Poisonous mushrooms as a resource of biomedical application

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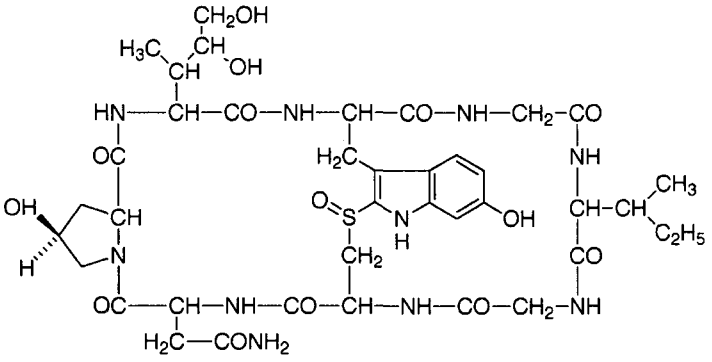
Abstract

Poisonous mushrooms have attracted much attention of scientists because of the unique chemical structures and the remarkable biological properties of their toxic components. A wide range of compounds has been characterized as toxic principles, and some of them have widened the horizons of chemical and biological research. Therefore, poisonous mushrooms can serve for a resource of biomedical application. We will describe the recent progress of such aspect of poisonous mushrooms.

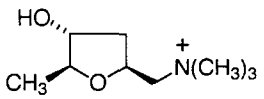
Introduction

Mushroom poisonings account for approximately 70% of natural poisonings. The symptoms of mushroom poisonings range from a weak gastrointestinal disorder to, in the worst case, death. Because of their remarkable biological properties, toxins responsible for mushroom poisonings have been extensively studied both in chemically and biologically. The chemistry and biology of the mushroom toxins have been reviewed1. They are not only toxic but also useful lead compounds for research tools or clinical therapeutic agents. For example, a-amanitin (**1**) and phalloidin (**2**), deadly poisonous peptide toxins from *Amanita phalloides*, have been used for years in various cell biological research, and muscarine (**3**), a neurotoxic alkaloid originally isolated from *Amanita muscaria*, played a crucial role in establishing receptor subtypes

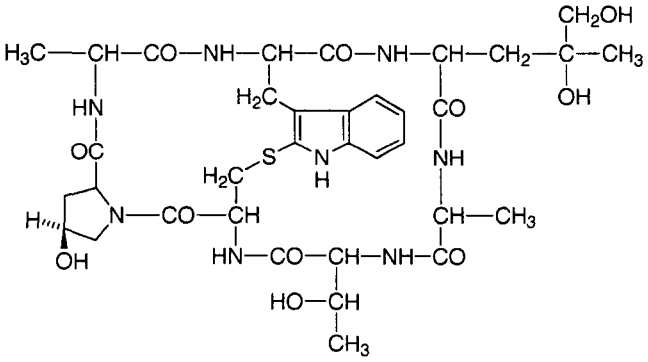
of acetylcholine receptors. Moreover, an analog of illudin S (**4**), a toxic principle of *Omphalotus illudens* and *Omphalotus japonicus*, was recently found to be a promising anti-tumor agen: and is currently in phase *II* clinical trials2. Therefore, chemical and biological studies on mushroom toxins are still extensively going on. We will describe the recent progress of such aspect of poisonous mushrooms, which we have been involved in recent years.



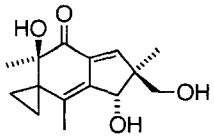
α-amanitin **1**



muscarine **3**



phalloidin **2**



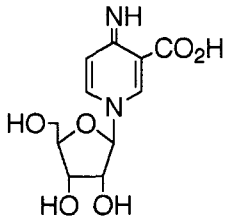
illudin S **4**

Toxins from *Clitocybe acromelalga*

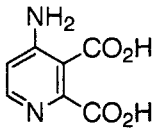
*Clitocybe acromelalga*is specifically distributed in certain areas of Japan and Korea. The symptoms are quite unique and among the most painful of those associated with mushroom poisonings. Eating this mushroom causes sever pain and a reddish edema in fingers and toes, conditions similar to those produced by acromelalgia and erythromelalgia, which persist for a month or so. A latent period of several days is another feature of this poisoning. Although

many cases have been reported in the past century, it is rarely fatal. Due to its specific distribution, it was once thought to be a local disease. This mushroom grows gregariously in bamboo grass under zelkova trees and has the typical appearance of the genus *Clitocybe* with medium-sized, brown-orange, funnel-like cap and decurent gills. Because of these features, it is often mistaken for the edible varieties of *Clitocybe gilbba*and *Lactarius hatsutake*. It also closely resembles *Lepista inversa*, an edible mushroom found in Europe.

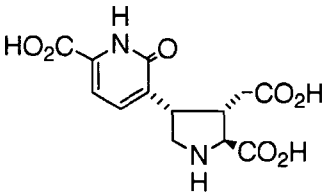
These characteristic biological properties prompted us to study the chemical constituents of the fungus. Fractionation monitored by lethal toxicity in mice led to the isolation of a variety of compounds. Clitidine (**5**)3is the most abundant toxic component. 4-Aminoquinolinic acid (**6**)4 shows no toxicity, but may be a biosynthetic precursor of clitidine. Acromelic acids A (**7**) and B (**8**) are quite minor components,5 and of particularly interest due to highly potent neurotoxic activity in central nervous systems. The new amino acids **9** also show neurotoxicity and may be a possible intermediate for biosynthesis of acromelic acids.6



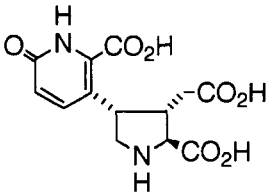
clitidine **5**



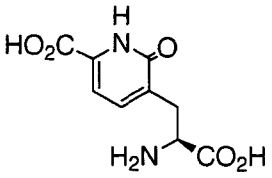
4-aminoquinolinic acid **6**



acromelic acid A **7**



acromelic acid B **8**



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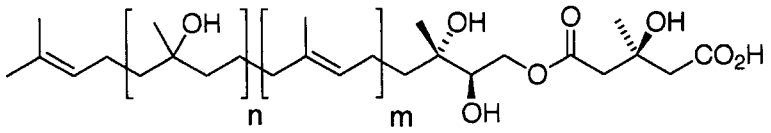
The chemistry and biology of these toxins are unique, but they did not prove to be responsible for the "erythromelalgia poisoning" of this mushroom. That is because of the lack of suitable bioassay. Initially we attempted to reproduce the human symptoms in laboratory animals, but all attempts resulted in failure. Recently, however, an animal model of this poisoning has been established in rats.7 A severe symptom resembling erythromelalgia in human appeared 3 days after feeding the niacin-free/tryptophan-limited diet to rats. This special diet is important for this assay and may be related to the biosynthesis of clitidine (**5**) and 4-aminoquinolinic acid (**6**), the tryptophan-niacin pathway.8 These results would contribute to elucidate the mechanism of toxicity of this mushroom poisoning.

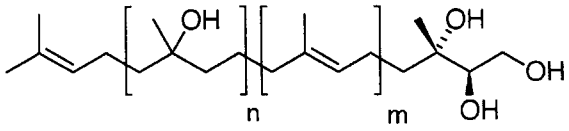
This mushroom poisoning has been reported only in Japan, but the same poisoning was first reported in Europe, France recently.9 Clinical features of erythromelalgia were observed in 7 cases seen over 3 years. All patients had eaten the same mushroom species, gathered in the same French alpine valley, which was identified to be *Clitocybe amoenolens*, originally described in Morocco.

Neurotoxic oligoisoprenoids of *Gymnopilus spectabilis*

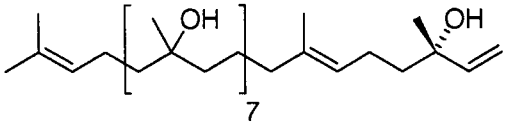
*Gymnopilus spectabilis*has long been known as a hallucinogenic mushroom in Japan. The odd behavior due to the intoxication of this mushroom is recorded in the Japanese classical literature "Konjaku Monogatari" of the 12th century. There have been, however, disputes concerning the hallucinogenic property and the presence of psilocybin, a hallucinogenic alkaloid in Psilocybe species, in this fungus. Although both the Japanese and the American specimens are reported to be hallucinogenic, psilocybin is found only in the latter; whereas the European specimen is not known to be hallucinogenic, nor it contains psilocybin. Assuming that this mushroom should contain the substance(s) acting on the centralnervous system as judged from the symptoms reported, we examined the neurobiological property of the Japanese specimen using the new-born rat spinal cord preparation. As a consequence, we found that the extract exhibited potent depolarizing activity, i.e., neurotoxicity. The activity turned out to reside in gymnopilin (**10**),10 which was originally obtained as bitter principle of this mushroom.11Besides this, gymnoprenol (**11**)12 and gymnopilene (**12**)13, not tasting bitter and being possible biogenetic precursors of gymnopilin, have also been obtained.



gymnopilin **10**

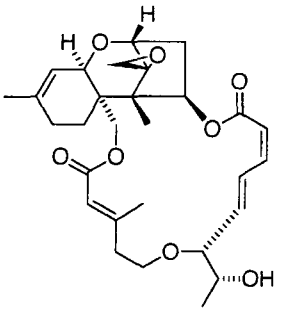


gymnoprenol **11**

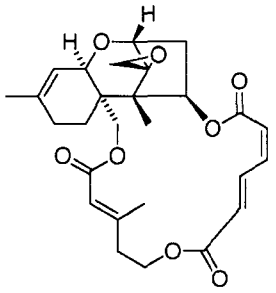


gymnopilene **12**

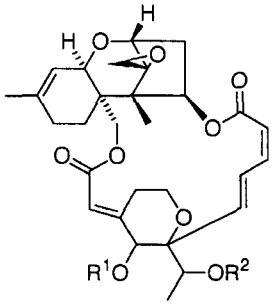
Although the gross structure of gymnopilin was already elucidated, it was obtained as a mixture of congeners consisting of various number of isoprene unit (m and n), and the mixture had never been separatedinto its components. In order to find which compound(s) are the real active principles, we further purified the gymnopilin mixture with HPLC, and then evaluated the activity of each component. Interestingly, the activity of the purified components was found to vary depending on the number of double bond and hydroxy group contained. Concerning the number of double bond (m), compounds having only one (m=1) was inactive; whereas those having two or three (m=2 or 3) were active, with the latter being more active than the former. Moreover, even within the compounds with m=2, the activity varied depending on the number of hydroxy group (n); it increased in the following order: n=7 < n=6 < n=5. These oligoisoprenoids seemingly interact with cell membrane in some way since the activity had been unaffected by any inhibitor of neuronal receptors.



roridin E **13**



verrucarin J **14**



satratoxin H **15**: $R^1 = R^2 = H$
satratoxin H 12',13'-diacetate **16**: $R^1 = R^2 = Ac$
satratoxin H 12'-acetate **17**: $R^1 = Ac$, $R^2 = H$
satratoxin H 13'-acetate **18**: $R^1 = H$, $R^2 = Ac$

Mycotoxins from *Podostroma cornu-damae*

In 1999, a lethal poisoning caused by the mushroom *Podostroma cornu-damae*happened in Niigata prefecture in Japan. Five people drank cups of sake containing some pieces of the fruit body and ate the soaked mushroom. The amount is estimated to be 1 g per person. One of them died after two days of ingestion. In 2000, the poisoning happened again in Gunma prefecture in Japan. A couple ate the fried mushroom and one of them died the following day. Poisoning attributable to the fungus has been known to have occurred six times in Japan. This mushroom grows in Japan and Java, but it is so rare fungus that these accidents have occurred very seldom.

Some of the following symptoms are observed in this poisoning: gastrointestinal disorder, erroneous perception, decrease in the number of leukocytes and thrombocytes, deciduous skin of face, loss of hair, and atrophy of the cerebellum which brings about a speech impediment and voluntary movement problems. These characteristic properties prompted us to study the toxic components of the mushroom.

From the culture broth of this fungus, roridin E (**13**), verrucarin J (**14**) and satratoxin H (**15**) were isolated as monitored by lethal effect on mice.14 On the other hand, satratoxin H (**15**), satratoxin H 12',13-diacetate (**16**), satratoxin H 12'-acetate (**17**) and satratoxin H 13'-acetate (**18**) were isolated from the fruit bodies. All these macrocyclic tricothecenes except for verrucarin J showed a lethal effect on mice by at least 0.5 mg per capita.14

Most of these macrocyclic tricothecenes have been previously isolated from several fungi.15Accordingly, some of the symptoms from the *Podostroma* poisoning are similar to those from the food poisoning contaminated with fungi producing these mycotoxins.16Interestingly,

these macrocyclic mycotoxins were also found as antimalarial principles in the plant extracts from *Ficus fistulosa* and *Rhaphidophora decursiva*.17

Toxic metalloendopeptidase from *Chlorophyllum molybdites*

The toadstool *Chlorophyllum molybdites* (one of the previous names, *Lepiota morganii*) is common in the tropics, and its poisoning has been known for more than 100 years. In recent years, however, this fungus have become widely distributed in Japan probably due to the global climate change, which resulted in the increase of this mushroom poisoning in this country.18 Although the poisoning is rarely fatal, the symptoms are severe; that is, vomiting, diarrhea, chill, intestinal pain, stomachache, and in some cases convulsions .

Early studies on the toxic components of this fungus suggested the presence of some cholinergic compounds. However, Eilers and Nelson later demonstrated that the toxic principle was proteinaceous, and reported partial purification and characterization of a toxic protein.19 Isolation of non-toxic alkaloids was also reported recently.

The recent increase of this mushroom poisoning prompted us to reinvestigate the toxic components of this fungus. Guided by lethal effect on mice, we have isolated a toxic protein named molybdophyllysin.20 SDS-PAGE analysis of the protein showed a single band and the molecular weight was estimated to be 23kDa. This result is in contrast to that previously reported by Eilers and Nelson.19They reported that the toxic component of this fungus is a polymeric protein with a molecular weight in excess of 400kDa, and the protein comprise some monomers with molecular weight of 40 ~ 60kDa. Molybdophyllysin is a relatively small protein, which could correspond to the Eilers' "monomer".

This toxic protein was found to be an enzyme as well. Sequence analysis of the tryptic digests showed this protein to be highly homologous to the metalloendopeptidases (MEPs) obtained from edible mushrooms, such as *Grifola frondosa* and *Pleurotus ostreatus*. Furthermore, molybdophyllysin is suggested to be a member of the deuterolysin family in the MEPs because of the presence of zinc binding motif aspzincin. Indeed, molybdophyllysin exhibited proteolytic activity on azocasein and azocollagen, and this enzymatic activity was kept under 60 C with the optimum pH at 7.0, and was inhibited both by 1,10-phenanthroline and *N*-bromosuccinimide.

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