 500 ml flasks containing 75 ml of main culture medium, followed by 168 h of cultivation at 30 °C.

### 2.4. Pigment extraction

Culture broths were extracted in ethanol for 24 h on a reciprocal shaking water bath. After supernatants were obtained by 15 min of centrifugation at 6000 rpm, they were concentrated by evaporation. The concentrated extracts were loaded on a prep-TLC (silica gel 60, Merck, Germany). Monascus pigment derivatives were obtained from the spots of TLC plates that were developed with a mixture of chloroform:methanol:water (65:25:4).

### 2.5. Purification and identification of monascus pigment derivatives

After major pigment spots on a prep-TLC (silica gel 60, Merck, Germany) were collected and dissolved in ethanol, the solutions were filtered through a 0.45 μm PTFE syringe filter (Whatman). The purity of pigment derivatives was analyzed by HPLC (HP-1100) with an ODS $C_{18}$ column ($250 \times 4.6$ mm, 5 μm and Hypersil; Kleinostheim, Germany) with a run time of 40 min, a flow rate of 0.8 ml $min^{-1}$, and an elution gradient of distilled water:methanol from 100:0 to 30:70. Purified pigment solutions were thus obtained. Identification of the pigment derivatives was performed by measurement of the molecular weight by LC–MS spectrometry.

### 2.6. Measurement of lipase-inhibitory activity

The enzyme used in inhibitory activity tests of monascus pigment derivatives was a porcine pancreatic lipase. *p*-Nitrophenyl butyrate (*p*-NPB) was used as a substrate. An enzyme-buffer was prepared by adding 30 μl of lipase solution (in 10 mM MOPS and 1 mM EDTA, pH 6.8) to 850 μl of Tris buffer(100 mM Tris–HC1 and 5 mM $CaCl_2$, pH 7.0). Pigment solutions were prepared by dissolving each pigment in a mixture (1:1) of ethanol and distilled water. Then, 100 μl of the pigment solution was mixed with 880 μl of enzyme-buffer. After the enzyme-pigment mixture was incubated for 15 min at 37 °C, 20 μl of the substrate solution (10 mM *p*-NPB in dimethyl formaide) was added and enzymatic reactions were carried out for 15 min at 37 °C. The hydrolysis of *p*-NPB to *p*-nitrophenol was monitored at 400 nm using a spectrophotometer (Nicaud et al., 2002). One unit of the enzyme was defined as the amount that liberated 1 μmol of *p*-nitrophenol under standard assay conditions.

### 2.7. Measurement of enzyme-inhibitory activity

#### 2.7.1. Inhibition assay of α-amylase activity

An enzyme-buffer solution was prepared by adding α-amylase to 600 μl of phosphate buffer (pH 6.9). After the solution was mixed with 100 μl of pigment solution, the mixture was incubated for 15 min at 37 °C. To the mixture, 400 μl of a 1% starch solution in phosphate buffer (pH 6.9) was added. Enzymatic reactions then proceeded during 15 min of incubation at 37 °C. The reaction was stopped by adding 2 ml of a DNS reagent (1% 3, 5-dinitrosalicylic acid and 30% potassium sodium tartrate in a 0.1 M NaOH solution). The reaction mixture was heated for 15 min at 100 °C, then diluted with 10 ml of cold distilled water. The activity of α-amylase was determined by measuring the absorbance at 540 nm using a spectrophotometer.

#### 2.7.2. Inhibition assay of α-glucosidase activity

An enzyme-buffer solution was prepared by adding α-glucosidase to 800 μl of PBS buffer (pH 6.8). After the solution was mixed with 100 μl of pigment solution, the mixture was incubated for 15 min at 37 °C. To the mixture, 100 μl of a substrate solution (2 mM *p*-nitrophenyl α-D-glucopyranoside in PBS buffer, pH 6.8) was added.

Enzymatic reactions then occurred during 15 min of incubation at 37 °C. The activity of α-glucosidase was determined by measuring the amount of *p*-nitrophenol released from *p*-nitrophenyl α-D-glucopyranoside at 400 nm using a spectrophotometer.

#### 2.7.3. Inhibition assay of protease activity

An enzyme-buffer solution was prepared by adding protease to 900 μl of Tris buffer (pH 7.5). After the solution

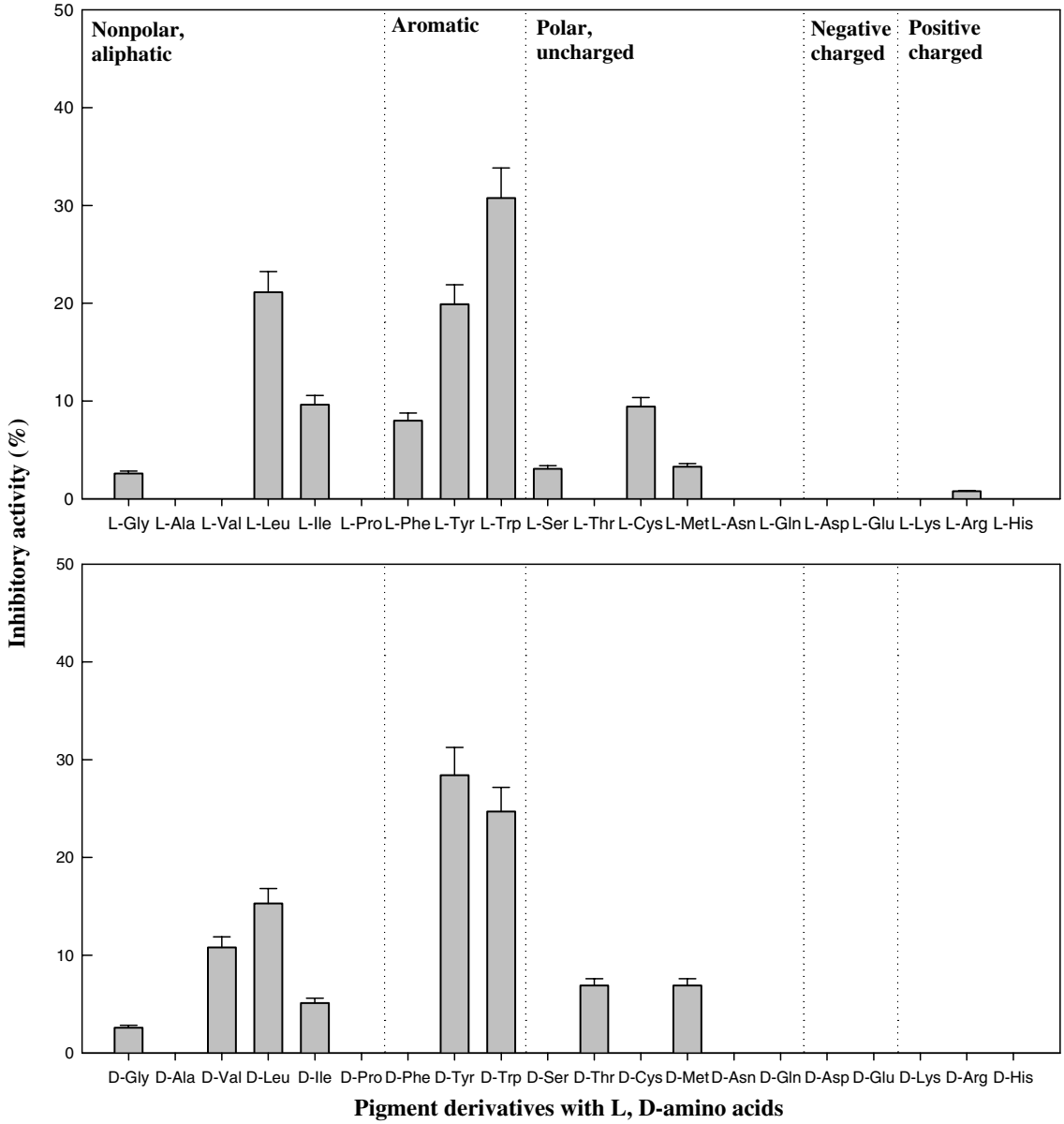


Fig. 1. Lipase-inhibitory activities of monascus pigments derived with L-, D-amino acids.

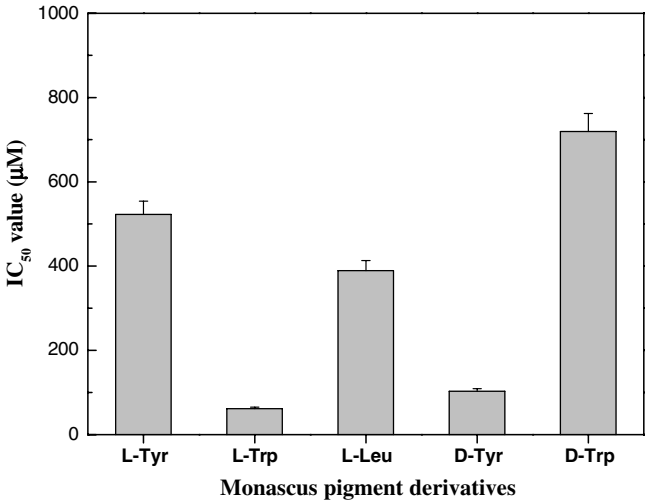


Fig. 2. $IC_{50}$ values of L-, D-amino acid derivatives of monascus pigments.

was mixed with 100 μl of pigment solution, it was incubated for 15 min at 37 °C. To the mixture, 1 ml of 0.2% casein solution was added. Enzymatic reactions then occurred during 15 min of incubation at 37 °C. The reaction was stopped by adding 2 ml of 0.5% trichloroacetic acid. The activity of protease was determined by measuring the protein concentration (Lowry, Rosebrough, Farr, & Rondall, 1951).

### 2.8. Statistical data analysis

Results were expressed as mean ± SD. Most determinations were performed in triplicate in at least three separate sets of experiments.

## 3. Results and discussion

### 3.1. Screening of lipase inhibitor from derivatives of monascus pigment

Various kinds of monascus pigment derivatives were produced by adding 39 L- and D-amino acids during cultivation of *Monascus* sp. J101. The derivatives were tested for lipase-inhibitory activity via in vitro assay using porcine lipase. Among them, 17 derivatives showed an inhibitory activity (Fig. 1). The L-Trp derivative had the highest inhibitory activity of more than 30% and the derivatives of L-Tyr, L-Leu, D-Tyr, and D-Trp had relatively high activities of 20–30%. The derivatives of D-Val and D-Leu showed inhibitory activities of 10–20% while all others were less than 10%.

The lipase-inhibitory activity of monascus derivatives is thought to be closely related to their amino acid moiety. The activity was highly enhanced by incorporation of amino acids containing an aromatic ring structure. Derivatives with incorporated nonpolar-aliphatic or

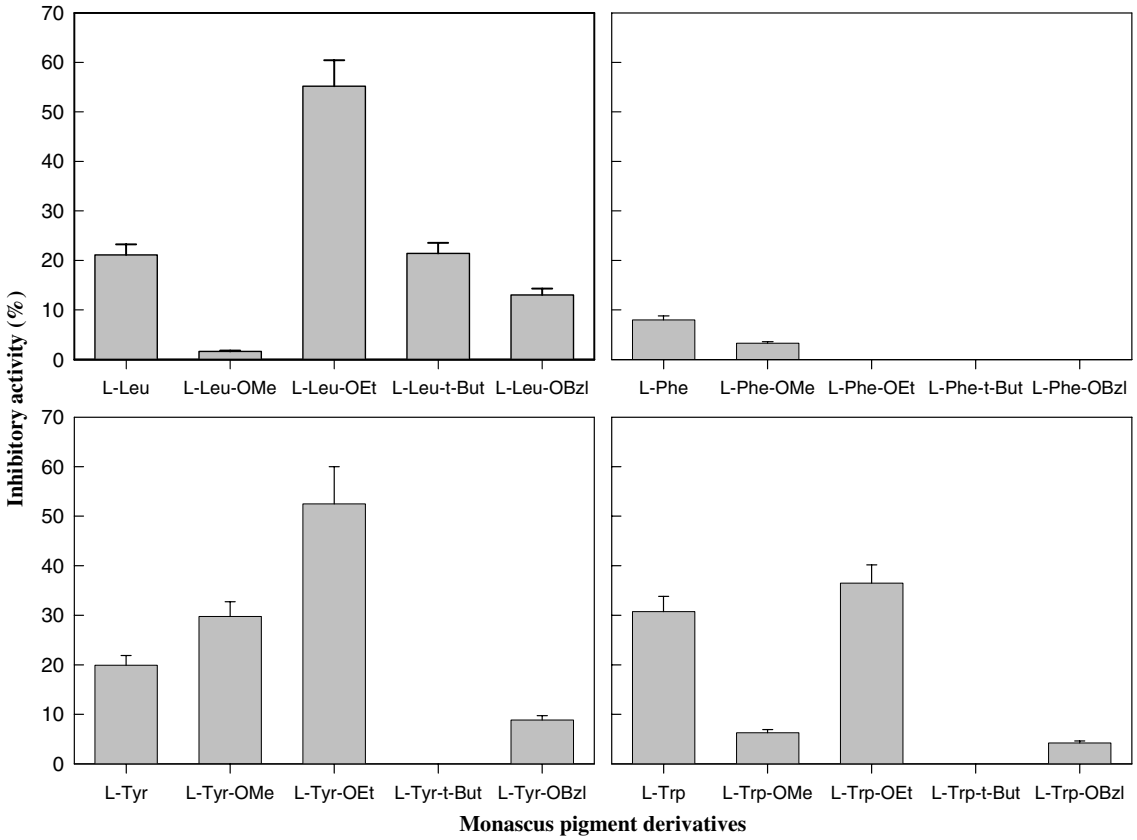


Fig. 3. Lipase-inhibitory activities of monascus pigments derived with L-amino acid esters.

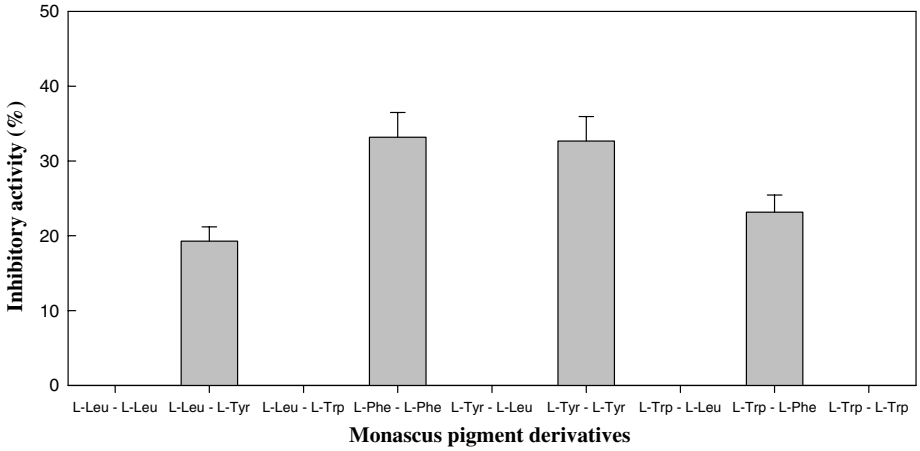


Fig. 4. Lipase-inhibitory activities of monascus pigments derived with dipeptides.

Table 1
Enhanced inhibitory activities due to structure modification of monascus pigment derivatives

| Type of pigment derivative | $IC_{50}$ (μM) | Type of pigment derivative | $IC_{50}$ (μM) |
|---|---|---|---|
| L-Phenylalanine | n.d.[a] | L-Tryptophan | $61.2 \pm 0.6$ |
| L-Phe–L-Phe | $24.7 \pm 0.3$ | L-Tryptophan ethyl ester | $41.2 \pm 2.9$ |
| | | L-Trp–L-Phe | $112 \pm 2.2$ |
| L-Tyrosine | $523 \pm 5.2$ | L-Leucine | $390 \pm 23.4$ |
| L-Tyrosine ethyl ester | $13.8 \pm 0.3$ | L-Leucine ethyl ester | $12.2 \pm 0.2$ |
| L-Tyrosine methyl ester | $71.0 \pm 0.7$ | L-Leucine tert-butyl ester | $184 \pm 1.8$ |
| L-Tyrosine benzyl ester | $575 \pm 5.8$ | L-Leucine benzyl ester | $2508 \pm 225.7$ |
| L-Tyr–L-Tyr | $32.1 \pm 1.0$ | L-Leu–L-Tyr | $163 \pm 3.3$ |

[a] n.d., not detected.

polar-uncharged amino acids showed a good activity. However, derivatives with negatively or positively charged amino acids exhibited little or no lipase-inhibitory activity. Considering that aromatic ring-containing, nonpolar-aliphatic and polar-uncharged amino acids are usually hydrophobic, the hydrophobicity of the amino acid moiety is apparently important for the activity. In this respect, derivatives of hydrophilic amino acids were expected to exhibit little or no activity. Generally, negatively and positively charged amino acids are relatively hydrophilic, and polar-uncharged amino acids (e.g., Asn and Gln) are also hydrophilic. Derivatives of Asn and Gln showed no lipase-inhibitory activity. The same trend that was observed for derivatives of L-amino acids was also seen for derivatives of D-amino acids. The derivatives of hydrophobic D-amino acids Leu, Ile, Tyr, Trp and Met also showed high activities. The relationship between the D-amino acid moieties of derivatives and the corresponding lipase-inhibitory activities is likely to be based on the L-type moieties.

The $IC_{50}$ values of active derivatives of L-Trp L-Tyr, L-Leu, D-Tyr and D-Trp were estimated. The L-Trp and D-Tyr derivatives exhibited the lowest $IC_{50}$ values (high activities) of 61.2, and 103 μM (Fig. 2). However, the other derivatives exhibited high $IC_{50}$ values (low activities).

### *3.2. Secondary modification of monascus pigment derivatives for enhancement of lipase inhibitory activity*

Since the lipase-inhibitory activity of pigment derivatives is thought to be closely related to the structure of the amino acid moiety, secondary modifications to the derivatives of aromatic and nonpolar aliphatic amino acids were attempted by incorporation of the methyl, ethyl, *t*-butyl, and benzyl esters of L-Trp, L-Tyr, L-Leu and L-Phe, and some dipeptides. Twenty-five new pigment derivatives were produced. The L-Leu-OEt and L-Tyr-OEt derivatives exhibited the high activities of more than 50% while the L-Trp-OEt and L-Tyr-OMe derivatives exhibited activities of greater than 30% (Fig. 3). The rest of the derivatives exhibited low inhibitory activities of less than 20%. Ethyl esters resulted in high activities while *t*-butyl and benzyl esters resulted in little or no activity.

The flexibility of derivatives is apparently closly related to the lipase-inhibitory activity. The derivatives of *t*-butyl ester and benzyl ester are thought to be too inflexible in structure to accommodate the lipase molecules. Derivatives of methyl ester are thought to have lower lipase-inhibitory activities than derivatives of ethyl ester, probably due to differences in flexibility. Among ester derivatives, the

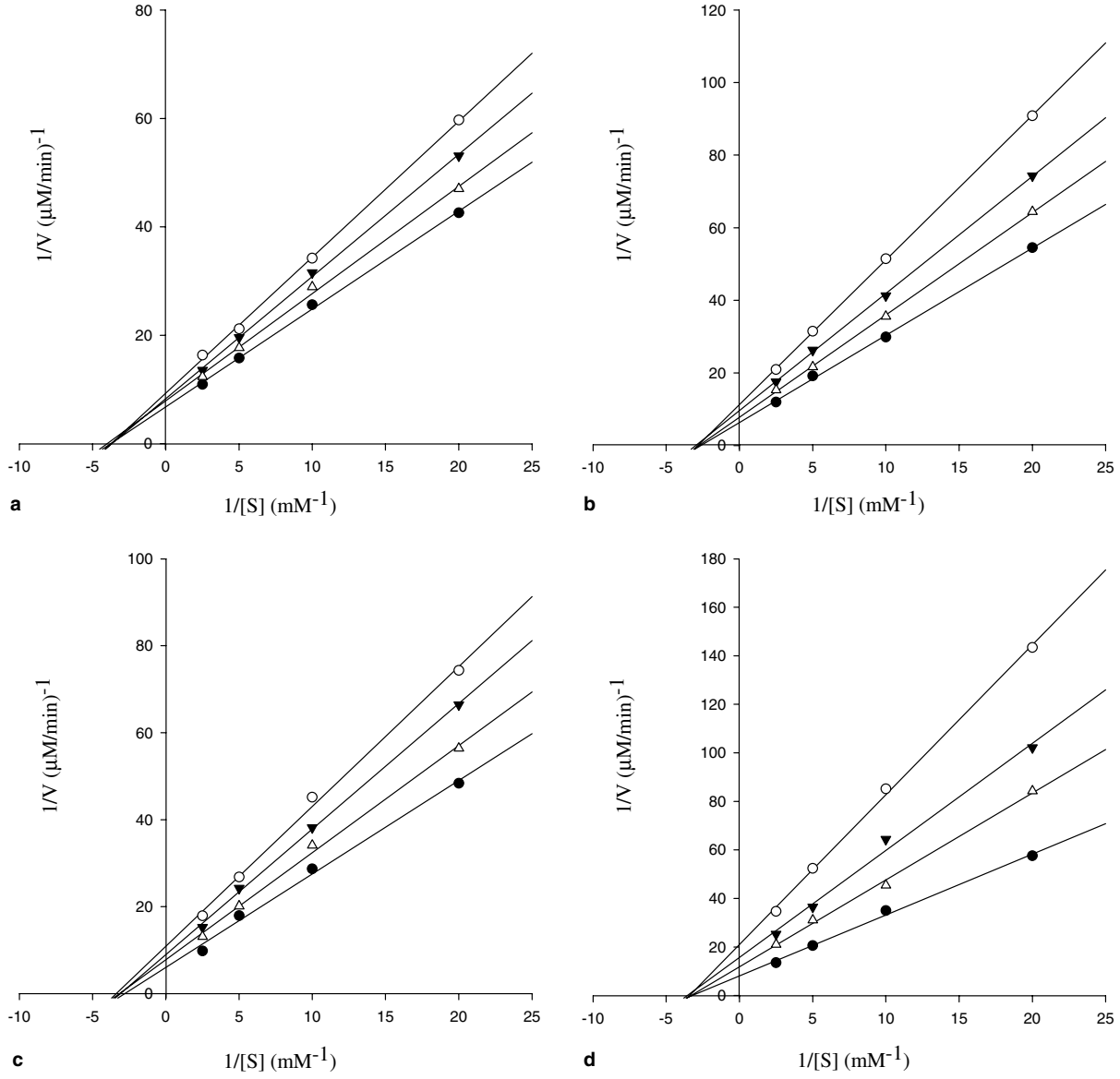


Fig. 5. Lineweaver–Burk plots of the inhibitory activities of pigment derivatives, (a) L-Leu derivative (μM): ○, 37.6; ▼, 18.8; △, 9.4; ●, no pigment; (b) L-Trp derivative (μM): ○, 31.2; ▼, 15.6; △, 7.8; ●, no pigment; (c) L-Leu-OEt derivative (μM): ○, 9.0; ▼, 4.5; △, 2.3; ●, no pigment; (d) L-Tyr-OEt derivative (μM): ○, 21.9; ▼, 11.0; △, 5.5; ●, no pigment.

derivatives of L-Phe ester did not exhibit an activity against lipase, indicating that the structure of the L-Phe ester derivative does not bind lipase.

Some dipeptide derivatives exhibited good lipase-inhibitory activities (Fig. 4). The derivatives of L-Phe-L-Phe and L-Tyr-L-Tyr exhibited greater than 30% inhibitory activities and derivatives of L-Leu-L-Tyr and L-Trp-L-Phe exhibited 20% inhibitory activities. The inhibitory activities of the dipeptide derivatives were not as good as the activities of the amino acid ester derivatives. Although it is difficult to make a definite correlation between the inhibitory activity and the pigment structure, some interaction between the lipase and the derivatives is apparently affected by the dipeptide moieties of the derivatives.

The $IC_{50}$ values of the 12 derivatives with the highest activities were then determined (Table 1). The L-Leu-OEt and L-Tyr-OEt derivatives showed the highest lipase-inhibitory activities with $IC_{50}$ values of 12.2 and 13.8 μM, respectively. The derivatives of the dipeptides L-Phe-L-Phe and L-Tyr-L-Tyr had relatively high lipase-inhibitory activities with $IC_{50}$ values of 24.7 and 32.1 μM, respectively.

By the further structure modifications, the lipase inhibitory activities of monascus pigment derivatives were improved. The ethyl ester modification of carboxylic acids

Table 2
Kinetic inhibition constants of monascus pigment derivatives

| Pigment derivative | Inhibition type | $K_i$ (μM) |
|---|---|---|
| L-Leucine | Noncompetitive | 82.7 ± 14.6 |
| L-Tryptophan | Noncompetitive | 34.6 ± 4.8 |
| L-Leucine ethyl ester | Noncompetitive | 9.4 ± 1.7 |
| L-Tyrosine ethyl ester | Noncompetitive | 12.5 ± 1.2 |

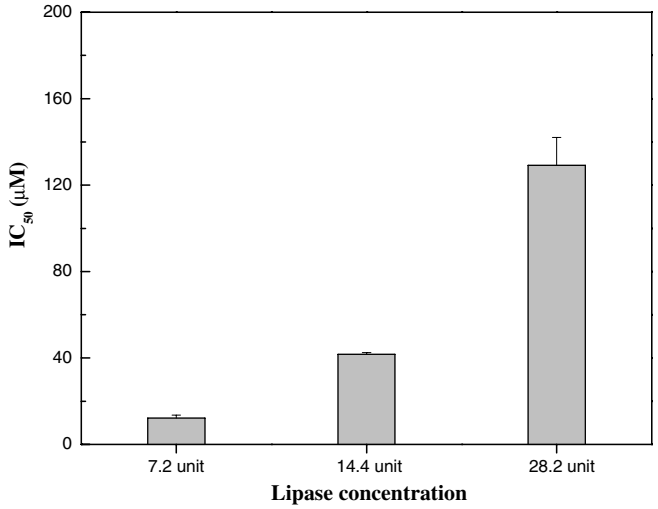


Fig. 6. $IC_{50}$ values of the L-Leu-OEt derivative at different lipase concentrations.

increased the activity by approximately five times, resulting in low $IC_{50}$ values. The essential structure of the pigment derivatives that causes a lipase-inhibitory activity is the ester moiety of the amino acid part. The aromatic ring and ethyl ester moieties of pigment Derivatives apparently enhance the affinity for the allosteric binding site of lipase.

### 3.3. Kinetic characteristics for inhibition of pigment derivatives

Inhibition data for the pigment derivatives were analyzed using a Lineweaver–Burk plot. Derivatives of L-Trp, L-Leu, L-Leu-OEt and L-Tyr-OEt were selected for kinetic analysis of inhibition. The pigment derivatives appeared to be noncompetitive inhibitors of lipase (Fig. 5). The apparent $V_{max}$ value decreased with an increasing inhibitor concentration and the $K_m$ value was constant, regardless of the inhibitor concentration. Although the inhibition mechanism was the same for all derivative structures, the inhibition constant was decreased by incorporation of the ethyl ester moiety. Inhibition constants ($K_i$) for the derivatives L-Leu, L-Trp, L-Leu-OEt and L-Tyr-OEt were estimated to be 82.7, 34.6, 9.4 and 12.5 μM respectively (Table 2). The L-Leu-OEt derivative appeared to be more effective than the others.

### 3.4. Inhibitory characteristics of the L-leucine ethyl ester derivative

The L-Leu-OEt derivative, which showed the highest activity, was used for analysis of inhibitory characteristics. The $IC_{50}$ value of the L-Leu-OEt derivative against a porcine pancreatic lipase was estimated at different enzyme concentration levels. The $IC_{50}$ value increased proportionally from 15 to 140 μM with a 4-fold increase in the lipase concentration (Fig. 6).

The inhibitory activities of the L-Leu-OEt derivative for other sources of microbial lipase were measured. The L-Leu-OEt derivative inhibited lipases from *Rhizomucor miehei* and *Candida rugosa* by approximately 20% and 14%, respectively (Fig. 7). Other digestive enzymes including protease, α-amylase, and α-glucosidase were also tested. The derivative showed little or no inhibitory against them. The L-Leu-OEt derivative apparently is a specific inhibitor against pancreatic lipase but not against other digestive enzymes.

It is generally known that pigments an ability to nonspecifically adsorb with other molecules. However, the inhibition of pigment derivatives seems to be accomplished by binding with allosteric sites on the enzyme. The monascus derivatives primarily inhibited porcine pancreatic lipase but did not significantly inhibit other types of lipases and

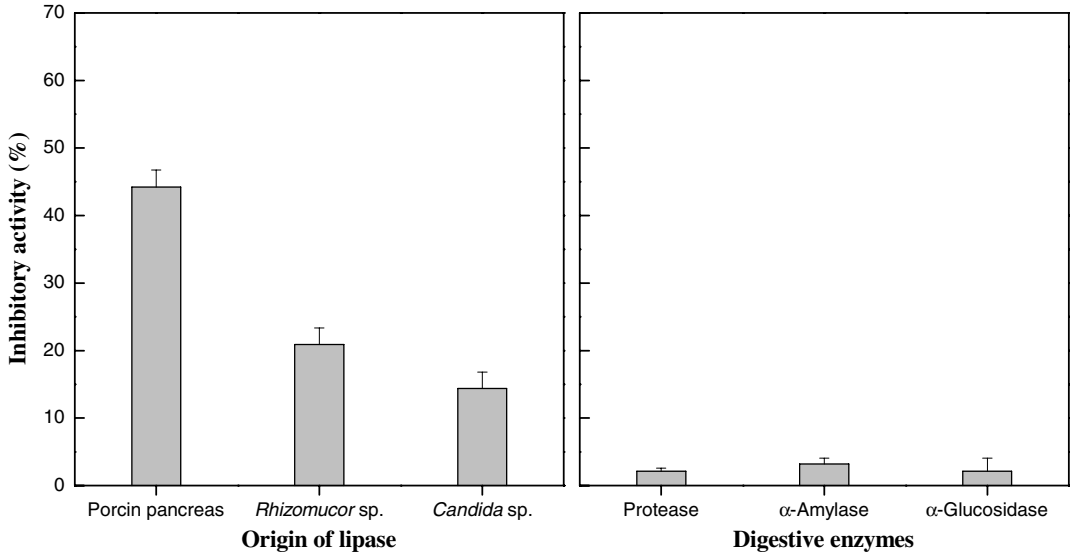


Fig. 7. Inhibitory activities of the L-Leu-OEt derivative against different types of lipases and other digestive enzymes. The applied concentration of the L-Leu-OEt derivative was 9.0 μM. The concentrations of all enzymes were adjusted to same unit.

other hydrolyzing enzymes, indicating that the monascus derivatives have enzyme specificity. In this respect, the derivatives of monascus pigment are good inhibitors of pancreatic lipase and have potential as a promising anti-obesity nutraceutical.

## 4. Conclusions

The aromatic ring and ethyl ester moieties of pigment derivatives apparently enhance inhibitory activities against a pancreatic lipase. L-Trp and D-Tyr derivatives of monascus pigment exhibited high activities but the original pigment showed little or no activity. The L-Leu-OEt and L-Tyr-OEt derivatives showed the highest activities. The pigment derivatives all functioned as noncompetitive inhibitors against lipase, regardless of the derivative structures. The inhibitory activity of the L-Leu-OEt derivative was specific to the pancreatic lipase not to other microbial sources of lipase, and other digestive enzymes.

## Acknowledgements

This study was supported by Grant Number 03-PJ1-PG3-22000-0058 from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea.

## References

Balsiger, B. M., Murr, M. M., Poggio, J. L., & Sarr, M. G. (2000). Bariatric surgery: surgery for weight control in patients with morbid obesity. *Medical Clinics of North America, 84*, 477–489.

Birch, A. J., Cassera, A., Fitton, P., Holker, J. S. E., Smith, H., Thompson, G. A., et al. (1962). Studies in relation to biosynthesis. Part XXX. Rotiorin, monascin, rubropunctatin. *Journal of Chemical Society, C*, 3583–3586.

Borgström, B. (1988). Mode of action of tetrahydrolipstatin: a derivative of the naturally occurring lipase inhibitor lipstatin. *Biochimica et Biophysica Acta, 962*, 308–316.

Bray, G. A. (2000). A concise review on the therapeutics of obesity. *Nutrition, 16*, 953–960.

Clapham, J. C., Arch, J. R. S., & Tadayyon, M. (2001). Anti-obesity drugs: a critical review of current therapies and future opportunities. *Pharmacology and Therapeutics, 89*, 81–121.

Endo, A. (1979). Monacolin K, a new hypocholesteolemic agent produced by a *Monascus* species. *Journal of Antibiotics, 32*, 852–854.

Fowell, A. D. G., Robertson, A., & Whelly, W. B. (1956). Monascorubramin. *Journal of Chemical Society (Special Publish), 5*, 27–35.

Hadfield, J. R., Holker, J. S. E., & Stanway, D. N. (1967). The biosynthesis of fungal metabolites. Part II. The β-oxo-lactone equivalents in rubropunctatin and monascorubrin. *Journal of Chemical Society, 19*, 751–755.

Hadváry, P., Sidler, W., Meister, W., Vetter, W., & Wolfer, H. (1991). The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. *Journal of Biological chemistry, 266*, 2021–2027.

Hiroi, T., Shima, T., Isobe, A., & Kimura, S. (1975). Studies on the structure of two pigment obtained from *Monascus* for sp.. *Journal of the Japanese Society of Food and Nutrition, 28*, 497–502.

Hiroi, T., Shima, T., Suzuki, T., Tsukioka, M., & Ogasawara, N. (1979). Hyperpigment-production mutant of *Monascus anka* for solid culture. *Agricultural and Biological Chemistry, 43*, 1975–1976.

Ju, J. Y., Nam, H. W., Yoon, J. C., & Shin, C. S. (1994). Extractive fermentation of red pigment using *Monascus* sp. J101. *Korean Journal of Applied Microbiology and Biotechnology, 22*, 85–91.

Jung, H., Kim, C., Kim, K., & Shin, C. S. (2003). Color characteristics of monascus pigments derived by fermentation with various amino acids. *Journal of Agricultural and Food Chemistry, 51*, 1302–1306.

Jůzlová, P., Martínková, L., & Křen, V. (1996). Secondary metabolites of fungus *Monascus*: a review. *Journal of Industrial Microbiology, 16*, 163–170.

Kurono, M., Nakanishi, K., Shindo, K., & Tada, M. (1963). Biosynthesis of monascorubrin and monascoflavin. *Chemical and Pharmaceutical Bulletin, 11*, 359–362.

Lin, T. F., Yakushijin, K., Büchi, G. H., & Demain, A. L. (1992). Formation of water-soluble *Monascus* red pigments by biological and semi-synthetic process. *Journal of Industrial Microbiology, 9*, 173–179.

Lowry, O. H., Rosebrough, N., Farr, A. L., & Rondall, R. L. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–273.

Ma, J., Li, Y., Ye, Q., Li, J., Hua, Y., Ju, D., et al. (2000). Constituents of red yeast rice, a traditional Chinese food and medicine. *Journal Agricultural of and Food Chemistry, 48*, 5220–5225.

Manchand, P. S., Whelly, W. B., & Chen, F. C. (1973). Isolation and structure of ankaflavin, a new pigment from *Monascus anka*. *Phytochemistry, 12*, 2531–2532.

Martínková, L., Jůzlová, P., & Veselý, D. (1995). Biological activity of polyketide pigments produced by fungus *Monascus*. *Journal of Applied Bacteriology, 79*, 609–616.

Martínková, L., Jůzlová, P., Křent, V., Kučerová, Z., Havíček, V., Olšovský, P., et al. (1999). Biological activities of oligoketide pigments of *Monascus purpureus*. *Food Additives and Contaminants, 16*, 15–24.

Mayes, P. A. (1983). Metabolism of lipids. In D. W. Martin, P. A. Mayes, & V. W. Rodwell (Eds.), *Harper's review of biochemistry* (19th ed.. *Fatty acids*, pp. 201–223). Singapore: Lange Medical Publications.

Moll, H. R., & Farr, D. R. (1976). Red pigment and process. US Patent 3, 993, 789.

Mukherjee, M. (2003). Human digestive and metabolic lipases – a brief review. *Journal of Molecular Catalysis B: Enzymatic, 22*, 369–376.

Nicaud, J. M., Madzak, C., Broek, P., Gysler, C., Duboc, P., Niederberger, P., et al. (2002). Protein expression and secretion in the yeast *Yarrowia lipolytica*. *FEMS Yeast Research, 2*, 371–379.

Pittler, M. H., & Ernst, E. (2004). Dietary supplements for body-weight reduction: a systematic review. *American Journal of Clinical Nutrition, 79*, 529–536.