Bioorganic Compounds Produced by the Fungus *Monascus* and their Use in Health Sciences and Medicine

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Abstract: The filamentous fungus '*Monascus*' has been used as a natural food coloring and folk medicine in East Asia for centuries. Several bioactive substances produced by *Monascus* species have been isolated and identified. Recent studies demonstrated their mode of action *in vitro* and effectiveness *in vivo*, and their utilization for health foods and medicine has advanced. This mini review will introduce the physiological functions and safety of bioorganic chemicals produced by *Monascus* species.

Keywords: *Monascus*, γ-aminobutyric acid, polyketide metabolites, dimerumic acid, hypertension, hypercholesterolemia.

1. INTRODUCTION

Monascus is a filamentous fungus belonging to the family Monascaceae, order Eurotiales, and is used in the making of fermented rice, '*Monascus*-rice' (also called red yeast rice, red mold rice, and red-*koji* or *beni-koji* in Japanese). More than 30 strains have been isolated and identified from food stuff or soil, and they have been classified into nine species: *M. ruber*, *M. pilosus*, *M. purpureus*, *M. barkeri*, *M. bisporus*, *M. kaoliang*, *M. floridanus*, *M. sanguineus*, and *M. lunisporas*. *Monascus*-rice has been used to make traditional fermented foods such as red-rice wine and fermented soybean curd (tofu-ru, furu in China; tofuyo in Okinawa, Japan) in East Asia, since *Monascus* species produce various useful enzymes like proteinases [1-6], peptidases [7-9], amylases [10, 11], and ribonucleases [12]. Another important characteristic of *Monascus* species is the production of vivid orange, red, and yellow pigments. Although these pigments have weak point of de-coloration by long-term exposure to light, they are stable at high temperatures and a wide range of pH, and good for dye against protein. Therefore, they are utilized as natural food colorings in products protected from light, such as ham, sausage, fish paste, and sweets.

Monascus-rice has been used not only in food processing but also in folk medicine for centuries. Records exist in the Chinese compendium of remedies ('Ben Cao Gang Mu' written by Li Shi-Zhen in 1578) using *Monascus*-rice to improve blood circulation, spleen and stomach health. These health-promoting effects of *Monascus*-rice have been scientifically verified, and multifunctional compounds have been isolated and identified, such as angiotensin-converting enzyme (ACE)-inhibitory peptides, γ-aminobutyric acid (GABA), monacolins, citrinin, pigments, and dimerumic acid. The present review will introduce the physiological functions and safety of bioorganic chemicals produced by *Monascus* species.

2. PROTEINASES AND ACE-INHIBITORY PEPTIDES

As for proteinases produced by *Monascus* species, an aspartic proteinase with a molecular mass of approximately 40 kDa (optimum pH, 3; optimum temperature, 55 ℃) was homogeneously purified from *M. anka* (synonym for *M. purpureus*) [1, 2], and two extracellular acid proteinases approximately 43 and 58 kDa (optimum pH, 2.5-3.0; optimum temperature, 55 ℃) were also homogeneously isolated from *M. pilosus* [3]. It has considered that these proteinases greatly contribute to food fermentation and maturation. For example, in making fermented soybean curd (tofuyo), proteinases hydrolyze soybean protein to soluble peptides and amino acids, producing a favorable smooth cream cheese-like texture and umami taste [9, 13, 14].

Proteinases are also able to produce bioactive compounds such as angiotensin I-converting enzyme (ACE, EC3.4.15.1) -inhibitory peptides. ACE is a dipeptidyl carboxy peptidase that plays an important role in the regulation of blood pressure. It converts angiotensin I into a powerful vasoconstrictor, angiotensin II, and also inactivates the vasodilator bradykinin. In this section, we will describe our previous investigation of ACE-inhibitory peptides in *Monascus*-rice and tofuyo (fermented soybean curd from Okinawa, Japan) and production of ACE-inhibitory peptides from soybean proteins, β-conglycinin and glycinin, using purified acid proteinase from *M. purpureus* [15-17].

Screening of ACE-inhibitory activities in *Monascus*-rice prepared by 24 strains of *Monascus* fungus was carried out, and we selected *M. purpureus* IFO4489 which was the most effective strain for ACE inhibition (Table **1**) [15]. Four ACE-inhibitory peptides were isolated from the extract by several column chromatographies and identified as IY, VVY, VF, and VW by protein sequencing (Table **2**). A computer sequence search of the SWISS-PROT protein sequence database showed that all peptide fragments exist in the primary structure of prolamin, a major rice protein, and VF and VVW also exist in glutelin. And thus, ACE-inhibitory peptides in *Monascus*-rice were considered to be produced from rice protein by the action of proteinases produced by the *Monascus* strain.

Table 1. ACE-Inhibitory Activity in *Monascus*-Rice Extracts Made with Various Strains of the Genus *Monascus*

	IC_{50} (mg/ml)
M. purpureus IFO 4478	1.64
M. purpureus IFO 4489	0.71
M. sp. C-1-1	1.66
M. sp. C-3	1.22
M. sp. C-4	1.68

Cited from Table **1** in Kuba *et al.* [15] with permission from Elsevier.

Proteinases such as pepsin, chymotrypsin and trypsin are frequently used in hydrolysis to obtain ACE-inhibitory peptides. Bacterial alkaline serine proteinases are also utilized in the production of ACE inhibitors from food protein. However, there are a small number of reports on the use of microbial acid proteinase in the production of ACE inhibitor. We investigated production of ACE-inhibitory peptides from soybean protein using *Monascus*-

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acid proteinase. Four ACE-inhibitory peptides (LAIPVNKP, LPHF, SPYP, and WL) were obtained from β-conglycinin and glycinin, which were directly hydrolyzed by purified acid proteinase from *M. purpureus* (Table **2**) [16]. WL has also been purified from tofuyo as mentioned below.

Table 2. ACE-Inhibitory Peptides Isolated from *Monascus*-Rice Related Materials and their Digestive Stability *in vitro*

	Amino acid sequence	Yield (%)	IC_{50} (μM)	IC_{50} after digestion (μM)[3)]
Monascus-rice[1)]	IY	7.0×10^{-2}	4.0	3.7
	VVY	2.5×10^{-2}	22.0	19.3
	VF	3.4×10^{-2}	49.7	51.2
	VW	8.0×10^{-3}	3.1	4.5
Tofuyo[1)]	IFL	2.4×10^{-2}	44.8	120.0
	WL	3.0×10^{-4}	29.9	110.3
Major soybean protein cleaved by an acid proteinase from *M. purpureus*[2)]	LAIPVNKP	1.5×10^{-3}	70	376
	LPHF	6.4×10^{-4}	670	610
	SPYP	2.7×10^{-3}	850	60
	WL	1.5×10^{-3}	65	77

[1)] ACE-inhibitory activity was measured by ACE obtained from rabbit lung [15, 17].

[2)] ACE-inhibitory activity was measured by ACE obtained from porcine lung [16].

[3)] Each inhibitor solution was successively treated with pepsin (0.05%, w/v), chymotrypsin and trypsin (0.25%, w/v for each concentration) followed by incubation for 6 h at 37°C as previously described [15-17]. The digests were used for measurement of ACE inhibition.

Modified from Tables from Kuba *et al.* [15-17] with permission from Elsevier and Japan Society for Bioscience, Biotechnology, and Agrochemistry.

The value of ACE-inhibitory activity in tofuyo was shown to be relatively similar to the other Japanese traditional fermented soybean foods (Table **3**) [17]. Two ACE inhibitors were isolated to homogeneity from tofuyo extract and identified as IFL and WL (Table **2**). IFL sequence is found in the α- and β-subunits of β-conglycinin, while WL sequence is in the B-, B1A-, and BX-subunits of glycinin from soybean. Since the α'-, α-, and β-subunits in β-conglycinin and the acidic subunit in glycinin are degraded to low-molecular-weight elements during tofuyo fermentation [18], it is likely that IFL was liberated from β-conglycinin by proteinases that were produced by *M. purpureus* and/or *Aspergillus oryzae* used in the fermentation process. Although the basic subunit in glycinin cannot be easily degraded by these enzymes [18] as compared with each subunit in β-conglycinin or the acidic subunit in glycinin, WL might have been liberated from the subunit during long-term fermentation (3 months).

Table 3. IC_{50} of Typical Fermented Soybean Foods Toward ACE

Fermented soybean food	IC_{50} (mg/ml)
Soy sauce	3.44
Miso paste	1.27
Natto	0.16
Tofuyo	1.77

Cited from Table **1** in Kuba *et al.* [17] with permission from Japan Society for Bioscience, Biotechnology, and Agrochemistry.

Cheung *et al.* [19] studied the structural relationships between ACE and its inhibitors, and concluded that ACE binding is highly specific for the terminal dipeptide residues of the inhibitors, with the C-terminal amino acid being the most important to overall binding to the active site of ACE. The most favorable C-terminal residues were W, Y, P, or F. Consistent with this report, LAIPVNKP that has P at the C-terminal residue showed strong inhibitory activities. Kobayashi *et al.* [20] pointed out that not only hydrophobicity but also the molecular size of aromatic amino acids is important for blocking the active site of ACE. Consistent with this point of view, VW, which included the largest aromatic amino acid at the C-terminus, had the highest inhibitory activity. Regarding the N-terminal amino acid residue, branched-chain aliphatic amino acids have been considered suitable for ACE inhibition (Fig. **1**). Six peptides isolated in our study had I or V at the N-terminus and exhibited relatively high inhibitory activity. Moreover, positively charged amino acids, R or K, between branched-chain aliphatic amino acids and aromatic amino acids have been reported to contribute to strong ACE inhibition. Although VVY had favorable amino acids at both ends, the ACE-inhibitory activity of the peptide was lower than that of the other tripeptides consisting of I or L + positively charged amino acids + aromatic amino acids because of the lack of positively charged residue.

The digestive stability of ACE inhibitors against gastrointestinal proteinases *in vitro* was examined in order to

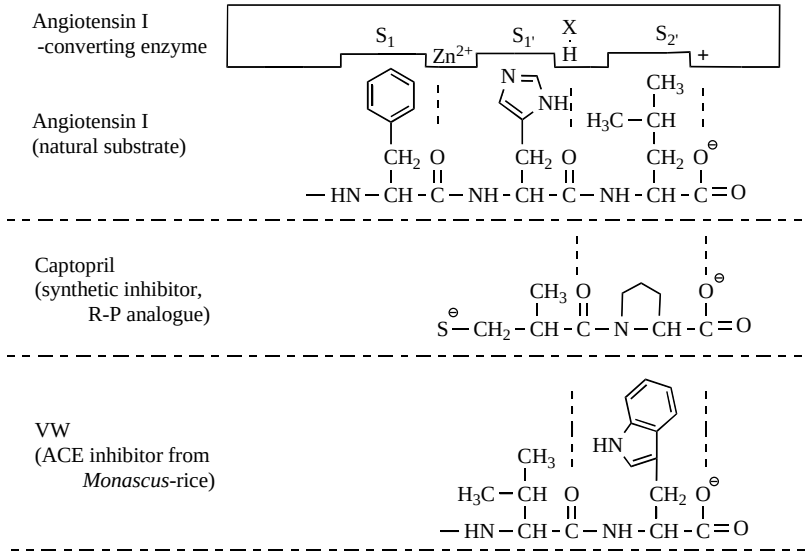

Fig. (1). Proposed binding of substrate and inhibitors to the active site of angiotensin I-converting enzyme.

predict their anti-hypertensive effect *in vivo* (Table **2**). Six peptides isolated from *Monascus*-rice and major soybean proteins showed almost completely preserved ACE-inhibitory activities. Interestingly, the IC_{50} value of LAIPVNKP increased significantly after digestion. The N- and C-terminal amino acid residues of this peptide (L and P, respectively) might be cleaved by pepsin and trypsin according to already known cleavage sites of these proteinases. In contrast, the IC_{50} value of SPYP decreased significantly after digestion. This peptide may be split into SPY, which was expected to have strong activity, and P by trypsin.

According to study on absorption and transport system of peptides, di- and tripeptides are more rapidly absorbed and reach a higher concentration in the blood than single amino acids [21]. In addition, short peptides (average residue length of 3.2) in a soybean hydrolysate were more rapidly absorbed than the long ones (average residue length of 5.2) when using a rat intestinal everted sac [22]. Matsui [23] found that 18 di- and tripeptides derived from β-conglycinin, including WL, were absorbed intact through the small intestine membrane of rats. Moreover, IY significantly decreased blood pressure in spontaneously hypertensive rats (SHR) by a single oral administration at a dose of 0.1 mg/kg [24]. These findings support the notion that ACE-inhibitory peptides, except LAIPVNKP, are expected to be easily absorbed and contribute to the anti-hypertensive effect *in vivo*.

Monascus-rice and proteinases from *Monascus* species are useful for the generation of ACE-inhibitory peptides derived from a variety of proteins. Recently, several physiological functions, such as antioxidation, nerve stimulation, and immunostimulation, are revealed in peptides, and it is anticipated that *Monascus*-rice and proteinases can be applied to the production of these functional peptides. For the practical use of physiological peptides as a health food, optimization of the orally effective dose would be required.

3. PHYSIOLOGICAL FUNCTION OF TOFUYO

Although ACE-inhibitory activity was confirmed in tofuyo or bioactive peptides prepared by *Monascus*-acid proteinase *in vitro*, the anti-hypertensive activity and other physiological function of tofuyo *in vivo* remain to be clarified. We examined the anti-hypertensive effect of tofuyo in SHR (male, 8 weeks of age) [25]. At 11 and 12 weeks of age, the systolic blood pressure (SBP) of rats fed experimental diets containing lyophilized tofuyo (17%, w/w) tended to be lower than controls, and at 13 weeks of age, the SBP in the tofuyo group was significantly lower than in the control group (Fig. **2**). Tofuyo would suppress a mild hypertension in the early stage. Because of the limited availability to prepare experimental diet containing tofuyo, we were unable to evaluate the effects of long term administration. For utilization of tofuyo as a health food, examination of the long term administration would be required. At the end of the experimental period, the ACE activities in tissues and serum were measured (Table **4**). Lung exhibited higher ACE activity than serum and other tissues, because vascular bed is abundant in lung. Although the ACE activity of lung tended to be higher in the tofuyo group than that in the control group, there were no significant differences. On the other hand, the ACE activity of kidney was significantly lower in the tofuyo group than that in the control group. ACE inhibition in peripheral tissues, especially vascular wall and kidney, is thought to be important for controlling blood pressure [26]. The ACE inhibition of kidney might contribute to reduction of blood pressure in tofuyo group. However, the ACE activities in this study were measured after a decline in SBP has been disappeared. Further study is necessary to elucidate the relationship between antihypertensive effect and ACE inhibition of tissues by tofuyo. Among the serum lipids, the total cholesterol and HDL cholesterol in the tofuyo group was significantly decreased, whereas the ratio of HDL to total cholesterol in the tofuyo group tended to be higher than that in the control group (Table **5**). These results indicate that tofuyo had serum hypocholesterolemic activity. Iwami *et al.* [27] reported the hypocholesterolemic effect of soybean protein digested by pepsin. Tofuyo is abundant in peptides and amino acids that are liberated from soybean protein by *Monascus* species during fermentation, which might contribute to the cholesterol reduction in serum.

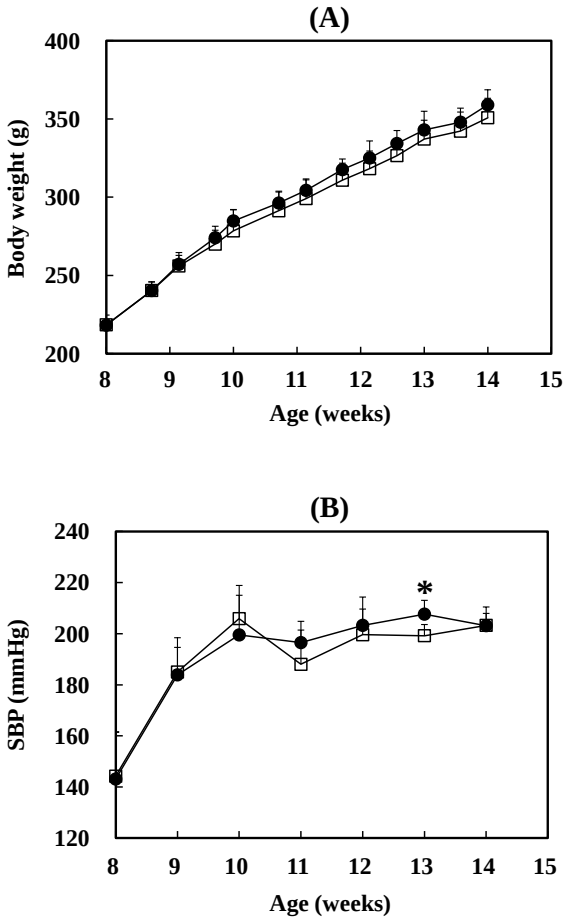

Fig. (2). Changes in body weight (**A**) and systolic blood pressure (**B**) during feeding the experimental diets in spontaneously hypertensive rats [25].
The data were expressed as mean ± S.D. (n=8). The experimental diets contained 1% sodium chloride to induce hypertension and 22% protein. Lyophilized tofuyo (17%, w/w) was added to the diet of the tofuyo group; that is, approximately 5% of total protein was supplied by tofuyo instead of by casein. *Asterisk indicates significant difference from control (p<0.05). ●, control group; □, tofuyo group. Cited from Fig. **1** in Kuba *et al.* [25] with permission from the Pharmaceutical Society of Japan.

Table 4. ACE Activities in Serum and Tissues of SHR at 14 Weeks of Age

	ACE activity (mU/mg protein)	
	Control	**Tofuyo**
Serum	1.4 ± 0.1	1.5 ± 0.1
Aorta	155.1 ± 51.0	144.9 ± 45.3
Lung	311.4 ± 132.0	442.9 ± 62.7
Kidney	4.9 ± 1.8	2.6 ± 0.8*

*Significant difference from the control group (t-test, p<0.05).

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Table 5. General Composition of Serum from SHR Fed the Experimental Diets for 6 Weeks

	Control	Tofuyo
Glucose (mg/dl)	114.8 ± 22.9	114.8 ± 15.9
Total protein (g/dl)	6.8 ± 0.3	6.6 ± 0.2
Albumin (g/dl)	2.7 ± 0.1	2.7 ± 0.1
AST (U/L)	212.8 ± 27.0	179.9 ± 31.2*
ALT (U/L)	67.5 ± 10.9	56.3 ± 13.0
Total-cholesterol (mg/dl)	53.8 ± 6.0	46.8 ± 5.0*
HDL-cholesterol (mg/dl)	18.9 ± 1.8	17.0 ± 1.6*
Triacylglycerol (mg/dl)	17.5 ± 11.2	13.6 ± 10.2
Phospholipid (mg/dl)	91.5 ± 11.4	82.6 ± 8.8

*Significant difference from the control group (t-test, $p<0.05$).

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4. γ-AMINOBUTYRIC ACID (GABA)

γ-Aminobutyric acid (4-aminobutanoic acid; GABA) is the chief inhibitory neurotransmitter in the mammalian central nerve system. It is produced by removing of the carboxylic acid at α-position of L-glutamic acid catalyzed by a glutamate decarboxylase (GAD). There are some reports on *Monascus* species has GAD, and *Monascus*-rice contains GABA at approximately 30-300 mg/kg [28, 29]. When GABA is exogenously derived, it cannot cross the blood-brain barrier and does not act as neurotransmitter. However, GABA is able to block peripheral autonomic ganglia and decrease blood pressure [30]. In animal experiments, high blood pressure in SHR was significantly decreased by feeding a chow containing *Monascus*-rice powder, and GABA was identified as the active substance [28, 29]. A small addition of *Monascus*-rice powder to the chow (0.03%; GABA content is 0.09 mg/100 g chow) was effective against hypertension. Moreover, a large addition of *Monascus*-rice powder (5%) also moderately lowered the blood pressure [31, 32].

In clinical study, mild and borderline hypertension patients drank *Monascus*-rice aqueous extract prepared with *M. pilosus* IFO4520 in two patterns: (i) at a dose equivalent to 27 g of *Monascus*-rice/day for 2 weeks; or (ii) at a dose equivalent to 9 g of *Monascus*-rice/day for 6 months [33]. It was observed that both patterns resulted in improved hypertension. In another study, the blood pressure of 91 mild hypertension patients was significantly decreased by *Monascus*-rice extract-drink for 12 weeks [34].

In order to increase GABA yield, optimal culture conditions for *Monascus* species have been investigated. Addition of sodium nitrate, dipotassium hydrophosphate, and 0.5% ethanol to the culture medium improved GABA yield to 1267.6 mg/kg, 1493.6 mg/kg, and 7453 mg/kg, respectively [35, 36]. Attempts to employ these conditions in the production of supplements and health foods have been made.

5. POLYKETIDE METABOLITES

5.1. Monacolin K (Mevinolin or Lovastatin, Mevacor) and Related Compounds

Endo [37] isolated and identified a cholesterol-lowering compound from *M. ruber*, and named it monacolin K. Monacolin K is identical to lovastatin (also known as mevinolin and mevacor) and is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (IC_{50} = 5 nM and Ki = 0.49 nM) [38]. Monacolin analogs (monacolin J, L, M, X, and dihydromonacolin L) that also have cholesterol-lowering activities have been identified from *Monascus* species (Fig. **3**) [39-41]. Compactin produced by *Penicillium citrinum* is also a monacolin-analog, and structural features of those molecules are (i) the lactone or the hydroxylic 3',5'-dihydroxyacid, (ii) the moiety bridging the lactone and the lipophilic groups, (iii) the decarine ring, and (iv) the side chain ester [42]. 3'- or 5'-Hydroxyl group in 3', 5'-dihydroxyacid portion is thought to play a crucial role for HMG-CoA reductase inhibition [42]. Although monacolin analogs are decomposed under the conditions of high humidity at high temperature and sunlight [43], the synthetic pathway in the fungus [44, 45] and several methods to increase the yield have been investigated [35, 36, 46-49]. Temperature is one of the important conditions for monacolin K production. In the case of *M. purpureus* NTU601, the highest production was observed at 30°C (530 mg/kg), while at lower temperature (25°C) gave less productivity [36]. In contrast, *M. pilosus* NBRC4520 produced much monacolin K (225 μg/g) and less citrinin with temperature-shift cultivation; i.e., the optimum temperature was shift from growth phase at 30°C to monacolin K production phase at 23°C [49].

Sviridov *et al.* [50] studied cholesterol-lowering effects of monacolin-related compounds *in vitro* using five kinds of human cells: umbilical vein endothelial cells, small intestine epithelial cells, hepatoma cell line HEP G2, normal skin fibroblasts, and skin fibroblasts from a patient with familial homozygous hypercholesterolemia. The most effective compounds were monacolin K and compactin, and their potencies were dose-dependent and tissue selective; IC_{50} values were 1.0-30 pg/ml for endothelial and epithelial cells, 0.01-66 ng/ml for HEP G2 cells, and 0.1-200 ng/ml for fibroblasts.

In *in vivo* experiments, anticholesterolemic and anti-arteriosclerotic effects of monacolin K were investigated in various animals [51-55]. Tang *et al.* [54] reported that monacolin K did not affect plasma and hepatic cholesterol levels in normal mice; however, it significantly reduced both plasma and hepatic cholesterol levels in transgenic mice overexpressing the ATP-binding cassette (ABC) transporter G5/G8 gene (*G5G8*^{Tg} mice), which is related to cholesterol biosynthesis and excretion. Individuals with higher baseline cholesterol synthesis rates are more sensitive to monacolin K, suggesting the reason for the interdividual variation of response to statin-drug treatment. Some experiments have investigated cholesterol-lowering mechanisms by monacolins. In the early study using rabbits and hamsters, it was reported that inhibition of cholesterol synthesis by monacolin K led to a compensatory increase in the amount of mRNA for HMG-CoA reductase [51]. This feedback regulation simultaneously caused an increase in mRNA for LDL receptors in liver, thereby decreasing in plasma cholesterol level [51]. The recent studies suggest another cholesterol-lowering mechanism which is not attributed to LDL receptors in liver. The protein levels of LDL and HDL receptors in *G5G8*^{Tg} mice, mentioned above, were unchanged by monacolin K treatment. Meanwhile, hepatic mRNA levels of ABC transporter G5/G8 and Niemann-Pick C1 Like1 (NPC1L1) were significantly increased. These two molecules are thought to modulate biliary cholesterol secretion, thus the upregulation of cholesterol excretion would result in reduction in plasma cholesterol level [54]. As for anti-arteriosclerotic effects, monacolin K showed an effect with the suppression of oxidative stress and down-regulation of apoptosis-related genes (Fas-ligand, caspase 8, and caspase 9) that deteriorate arteriosclerosis in rabbits [55].

Additionally, HMG-CoA reductase inhibitors including monacolin K suppressed smooth muscle cell migration and proliferation and cholesterol phagocytosis of macrophages, indicating that they exert a direct anti-atherosclerotic effect in the arterial wall, independent of their lipid-lowering properties [56]. These beneficial effects were not only detected in pure monacolin K but also in crude *Monascus*-rice powder [57-60], and the

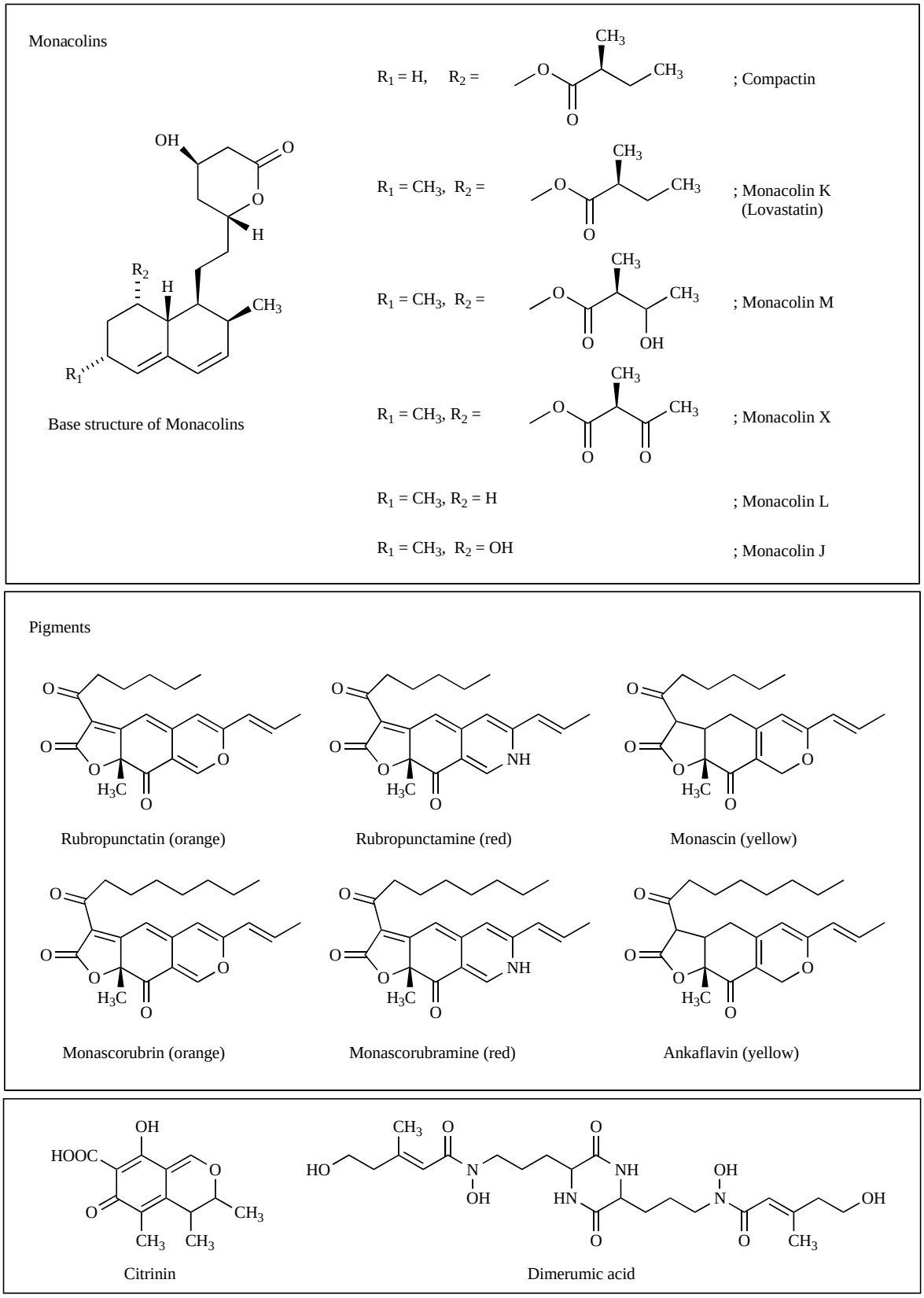

Fig. (3). Structures of bioactive compounds in *Monascus*-rice.

minimum effective dose was reported as 0.5 mg/kg/day (content of monacolin K is 4.75 mg/kg) in rabbits [58].

In clinical data, statins including monacolin K are therapeutically equivalent in reducing LDL-cholesterol at comparable doses [61]. While there are some reports on the cholesterol-lowering effect of *Monascus*-rice supplements to hypercholesterolemic patients, discussions of clinical efficacy and safety are ongoing [62, 63]. Problems encountered with *Monascus*-

rice supplements are the variable content of monacolin K, contamination with citrinin, and side effects like rhabdomyolysis [64] and nephropathy; thus, these risks must be taken into consideration when using these supplements.

Recently, in addition to the cholesterol-lowering effect, other effects such as neuroprotection [65], dementia prevention [66], anticancer effect [67-69], and osteogenesis promotion [70] in statins and *Monascus*-rice have been reported, and further studies are necessary to maximize health-promoting applications.

5.2. Pigments (Azaphilones)

The major *Monascus* pigments (Fig. **3**) are six azaphilones classified into the following three groups: (i) orange pigments, rubropunctatin and monascorubrin (they have different aliphatic side chains); (ii) red pigments, rubropunctamine and monascorubramine (the pyronoid oxygen atom of orange pigments is replaced by a NH group at high pH); and (iii) yellow pigments, monascin and ankaflavin (reduced forms of orange pigments). Orange pigments are biosynthesized first, and red and yellow pigments are considered to be derived from orange pigments depending on culture conditions [71]. *Monascus* pigments are stable at a wide range of pH and high temperatures and are used as natural food colorants [72].

Martinkova *et al.* [73] reported that *M. purpureus* cultured with ammonium chloride mainly produced orange pigments, which exhibited antibiotic effects not only against bacteria but also against yeasts and certain filamentous fungi (*Myrothecium* and some *Monascus* strains). In an embryotoxicity test, ED_{50} values of the orange pigments were around 10 μg, comparable with mycotoxins such as penicillic acid, cyclopiazonic acid, tenuazonic acid or citrinin.

Recently, the anticancer effects of *Monascus* pigments have been investigated. Zheng *et al.* [74, 75] studied cytotoxic activities of rubropunctatin against human cancer cells *in vitro* and tumor-bearing nude mice *in vivo*. This pigment inhibited the proliferation of human gastric adenocarcinoma BGC-823 cells with IC_{50} of 12.57 μM, while it exhibited no significant toxicity to normal gastric epithelial GES-1 cells at the same concentration [74]. Tumor weight in BGC-823-bearing nude mice was reduced 23.5% and 37.7% after five-time intravenous injection of rubropunctatin at a dose of 8 mg/kg and 32 mg/kg, respectively. Moreover, the expression of 30 genes related to the induction of apoptosis, especially tumor necrosis factor (TNF) and DNA-damage inducible transcript 3, were found to be up-regulated significantly *in vitro*, and these factors contribute to the anticancer activities [74]. By the analysis of the relationship between pharmaceutical activity and the chemical structure of rubropunctatin, it is concluded that 6-internal ether, 4-carbonyl, and conjugated double bonds in the tricyclic structure is necessary for the anticancer effect [74].

Six *Monascus* pigments also exhibited anti-inflammatory activities on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema in mice by topical administration (ID_{50} = 0.11-0.4 mg/ear) [76]. Yasukawa *et al.* [77, 78] reported that oral administration of monascorubrin suppressed the tumor promotion in two-stage carcinogenesis, i.e., initiation by 7, 12-dimethylbenz anthracene (DBA) and promotion by TPA, in mice. Four *Monascus* pigments (rubropunctatin, monascorubrin, rubropunctamine, and monascin) inhibited Epstein-Barr virus early antigen (EBV-EA) activation, which was almost equivalent to what is observed with β-carotene, except without its cytotoxic activity in Raji cells [76].

Similar to those results, Su *et al.* [79] reported that ankaflavin inhibited the proliferation of human hepatocellular carcinoma Hep G2 and human lung epithelial carcinoma A549 with both IC_{50} values of 15 μg/ml, while it exhibited no significant toxicity to normal cells (MRC-5 and WI-38). By cell cycle analysis, it was found that ankaflavin induced a distinct sub-G1 peak. Apoptosis was therefore suggested as the possible mechanism of cell death. Both ankaflavin and monascin contain the same basic chromophoric structure and differ from each other only in the length of the saturated side chain (C_7 in ankaflavin and C_5 in monascin). However, monascin did not have anticancer activity, indicating that the length of the saturated side chain on the ketonic carbonyl group of ankaflavin is an important factor in its anticancer activity.

5.3. Citrinin

Citrinin (also known as monascidin, Fig. **3**) was originally identified as a yellow pigment produced by *Penicillium citrinum* [80], and was subsequently isolated from *Monascus* and *Aspergillus* fungi [81, 82]. The biosynthesis pathways of citrinin and other pigments differ; the former is produced from tetraketide and the later is produced from hexaketide. Although it has antibiotic activities against several bacteria, including *Staphylococcus aureus*, it is regarded as a mycotoxin because of its nephro- and hepato-toxicity [83, 84]. Citrinin-induced renal dysfunction has been recognized in various animals, and the dose-dependent ultrastructural lesions were accompanied by proteinuria and glucosuria [83, 84]. Cellular analysis suggested that citrinin accumulated in mitochondria and induced an increase in superoxides and inhibition of the electron transport system [85]. Macromolecule biosynthesis (protein, RNA, and DNA) was also inhibited [86, 87], and these multiple effects of citrinin led to cell death. The LD_{50} value for intraperitoneal administration in mice and rats was 35-67 mg/kg body weight [88].

The mutagenicity of citrinin remains controversial. Citrinin did not show mutagenicity in the Ames test with or without S9-mix in *Salmonella typhimurium* (TA-98, TA-100, TA-1535, TA-1538, TA-97, and TA-102) [89-91]. However, citrinin pretreated with primary hepatocyte culture induced mutagenicity in TA-98 and TA-100 strains [92], indicating that citrinin requires a complex cellular biotransformation to become mutagenic.

There are some reports on the genotoxicity of citrinin *in vitro* and *in vivo*. In a study of Chinese hamster ovary cells and HEK293, citrinin did not affect the frequency of sister chromatide exchange (SCE) and DNA gaps and breaks [92]. On the other hand, at high concentration of 5×10^{-4} M of citrinin induced a significant increase in complex translocation and chromosomal coiling disorder in V79-E cells originally from Chinese hamster [93]. Aneuploidic potential of citrinin was also observed in the same cell line. In a study of mice, citrinin induced chromosome abnormalities and breaks in bone marrow cells both of young weanling and adult mice [94, 95]. In addition, citrinin caused a significant concentration-dependent increase in micronucleus frequency in human lymphocytes [96]. The genotoxicity is related to tumorigenicity; oral administration of citrinin to Fischer 344 rats for 80 weeks caused renal adenomas [97].

Sabater-Vilar *et al.* [91] reported that citrinin content in commercial *Monascus*-rice varied between 0.2 to 17.1 μg/g. Blanc *et al*. [82] also reported citrinin production by *M. purpureus* and *M. ruber*, where the concentrations of citrinin were 270 and 340 mg/L in submerged culture and 100 and 300 mg/kg in solid state culture. In rat repetitive dosing toxicity test for 90 days, no abnormalities were detected in biochemical and pathological indexes of kidney and liver. Safe dosage for daily consumption in human is estimated to be 2 g of *Monascus*-rice (citrinin content is less than 4 ppm) [98].

Culture conditions of *Monascus* species have been studied to reduce citrinin content for its use in food colorants or supplements [36]. Carbon and nitrogen sources are considered to be the most important factors, and other conditions such as oxygen, metal ions, and temperature influence polyketide production including citrinin [99]. UV light and chemical mediators were used to obtain low citrinin-producing mutants [100]. Genes responsible for citrinin and other polyketide biosynthesis (*pksCT*, *ctnA*, and *Mga1*, etc.) have

been identified [101-103], thereby employing genetic engineering in the inhibition of citrinin expression [104, 105].

6. DIMERUMIC ACID

Dimerumic acid (Fig. **3**) is one of the siderophores secreted by microorganisms, and has high-affinity for iron (III) ions and contributes to the microbial iron transport system [106]. Recently, the antioxidant and hepatoprotective actions of dimerumic acid have been elucidated [107-111]. Dimerumic acid is considered to scavenge radicals by one electron donation of hydroxamic acid group in its molecule toward oxidants such as 1,1-dipheny-2-picrylhydrazyl (DPPH), hydroxyl radical, superoxide anion, ferrylmyoglobin, and peroxyl radical, resulting in inhibition of lipid peroxidation [109, 110].

When rats were pretreated with *Monascus*-rice or pure dimerumic acid, liver injuries induced by chemical mediators like acetaminophen (AAP), galactosamine, lipopolysaccharide, or carbon tetrachrolide were significantly depressed by the radical scavenging effect of dimerumic acid. Some reports suggested possible mechanisms for the hepatoprotective action [107-110]. Aniya *et al.* [107] reported that both of the antioxidative and cytochrome P450 inhibitory effects disturbed the generation of N-acetyl-p-benzoqunone imine, hepatotoxin, from APP in hepatic metabolism. Meanwhile, Yamashiro *et al.* [111] suggested that the hepatoprotective effect of dimerumic acid was mainly caused by suppressing oxidative stress without affecting the cytochrome P450 enzyme system.

7. PHYSIOLOGICAL FUNCTIONS OF *MONASCUS*-RICE-PRODUCTS

In addition to the bioactive compounds mentioned above, *Monascus*-rice contains many useful ingredients, such as phytosterol (β-sitosterol, campesterol, and stigmasterol), saponin and sapogenin, isoflavones and isoflavone glycoside, selenium, and zinc [112]. In this section, we will introduce some findings on the physiological functions of *Monascus*-rice and processed foods where the active substances have not yet been identified.

With respect to anti-diabetic effects, Chen *et al.* [113] demonstrated that a single oral administration of *Monascus*-rice to Wistar rats (the upper limit is 150 mg/kg) caused a significant decrease in blood glucose level and increases in blood insulin and C-peptide. They suggested that *Monascus*-rice would have the ability to raise the release of acetylcholine from nerve terminals, which in turn stimulates muscarinic M_3 receptors in pancreatic cells and augments insulin release, resulting in a plasma glucose lowering action. Shi *et al.* [114] also found that blood glucose, urine sugar, and urine protein in streptozotocin-induced diabetic rats were decreased by feeding of *Monascus*-rice, *Monascus*-fermented dioscorea, and *Monascus*-fermented adlay at 200 mg/kg for 8 weeks. Among these three *Monascus*-fermented products, dioscorea had the highest anti-diabetic activity and larger amounts of secondary metabolites such as monascin, ankaflavin, and GABA, than rice and adlay. Specifically, GABA acts as a blood glucose-lowering factor by repressing glucagon release [115], thereby contributing to the anti-diabetic effect of the products.

As for anti-obesity effects, Jeon *et al.* [116] reported that *Monascus*-rice had an inhibitory effect on adipocyte differentiation, as indicated by a decrease in GPDH activity and TG content. This might be mediated through the down-regulated expression of adipogenic transcription factors (C/EBP α and PPAR γ), adipocyte fatty acid binding protein (aP2), and leptin. Recently, inhibitory effects of statin analogs on adipocyte differentiation have been elucidated [117, 118]; however, monacolin K obtained from *Monascus*-rice showed no effect [116]. Further study is necessary to identify the active substance.

In addition to its actions related to energy metabolism, *Monascus*-rice exhibits macrophage-stimulating activity [119], improvement of erythrocyte deformability [120], anti-osteoporosis activity [121], and amelioration of memory and learning ability by repressing amyloid β accumulation [122]. It is anticipated that elucidation of the bioactive substances and mechanisms will lead to future advances.

CONCLUSIONS

In this review, we described that bioorganic chemicals produced by *Monascus* species are very useful for health foods and medicine. Recent studies demonstrated their mode of action and *in vivo* effectiveness in detail. In continued application of these bioactive compounds and *Monascus*-rice-products for health promotion in the future, it is important to pay attention to (i) any side effects and interactions between the bioactive compounds and therapeutic drugs, (ii) risk factors such as citrinin and, (iii) the latest reports on bioactive mechanisms and effective intake, as the medicinal functions of these compounds, except for monacolin K, require further elucidation.

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