Amanitin Content and Toxicity of *Amanita verna* Bull.

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The α-, β-, and γ-amanitin content of 11 samples of *Amanita verna* Bull., collected during 1975–1978 in Germany and Switzerland, has been determined by high performance thin-layer chromatography (HPTLC) of crude methanolic extracts. The toxicity (i. v. LD_{50} for mice of defatted, lyophilized, methanolic extracts) of 3 samples has been compared with that of *A. phalloides* from the same site of collection.

The amanitin content of *A. verna* ranged from 2250 to 4570 mg/kg dry weight; the fungi contained almost as much β- as α-amanitin, whereas the γ-amanitin content amounted to about 12% of the total amanitin. *A. verna* contained less amanitin (65% on the average) than *A. phalloides* from the same collection site, but it was not significantly less toxic, since the phallotoxins contributed to the toxicity of either species in our tests.

Introduction

Amanita verna Bull. belongs to the "deadly Amanitas". Morphologically it largely resembles *A. phalloides* (Vaill ex Fr.) Secr.; however, it is white, generally smaller, and its volva surrounds the stalk more closely. As a thermophilic species it is rarely encountered in Central Europe. It is occasionally found in France, but it occurs preferentially in Southern Europe and North Africa. Furthermore, it grows in Japan and Australia, and it is widespread in the temperate zone of North America [1–8].

A. verna contains the amatoxins α-amanitin [9], β-amanitin [9], and γ-amanitin [10], the phallotoxins phallisacin [10], phallacidin [10], and phalloidin [11], as well as a labile haemolysin [12]. The high toxicity of the death cap fungi is mainly due to their amatoxin content [13].

Apparently, the amanitin concentration in *A. verna* of American origin is subject to considerable variation: while a maximum content of 1.7 mg amanitin per g dry weight has been reported, occasionally amanitin-free [9, 14–17] and non-toxic [9] single fruit-bodies have been described. The data available for Germany [10, 11] are based on the examination of only one carpophore each.

The purpose of the present investigation was to obtain, for a larger number of samples, a more precise knowledge of the amanitin content and toxicity of the *A. verna* Bull. occurring in Central Europe.

Materials and Methods

The fungi were collected in 1975 and 1977 at two places in Unterfranken, FRG, and in 1978 at one site in Switzerland (Table I). If not otherwise specified, each sample consisted of several fruit-bodies; since the collection sites were small and the species rare, they may stem from the same mycelium. For comparative purposes, also *A. phalloides* specimens were collected at about the same time at the afore-mentioned sites in Germany. The fungi were cleaned mechanically, lyophilized, ground to fine powder on a crushing mill and kept in tight glass bottles protected from light until use.

The amanitin content was determined by high performance thin-layer chromatography (HPTLC) of crude methanolic extracts on 10 × 10 cm silicagel 60 plates for nano-TLC (Merck No 5628), with

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pure α- [18], β-, and γ-amanitin [19] as reference compounds. After development in chloroform-methanol-acetic acid-water 75-33-5-7.5 v/v, the chromatograms were sprayed with cinnamaldehyde-hydrochloric acid and the visualized amanitin spots were quantified densitometrically as described elsewhere [20].

For toxicity determinations 1 g mushroom powder was extracted 3 times for one hour at room temperature with 15 ml methanol (analytical grade, Merck). The combined extracts were concentrated under reduced pressure and freeze dried. The lyophilized material was taken up in 25 ml isotonic, phosphate-buffered saline (PBS: 4 parts 0.85% sodium chloride and 1 part 0.15 M sodium phosphate buffer (Merck) pH 7.0 with 10^{-3} M sodium azide (Merck)). To remove the fat, this liquid was shaken twice with the same volume of diethylether (DAB 7, Merck). The aqueous phase was again lyophilized and dissolved in 25 ml water. The test animals were female NMRI mice (Zentralinstitut für Versuchstiere, Hannover, FRG) weighing 20 – 30 g; they were fed on Altromin R chow and water ad libitum. For the determination of the LD_{50} the extracts were diluted with PBS and 0.1 ml per 10 g body weight was injected intravenously. The animals were observed for two weeks. The LD_{50} was calculated as described by Litchfield and Wilcoxon [21].

Results

The amanitin content (Table I) of all 11 samples was between 2250 and 4570 mg/kg dry weight. On the average, the mushrooms contained almost as much β- as α-amanitin. The γ-amanitin content amounted to about 12% of the total amanitin.

Table I. Amanitin concentrations [mg/kg dry wt.] in *Amanita verna* carpophores collected near Gamburg/Nordbaden and Würzburg/Unterfranken, FRG during 1975 and 1977 and Zürich, Switzerland 1978.

Sample Nr.	Site and date of collection		Fresh weight of sample	α-Amanitin	β-Amanitin	γ-Amanitin	Total Amanitin content
1	Gamburg	20. 7. 1975	15.4 ***	1550	1700	260	3510
2	Gamburg	27. 7. 1975	15.8 **	1450	1750	320	3520
3	Gamburg	15. 9. 1975	33.5 *	1550	1350	300	3200
4	Gamburg	16. 9. 1975	161.1	1750	1250	450	3450
5	Würzburg [23]	16. 9. 1975	40.8	1700	1100	560	3360
6	Gamburg	20. 9. 1975	220.6	1500	1100	340	2940
7	Gamburg	24. 9. 1975	20.0	1300	850	260	2410
8	Gamburg	28. 9. 1975	5.5 *	1200	1000	220	2420
9	Gamburg	4. 9. 1977	66.3	1750	1400	510	3660
10	Gamburg	11. 9. 1977	98.9	1850	2150	570	4570
11	Zürich [24]	7. 1978	*	1500	500	250	2250
			$\bar{x} \pm$ S. E.	1550 ± 60	1290 ± 140	370 ± 40	3210 ± 200

* 1 Specimen.

** 4 Very young carpophores, velum universale still closed.

*** Very young specimens.

Table II. Amanitin content and toxicity (for mice i. v.) of *A. phalloides* and *A. verna* collected at the same place at about the same time.

Origin	Species					
	Amanita phalloides			*Amanita verna*		
	Amanitin conc. [mg/kg dry wt.]	LD_{50} (confid. lim.) [mg mushroom powder per kg body weight]	Cause of death	Amanitin conc. [mg/kg dry wt.]	LD_{50} (confid. lim.) [mg mushroom powder per kg body weight]	Cause of death
Gamburg 16. 9. 1975/21. 9. 1975	7390	138 (90 – 211)	Amatoxins	3450	154 (92 – 259)	Amatoxins
Würzburg 16. 9. 1975	5490	162 (122 – 215)	Amatoxins	3360	170 (125 – 231)	Phallotoxins Amatoxins
Gamburg 4. 9. 1977/11. 9. 1977	5240	140 (102 – 192)	Phallotoxins Amatoxins	4570	152 (96 – 240)	Amatoxins

Interestingly, the total amanitin content of all samples varied only within a factor of two. Among the individual amanitins, only the β-compound varied more strongly, namely by a factor of 4.3.

In the 8 samples gathered during 1975 the fluctuation range is even more narrow, *i. e.* from 1 to 1.5. The total amanitin content of very young mushrooms was not different from that of the other samples, but they seemed to contain relatively more β-amanitin.

On the average, *A. verna* contained only 65% of the amanitin of *A. phalloides* specimens collected at the same site at about the same time. However, as to the toxicity, the two species did not differ significantly (Table II).

Discussion

The amanitin concentrations of all our *A. verna* samples were higher than those reported by others (Table III). This might mean that our samples are richer in amanitin, but the difference may at least be partially due to a more exhaustive extraction method and to the better performance of the HPTLC technique. The latter is superior to the traditional thin-layer chromatography, since it gives a better separation and has a higher sensitivity. Where amatoxins were determined in purified extracts, possible losses during purification should also be considered.

Unlike various American authors [9, 14 – 17] we have not found any amanitin-free *A. verna* samples. Since we examined mostly pooled samples, it is not impossible, that isolated amanitin-free carpophores were included. However, this is not likely in view of the small differences in the amanitin content of the various samples. A few scattered results of earlier authors suggested that the amanitin concentration of *A. verna* might be lower than that of *A. phalloides*. We could definitely confirm this by comparative examination of both species collected at about the same time at the same site in Unterfranken.

That methanolic extracts of *A. verna* were not less toxic than those of *A. phalloides* can be explained by the fact that, in part of the samples of either species, the phallotoxins contributed to the toxicity (Table II). It cannot yet be decided whether these compounds also play a role in cases of death cap poisoning in man, because no data are available on the absorption of phallotoxins.

European *A. verna* Bull. is also considered to be a subspecies of *A. phalloides* [22]. Corroborating evidence for this assumption was obtained during HPTLC of the methanolic extracts: the chromatographic patterns of both species were indistinguishable, not only with regard to the amanitin spots, but also because of the identical coextractives.

A. verna Bull. may even be regarded as an inferior variety of the green death cap: it needs obviously a warmer climate for its development; its fruit-bodies remain smaller and produce no colorants and also less amanitins.

Table III. Amatoxin concentrations of *A. verna* as reported by various authors.

Amatoxin concentration [mg/g dry weight]	Material	Author	Reference No.
0 – 1.7	10 samples, USA	Tyler *et al.*, 1966	[14]
0.45 *	1 small specimen, Southern Germany	Faulstich *et al.*, 1974	[10]
0 – 1.05	10 specimens, Florida, USA	Preston *et al.*, 1975	[16]
0 – traces *	4 specimens, Delaware, USA	Yocum and Simons, 1977	[17]
2.41 – 4.57	11 samples, Southern Germany and Switzerland	Seeger and Stijve, present study	

* Determination performed on purified extracts.

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