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Analysis of hallucinogenic constituents in *Amanita* mushrooms circulated in Japan

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Abstract

The constituents of seven mushrooms sold as *Amanita muscaria* or *Amanita pantherina* (five *A. muscaria* and two *A. pantherina*) and four ''extracts purported to contain *A. muscaria*'' products that are currently circulated in Japan were determined. All mushroom samples were identified as *A. muscaria* or *A. pantherina* by macroscopic and microscopic observation. The dissociative constituents, ibotenic acid (IBO) and muscimol (MUS), were extracted with 70% methanol twice and determined by gas chromatography/mass spectrometry. The IBO (as the hydrate)/MUS contents were in the range of <10–2845 ppm/46–1052 ppm in the cap of *A. muscaria* and 188–269 ppm/1554–1880 ppm in the cap of *A. pantherina*. In the caps, these compounds had a tendency to be more concentrated in the flesh than in the cuticle. On the other hand, the IBO/MUS contents in the stem were far lower than in the caps. In the ''extracts purported to contain *A. muscaria*'' products, IBO/MUS were detected below the lower limit of calibration curve (<10 ppm/<25 ppm) or not detected. However, these samples contained other psychoactive compounds, such as psychoactive tryptamines (5-methoxy-*N,N*-diisopropyltryptamine and 5-methoxy-*N,N*-dimethyltryptamine), reversible monoamine oxidase inhibitors (harmine and harmaline) and tropane alkaloids (atropine and scopolamine), which were not quantified. This is the first report of the chemical analysis of *Amanita* mushrooms that are circulated in the drug market.
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Keywords: *Amanita muscaria*; *Amanita pantherina*; Ibotenic acid; Muscimol; GC/MS

1. Introduction

Amanita muscaria, known by the name ''fly agaric,'' is a psychotropic mushroom that is traditionally used for religious or recreational purposes in Siberia, North-East Asia and India [1,2]. This mushroom has been recently reported as being used as an intoxicant in several countries [3,4]. In Japan, not only *A. muscaria* but also *Amanita pantherina*, a dissociative mushroom similar to *A. muscaria*, are circulated via the Internet or in ''smoke shops.'' In addition, ''extracts purported to contain *A. muscaria*'' products are also in circulation. In 2003, the Japan Poison Information Center received four cases of intoxication caused by ''extracts purported to contain *A. muscaria*'' products [5].

A. muscaria and *A. pantherina* contain two dissociative constituents, ibotenic acid (IBO) and muscimol (MUS) (Fig. 1). IBO is a powerful agonist of the *N*-methyl-D-aspartic acid (NMDA) receptor [6]. Nielsen et al. reported that IBO was converted by decarboxylation to MUS in mouse brain homogenates [7]. MUS, which acts as a potent $GABA_A$ agonist [8], has more potent neuropharmacological activity [9–11].

There are several reports on the contents of IBO/MUS in *A. muscaria* and *A. pantherina* in natural products. Determination of IBO/MUS in mushrooms was performed using paper chromatography [12], high performance liquid chromatography [13–15], single-column chromatography [16] and gas chromatography/mass spectrometry (GC/MS) [17]. However, an analysis of samples that are circulated in the drug market has not yet been reported. In this study, we report on the chemical analysis of *Amanita* mushrooms and ''extracts purported to contain *A. muscaria*'' products that are circulated in Japan.

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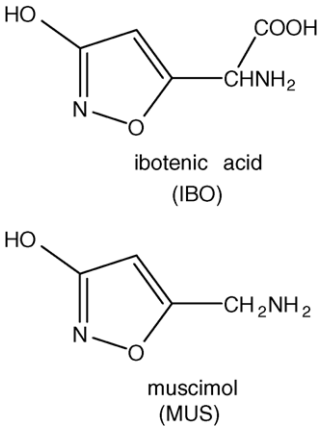

Fig. 1. Chemical structures of ibotenic acid (IBO) and muscimol (MUS).

2. Experimental

2.1. Samples and chemicals

Eleven samples were used in this study; seven were dried mushrooms sold as *A. muscaria* or *A. pantherina* (five *A. muscaria* (see Fig. 2A) and two *A. pantherina* (see Fig. 2B)), and four were "extracts purported to contain *A. muscaria*" products (see Fig. 2C and D). These samples were obtained from "smoke shops" or via the Internet in Japan.

IBO hydrate was obtained from Biosearch Technologies (Novato, CA, USA). MUS was obtained from Sigma (St. Louis, MO, USA). *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 10% trimethylchlorosilane (TMCS) was obtained from Pierce Chemical Co. (Rockford, IL, USA). All other chemicals used in the experiments were of analytical grade.

2.2. Optical microscopic examination

A microscopic examination was performed using the method reported by Walting [18]. A 2.5% potassium hydroxide solution was used as a swelling agent and to return the dried tissues to their previous state. Small pieces of the gills were cut from the fruit-body and mounted on a glass slide, while directly in the 2.5% potassium hydroxide solution. After covering with a glass cover-slip, it was tapped with a rubber-tipped pencil to separate the tissues from each other. Observation was carried out using a biological microscope. The sizes of the spores were measured with Image J (Wayne Rasband, National Institute of Health, USA) and an average of 12 spores was measured for each sample.

Melzer's staining reaction was used for detecting amyloid, pseudoamyloid, or nonamyloid of the spores. Staining was performed using the method described in Ref. [19]. Small pieces of the gills were cut from the fruit-body and mounted on a glass slide, while directly in the 2.8% ammonia solution. After washing by water, the samples were swelled in the Melzer's reagent (composition as follows: 0.5 g of iodine, 1.5 g of potassium iodide, 22 g of chloral hydrate and 20 g of water) and were observed under a biological microscope.

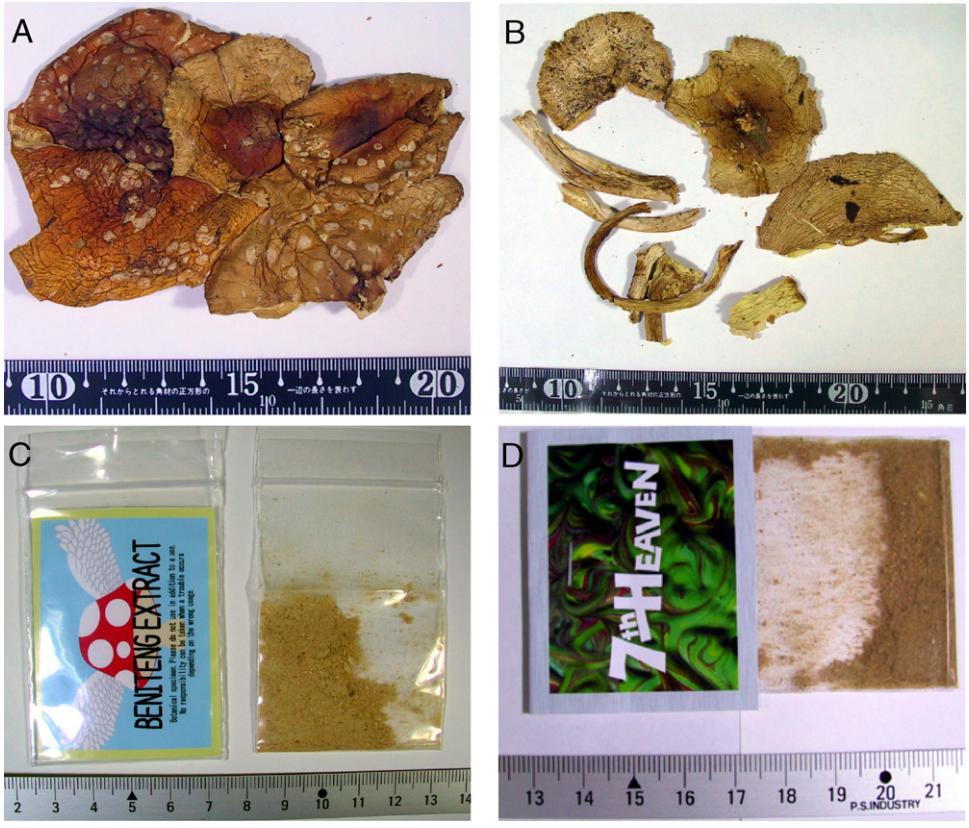

Fig. 2. Representative photographs of the samples: (A) *A. muscaria*; (B) *A. pantherina*; (C and D) "extracts purported to contain *A. muscaria*" products.

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