

Toxicological evaluation of red mould rice

(shortened version)

At present red mould rice is offered for sale under various trade names¹ primarily through the internet, mainly as a nutritional supplement with a cholesterol-lowering action, i.e. without legal approval as a drug. The DFG-Senate commission on food safety (SKLM) has used this development as a reason for a first evaluation of red mould rice from the point of view of its safety to health. The German version of the opinion was adopted on 26th October 2004, the English version was accepted on 8th April 2005. Deletions in the original text are labelled by “[...] “. For more information, please refer to the original document. Also references to the literature of the original document are not included.

1 Introduction

Red mould rice is the fermentation product of ordinary rice with certain mould species of the genus *Monascus*. The use of red mould rice for the colouring, flavouring and preservation of foods as well as its use as a medicament for the stimulation of the digestion and the circulation of the blood dates back over several centuries in East Asia [1]. In China, in 1982, red mould rice was included in a Directive for Food Additives as a food additive for the colouring of meat, fish and soya products [2]. In Japan, on the contrary, only the pigments of the red mould rice species *Monascus purpureus* are permitted for use in foodstuffs. There the production of red mould rice already had reached 100 tons/year in the year 1977 [3].

[...]

In the USA, a red mould rice preparation (cholestinTM) has been marketed as a food supplement [10]. In the year 2000 this was declared by the Food and Drug administration (FDA) to be an unapproved medicament because of its drug-like action and thus its marketing was prohibited [11].

In the EU, red mould rice is offered as a so-called food supplement for the lowering of the blood cholesterol level. In an official press notice the Federal Institute for Drugs and Medical Devices warned of the consumption of such products [12], because the constituent monacolin K, responsible for this effect, is identical with lovastatin, a potent statin. Statins inhibit the cholesterol synthesis at the stage of the hydroxymethyl-glutaryl co-enzyme A reductase (HMG-CoA). The simultaneous ingestion of red mould rice and statins as drugs can lead to an increase in the inhibitory effect with consequent disadvantageous effects on health.

2 Constituents and their toxicology

Red mould rice is produced through fermentation of ordinary rice with certain mould species of the genus *Monascus* (*M. ruber*, *M. purpureus*, *M. pilosus*, *M. floricornis*) [13]. [...]

Over several days to weeks, numerous products of the secondary metabolism of moulds are formed during the fermentation process, among them various pigments, pharmacologically active monacolins (HMG-CoA reductase inhibitors) and monancarinones (inhibitors of monoamine oxidase), the mycotoxin citrinin as well as other non-colouring substances [17,13]. Additionally, *Monascus* species from other metabolites which as yet have been identified only partially [18].

2.1 Pigments

Monascus species form not only the free pigments but also those bound as complexes with proteins, amino acids and peptides [19]. Apart from the two red colours rubropunctamine and monascorubramin, the orange-red pigments rubropunctatin and monascorubrin as well as the yellow pigments monascin and ankaflavin are also main colouring components [20-24] (see Fig. 1).

Fig.1: Main pigments of *Monascus* spp.(after [13])

¹ among others as Red Rice, Red yeast rice, red mould rice, Angkak, Hongqu and Red Koji as well as CholestinTM, HypoColTM, CholestolTM, CholesteSureTM and CholestOutTM

Red	Rubropunctamine ($C_{21}H_{23}NO_4$) 	Monascorubramin ($C_{23}H_{27}NO_4$)
Orange-red	Rubropunctatin ($C_{21}H_{22}O_5$) 	Monascorubrin ($C_{23}H_{26}O_5$)
Yellow	Monascin (=Monascoflavin $C_{21}H_{26}O_5$) 	Ankaflavin ($C_{23}H_{30}O_5$)

The content of pigments in red mould rice varies depending on the culture conditions such as humidity, pH, nutrient supply and oxygen provision [25,26]. A red mould rice product manufactured traditionally with *Monascus purpureus* had a pigment content of 0.3% in rice flour [1]. No data are available on the proportions of individual pigments and the limits of their natural variation in this traditional product.

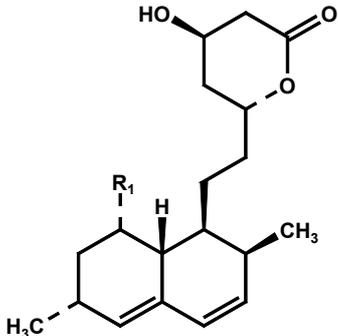
Pigments purified by chromatography (HPLC) from the mycelium, monascorubrin, rubropunctatin, monascin and ankaflavin, have caused embryonal malformations respectively embryo lethality after treatment of 3- day old chicken embryos following incubation over 9 days. The doses causing this effect in 50% of treated embryos [ED 50] were monascorubrin, 4.3 $\mu\text{g}/\text{embryo}$, rubropunctatin, 8.3 $\mu\text{g}/\text{embryo}$, monascin, 9.7 $\mu\text{g}/\text{embryo}$, and ankaflavin, 28 $\mu\text{g}/\text{embryo}$. The C_5H_{11} -sidechain homologues rubropunctatin and monascin, in contrast to the C_7H_{15} -sidechain homologues monascorubrin and ankaflavin, showed teratogenic properties at doses above 3 $\mu\text{g}/\text{chicken embryo}$. Studies on embryotoxicity and teratogenicity in relevant mammalian systems are not available. Reports exist on the antibacterial and fungicidal properties of several pigments [27].

[...]

2.2 Monacolins

Monacolins are polyketides formed, among others, by species of the genus *Monascus* [Fig. 3]. The biosynthesis of monacolin K proceeds in *Monascus ruber* via the derivatives monacolin L, J and X. *Monascus* species producing monacolin K are rather poor pigment producers [13].

Fig. 3: Structure of the monacolins

Monacolins		R ₁
	Monacolin J	OH
	Monacolin K	OOCCH(CH ₃)C ₂ H ₅
	Monacolin L	H
	Monacolin M	OOCCH ₂ C(OH)CH ₃
	Monacolin X	OOCCH(CH ₃)(OC)CH ₃

Numerous monacolins have been identified as inhibitors of cholesterol biosynthesis. The reversible competitive inhibition of the microsomal hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase prevents the reduction of HMG-CoA to mevalonic acid and thereby the formation of cholesterol as well as other compounds such as ubiquinones [29-31].

This effect forms the basis for the application of monacolin K as a drug in Japan and in the USA. Today the usual registered name of the active pigment monacolin K is lovastatin. The therapeutic dose of this statin for the treatment of hypercholesterolaemia amounts in adults on average to 40 mg daily with a usual initial dose of 20 mg/day. The oral bioavailability of lovastatin is rather low at 5% [32]. Lovastatin is metabolised in the liver and the small gut mainly by proteins of the cytochrome P 450(CYP)3A family and is excreted in the bile [33].

[...]

In man the most important undesirable effect of lovastatin is its muscle toxicity, which only rarely occurs during a monotherapy but frequently after simultaneous administration of drugs

which act either as substrates or inhibitors of CYP3A isoenzymes. To these belong representatives of the class of immunosuppressive agents of the ciclosporin type [35], other statins and other cholesterol-lowering agents such as fibrates (clofibrate), antimycotics like itraconazol [36], certain antibiotics such as erythromycin, clarithromycin, troleandomycin, antidepressants such as nefazodon, anticoagulants of the coumarin type, and certain protease inhibitors. Similarly, the simultaneous intake of grapefruit juice can inhibit the metabolism of lovastatin [37]. The blood levels of lovastatin and its active metabolite lovastatinic acid rise considerably through blockage of the CYP-mediated degradation of statins [37, 38]. In several cases this has resulted in a rhabdomyolysis (serious muscle damage) with lethal outcome [39].

Food supplements derived from red mould rice contain monacolins at concentrations of up to 0.5% [40]. Up to 75% of the total amount of monacolins is represented by monacolin K. Red mould rice products in capsular form contain 0.15 to 3.37 mg monacolin K/capsule. At a capsule filling of 600 mg and a mean monacolin content of 0.4% the typical recommended dose of 4 capsules per day [63] would result in a daily consumption of 10 mg monacolin [1].

2.3 Citrinin

The mycotoxin citrinin (Fig. 4) is formed by various *Penicillium*-, *Aspergillus*- and *Monascus* species (*M. purpureus*, *M. ruber*) [41]. The formation of citrinin (identical with monascidine A) by *Monascus* species depends on the culture conditions [26], for example, the fermentation of foodstuff-relevant *Monascus* species on rice produces citrinin contents up to about 2.5 g/kg dry matter, while liquid cultures have reached values up to 56 mg/kg dry matter [42,17].

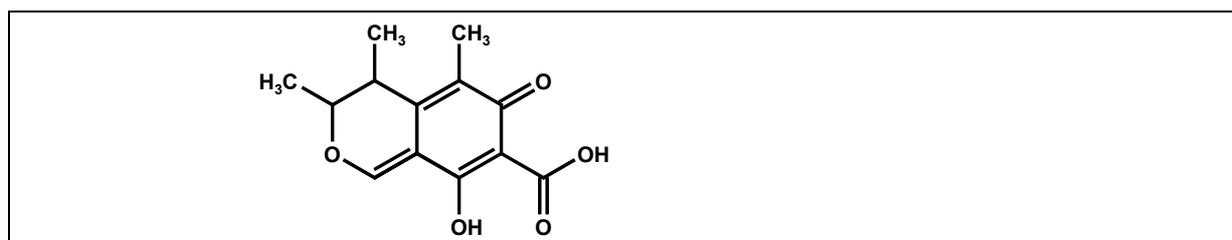
In commercial samples of *Monascus* fermentation products, such as red mould rice, up to 17 µg citrinin/g dry matter have been detected [43], in the usual marketed food supplements up to 65 µg/capsule [40] and in vegetarian sausages up to 105 µg/kg [44]. Citrinin has also been found in silage [45].

Citrinin was found to be nephrotoxic by repeated administration to various animal species. Administration of 0.1% citrinin in the diet of male Fischer 344 rats (equivalent to 50 mg/kg b.w./day) resulted in the appearance of focal hyperplasias of the renal tubular epithelium and of adenomas at 40 weeks in all treated animals. Benign renal tumours were observed after 60 weeks, which were described histopathologically as clear cell adenomas. After administration to male Sprague-Dawley rats of up to 0.05% citrinin in the diet (equivalent to 25 mg/kg

b.w./day) for 48 weeks all animals were killed for histopathological examination. At that time only damage to the epithelial cells of the renal tubules, without any tumour formation, was observed. None of the studies permitted the derivation of a dose without effect (NOEL). Citrinin is classified by the International Agency for Research on Cancer (IARC) in Group 3. (Definition according IARC: Group 3 -The agent is not classifiable as to its carcinogenicity to humans. This category is used most commonly for agents, mixtures and exposure circumstances for which the *evidence of carcinogenicity is inadequate* in humans and *inadequate or limited* in experimental animals.) [46-48]. A potential role is being discussed for citrinin and ochratoxin A, respectively for their interaction in the aetiology of the so-called endemic Balkan nephropathy, in which fibrosis of the renal cortex, necrosis of the tubular epithelium and tumours of the descending urinary tract occur. The consumption of mouldy cereals in endemic areas has been considered as possibly causative of this mycotoxin-induced nephropathy [49-51].

No mutagenic activity of citrinin was found in the Ames test with *Salmonella typhimurium* either with or without the presence of S9 mix. In the so-called Salmonella hepatocyte assay, in which the cell-free culture supernatant of an incubation of rat hepatocytes with citrinin is incubated with *Salmonella typhimurium* (TA 98 and TA 100), there was, in the course of the subsequent culturing of the treated bacteria, a concentration-dependent mutagenic activity. CYP3A4-dependent phase I metabolism, with a possible subsequent biotransformation by phase II enzymes, is being discussed as an activation process [43]. In transgenic NIH-3T3 cells expressing CYP3A4 in contrast to wild-type cells a dose-dependent rise in mutation frequency could be demonstrated. Citrinin shows an aneuploidogenic effect in Chinese hamster V-79 cells [52]. Teratogenic effects in chicken embryos have also been described with a teratogenicity percentage of 46% in survivors at doses of 50 µg/embryo and higher [53].

Fig.4. Structure of citrinin/ monascidine A

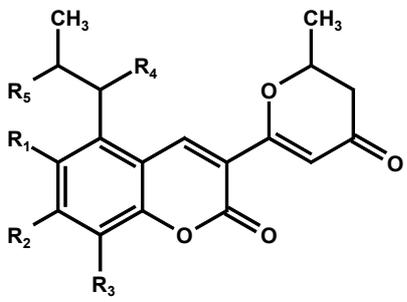


2.4 Other products of the secondary metabolism of *Monascus* species .

2.4.1 Monankarins

The monankarins A-F (Fig.5) are compounds with a pyrano-coumarin structure produced by *Monascus anka* (*M. purpureus*) that are not considered to be pigments despite their yellow colouration. From the mycelium some 0.003% monankarine A, 0.0005% monankarin B, 0.003 % monankarin C and 0.0007% monankarin can be extracted. No data are available on the concentrations of monankarins in red mould rice. The diastereomeric monankarins A and B as well as C and D are inhibitory to the monoamineoxidase of mouse brain and liver preparations at micromolar concentrations [54]. Monankarin C possesses the highest activity with an IC₅₀ value of 11 μM.

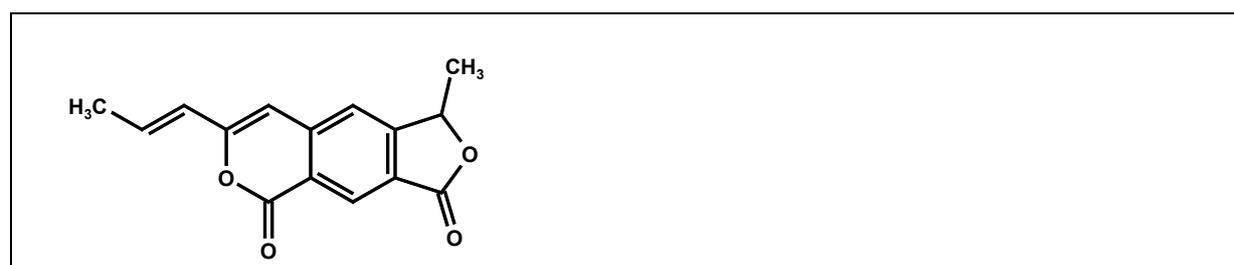
Fig. 5: Structure of monankarins A-F

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

2.4.2 Monascodilone

Monascodilone (Fig. 6) has been demonstrated to be present in 6 out of 12 untreated samples of red mould rice at concentrations up to 0.4 mg/g. Additional amounts are formed during heating, in this process the so-far unidentified precursors are neither pigments nor citrinin. Under the conditions chosen in the laboratory (121° C, 20 min.) contents of up to 5 mg/g were detected in red mould rice [18]. So-far nothing is known about its pharmacological or toxic properties.

Fig. 6: Structure of monascodilone

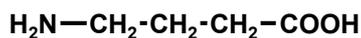


[...]

2.4.4 γ -Aminobutyric acid (4-aminobutanoic acid, GABA)

Up to 1.5 g/kg of γ -aminobutyric acid (4-aminobutanoic acid, GABA, Fig. 8) are formed during the fermentation of *Monascus purpureus* CCRC 31615 in rice [56]. GABA has several physiological functions, e.g. as neurotransmitter in inhibitory neurones of the brain and spinal cord, and also produces blood pressure lowering and diuretic effects [57]. Intravenous administration of 250 μ g/kg body weight of GABA, isolated by HPLC from red mould rice, resulted in a lowering of the blood pressure in rats with spontaneous high blood pressure [58]. Further data are not yet available.

Fig. 8: Structure of GABA



[...]

3 Toxicological studies with red mould rice

Studies on the toxicity of red mould rice in relevant systems have not yet been provided. Tests for embryotoxicity of red mould rice extracts in chicken embryos showed a much weaker teratogenic and lethal effect than would be expected on the basis of the pigment concentration. This was thought to be due to the fact that the more strongly embryotoxic orange-red pigments react during the fermentation process with amino groups in the matrix, while the weakly embryotoxic yellow pigments monascin and ankaflavin remain intact. A comparison of the effect of red mould extract with that of citrinin on chicken embryos has not been carried out. However, a concentration-dependent mutagenic action of *Monascus* extract in the *Salmonella*/hepatocyte assay has been reported in connection with the testing for citrinin.

In connection with tests on the blood pressure-lowering effect of intravenously administered GABA a similar effect has been reported after oral administration to rats of wheat fermented by *Monascus pilosus* [60].

Studies have been described on the clinical effectiveness of monacolin K-containing red mould rice in patients suffering from hypercholesterolaemia. The concentration of total cholesterol as well as those of low-density lipoprotein (LDL)-cholesterol and triglycerides were distinctly reduced after 12 weeks of daily consumption of 2.4g red mould rice (corresponding to a daily dose of 10 mg total monacolins or 5 mg monacolin K), while the level of high-density lipoprotein (HDL)-cholesterol remained significantly unchanged [61].

Toxic effects on muscles can occur during the consumption of red mould rice together with the simultaneous ingestion of drugs having CYP3A inhibitory properties. The ingestion of a red mould rice-containing product caused rhabdomyolysis in one patient treated with ciclosporin after a renal transplantation [62].

Furthermore, there exist individual reports of allergic reactions after contact with red mould rice during the manufacture of sausage products. The exposure occurred via the respiratory tract as well as through the skin and expressed itself in symptoms such as rhinitis, conjunctivitis, asthma and dermal eczema. Investigations on patients demonstrated a reaction to *Monascus purpureus* mediated through immunoglobulin E [7-9]. Systematic studies on the allergenic potential of red mould rice are not available.

4 Summary

Besides a series of known biologically active substances, red mould rice also contains other hitherto little or un-investigated constituents. Depending on the genus and the chosen production conditions variable contents of the individual constituents are to be expected. For marketed red mould rice products (e.g. loose goods, products in capsules) there exist neither data on identity and content of constituents nor product specifications and purity criteria. Basic toxicological data are not available for a scientifically-based safety evaluation of red mould rice.

A typical recommended dose as a food supplement based on red mould rice consists of 4 capsules, each of 600 mg, per day which provides an uptake of 2.4 g/day. The consumption of larger amounts is, however, feasible, e.g. through consumption of loose products.

On the basis of the known constituents there is a need for the critical evaluation of, primarily, citrinin and monacolin K as well as the pigments.

Citrinin is described as nephrotoxic and teratogenic and produces renal tumours in chronic toxicity studies in rats at a dosage of 50 mg/kg body weight/day after 60 weeks in 100% of

the test animals. Citrinin can be formed by all foodstuff-relevant *Monascus* species. Contents up to 17 mg/kg dry matter were found in commercial samples of red mould rice. In marketed food supplements 65 µg/capsule were found. At a typical dosage of 4 capsules of 600 mg/day citrinin exposure amounts to 260 µg/day or 4.3 µg/kg body weight/day (based on 60 kg body weight).

The **monacolins** that have been identified as inhibitors of cholesterol biosynthesis were found in marketed food supplements in concentrations up to about 0.6%, of which monacolin K (lovastatin) represented up to 75% of the total monacolins. Contents of up to 3.37 mg/capsule were found. The recommended consumption of up to 4 capsules/day could, in certain circumstances, approach the therapeutic dose range for lovastatin. An increased risk for muscle toxicity exists if there is a simultaneous intake of substances with CYP-inhibitory effects.

The orange-red **pigments** rubropunctatin and monascorubrin as well as the yellow pigments monascin and ankaflavin caused embryotoxic respectively teratogenic effects in chick embryos in the lower micromolar dose range. There are no data on possible teratogenic action in mammalian species. The pigment content of red mould rice is about 0.3% in dry products but varies with the culture conditions. With a typical dosage one can assume an ingestion of about 7 mg/day corresponding to 120 µg/kg body weight.

5 Final evaluation

Red mould rice contains the constituents monacolin K (lovastatin) and citrinin of particular toxicological relevance, in addition to a large number of other constituents of which the toxicological relevance is only inadequately known. Monacolin K is a potent agent in medicines for the lowering of blood cholesterol levels, it should only be administered under medical supervision. According IARC, there is limited evidence for the carcinogenicity of citrinin to experimental animals. Citrinin was adequately tested for carcinogenicity in one strain of male rats by oral administration in the diet; it produced renal tumours. In another experiment in rats, citrinin was administered in the diet after *N*-nitrosodimethylamine or *N*-(3,5-dichlorophenyl)succinimide; an increased incidence of renal tumours was observed as compared to that in animals receiving *N*-nitrosodimethylamine or *N*-(3,5-dichlorophenyl)succinimide alone [46]. Generally, basic toxicological data for a safety evaluation of red mould rice or its constituents are missing. Standards and specifications to

ensure purity and identity as well as the absence of toxic constituents are completely missing. For the above reasons red mould rice is not suitable for use as a foodstuff/food supplement.

References

[...]

The original version of this opinion can be obtained through the Scientific Office.
S. Guth, M. Kémeny, M. Habermeyer and D. Wolf:
E-mail: sklm@rhrk.uni-kl.de
