Inorganic Bromide in Higher Fungi

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A total of 138 samples of higher fungi, representing 35 species (15 belonging to the genus *Amanita*), were analysed for the trace element bromine using spectrophotometric and gas chromatographic methods. High concentrations of bromine, up to 100 mg/kg on dry weight, were encountered in Amanitaceae, especially in members of the subsections Phalloideae and Eu-Amanita, whereas in other fungi the mean value for this element was only 3,3 mg/kg.

Some bromine-accumulating Amanitas also contained unusually high chlorine levels. A maximum concentration of 3 percent on dry weight was measured in *A. phalloides*.

It could be demonstrated that most, if not all of bromine and chlorine was present in the fungi as ionisable inorganic salts. Two organochlorine metabolites, previously reported in some mushrooms, were absent in *A. phalloides* and *A. muscaria*.

Introduction

During the mid seventies it was discovered that many species of higher fungi are capable of accumulating trace elements, among which are several potentially toxic metals.

Reviews on this subject were recently published by Seeger [1] and by the present author [2].

There is still a lack of data on the occurrence of non-metals, although Byrne and Ravnik [3] reported the results of a survey on bromine, iodine, and selenium concentrations in a small number of mushrooms. Their values for the latter element have been amply confirmed [4, 5], but the observations made by these authors on the bromine content of fungi have not yet been subjected to critical examination.

Upon analysing 21 samples involving 12 species, Byrne and Ravnik found high levels (up to 200 mg/kg on dry weight) in four members of the genus *Amanita*, *i.e. A. muscaria*, *A. phalloides*, *A. rubescens* and *A. pantherina*, and very little in other fungi. Although it cannot yet be concluded that all Amanitaceae are bromine accumulators, it seems that there are members of this genus that have a special affinity for the element. For example, carpophores of *A. muscaria*, the fly agaric, gathered at two different sites, contained more than 100 mg/kg of bromine.

Considering that the above-mentioned authors investigated a small number of fungi, which were all from Slovenia, Yugoslavia, it seemed to us worthwhile to make a survey of the bromine content of a much larger number of species, gathered in various European countries with special emphasis on Amanitaceae. In addition, the chemical form in which bromine occurs in fungi, and the possible affinity of bromine-accumulating species to the other halogens fluorine, chlorine, and iodine, was also investigated.

Experimental

The mushrooms studied were gathered between 1975 and 1982 at various sites in the German Federal Republic, Switzerland, France, and the Netherlands.

The species were identified using determinative manuals of the mycologists Moser [6], Kühner-Romagnesi [7] and Singer [8]. Depending on size and availability, several specimens were collected at each site. The carpophores were cleaned manually, lyophilised and ground to a fine powder, which was stored in glass bottles at room temperature.

Total bromine was determined by alkaline treatment, ashing at 500 °C and conversion of the resulting inorganic bromide to tetrabromofuchsine which was determined by colorimetry according to Hunter and Goldspink [9]. Bromide ion in the mushroom tissues was determined directly (without ashing) by gas chromatography of 2-bromoethanol formed by reaction with ethylene oxide in an acid sample slurry as described by Stijve [10].

Chlorine and fluorine were estimated according to standard methods [11], whereas iodine was deter-

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mined by electron capture gas chromatography after conversion to iodobutanone [12].

Results and Discussion

A total of 138 samples representing 35 higher fungi were analysed. The fifteen *Amanita* species contained far more bromine than mushrooms belonging to other genera (Tables I and II). Only *A. strobiliformis* had a bromine level close to the average value of 2.3 mg/kg on dry matter reported for non-accumulating species by Byrne and Ravnik [3].

The bromine-accumulating ability of *A. phalloides, A. muscaria, A. pantherina*, and *A. rubescens* was amply confirmed. It is interesting to note that *A. verna* contains on the average as much bromine as *A. phalloides*, indicating the close relationship between the two species, already suggested by Seeger and Stijve [13].

The concentration range in both Phalloideae is narrower than that measured in *A. muscaria* or *A. pantherina*, where differences of a factor 7 between the highest and the lowest level were observed.

The sections Vaginaria and Mappae contained only moderate bromine levels.

Comparative analyses of inorganic bromide and total bromine yielded virtually the same results (Table III), suggesting that all bromine is present as inorganic water-soluble salts.

The inorganic bromide content of humus samples collected at the sites where the fungi were gathered was very low, *i.e.* on the average 0.35 mg/kg. This suggests an accumulating factor for *Amanita phalloides* of not less than 74/0.35 = 210!

Among the species listed in Table II, *Boletus edulis* seems to be a special case. Some samples contained very little bromide, but in others appreciable levels were encountered. It should be pointed out that for studies on the bromine content of this species, one should use carpophores gathered in the

Table I. Bromine concentrations in fungi belonging to the genus *Amanita*.

Species	Number of samples	Range mg/kg	Mean value mg/kg dry weight
Section Vaginaria			
Amanitopsis vaginata (Bull. ex Fr.) Vitt.	6	4.7–24	10.9
A. crocea (Quel.) Singer	3	4.9–14	9.3
A. inaurata Secr.	1	–	19
Section Eu-Amanita			
A. pantherina (DC ex Fr.) Secr.	6	8–57	26
A. muscaria (L. ex Fr.) Hooker	11	14–103	37
Section Phalloideae			
A. phalloides (Vaill. ex Fr.) Secr.	26	40–113	74
A. verna Bull.	5	58–87	74
A. virosa Lam ex Secr.	1	–	13
Section Mappae			
A. citrina (Schff.) S. F. Gray	15	6–29	16
A. citrina (Schff.) S. F. Gray var. *alba* Gill	2	8–17	12
A. porphyrea (A. & S. ex Fr.) Schummel	1	–	10
Section Validae			
A. spissa (Fr.) Kummer	5	13–22	18
A. spissa var. *excelsa* Fr.	1	–	35
A. rubescens (Pers. ex Fr.) S. F. Gray	8	15–91	54
A. aspera (Fr.) Hooker	1	–	11
Section Lepidella			
A. strobiliformis (Paul. ex Vitt.) Bertillon	2	2–3	2.5

Table II. Bromine concentrations in other fungi in mg/kg on dry weight.

Species	Number of samples	Range mg/kg	Mean value mg/kg
Boletus edulis	12	1.4–43	8.8
Suillus luteus	5	1.4–3.7	2.3
Agaricus edulis	2	2.9–3.3	3.1
Agaricus augustus	1	–	8.2
Macrolepiota procera	3	1.2–4.0	2.6
Cystoderma carcharias	1	–	3.1
Cantharellus cibarius	2	1.1–2.5	1.8
Rozites caperata	1	–	2.4
Lycoperdon gemmatum	2	2.3–5.6	4.0
Langermannia gigantea	1	–	3.8
Lactarius deliciosus	3	1.8–3.6	2.7
Hygrophorus coccineus	2	1 –1.4	1.2
Laccaria laccata	2	2.6–2.8	2.7
Panaeolina foenisecii	2	–	< 1
Psathyrella stipatissima	1	–	< 1
Stropharia aeruginosa	1	–	5.5
Sarcodon imbricatum	1	–	1.1
Peziza vesiculosa	1	–	2.6
Auricularia polytricha	2	–	< 1

woods. Commercially available dried boletes often have been fumigated with methyl bromide against insect infestation, and consequently contain hundreds of mg/kg of inorganic bromide [14].

When the analyses were extended to other halogens, it was found that the two most potent bromide-accumulators, *A. phalloides* and *A. verna* also had a marked affinity for chlorine: these species contained as much as 2.5–3 percent Cl^- (dry weight), which is considerably more than the average concentration in mushrooms (0.1 percent). Both fungi also contained some iodine and fluorine, but the levels for these elements were not significantly different from those measured in other species (Table IV).

Considering the unusually high chlorine content, and the reports of organochlorine compounds in some fungi [15–17], it was considered worth investigating whether some of it would be in the covalent form.

Table III. Comparative analyses of inorganic and total bromide in several mushrooms.

Species	Origin	Inorganic Br′ as determined by GLC	Total bromide as determined by colorimetry
Boletus edulis	Market Vevey, CH, 1980	43	38
Amanita phalloides	Emmental, CH, 1979	64	67
A. phalloides	Bern, CH, 1979	84	87
A. citrina	Bern, CH, 1979	13	11
A. muscaria	Saanen, CH, 1979	23	22
A. rubescens	Gamburg, GFR, 1977	65	62
Amanitopsis vaginata	Puidoux, CH, 1977	5	6

All values in mg/kg on dry weight.

Table IV. Halogen concentrations in *Amanita* species and in some other fungi.

Species	Origin	Cl	Br	I	F
Amanita muscaria	Lally, CH, 1979	5 200	16	0.69	13.5
A. phalloides	Emmental, CH, 1979	25 400	64	0.30	16.8
A. phalloides	Monthey, CH, 1975	29 600	91	0.60	19.5
A. phalloides	Bern, CH, 1979	29 500	86	0.75	17.2
A. verna	Gamburg, GFR, 1977	26 500	74	0.12	8.3
A. citrina	Bern, CH, 1979	18 100	12	0.24	10.8
A. citrina	Mont Pélerin, CH, 1975	16 600	23	0.36	15.5
Boletus edulis	Market Vevey, CH, 1980	5 300	43	2.35	12.4
Boletus edulis	Bex, CH, 1976	3 000	12	0.50	6.4
Peziza vesiculosa	Vevey, CH, 1978	6 100	2.6	0.63	18

All values in mg/kg on dry weight.

A. solitaria (Bull. ex Fr.) Merat, also known as *A. cokeri* (Gilb. and Kuehn) Gilb., has a chloride ion concentration of 0.2 percent and contains about 300 mg/kg of *trans*-2-amino-5-chloro-4-hexenoic acid. Since this mushroom is related to *A. phalloides*, we investigated the possible presence of the chloroamino acid in the latter species using the method described by Chilton and Tsou [15], but found none.

Tetrachloro-1,4-dimethoxybenzene is a known metabolic product of several Basidiomycetes, which has not only been found in mycelium [16], but also in mature sporocarps of *Agaricus bisporus* [17]. Upon subjecting *A. phalloides* and *A. muscaria* samples to entrainment distillation with continuous extraction of the distillate with toluene [18], followed by electron capture gas chromatography of the extract, we measured concentrations at or about the limit of detection of 5 mcg/kg (ppb). No other volatile chlorinated hydrocarbons were observed either.

Although the presence of trace amounts of unidentified organochlorine compounds in the *Amanita* species cannot be excluded, it is highly likely that virtually all chlorine is present as inorganic ionisable chloride. In fact, when analysing *A. phalloides*, the difference between total chlorine, determined volumetrically after ashing, and inorganic chloride, estimated similarly in an aqueous extract, was of the same order of magnitude as the fluctuations observed between duplicate analyses by either method.

[1] R. Seeger, Deutsche Apotheker Zeitung **37,** 1835 (1982).
[2] T. Stijve, Coolia **23,** 92 (1980).
[3] A. R. Byrne and V. Ravnik, Sci. Total Environm. **6,** 65 (1976).
[4] T. Stijve, J. M. Diserens, and M. Pletikosa, Z. Lebensm. Unters. Forsch. **164,** 201 (1977).
[5] S. Piepponen, H. Liukkonen, Lilja, and Taina Kuusi, Z. Lebensm. Unters. Forsch. **177,** 257 (1983).
[6] M. Moser, Die Röhrlinge und Blätterpilze, in Kleine Kryptogamenflora, **Bd. IIb/2,** Basidiomyceten II. Teil (W. Gams, ed.), VEB Gustav Fischer Verlag, Jena 1967.
[7] R. Kühner and H. Romagnesi, Flore Analytique des Champignons Supérieurs, Ed. Lechavalier, Paris 1953.
[8] R. Singer, The Agaricales in Modern Taxonomy, A. R. Ganter Verlag, Vaduz 1975.
[9] G. Hunter and A. G. Goldspink, Analyst **79,** 467 (1954).
[10] T. Stijve, Deutsche Lebensm. Rundschau **77,** 99 (1981).
[11] W. Horwitz (ed.), Official Methods of Analysis of the Association of Official Analytical Chemists, 13th Edition, Washington DC, 1980.
[12] H. J. Bakker, Journal of the A.O.A.C. **60,** 1307 (1977).
[13] R. Seeger and T. Stijve, Z. Naturforsch. **34c,** 330 (1979).
[14] T. Stijve, Deutsche Lebensm. Rundschau **73,** 321 (1977).
[15] W. S. Chilton and G. Tsou, Phytochemistry **11,** 2853 (1972).
[16] J. F. Grove, Phytochemistry **20,** 2021 (1981).
[17] H. Buss and L. Zimmer, Chemosphere **3,** 123 (1974).
[18] T. Stijve and E. Cardinale, Mitt. Gebiete Lebensm. Unters. Hyg. **64,** 415 (1973).