AMATOXINS IN EDIBLE MUSHROOMS

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1. Introduction

The toxic cyclopeptides in *Amanita phalloides* mushrooms weigh about 0.05% of the fresh tissue. Two-thirds of this weight are phallotoxins, mostly the acidic compounds phallacidin and phallisacin [1,2], the rest being amatoxins, predominantly α-amanitin. A combination of chromatographic procedures [3] permits the spectrophotometric determination of up to 11 toxic compounds [4] in single mushrooms. The sensitivity of this analysis is limited to 0.1 μg of each toxin, this being sufficient, however, to determine 5 different amatoxins in *A. phalloides* and 3 amatoxins in *A. verna*. In *A. virosa* and in *Galerina marginata* only α-amanitin was found. No amatoxins were detected in either *A. pantherina* or *A. citrina* by this procedure.

The sensitivity of the determination of amatoxins was much improved by a radioimmunoassay [5]. This permits the detection of as little as 50 pg of amatoxins. Serum was raised against an albumin derivative of β-amanitin. However, the antibodies obtained cross-reacted fully with the other naturally occurring amatoxins [4], i.e. α-, γ- and ε-amanitin, amanin, and the nontoxic amanullin. There was no cross-reaction with the phallotoxins. Hence the radioimmunoassay provides a specific and sensitive method of determining the total amount of all the amatoxins present in mushroom tissues.

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2. Materials and methods

All mushrooms of the Amanita species, except *A. rubescens* and *A. pantherina*, were harvested near Trento (Italy) in 1974. The others were collected near Heidelberg in 1975. The tissues (20–80 g) were homogenized in a Star mixer together with a two-fold volume of methanol and then kept under stirring at 70°C for 1 h. After centrifugation the supernatant was evaporated in vacuo. The residues were thoroughly dried, washed with dry ether and dissolved in 2 ml of water. The amatoxins were extracted from these solutions with *n*-butanol, evaporated and redissolved in water. Diluted samples from the latter solutions were used in the radioimmunoassay according to [5]. Inhibition of RNA-polymerase B was measured according to [7].

3. Results

The results compiled in the table indicate that all species investigated in this study contain amatoxins at least in minimal amounts (table 1).

4. Discussion

Originally the aim of this investigation was to confirm the values of amatoxin concentrations which had been determined in the green and white Amanita species by other means. For *A. phalloides* the value obtained here (4.4 mg/25 g fresh tissue) agrees very

Table 1

Species	By radioimmunoassay	By inhibition of RNA-polymerase B
Amanita phalloides	17.5×10^4	–
Amanita virosa	25.7×10^4	–
Amanita pantherina	16.3	11.0
Amanita citrina	0.6	1.4
Amanita muscaria	1.1	0.9
Amanita rubescens	7.5	6.5
Agaricus silvaticus	8.5	9.1
Boletus edulis	< 0.2	3.1
Cantharellus cibarius	5.5	4.8

Concentration of amatoxins [nanogram/g fresh tissue] of various toxic, inedible, or edible Amanita species, and of some other highly estimated species of edible mushrooms as determined by the two independent methods.

well with that measured earlier (4.1 to 4.5 mg/25 g fresh tissue) [1,3]. For the white species, *A. virosa* (6.4 mg/25 g fresh tissue) the value measured by radioimmunoassay was 2.5-fold higher. However, the mushrooms were of different sources. Moreover it is not excluded that the newly detected toxin in *A. virosa,* viroidin [3] may give a cross-reaction with the amanitin-induced antibodies.

The yellow species *A. citrina* (or *mappa*) and according to some authors also *A. pantherina,* were for a long time regarded as deadly poisonous. An examination in Wieland's laboratories [6] had revealed the presence of bufotenin in *A. citrina,* not however, of amatoxins and phallotoxins. *A. pantherina* on the other hand is believed to contain muscarin, like *A. muscaria,* but to be likewise devoid of amatoxins. The radioimmunoassay now indicated that *A. pantherina* as well as *A. citrina* contain amatoxins. Compared to the green or the white species of Amanita, however, the concentration of the amatoxins in *A. pantherina* is 10^4 times lower, that in *A. citrina* more than 10^5 times.

These data prompted us to examine more Amanita species including an edible one. Indeed we found a small amount of amatoxins in *A. muscaria* and a relatively high amount of amatoxins in *A. rubescens,* which is a highly estimated edible mushroom. The toxin concentration in *A. rubescens,* however, is far from being threatening to consumers. A meal of 1 kg of these mushrooms contains only 0.1% of the supposed lethal dose for an adult human.

Since amatoxins were determined without exception in all Amanita species, we extended these investigations to mushrooms of species other than Amanita, preferably those, which are widely consumed. Indeed, we found the toxins in all mushrooms investigated. As the table indicates, *Agaricus silvaticus* (Champignon) contains a relatively high amount of amatoxins, and considerable amounts of amatoxins were determined in *Cantharallus cibarius* (Pfifferling), and *Boletus edulis* (Steinpilz).

To make sure that the effect in the radioimmunoassay was not occasioned by any unknown component, which by chance crossreacted with the amanitin-induced antibodies, we examined the mushroom extracts for their capacity to inhibit calf thymus RNA-polymerase B according to Cochet-Meilhac et al. [7]. A similar approach for the determination of the amatoxins in *A. verna* was recently published by Preston et al. [8]. The results obtained (table 1) are in good agreement with those of the radioimmunoassay. Since the two assays evaluate different features of the amatoxins, namely affinity to antibodies and affinity to an enzyme, we conclude that the effects observed are really caused by amatoxins.

The fact that all mushrooms investigated up to now contain at least small amounts of amatoxins, suggests that these cyclopeptides might be of some significance for the development of basidiomycetes. It is possible that the concentration of 1–10 ng/g tissue measured in the nontoxic species represents the norm; the deadly toxic species of Amanita as well as those of

Galerina described by Tyler et al. [9] may have acquired an overproduction of these compounds balanced during their development by some hitherto undetermined compartmentation of the toxins. In the nontoxic species the amount of toxins present can provide a 5×10^{-9} M concentration of amanitin in the whole tissue. Provided the sensitivity against amanitin of mushroom RNA-polymerase is comparable to that of the calf-thymus enzyme, the amatoxins could play a regulatory rôle in the protein synthesis of mushrooms.

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