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SKLM

Toxicological evaluation of red mould rice:

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Red mould rice is presently offered under various trade names[1] primarily through the internet, mainly as a food supplement with a cholesterol-lowering effect, i.e. without legal approval as a drug. This development caused the DFG Senate Commission on Food Safety to evaluate red mould rice with respect to its effects on human health in a first opinion in 2004. The European Food Safety Authority published an assessment of the health claims regarding monacolin K from red mould rice. The SKLM updated its opinion on December 18th 2012. The English version was agreed on November 13th/14th 2013.

Toxicological evaluation of red mould rice

1 Introduction

Red mould rice is the fermentation product of ordinary rice with certain mould species of the genus *Monascus*. The fermentation leads to the formation of red colour constituents and also various potentially active constituents, such as monacolins, monankarins, ankalactones and citrinin are formed. The use of red mould rice for the colouring, flavouring and preservation of foods and as medication to stimulate digestion and blood circulation in East Asia dates back various centuries [1]. In China, red mould rice was included as a food additive in a Directive for Food Additives for the colouring of meat, fish and soy products in 1982 [2]. In contrast in Japan, only the pigments of the red mould rice species *Monascus purpureus* are allowed to be used in foodstuffs and the production of red mould rice already reached 100 tons/year in 1977 [3].

The product „red rice flour“, provided that it is used for colouring of foodstuffs i.e. for technological purposes, is classified in the EU as a food additive and not as a food with colouring properties. Accordingly, it requires approval as a food colouring agent which has not yet been given [4, 5]. The potential use of “red rice flour” as food is not uniformly evaluated in the EU.

Although red mould rice is not approved as a food additive, its use in vegetarian sausage-like products has been detected [6]. Moreover, in connection with reports on allergic reactions unapproved use in the production of sausage products has been uncovered [7-9].

[1] Among others as red rice, red fermented rice, red yeast rice, red mould rice, Angkak, Hongqu and Red Koji as well as Cholestin™, Xuezhikang™, HypoCol™, Cholestol™, CholesteSure™ and CholestOut™

In the USA a red mould rice preparation (Cholestin™) has been marketed as a food supplement (FS) [10]. In 2000 it was classified as an unapproved drug by the Food and Drug Administration (FDA), based on its drug-like action [11].

In the EU red mould rice is offered as a food supplement for lowering the blood cholesterol level. The „Novel Food“ catalogue of the European Commission confers to the product „red yeast fermented rice“ the „FS“ status, i.e. the product was only used as or in food supplements before 15th May 1997, any other use requiring approval according to the Novel Food Regulation [http://ec.europa.eu/food/committees/regulatory/scfcah/general_food/sum_26012010_en.pdf]. The Federal Institute for Drugs and Medical Devices (BfArM, Germany) issued a press release warning against the consumption of such products [12], since the active constituent monacolin K is identical with lovastatin, a potent statin used as a drug. Statins inhibit the synthesis of cholesterol at the level of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. The simultaneous ingestion of red mould rice and statins and numerous other drugs may lead to an increase in the inhibitory effect on the above-mentioned enzyme with subsequent adverse health effects.

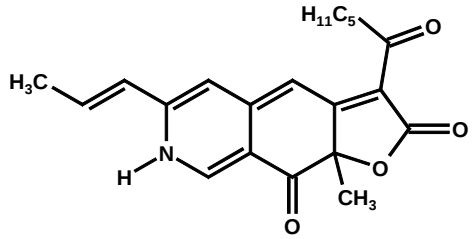
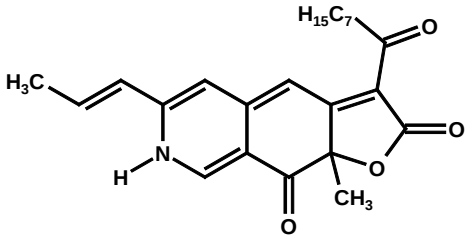
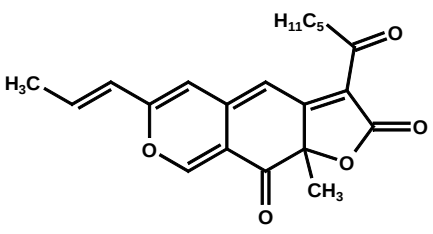
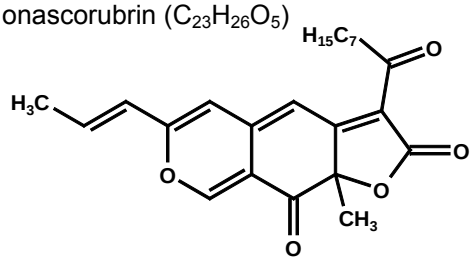
Upon request of the European Commission, the European Food Safety Authority (EFSA) recently published a Scientific Opinion on the substantiation of health claims related to monacolin K from red yeast rice and the maintenance of normal blood LDL-cholesterol concentrations [13]. The evaluation of EFSA is based on two clinical studies [14, 15], which showed an effect of red mould rice preparations at a daily dose of about 10 mg monacolin K on the LDL cholesterol levels in individuals with hypercholesterolaemia. It is based as well on the known pharmacological effect of lovastatin as an inhibitor of the HMG-CoA reductase. However, this evaluation of EFSA does not represent a classification or an approval of red mould rice as a foodstuff and no risk assessment has been carried out. According to EFSA a cause-effect relationship has been established between the consumption of monacolin K from red mould rice and maintenance of a normal blood LDL-cholesterol concentration. In order to accomplish the claimed effect, 10 mg of monacolin K from fermented red yeast rice preparations need to be consumed daily. Further clinical studies have confirmed the cholesterol-lowering effect of red mould rice [16-18].

2 Constituents and their biological effects

Red mould rice is produced by fermentation of ordinary rice (*Oryza sativa*) with certain mould species of the genus *Monascus* (*M. ruber, M. purpureus, M. pilosus, M. floridanus*) [19]. Taxonomically, *Monascus* species belong to the *Monascaceae* family [20, 21], for which terminal cleistothecia surrounded by hyphae are characteristic. Depending on the fermentation process, the main constituents of red mould rice are carbohydrates (25-73%), proteins (14-31%), water (2-7%) and fatty acids (1-5%) [1, 22]. During fermentation over several days to weeks, numerous products of the secondary metabolism of moulds are formed. Among the compounds that may be produced by the organisms are various pigments, pharmacologically active monacolins (HMG-CoA reductase inhibitors) and monankarins (inhibitors of monoamine oxidase), the mycotoxin citrinin and other non-colouring substances [19, 23].

2.1 Pigments

Pigments formed by *Monascus spp*. are not only present in free form but also bound as complexes to proteins, peptides and amino acids [24]. The main colouring components are the two red pigments rubropunctamine and monascorubramine, the orange-red pigments rubropunctatin and monascorubrin, as well as the yellow pigments monascin und ankaflavin [25-29] (Fig. 1).

Red	Rubropunctamine ($C_{21}H_{23}NO_4$) 	Monascorubramine ($C_{23}H_{27}NO_4$) 
Orange-red	Rubropunctatin ($C_{21}H_{22}O_5$) 	Monascorubrin ($C_{23}H_{26}O_5$) 

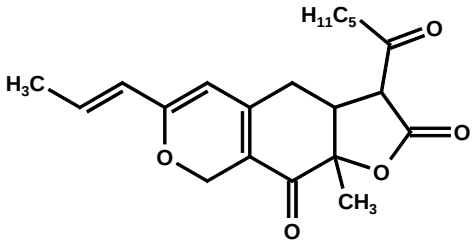
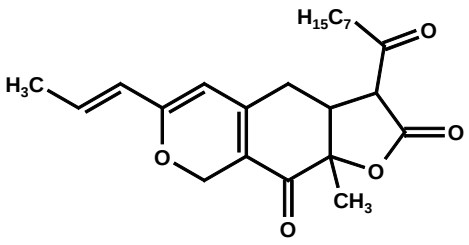
	Monascin (= Monascoflavin, $C_{21}H_{26}O_5$)	Ankaflavin ($C_{23}H_{30}O_5$)
Yellow		

Fig. 1: Main pigments of *Monascus* ssp. (according to [19])

The content of pigments in red mould rice varies depending on the culture conditions such as humidity, pH as well as nutrient and oxygen supply [30, 31]. A red mould rice product traditionally manufactured with *Monascus purpureus* had a pigment content of 0.3% in rice flour [1]. However, data on the proportion of individual pigments and their natural variability in the traditional product are not available.

The incubation of 3-day old chicken embryos for 9 days with the mycelium pigments monascorubrin, rubropunctatin, monascin and ankaflavin purified by HPLC led to embryonic malformations and lethality. The doses causing these effects in 50% of the treated embryos (ED_{50}) were 4.3 µg monascorubrin/embryo, 8.3 µg rubropunctatin/embryo, 9.7 µg monascin/embryo and 28 µg ankaflavin/embryo. Studies on embryotoxicity and teratogenicity according to OECD Guidelines are not available. Reports on the antibacterial and fungicide properties of several pigments have been published [32]. Ankaflavin was reported to induce chromosomal condensation and fragmentation in the human tumour cell lines HepG2 and A549 (IC_{50} 15 µg/ml, [33]).

Another yellow pigment, xanthomonascin A (Fig. 2), has been described. However, toxicological data are not available up to now [34].

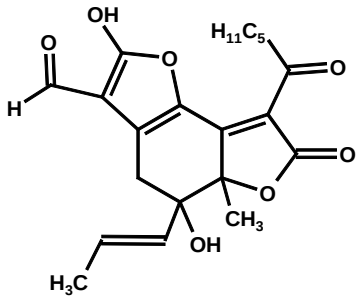
Yellow	Xanthomonascin A 

Fig. 2: Xanthomonascin A in *Monascus* ssp.

2.2 Monacolins

Monacolins are polyketides, which are formed, among others, by species of the genus *Monascus* (Fig. 3). In *Monascus ruber* the biosynthesis of monacolin K proceeds via the derivatives monacolin L, J and X. *Monascus*-species producing monacolin K are rather poor pigment producers [19]. In red mould rice at least 14 monacolins, among others monacolin K (mevinolin), J, L, M, X, dehydromonacolin K, dihydromonacolin L and compactin, have been found [35].

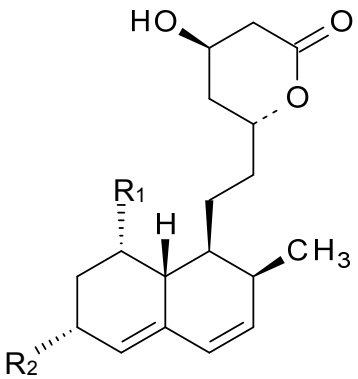
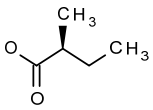
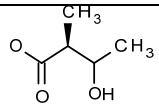
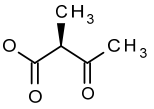
Monacolins		R1	R2
	Monacolin K		CH_3
	Compactin		H
	Monacolin M		CH_3
	Monacolin X		CH_3
	Monacolin J	CH_3	OH
	Monacolin L	CH_3	H
	ML-236A	OH	H
	ML-236C	H	H

Fig. 3: Structure of the monacolins [35]

Numerous monacolins have been identified as inhibitors of cholesterol biosynthesis. The reversible competitive inhibition of the microsomal HMG-CoA reductase prevents the reduction of HMG-CoA to mevalonic acid and thereby the formation of cholesterol as well as that of other compounds such as ubiquinones [36-38].
This effect is the basis for the use of monacolin K as a drug. The active compound monacolin K is usually referred to as lovastatin. In adults the therapeutic dose of this statin for the treatment of hypercholesterolaemia corresponds to an average of 40 mg daily with a usual initial dose of 20 mg/day. The oral bioavailability of lovastatin amounts to about 31% in humans [39]. Lovastatin is mainly metabolized in the liver and small intestine by members of the cytochrome P450 (CYP) 3A family [40]. Lovastatin itself is ineffective. It is metabolized under alkaline conditions [35] or enzymatically [41] by opening of the lactone ring to the active form, the hydrophilic 6´-beta-hydroxy-lovastatin (lovastatin hydroxy acid). In animal experiments, lovastatin hydroxy acid is very well absorbed (80-100%) [39]. After oral administration of lovastatin, similar concentrations of lovastatin and lovastatin hydroxy acid are detected in blood [42].

In studies on the subacute toxicity of lovastatin, oral doses of 100-200 mg/kg b.w. per day were lethal to rabbits. In contrast, such doses were tolerated by dogs, rats and mice. Lovastatin led to the development of liver and kidney necroses in rabbits, which is due to a specific, extremely strong inhibition of the mevalonate biosynthesis in this animal species. This effect could be prevented completely by the administration of the cholesterol precursor mevalonate, but not by the administration of cholesterol. Based on this observation it was concluded that the specific toxicity of lovastatin in rabbits was the result of the depletion of a mevalonate metabolite, essential for the survival of cells [43].

In man, the most important adverse effect of lovastatin is its muscle toxicity, which rarely occurs during a monotherapy but is frequently observed after the simultaneous administration of drugs that act either as substrates or inhibitors of CYP3A isoenzymes. Immunosuppressive agents of the cyclosporin type belong to this class of compounds [44], furthermore statins and other cholesterol-lowering agents, such as fibrates (clofibrate), antimycotic agents like itraconazole [45], certain antibiotics

such as erythromycin, clarithromycin and troleandomycin, antidepressants such as nefazodone, anticoagulants of the coumarin type and certain protease inhibitors. Similarly, the simultaneous intake of grapefruit juice can inhibit the metabolism of lovastatin [46]. The blood levels of lovastatin and its active metabolite lovastatin hydroxy acid rise considerably following the blockage of the CYP-mediated degradation of statins [46, 47]. This led in several cases to rhabdomyolysis (severe muscle damage) with lethal outcome [48].

Food supplements derived from red mould rice contain monacolins at concentrations up to 1.9% [49-51]. Red mould rice products in capsular form contained 0.31 to 11.15 mg monacolin K per capsule [49]. Moreover, the ratio of lactone to acid strongly varies, the acid being the active one. The acid form may account for 5-100% of the total amount of monacolin K [35, 49], so that bioavailability and biological effect are difficult to estimate. Therefore, the effect of red mould rice on cholesterol biosynthesis is not only determined by the monacolin K content. Optimization of the fermentation conditions and selection of the rice variety can lead to significantly higher concentrations of HMG-CoA reductase inhibitors. As an example, soluble red mould rice powder has been offered in the internet with up to 30 mg monacolin K/g powder. In cultures with Thai rice (*Oryza sativa* L. cv. RD6), in addition to other monacolins, up to 34 mg monacolin K as well as 22 mg compactin (desmethyl-monacolin K, mevastatin) per g powder were reported [52]. The inhibitory effect of the active form of compactin on the HMG-CoA reductase is about half that of monacolin K (K_I=1.4 and 0.64 nM for compactin and monacolin K, respectively [36]).

2.3 Citrinin

The mycotoxin citrinin (Fig. 4) is formed by various *Penicillium*-, *Aspergillus*- and *Monascus* species (*M. purpureus*, *M. ruber*) [53]. The formation of citrinin (identical with monascidine A) by *Monascus* species depends on the culture conditions [31]. For example, the fermentation of food-relevant *Monascus* species on rice leads to citrinin contents of up to about 2.5 g/kg dry matter, while in liquid cultures values of up to 56 mg/kg dry matter have been reached [23, 54].

In commercial samples of *Monascus* fermentation products such as red mould rice up to 17 µg citrinin/g dry matter [55] have been detected and in vegetarian sausages

up to 105 µg/kg [56]. Heber et al. (1999) measured citrinin concentrations of up to 65 µg/capsule via enzyme immunoassay in seven out of nine food supplements on the basis of red mould rice [51]. Gordon et al. (2010) analyzed 12 commercially available food supplements by LC-MS/MS and found citrinin amounts of 24 to 189 mg/kg resp. 14 to 114 µg citrinin/capsule in about one third of the samples [49].

Citrinin/Monascidine A

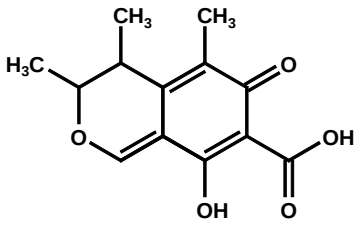


Fig. 4: Structure of citrinin [57]

Red mould rice produced with a citrinin-synthesizing mutant of *Monascus ruber* (NTU 505) was fed to male Wistar rats in a 90-day study [58]. The applied red mould rice contained 1, 2, 10, 20 and 200 mg/kg citrinin as well as 0.24 mg/kg b.w. lovastatin. Control groups received unfermented rice without added citrinin or with a citrinin content of 2 and 200 mg citrinin/kg. Even at the highest (fed) dose corresponding to a citrinin concentration of 200 mg citrinin/kg (20 µg citrinin/kg b.w./day) no toxicologically significant alterations of body weight, feed intake, organ weights, clinical chemistry (serum) and liver as well as kidney histopathology were observed. Based on this study EFSA identified a no observed adverse effect level (NOAEL) of 20 µg/kg b.w./day for nephrotoxicity [57]. At present, further subchronic toxicity studies to be used to derive a NOAEL are not available [57].

The chronic administration of citrinin resulted in nephrotoxicity in various animal species [59, 60]. Six-week old male Fischer-344 rats were fed 0.1% citrinin ad libitum (initial dose corresponding to about 70 mg/kg b.w./day [57]) for up to 80 weeks. Focal hyperplasia of the renal tubular epithelium and renal adenomas developed in all treated animals after 40 weeks. After 60 weeks benign renal tumours, histopathologically described as clear cell adenomas, were observed [59].

In a pilot study, in which male Sprague-Dawley rats were fed 0.02 or 0.05% citrinin (corresponding to 25 or 70 mg/kg b.w./day [57]) for 48 weeks, damage of epithelial cells in renal tubuli but no renal tumours were observed [60]. In a subsequent initiation-promotion experiment, rats were first treated orally with *N*-nitrosodimethylamine or *N*-(3,5-dichlorophenyl)succinimide for two weeks and were then fed 25 or 75 mg citrinin/kg b.w./day for 20 weeks. In these rats an enhanced number of renal tumours was found when compared to the animals only treated with *N*-nitrosodimethylamine or *N*-(3,5-dichlorophenyl)succinimide [60].

Adequate long-term carcinogenicity studies with citrinin over 2 years have not been performed up to now.

The *International Agency for Research on Cancer* (IARC) classified citrinin in Group 3 (i.e. the agent is not classifiable as to its carcinogenicity to humans) [61]. Associations of citrinin and/or ochratoxin A with the so-called endemic Balkan nephropathy, showing fibrosis of the renal cortex, necrosis of the tubular epithelium and tumours of the descending urinary tract have been discussed for the individual compounds as well as for their combination. The consumption of mouldy cereals in endemic areas has been considered as a potential cause of this mycotoxin-induced nephropathy [62-64].

A mutagenic effect of citrinin in the Ames test has not been observed either in the absence or presence of rat / human liver homogenate as metabolic activation system [55, 65]. Only in the so-called *Salmonella*-hepatocyte assay, in which primary rat hepatocytes are used as a metabolic activation system, a concentration-dependent mutagenic effect of citrinin was observed. CYP3A4-mediated activation and subsequent conjugation reactions catalyzed by phase II enzymes have been discussed as metabolization pathways [55]. A concentration-dependent increase of the mutation frequency was observed in CYP3A4-expressing NIH 3T3 cells when compared to wild-type cells. Citrinin did not induce single-strand breaks in HepG2 cells [65], oxidative DNA damage in human embryonic kidney cells (HEK293) or sister-chromatid exchanges in Chinese hamster ovary cells and human lymphocytes [66]. In contrast, citrinin led to an aneuploidogenic effect in the Chinese hamster lung fibroblast cell line V79 [67], induced micronuclei in the human hepatoma cell line

HepG2 [65] and isolated peripheral human lymphocytes [68] and led to chromosomal aberrations in human peripheral mononuclear blood cells [69]. Chromosomal aberrations and hypoploidy were observed in bone marrow cells of mice fed 5 or 20 mg citrinin/kg b.w./day for 8 weeks [70].

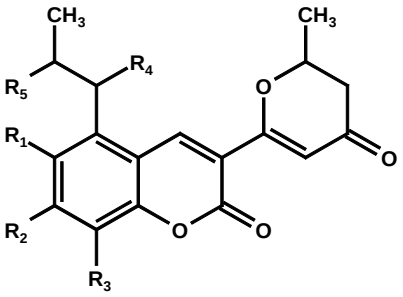
Furthermore, citrinin induced reproductive toxicity, malformations and embryotoxic effects *in vitro* [71, 72] and *in vivo* [73-76]. Malformations were induced in chicken embryos (46% in the surviving animals at a dose of ≥ 50 µg citrinin/embryo) [77, 78]. It should be noted that the doses used in the *in vivo* studies led to maternal toxicity and which may have been the cause of the observed foetal effects [57].
The available *in vitro* studies show that the toxic effect of citrinin is mediated by different mechanisms, e.g. by inhibition of DNA and RNA synthesis, inhibition of microtubule assembly and of tubulin polymerization, alteration of mitochondrial functionality with enhanced production of reactive oxygen species, inactivation of the heat shock protein 90 multichaperone complex and activation of apoptotic signal transduction pathways such as the caspase cascade [57].

A NOAEL for citrinin of 20 µg/kg b.w./day was derived from a subchronic study in rats. Applying an uncertainty factor of 100, EFSA deduced a level of no concern for nephrotoxicity in humans of 0.2 µg citrinin/kg b.w. per day [57]. Based on the available data, a concern for genotoxicity and carcinogenicity could not be excluded at this dose level. Thus, considerable uncertainties still exist regarding the risk assessment of citrinin [57].

2.4 Further products of the secondary metabolism of *Monascus ssp*.

2.4.1 Monankarins

The monankarins A-F (Fig. 5) are compounds with a pyranocoumarin structure produced by *Monascus anka* (*M. purpureus*) and are not considered as pigments despite their yellow colour. No data are available on the concentrations of monankarins in red mould rice. The diastereomeric monankarins A/ B and C/ D inhibit the monoamino oxidase activity in mouse brain and liver preparations at micromolar concentrations [79]. Monankarin C showed the highest activity with an IC_{50} value of 11 µM.

Monankarins		R_1	R_2	R_3	R_4	R_5
	Monankarin A and B*	CH_3	OH	H	CH_3	OH
	Monankarin C and D*	CH_3	OH	CH_3	CH_3	OH
	Monankarin E	H	OH	CH_3	H	OH
	Monankarin F	CH_3	OH	CH_3	H	OH

*diastereomers

Fig. 5: Structures of monankarins A-F [79]

2.4.2 Monascodilone

Monascodilone (Fig. 6) was detected in 6 out of 12 untreated red mould rice samples at concentrations up to 0.4 mg/g. Heating was found to increase monascodilone contents, yet, precursor compounds have not been identified. Under the chosen experimental conditions (121°C, 20 min) contents of up to 5 mg/g were determined in red mould rice [80]. Pharmacological and toxicological characteristics remain unknown at the present time.

Monascodilone

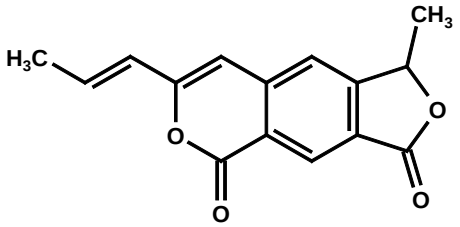


Fig. 6: Structure of monascodilone [80]

2.4.3 Monascopyridines

The colourless monascopyridines A and B (Fig. 7) were detected at concentrations up to 6 mg/g in preparations of red mould rice after the fermentation with *Monascus purpureus* DSM 1379 and DSM 1603 [81]. Furthermore, the monascopyridines C to F have been identified in fermented red mould rice [82-84]. Only few data on the pharmacological or toxicological properties of these compounds are available up to

now. Monascopyridines were cytotoxic in the micromolar concentration range in immortalized human renal epithelial cells [83, 84]. Monascopyridine C and D have been discussed to display an aneuploidogenic potential; however, both compounds were not found to trigger apoptosis [82, 83].

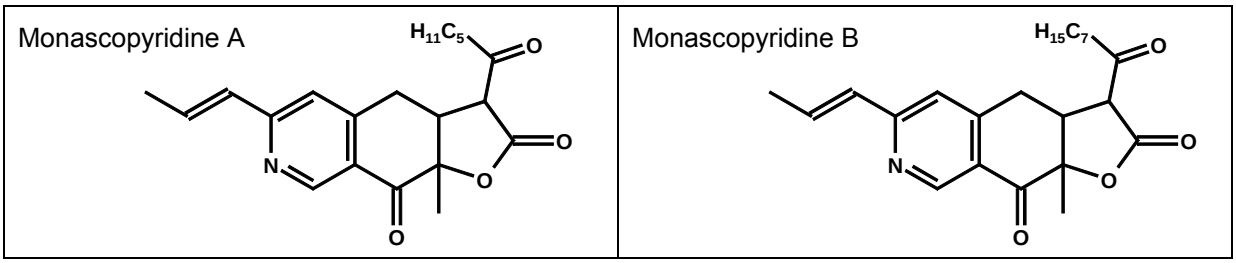


Fig. 7: Structures of monascopyridine A and B [81]

2.4.4 γ-Aminobutyric acid (GABA)

Up to 1.5 g γ-aminobutyric acid (GABA) per kg are formed during the fermentation of *Monascus purpureus* CCRC 31615 on rice [85]. GABA has several physiological functions, e.g. as neurotransmitter in inhibitory neurones of the brain and spinal cord, and also shows a blood pressure lowering as well as a diuretic effect [86]. The intravenous administration of GABA (250 µg/kg b.w.), isolated by HPLC from red mould rice, resulted in a decrease of the blood pressure in rats with spontaneous hypertension [87].

2.4.5 Ankalactone

The colourless ankalactone (Fig. 8) of *Monascus anka* (*M. purpureus*) inhibits the growth of *Escherichia coli* and *Bacillus subtilis* [88]. Up to now no further pharmacological or toxicological data are available.

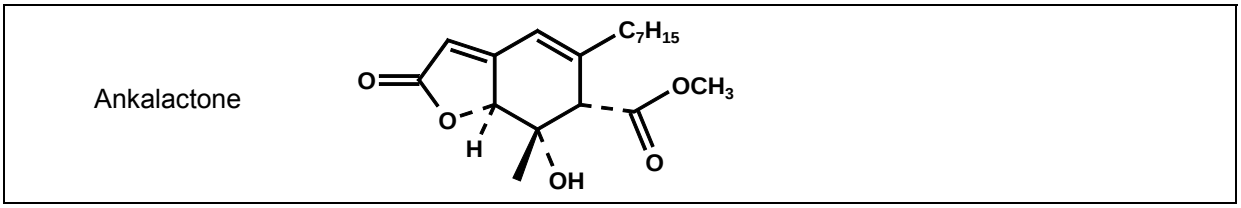


Fig. 8: Structure of ankalactone [88]

2.4.6 Further identified compounds

In the last years, a number of further compounds have been identified in products fermented with different *Monascus* species, e.g. stigmast-4-en-3-one [89], monapurfluorene A and B [90], peroxymonascuspyrone [91], monascuspyrrole [92], monasnicotinates A-D [93] and cytotoxic steroids [94].

Only very few data are available on the toxicological characteristics of these compounds. Basically, cytotoxicity has been investigated in a number of different cell lines up to now. Different steroidal compounds showed a cytotoxic effect in human lung adenocarcinoma and ovarian carcinoma cells with IC_{50} values in the micromolar range [94]. Monapurfluorenes A and B showed moderate antiproliferative effects in the micromolar concentration range in human laryngeal cancer and colon adenocarcinoma cells [90].

2.4.7 Not identified compounds

Fung et al. [95] extracted a polar fraction from red mould rice which increased the P-glycoprotein activity and also inhibited a number of drug metabolizing cytochromes P450 (CYP 1A2, 2C9 and 3A4).

3 Biological effects of red mould rice

Studies on the toxicity of red mould rice in relevant test systems are not available up to now. Analyses on the embryotoxicity of red mould rice extracts in chicken embryos showed a much weaker teratogenic and lethal effect than expected on the basis of the pigment concentration. It was speculated that the stronger embryotoxic orange-red pigments may preferentially react with matrix amino groups during fermentation. A comparison of the effect of red mould rice extracts with that of citrinin in chicken embryos has not been performed. However, a concentration-dependent mutagenic effect of *Monascus* extracts in a *Salmonella*-hepatocyte assay has been reported in connection with citrinin toxicity studies [55].

Studies showed a blood pressure lowering effect of intravenously administered GABA, a corresponding effect was reported after feeding rats with *Monascus pilosus*-fermented wheat [96].

3.1 Pharmacological effects of red mould rice

Pharmacological efficacy of red mould rice has been reported in several clinical studies with different commercial red mould rice products. The daily doses used in these studies ranged between 1.2 and 4.8 g of red mould rice, corresponding to an amount of monacolin K (the sum of monacolin K and monacolin K-hydroxy acid) between 7.2 mg [18] and 10-12.8 mg [97]. In these studies a decrease in the LDL-cholesterol concentration of up to 30% and a decrease in the total cholesterol concentration of up to 23% were reported [15, 17, 18, 97, 98]. In one study red mould rice (2 x 2400 mg daily) was reported to be as effective as 2 x 20 mg pravastatin [18]. In addition to lowering blood levels of LDL-cholesterol and total cholesterol, reduced mortality in patients with coronary heart diseases and in patients after a myocardial infarction has also been reported [16].

The use of these HMG-CoA reductase inhibitors in combination with grapefruit ingestion [46] or with application of numerous drugs may lead to well-known food drug interactions with the CYP3A-mediated metabolism [99]. This applies, for example, to further lipid-lowering agents (gemfibrozil and other fibrates [48, 100]), niacin (nicotinic acid) in amounts of 1 g or more per day, amiodarone [101], coumarin derivates [48], cyclosporin [48], mibefradil [48], verapamil, antimycotic agents such as itraconazole and ketoconazole [48], macrolide antibiotics (erythromycin, clarithromycin, azithromycin) [48], HIV protease inhibitors [102] and nefazodone [103]. When consuming red mould rice and simultaneously drugs with CYP3A-inhibiting properties, myotoxic effects may occur because of markedly reduced metabolic clearance of HMG-CoA reductase inhibitory constituents. A case of rhabdomyolysis in a patient treated with cyclosporin after kidney transplantation who concomitantly ingested a red mould rice-containing product was first noted in 2002 [104]. Since then, additional cases of myopathies following the consumption of red mould rice have been published [105-108].

Furthermore, there are individual reports on allergic reactions after contact with red mould rice during the production of sausage products. The exposure occurred via the respiratory tract as well as through the skin and was characterized by rhinitis, conjunctivitis, asthma and dermal eczema. Studies of the affected patients

demonstrated an immunoglobulin E-mediated reaction to *Monascus purpureus* [7-9]. Systematic studies on the allergenic potential of red mould rice are not available.

4 Summary

Besides a number of known pharmacologically active constituents, red mould rice also contains other constituents not adequately characterized yet. Depending on genus and production conditions marked variations in contents of individual constituents are to be expected. For commercially available red mould rice products (e.g. loose goods, products in capsules) frequently specifications for purity, identity and content of the constituents are not given or unavailable. Basic toxicological data for a scientifically-based safety evaluation of red mould rice are largely lacking.

Among the known constituents, citrinin and constituents inhibiting HMG-CoA reductase, particularly monacolin K and compactin, as well as the pigments need to be evaluated.

Citrinin is described to be nephrotoxic and teratogenic and to induce renal tumours in chronic toxicity studies in rats. A dose of 50 mg/kg b.w./day after 60 weeks caused tumours in 100% of the test animals. Adequate long-term carcinogenicity studies over 2 years are missing up to now. Based on a NOAEL derived from a subchronic study in rats at a citrinin dose of 20 µg/kg b.w./day and by applying an uncertainty factor of 100, EFSA deduced a level of no concern for nephrotoxicity of 0.2 µg citrinin/kg b.w. per day for humans [57]. However, based on the available data a concern for genotoxicity and carcinogenicity could not be excluded at this level, and many uncertainties still exist regarding the risk assessment of citrinin [57].

Citrinin can be formed by all food-relevant *Monascus* species. In commercially available food supplements up to 114 µg citrinin/capsule were detected [49]. At a typical recommended dosage of 4 capsules, exposure to citrinin may reach 456 µg/day or 7.6 µg/kg b.w./day (based on a body weight of 60 kg), thus largely exceeding the level of no concern for nephrotoxicity in humans of 0.2 µg/kg b.w./day derived by EFSA.

Monacolins, which have been identified as inhibitors of cholesterol biosynthesis, were detected in commercially available food supplements in concentrations of up to

~1.9%, monacolin K (lovastatin) representing up to 90% of the total monacolin content. Contents of 0.31 to 11.15 mg/capsule have been found. As a consequence, a recommended ingestion of 4 capsules per day may be anywhere between close to and clearly exceeding the recommended therapeutic dose of lovastatin. Simultaneous ingestion of compounds with CYP-inhibiting properties, which can also be present in red mould rice, may increase the risk for myotoxic effects. The highly varying monacolin K to monacolin K hydroxy acid ratio in commercially available products is an additional uncertainty factor. Monacolin K hydroxy acid is the active form of monacolin K and is much better absorbed than monacolin K. Hence, just labelling the monacolin content is not sufficient to ascertain safe therapeutic dosing. Moreover, the biological activity of other monacolins such as e.g. compactin, which is half as effective in inhibiting HMG-CoA reductase as monacolin K, also needs to be taken into consideration.

The orange-red pigments rubropunctatin and monascorubrin as well as the yellow pigments monascin and ankaflavin led to malformations in chicken embryos in the lower micromolar dose range. No data are available on a possible teratogenic effect of these compounds in mammalian species. Based on a pigment content of red mould rice of about 0.3% in dry products a typical dosage may lead to ingestion of about 7 mg/day or 120 µg/kg body weight (60 kg).

5 Final evaluation

Red mould rice contains pharmacologically and toxicologically relevant constituents including monacolin K (lovastatin) and other monacolins as well as citrinin and further constituents with largely unknown biological effects. Monacolins are potent cholesterol lowering drugs to be administered under medical supervision. However, its use as food supplement may be questioned by the proven pharmacological effect of the individual red mould rice constituents. Moreover, the variability in contents and types of constituents many of them inadequately characterized or tested for safety, precludes an adequate safety evaluation.

The available data regarding the safety of red mould rice and its constituents are insufficient. Furthermore, standards and specifications to ensure purity and identity of the preparations as well as information on the absence of constituents potentially detrimental to human health are missing. In addition, adequate monitoring and

assessment of side effects observed on ingestion of red mould rice products appears mandatory. Taken together, the Senate Commission is of the opinion that red mould rice is not a safe food/food supplement.

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