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REVIEW ARTICLE

PHARMACOLOGICALLY AND TOXICOLOGICALLY RELEVANT COMPONENTS OF *Amanita muscaria*

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Summary

Amanita muscaria, the red fly agaric, is the most famous of all Amanita. The initial history of this fascinating mushroom dates back to at least the 13th century. The use of mushrooms began in antiquity and is associated with mysticism. The collection and consumption of mushrooms and other plants containing psychoactive substances is now very popular, especially among young people who are experimenting with drugs. Ibotenic acid and muscimol are the main active ingredients of this mushroom, but other substances are likely to be involved in the psychotropic effects. *A. muscaria* also contains some other non-psychotropic substances that are interesting not only for their chemical structure but also for their biological activity. Current knowledge about chemistry, pharmacology and toxicology regarding this fungus is reviewed in this article.

Key words: Amanita muscaria; fly agaric; ibotenic acid; muscimol; stizolobic acid; muscarufine; betalains

INTRODUCTION

Amanita muscaria (L. ex Fr.) Hooker, the "fly agaric", is the best known of all the *Amanitas*. The early history of this fascinating fungus dates back at least to the 13th century. (Fly Agaric) is a mycorrhizal basidiomycete fungus and it is perhaps the most fascinating mushroom on this planet. This inedible, neurotropic mushroom (Nyberg, 1992) is native to temperate and boreal regions of the Northern Hemisphere; however, it has also been unintentionally introduced to many countries in the Southern Hemisphere, and it seems to have become a cosmopolitan species (Michelot and Melendez-Howell, 2003).

A. muscaria was originally described in Europe but recent genetic and mophological evidence suggests that there are a number of distinct species worldwide that have been lumped under this name (Geml et al., 2006). The *Amanita* and its infamous red and white spotted cap has a long history unlike any other mushroom, yet has been veiled in mystery for thousands of years. *A. muscaria* is one of the most beautiful and mysterious mushrooms (Fig. 1), showing strange chemical and pharmacological properties. Centuries have passed since observations of some of the unusual characteristics, notably, physiological effects, of these mushrooms were first documented. These characteristics include insecticidal properties, deadly toxic capabilities and the ability to cause hallucinations, narcosis and other intoxications. For over 100 years, chemists, pharmacologists and ethnobotanists, among others,

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have deployed great efforts in attempts to resolve the curious chemical composition of these mushrooms and to explain fully some of the peculiarities attributed to them. Despite these efforts, there remains much to be clarified. This striking macroscopic mushroom has become an inspiration for countless religious sects including Buddhism, Hinduism, and even Christianity. Can *A. muscaria* be an ancient Soma, about which Indian Vedas and other historical sources speak (Brough, 1971; Hajicek-Dobberstein, 1995)? In 1968 R. Gordon Wasson first proposed his ground-breaking theory identifying Soma, the hallucinogenic sacrament of the Vedas, as the *A. muscaria* mushroom (Feeney, 2010). *A. muscaria* is known to contain several toxic, psychoactive compounds and some other biologically active substances (Waser, 1967; Michelot and Melendez-Howell, 2003). An overview of hitherto isolated compounds from this biological source and their pharmacological and toxicological properties are of interest in this review.

Figure 1. Fly Agaric (*Amanita muscaria*). Photo © Ing. Barbora Kočandrlová

AMANITA MUSCARIA IS PSYCHOACTIVE MUSHROOM

Mushrooms have long been a part of the human diet, as well as knowledge of their special effects on human health and behavior. Mushroom ingestion can produce a variety of clinical pictures and psychoactive mushrooms have been and are still very popular (Soković et al., 2017). The ingestion of hallucinogenic mushrooms by Siberian tribes of the Kamchatka peninsula and by Indians of the Mexican highlands has been carried out in ritual and orgy for centuries. These mushrooms still attract a certain part of society and growing interest in hallucinogenic mushrooms among young people has become a serious medical problem of our time (Marciniak et al., 2010). Websites make it incredibly easy for people to obtain information on the morphology and appearance of mushrooms with psychoactive properties, which leads inexperienced pickers to misidentification, resulting frequently in a fatal outcome.

Psychoactive properties, especially hallucinations and other central activities, have been known for a very long time. The psychotropic effects were widely used by the inhabitants of Siberia, Kamchatka, Vikings, as well as some North American Indian tribes and the Mayan Guatemalans (Lowy, 1974; Saar, 1991). However, attempts to isolate

and identify the active components, dating back almost as far, have been only partly successful. Thus the chemical knowledge to date is not sufficient to explain fully all the pharmacological actions attributed to this mushroom. The first reported substance believed to be responsible for the central-active properties of this mushroom, was muscarine (Corrodi et al., 1957).

Although muscarine, as a potent parasympathomimetic, has a direct effect on the nervous system, it could not possibly be responsible for the reputed psychotropic actions of this mushroom. In addition, its total content in *A. muscaria* is very low. Muscarine, as the responsible agent, was further precluded when it was determined that several *Inocybe* species, containing much larger amounts of this compound, produced toxicities unlike those of *A. muscaria* and never produced the hallucinations and other central effects noted for the mushroom in question (Lurie et al., 2009).

The search for new, centrally active substances in *A. muscaria* lasted a long time (Salemink et al., 1963; Levenberg, 1964; Bowden and Drysdale, 1965), until finally the substances with the structure of isoxazole were discovered, which are considered today the main cause of psychoactive poisoning with this mushroom (Müller and Eugster, 1965). In addition to psychoactive isoxazoles, other biologically active substances have also been isolated from *A. muscaria*, which are also a part of this review article.

Hypothesis have been made that relatively high level of mannitol present in the tissues of *A. muscaria* enables more efficient transportation of these active substances into the brain and thus enhance their total activity. It may have been supported by the fact that hallucinogenic effect after *A. muscaria* consumption is greater than after ingestion of an active substance quantity which the eaten fungi dose contain (Maciejczyk and Kafarski, 2013).

CLINICAL SYMPTOMS OF POISONING BY *AMANITA MUSCARIA*

Mushroom poisonings are quite common, especially in summer and autumn, but fly agaric is a rather rare cause of these intoxications. Fly agaric is a cause of deliberate poisoning. It can be consumed for suicidal purposes or its psychedelic effect. The ingestion of these mushrooms produces a distinctive syndrome consisting of alternating phase of drowsiness and agitation with hallucinations, and sometimes with convulsions (Tupalska-Wilczyńska et al., 1997). The main toxins of this mushroom is ibotenic acid, muscimol, muscazone and muscaridine. The other bioactive substances are stizolobic and stizolobinic acids. All these compounds are responsible for diverse picture of intoxication. An analysis of patients with *A. muscaria* (and *A. pantherina*) poisoning hospitalized in the Poznan Departament of Toxicology revealed that symptoms occurred after 30 minutes to 2 hours with vomiting, hallucinations, restlessness, increased psychomotor drive and central nervous system depression. Other anticholinergic symptoms like tachycardia and increased blood pressure, mydriasis, dry and red skin were seen only in a few cases (Patocka and Frynta, 2010). Acute respiratory failure was the most dangerous symptom observed in the course of poisoning (Łukasik-Głebocka et al., 2011). In some cases, delirium has been observed (Roch and Mach, 1960; Brvar et al., 2006).

The first (excitation) period begins to show 1 to 4 hours after ingestion. The patient has a feeling of heat, tingling all over the body surface, feeling unusual lightness and desire for movement. However, the movement is soon uncoordinated, the limbs seem intangible, floating and there is a feeling of flying and dizziness. There is loss of strength, inability to keep light objects in hand, and fainting disorders. Psychic excitement increases and hallucinations appear. Motor agitation (spasms, facial grimaces) is also aggravated. Disorders of perception are manifested by black and white, yellow, blue or purple vision. Even in low light, you can see unusually sharp, close objects are getting bigger and the picture seems plastic (Michelot and Melendez-Howell, 2003).

Audible hallucinations also occur. Intoxicated person is multi-spoken, repeats words that hears in the neighborhood and has an excited mood. Gradually poisoned loses consciousness and contact with the environment. Hallucinatory dreams are experienced either with a feeling of transition to a post-mortem life or with a feeling of depersonalization. The excitement period passes into the comatose period in a few hours. The second (comatose) period lasts several hours. The patient is in a different deep coma, from which he can spontaneously awaken. The coma is accompanied by increased neuromuscular irritation and decreased blood pressure (Riedl and Vondráček, 1980).

The entire course of poisoning takes about 24 hours. Upon awakening, the intoxicated person often has the feeling of reincarnation.The affected person feels headaches, weakness and depressive states. Disorders of coordination of movement, speech and vision sometimes persist for several days. The most common cause of death is heart failure and arrest of breath. Random poisoning with red-toed redhead ends in death in only 2-5% of cases. Children and elderly people are at risk (Benjamin, 1992).

The treatment is only symptomatic, and the prognosis is usually good. Recovery was rapid and complete in all patients. After ingestion of the *A. muscaria*, it is necessary first to remove the mushrooms from the digestive tract as quickly as possible by vomiting, stomach rinsing or by the administration of activated charcoal (Benjamin, 1992). The trouble is that the affected person does not cooperate in the excitatory or comatose stage and can defend himself. If gastric lavage is achieved, saline laxatives and adsorption are applied. Sometimes the patient needs to be sedated by injection of barbiturates. In the comatose state, it is important to control respiration and circulation. If symptoms of muscarinic syndrome are observed at the onset of poisoning, small doses of atropine (0.25-0.5 mg) are administered subcutaneously (Riedl and Vondracek, 1980; Michelot and Melendez-Howell, 2003).

Satora et al. (2005) described the acute poisoning of five young persons (18-21 years of age) who ate dried fly agaric fruiting bodies at a party in order to evoke hallucinations. Visual and auditory hallucinations occurred in four of them, whereas an 18-year-old girl lost consciousness. The following morning, she went to the Clinic of Toxicology. Due to the fact that not all the active substances present in the fly agaric have been identified, and some of them have an effect after a period of latency, the patient was admitted for several days of observation during which check-up examinations were performed. After four days without any problems, she was discharged. The poisoning regressed with no organ complications. The remaining persons who had eaten the fly agaric were free from any complaints.

CHEMICAL COMPONENTS OF *AMANITA MUSCARIA*

Mushrooms contain a variety of biologically active substances and can be their valuable source. Such a mushroom is also *A. muscaria*. From the isolated compounds to date, they are in particular central active isoxazoles, choline derivatives, alkaloids, pigments, polysaccharides, and some other compounds (Michelot and Melendez-Howell, 2003). The structural formulas of the most important biologically active substances in *A. muscaria* are summarized in Fig. 2.

Isoxazoles

A. muscaria is a mushroom containing centrally effective isoxazoles, as well as *A. pantherina*. Bioactive isoxazoles were identified as ibotenic acid, muscimol and muscazole. These substances are responsible for the psychoactive effects of both mushrooms. Muscimol and ibotenic acid are most likely to be the biologically active principles of the species of concern. Muscazone is less active but other active components are suspected. However, the ratio of isoxazoles is different in both mushrooms, which explains the different clinical symptoms of poisoning by these two species of mushroons (Łukasik-Głebocka et al., 2011; Vendramin and Brvar, 2014).

Ibotenic acid

Ibotenic acid (**I**), α-amino-3-hydroxy-5-isoxazol acetic acid (CAS Number 2552-55-8), is colourless crystals, mol wt 158.11, m.p. 150–152 °C (decomposition), readily soluble in water. It is a conformationally-restricted analogue of the neurotransmitter glutamate, and due to its structural similarity to this neurotransmitter, acts as a non-selective glutamate receptor agonist (Liljefors et al., 2002). Ibotenic acid is a strong neuronal excitant, structurally related to L-glutamic acid (Krogsgaard-Larsen et al., 1980; Ji et al., 2009) and a powerful neurotoxin (Becker et al., 1999), as well as kainic acid, but ibotenic acid is approximately five times less potent than kainic acid in causing degeneration of hippocampal neuronal cell bodies (Köhler et al., 1979). Ibotenic acid is decarboxylated to muscimol in the body (Nielsen et al., 1985), and in older literature it is sometimes referred to as premuscimol (Good et al., 1965).

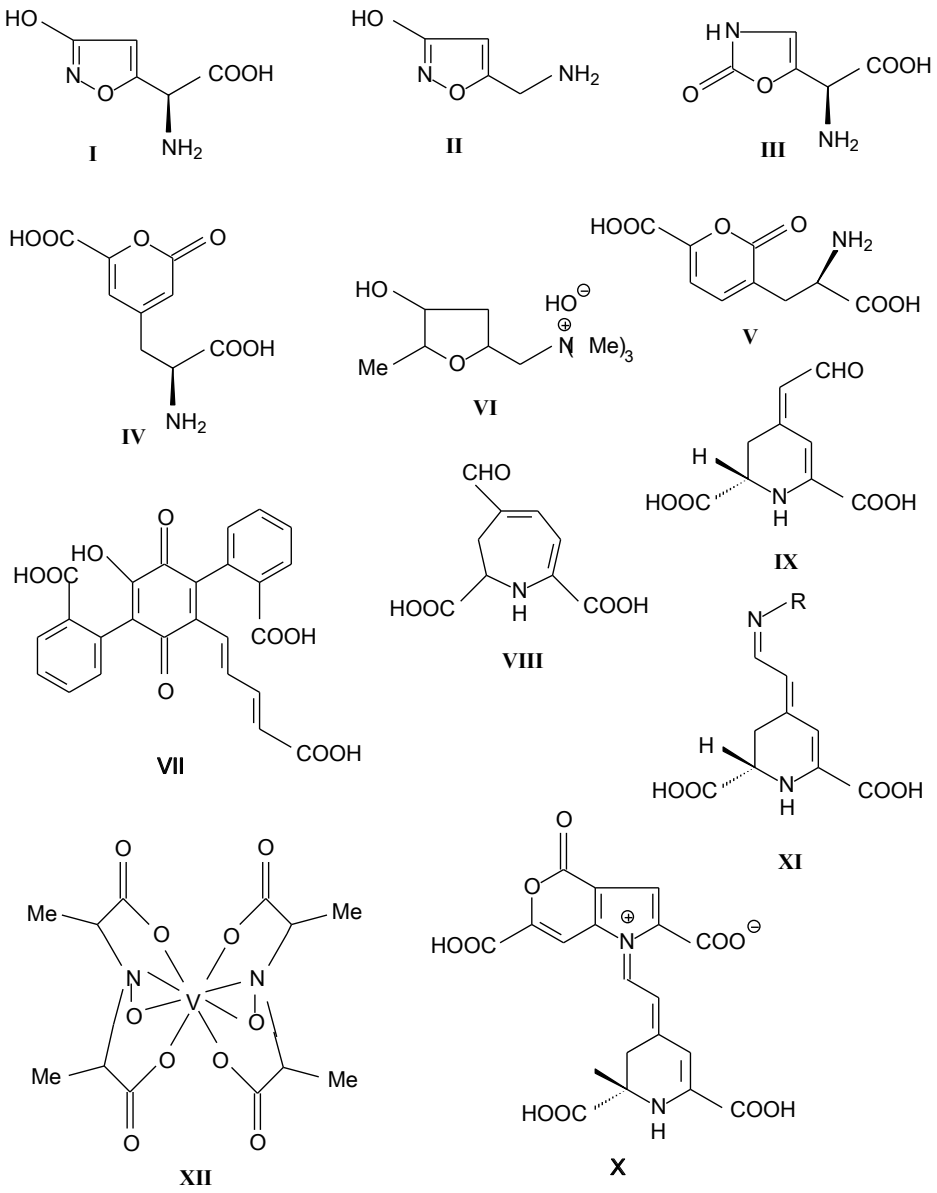

Figure 2. The structural formulas of the most important biologically active substances of *Amanita muscaria*. **I** - ibotenic acid, **II** - muscimol, **III** - muscazone, **IV** - stizolobic acid, **V** - stizolobinic acid, **VI** - muscarine, **VII** - muscarufin, **VIII** - muscaflavin, **IX** - betalamic acid, **X** - muscapurpurin, **XI** - muscaaurins (R can be ibotenic acid, stizolobic acid, aspartic acid, glutamic acid, histidine, and other aminoacids), **XII** - amavadine.

The effects of ibotenic acid on fibres of the coxal adductor muscle of the locust *Schistocerca gregari* showed two populations of glutamate receptors in insect muscle-fibres. The combined effects of glutamate and ibotenic acid suggest the existence of non-synaptic glutamate receptors with different pharmacological properties and ion specificities from those found on the excitatory postsynaptic membrane (Lea and Usherwood, 1974).

Ibotenic acid is a potent NMDA receptor agonist that elicits severe injury and even death in neurons by inducing excessive calcium influx. Injecting ibotenic acid into the cerebroventricle leads to a direct exposure of the paraventricular areas to this toxin; resulting in severe neuronal lesions in these regions, which may spread to the cerebral cortex. Intracerebral injection of ibotenic acid in animals such as rats and primates can elicit symptoms and pathological changes similar to those observed in human Alzheimer's disease (Clark et al., 2000; Zola et al., 2000; Ji et al., 2009).

Stereotactic intrahippocampal administration of ibotenic acid (5μg/μl) lesioned rats impairs cholinergic transmission, learning and memory performance that is rather related to Alzheimer's disease and thus chosen as a suitable model to understand the drug efficacy in preventing Alzheimer's disease pathophysiology. Since ibotenic acid is an agonist of glutamate, it is expected to exhibit an excitotoxic effect by altering glutamatergic receptors like NMDA receptor. The study of Kathrick et al. (2016) also displayed significant alterations in the mRNA expression of NR2A and NR2B subunits of NMDA receptors, and it is surprising to note that cholinergic receptors decreased in expression particularly α7-nAChR with increased m1AChR.

Muscimol

Muscimol (**II**), pantherine, agarin, 3-hydroxy-5-aminomethylisoxazole (CAS Number 2763-96-4), is colourless crystals, mol wt 114.10, m.p. 174-175° (waterfree) and 155-156° (hydrate), readily soluble in water. Muscimol is the most important psychoactive isoxazole from *A. muscaria* and related mushrooms, and remarkably selective agonist at ionotropic receptors for the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Johnston, 2014). GABA neurotransmission is mediated primarily by $GABA_A$ receptors. $GABA_A$ receptor is a pentameric transmembrane receptor that consists of five subunits arranged around a central pore (Fig. 3). Each subunit comprises four transmembrane domains with both the N- and C-terminus located extracellularly. The receptor sits in the membrane of its neuron, usually localized at a synapse, postsynaptically. However, some isoforms may be found extrasynaptically (Wei et al., 2003). Muscimol can activate both postsynaptic and extrasynaptic $GABA_A$ receptors (Walton et al., 2017).

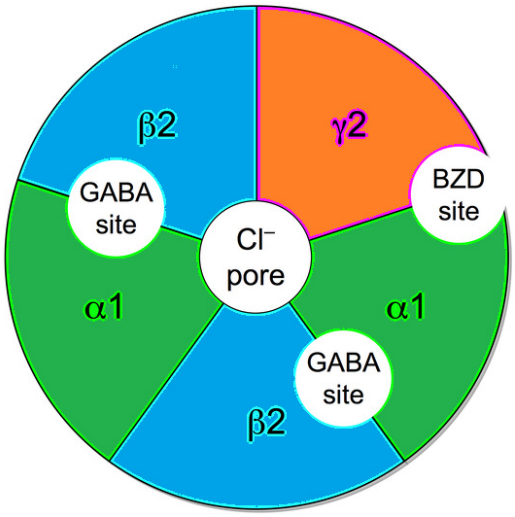

Figure 3. Schematic diagram of a $GABA_A$ receptor protein ((α1)2(β2)2(γ2)) which illustrates the five combined subunits that form the protein, the chloride (Cl^-) ion channel pore, the two GABA active binding sites at the α1 and β2 interfaces, and the benzodiazepine (BDZ) allosteric binding site (Richter et al., 2012).

Acute toxicity of muscimol was studied by German authors (Theobald et al., 1968) in mouse, rat, and rabbit, and the results are summarized in Table I. Low minimal toxic dose (TDLo) for rabbit is estimated at 10 mg/kg (intravenously) and for humans is estimated at 0.109 mg/kg (unreported route of administration).

Muscazone

Muscazone (**III**), α-amino-2,3-dihydro-2-oxooxazole-5-acetic acid (CAS Number 2255-39-2) (Fritz et al., 1965). This heterocyclic substituted glycine derivative is to date found only in the *A. muscaria* (Good et al., 1965). It is optically inactive and is, therefore, as ibotenic acid, racemic in character. It is possible that in the organism muscazone is formed from ibotenic acid. Supporting this idea is the racemic character which would be hard

to understand otherwise. But, it cannot be completely excluded that muscazone is an artefact generated during extraction and processing of ibotenic acid. In pharmacological investigations, muscazone was found to be less active than either ibotenic acid or muscimol (Catalfotmo and Eugster, 1970).

Table I. Acute toxicity of muscimol in mouse, rat, and rabbit

Organism	Test Type	Route *	Reported Dose mg/kg	Effect	Source
Mouse	LD_{50}	i.p.	2.5	Respiration dyspnea, Behavioral change in motor activity, ataxia	Theobald et al., 1968
Mouse	LD_{50}	i.v.	5.62		U.S. Army Armament Research & Development Command, Chemical Systems Laboratory, NIOSH Exchange Chemicals. Vol. NX#11824
Mouse	LD_{50}	oral	22.0		European Patent Application. Vol. #0000338
Mouse	LD_{50}	s.c.	3.8	Respiration dyspnea, Behavioral change in motor activity, ataxia	Theobald et al., 1968
Rat	LD_{50}	i.v.	4.5		Theobald et al., 1968
Rat	LD_{50}	oral	45.0		Theobald et al., 1968
Rabbit	TDLo	i.v.	10.0	Respiration dyspnea, Behavioral change in motor activity, ataxia	Theobald et al., 1968
Human	TDLo	unreported	0.109	Sleep behavioral, halllucinations, Distorted perceptions, Gastrointestinal nausea or vomiting	Theobald et al., 1968

* i.p. intraperitoneal, i.v. intravenous, s.c. subcutaneous

Non-proteinogenic 2-oxo-pyran amino acids

Small amounts of some non-proteinaceous amino acids have also been found in *A. muscaria*. More important are two heterocyclic 2-oxo-pyran amino acids, stizolobic acid and stizolobinic acid (Chilton and Ott, 1975), although these amino acids are found especially in higher plants (Hatori and Komamine, 1959; Saito and Komamine, 1976, 1978). As demonstrated by Japanese researchers Ishida and Shinozaki (1988), stizolobic and stizologinic acids are excitatory amino acids in the mammalian central neurons which bind preferably to other receptors than the NMDA-type receptor. Both amino acids reduced responses to glutamate and quisqualate in a competitive manner at the crayfish neuromuscular junction, without affecting responses to GABA (Shinozaki and Ishida, 1988).

Stizolobic and stizolobinic acids

Stizolobic acid (**IV**), (S)-4-(2-Amino-2-carboxyethyl)-2-oxo-2H-pyran-6-carboxylic acid (CAS Number 15911-87-2), mol wt 227.17, melting point 394.6 °C, boiling point 528.2 °C at 760 mm Hg, readily soluble in water. Stizolobinic acid (**V**), 3-[(2S)-2-amino-2-carboxyethyl]-2-oxo-2H-pyran-6-carboxylic acid (CAS Number 17388-96-4), mol wt 127.17. Stizolobic acid and stizolobinic acid were isolated from the poisonous mushroom, *Clitocybe acromelalga*. The co-occurrence of these compounds and acromelic acids in the same mushroom strongly suggests that the pyridone moieties of acromelic acids are derived biosynthetically from d-DOPA (Fushiya et al., 1992).

As Shinozaki and Ishida (1988) found, stizolobic acid reduced responses to glutamate and quisqualate in a competitive manner at the crayfish neuromuscular junction, without affecting responses to GABA. Excitatory junctional potentials were decreased in the presence of stizolobic or stizolobinic acid in a concentration dependent

manner. Stizolobinic acid was about 5 times less potent than stizolobic acid (Shinozaki and Ishida, 1988; Ishida and Shinozaki, 1988).

Muscarine Alkaloids

Muscarine (**VI**), (4-hydroxy-5-methyl-tetrahydrofuran-2-ylmethyl)-trimethyl-ammonium (CAS Number 300-54-9) is a toxic alkaloid found in *A. muscaria* and other fungi of the *Inocybe* species. It is the first parasympathomimetic substance ever studied and it causes profound parasympathetic activation that may end in convulsions and death. Nevertheless, muscarine is only a trace compound in the *A. muscaria* (King, 1922). Therefore this toxin with the structure of quaternary amine is only a minor component in this mushroom and the central activity of fly-agaric presumably does not involve muscarine (Catalfomo and Eugster, 1970). Muscarine is a chiral molecule which exists in eight streoisomers. Enantiopure (2S)-configured muscarines are natural and (2R)-configured muscarines are non-natural (Carnero et al., 2017). The stereoisomers of muscarine are not easy to distinguish from one another because of their similar chemical characteristics. Former investigations showed that main stereoisomer of the *A. muscaria* is (2S, 4R, 5S)-muscarine (Stadelmann et al., 1976).

Also other quaternary ammonium substances in *A. muscaria* (choline, acetylcholine, betaine, hercynine, butenyl-trimethyl ammonium salt) have been reported, but only in a trace amount and their contribution to the biological effects of *A. muscaria* is apparently negligible (Stadelmann et al., 1976). In addition, quaternary ammonium substances do not penetrate the haemato-encephalic barrier and therefore have no central effects (Pechenkin, 1966).

Pigments

The yellow and red pigments of *A. muscaria* are a complicated mixture of very labile compounds (Stintzing and Schliemann, 2007). Principal component is muscarufin, a derivative of terphenylquinone, responsible for the yellow colour of fly agaric (Depovere and Moens, 1984).The most numerous group of pigments are betalains, mainly muscaaurins, which are responsible for the characteristic red-orange colour of the caps of several of other *Amanita* species (Michelot and Melendez-Howell, 2003). Muscapurpurin (purple) and muscarubin (red-brown), whose structures are closely related to the muscaaurins, also have been isolated from *A. muscaria* (Stintzing and Schliemann, 2007).

Muscarufin

Muscarufin (**VII**), (2E,4E)-5-[3,6-dioxo-2,5-di(2-carboxyphenyl)-4-hydroxy-1,4-cyclohexadien-1-yl]-2,4-pentadienoic acid (CAS Number 602-39-1), mol wt 460.39, melting point 275.5 °C (Watt and Breyer-Brandwijk, 1962), the unusual terphenylquinone pigment of the cap of A. muscaria, was assigned to the structure by Kögel and Erxleben in 1930 (Edwards and Lewis, 1959). Muscarufin is responsible for the bright color of *A. muscaria* (Li and Oberlies, 2005).

Muscaflavin

Muscaflavin (**VIII**), (S)-4-formyl-2,3-dihydro-1H-azepine-2,7-dicarboxylate (CAS Number 12624-18-9), mol wt 209.16 is yellow pigment isolated from fly agaric (Stintzing and Schliemann, 2007). Muscaflavin and similar pigments have also been identified in *Hygrocybe* mushrooms. The presence of muscaflavin pigments is a remarkable chemotaxonomical character (Ardenne et al., 1974). Bresinsky and Kronawitter (1986) detected muscaflavins in 42 out of 53 studied *Hygrocybe* species. Muscaflavin was subjected to screening of pharmacological activities such as antimicrobial, anti-inflammatory, antianalgesic and wound healing activities. Chittaragi (2016) showed a significant activity of muscaflavin against more human and plant pathogenic bacteria and fungi and the pharmacological properties are more or less similar to those of standard drugs.

Betalains

Betalains are a class of red and yellow indole-derived pigments found in plants and in some higher order fungi (Gengatharan et al., 2015).The name "betalain" comes from the Latin name of the common beet (*Beta vulgaris*),

from which betalains were first extracted. Betalains represent a new class of dietary cationized antioxidants (Kanner et al., 2001). Betalains are divided into two classes, the betaxanthins and betacyanins, which produce yellow to orange or violet colours, respectively. These pigments are derived from the betalamic acid (**IX**) (4-(2-oxoethylidene)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid, CASS Number 18766-66-0). Betalamic acid condenses with imino compounds, or amino acids/derivatives to form variety of betacyanins (violet) and betaxanthins (yellow), respectively. The mixture or suppression of these chemical components can determine what color prevails in a mushrooms.

Muscapurpurin (**X**) and numerous muscaaurins (**XI**) have been found in *Amanita muscaria*, in which betalamic acid is condensed, for example, with glutamine, valine, proline, histidine, asparagine, but also with ibotenic acid or stizolobic acid (Michelot and Melendez-Howell, 2003).

Betalains replace the anthocyanins in flowers and fruits of plants of most families of the *Caryophyllales*. Unexpectedly, they were also found in some higher fungi. Whereas the anthocyanin-analogous functions of betalains in flower and fruit colouration are obvious, their role in fungi remains obscure. Betalains have attracted workers in applied fields because of their use for food colouring and their antioxidant and radical scavenging properties for protection against certain oxidative stress-related disorders (Strack et al., 2003).

Polysaccharides

Japanese scientists Kiho et al. (1992) isolated β-D-glucan (AM-ASN) from the alkaline extract of the fruiting bodies of *A. muscaria*. Its estimated molecular weight was 95,000 in this alkaline solution and 260,000 in a neutral solution. The branches in the glucan were primarily single, (1→6)-linked D-glucopyranosyl groups, two for every seven residues in the (1→3)-linked main chain. AM-ASN exhibited significant antitumor activity against sarcoma 180 in mice, and a mixture of AM-ASN with mitomycin C was more effective against the tumor than mitomycin C only.

Somewhat different water-insoluble but alkali-soluble glucan (AM-APP) was isolated from the alkaline extract of the fruiting bodies of *A. muscaria* by Kiho et al. (1994). The results of chemical and spectroscopic investigations indicate that AM-APP is a linear (1→3)-α-*D*-glucan with a molecular weigh estimated by gel chromatography of about 42000. Its carboxymethylated product (AM-APP-CM) showed potent antitumor activity against sarcoma 180 in mice, although the native polysaccharide (AM-APP) had little effect. The distribution of carboxymethyl groups in the molecule was analyzed by gas chromatography and mass spectrometry. The degree of substitution of carboxymethyl groups was 0.95 and the substituents were located at O-2, at O-4, at O-6, at O-2 and O-6, and at O-4 and O-6 on glucose.

A fucomannogalactan (FMG-Am) was isolated from *A. muscaria* fruiting bodies by Ruthes et al. (2013), like β-*D*-glucan (βGLC-Am). FMG-Am was shown to be a heterogalactan formed by a (1→6)-linked α-D-galactopy ranosyl main chain partially substituted at O-2 mainly by α-L-fucopyranose and a minor proportion of β-*D*-mannopyranose non-reducing end units. βGLC-Am was identified as a (1→3)-linked β-*D*-glucan partially substituted at O-6 by mono- and a few oligosaccharide side chains. Both the homo- and heteropolysaccharide were evaluated for their anti-inflammatory and antinociceptive potential, and they produced potent inhibition of inflammatory pain.

Metallic elements

As any other biota, mushrooms also need certain quantities of many minerals for balanced growth and reproduction. Mycelia mobilize and up-take minerals from substratum and translocate them to fruit-bodies. As a result of these, nacrofungi fruit bodies (carpophores) can be enriched in many metallic elements (Borovička and Řanda, 2007; Arce et al., 2008; Boorovička et al., 2010, 2011). A. muscaria also has the ability to accumulate metalloid elements from the soil in its body (Lipka and Falandysz, 2017). Amanita muscaria accumulates higher concentration levels of Cd, Cu, Hg and Mn in caps, but the content of these metals is strongly dependent on their amount in the soil or substrate on which the fly agaric is growing (Mędyk et al., 2017).

The toadstool A. muscaria is relatively rich in vanadium and contains up 120 ppm vanadium per g dry weight (Bayer and Kneifel, 1972). Vanadium in A. muscaria is present in the form of a complex organo-vanadium pale-blue color compound called amavadine (**XII**) (Berry et al., 1999, Garner et al. 2000). It is a complex of tetravalent

vanadium with two molecules of N-hydroxyimino-2,2'-dipropionic acid. The measured vanadium concentration in the toadstools was 400 times higher than its concentration in the surrounding vegetation, irrespective of the age of the fruit and the vanadium content of the forest soil, and reached values of 32-192 mg/kg of dry weight of the fruit (Meisch et al., 1979). Although the role of vanadium and its remarkable complex compounds in the toadstool remains unclear, in any case, amavadine is an extremely interesting bio-organic molecule (Hubregtse et al., 2007; Patočka, 2010). The selective enrichment of metals in nature is of great interest. Therefore, the study of amavadine and its still unknown ligand N-hydroxyimino-2,2'-dipropionic acid is very significant.

CONCLUSION

The fly agaric is a remarkable mushroom in many respects. It has a long history of hallucinogenic mushroom. Ibotenic acid and muscimol are the main active ingredients, but other substances found in this fungus are likely to be involved in the psychotropic effects.The use of the mushroom started in ancient times and is connected with mysticism. Current knowledge on the chemistry, toxicology, and biology relating to this mushroom is reviewed in this time, together with distinctive features concerning this unique species.

DISCLOSURE STATEMENT

The authors proclaim that they have no competing interests.

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