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SHORT COMMUNICATIONS

TOXIN CONTENT, PHALLOTOXIN AND AMATOXIN COMPOSITION OF *AMANITA PHALLOIDES* TISSUES

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F. ENJALBERT, C. GALLION, F. JEHL and H. MONTEIL. Toxin content, phallotoxin and amatoxin composition of *Amanita phalloides* tissues. *Toxicon* **31**, 803–807, 1993.—The toxin content and composition of *Amanita phalloides* tissues were determined in three specimens at two carpophore development stages. The carpophore was subdivided into six parts, namely, the cap, gills, ring, stipe, volva and bulb. To our knowledge, this is the first report of such an investigation on the ring and the bulb. Substantial differences in the tissue toxin content were revealed. The ring displayed a very high amount of toxins, whereas the bulb had the lowest toxin content. Compositional differences in relation to the nature of the tissue were also noted. The highest amatoxin content was found in the ring, gills and cap, whereas the bulb and volva were the richest in phallotoxins. Furthermore, variability in the toxin composition was observed. The differences in the distribution of individual toxins in the tissues might be related to the carpophore developmental stage.

Amanita phalloides (Fr.) Link. is the fungus responsible for the great mortality from mushroom poisoning. This species contains two classes of hepatotoxic peptides: amatoxins and phallotoxins. The amatoxins are dicyclic octapeptides which act as inhibitors of the nuclear RNA polymerases of most eukariotic organisms. The main amatoxins are β-amanitin (β-Ama) (acidic toxin), α- and γ-amanitin (α- and γ-Ama) (neutral amatoxins) (reviewed in WIELAND and FAULSTICH, 1983; WIELAND, 1986). Phallotoxins are not taken up by intestinal cells and do not play a role in *Amanita phalloides* poisoning. After i.p. administration, they inhibit the conversion of actin-F into actin-G and disturb the dynamic equilibrium of these forms necessary for cell functions (FAULSTICH and MÜNTER, 1986; WIELAND, 1987). Phallotoxins are dicyclic heptapeptides which are divided into two groups. Phalloidin (PHD), phallisin (PHS), phalloin (PHN) are the main neutral phallotoxins and phallacidin (PCD) and phallisacin (PSC) are the main representatives of the acidic phallotoxins (reviewed in WIELAND and FAULSTICH, 1983; WIELAND, 1986). The amounts of amatoxins and phallotoxins in *A. phalloides* carpophore have been determined by different analytical methods (reviewed in WIELAND, 1986). Various parts of the mushroom have also been investigated (ANDARY *et al.*, 1977). The results revealed differences in the concentration of the carpophore tissues. When cap and stipe were analysed separately, the amatoxin content in the cap was found to be three times higher

than in the stipe (ANDARY *et al.*, 1977). In a more detailed analysis by BODENMULLER *et al.*, 1980, the highest amount of amatoxins was found in the gills, while the volva contained only 9% of the total amount of toxins; a similar amatoxin content was observed in the cap and the stipe. A qualitative and quantitative evaluation of the phallotoxins in the three organs of the carpophore have also been performed, and the volva was found to be the most toxic organ (ENJALBERT *et al.*, 1989).

In a previous paper (ENJALBERT *et al.*, 1992) we described the measurement of the amounts of the main amatoxins (α- β- and γ-Ama) and phallotoxins (PCD, PSC, PHD, PHN and PHS) in a single *Amanita phalloides* specimen using a sensitive and reproducible high-performance liquid chromatographic (HPLC) assay. Recently, we found that there were variations in the toxin content of mushrooms collected in the same year. Furthermore, the toxic peptides were not equally distributed in the carpophore. The major components were β- and α-Ama, PCD, PSC and PHD, whereas the quantity of γ-Ama, PHS and PHN was very small (unpublished data).

In the present report, the HPLC method was used to analyse the toxin content in six different parts of the mushroom. This fine subdivision of the carpophore revealed marked differences in the toxin content of the tissues. Based on the toxin composition, the cap, ring and gills could be distinguished from the stipe, volva and bulb.

Three *A. phalloides* specimens were collected in different sites of Alsace-Lorraine in France, in October 1990. One young carpophore (weighing 12.4 g), with the cap completely free from the universal veil but the gills concealed by the partial veil, was designated specimen *a*. Two fully developed carpophores (weighing 7.9 and 47.3 g, respectively) displaying a completely expanded cap and a typically membranous ring, left by the partial veil, surrounding the stipe, represented specimens *b* and *c*. The different tissues constituting the carpophore, namely, the cap (flesh and cuticle), gills, the partial veil in specimen *a* or the ring in specimens *b* and *c*, volva and stipe were separated. We use the word 'ring' to indicate the intact or broken partial veil. The stipe base corresponds to the swollen part surrounded by the volva and was called the bulb; the thin, straight part was taken as the stipe. The average weight of the different tissues from the three carpophore specimens was found to be 8.67 ± 7.6 g, 4.64 ± 6.3 g, 0.03 ± 0.01 g, 5.54 ± 5.6 g, 0.88 ± 0.8 g, and 2.79 ± 1.6 g, for the cap, gills, ring, stipe, volva and bulb, respectively. Extraction of the toxins from each fresh tissue of specimens *a*, *b* and *c* was carried out according to a published procedure (ENJALBERT *et al.*, 1992). A 20-μl aliquot of the crude extract was analysed by a reversed-phase high-performance liquid chromatographic method (ENJALBERT *et al.*, 1992) that allows the simultaneous determination of amatoxins (α- β- and γ-Ama) and phallotoxins (PCD, PSC, PHD, PHS and PHN). Measurements were made in triplicate for each tissue extract and the mean value was determined.

The total toxin content and phallotoxin and amatoxin distribution in the various tissues of the three *A. phalloides* specimens are reported in Table 1. The highest amount of toxin was found in the ring then in the gills and volva, while the toxin content of the cap, stipe and mainly the bulb was low. The results indicate that the variation range of the toxin concentration in the different tissues was particularly large. Based on the differences between the amounts of the toxins in the tissues three groups can be formed: first, the ring, then the gills, volva and cap and finally the stipe and bulb. Furthermore, this study shows the toxin levels in two parts of the mushroom not investigated until now, namely, the ring and the bulb. The very high amount of toxins in the ring suggests that this tissue could be either the site of toxin metabolism or a storage compartment. The variation in toxin content in the stalk according to sampling site should be mentioned. Histologically, the

TABLE 1. PHALLOTOXIN AND AMATOXIN CONTENTS IN THE VARIOUS TISSUES AND WHOLE CARPOPHORE OF THREE *Amanita phalloides* SPECIMENS

Tissue	Total toxin content (mg/g)* Mean S.D.	Phallotoxins (P) (%) Mean S.D.	Amatoxins (A) (%) Mean S.D.	P/A
Ring	6.497 ± 4.16	54.2 ± 0.2	45.8 ± 0.3	1.2
Gills	1.632 ± 0.83	55.8 ± 3.6	44.2 ± 3.7	1.3
Volva	1.387 ± 0.69	78.6 ± 2.9	21.4 ± 2.9	3.8
Cap	0.830 ± 0.36	57.0 ± 3.7	43.0 ± 3.7	1.3
Stipe	0.454 ± 0.21	67.8 ± 6.2	32.2 ± 6.2	2.2
Bulb	0.253 ± 0.37	80.8 ± 4.6	19.2 ± 4.7	4.3
Carpophore	0.781 ± 0.37	62.1 ± 1.5	37.9 ± 1.6	1.6

*The data are expressed in terms of mg total toxin/g wet weight of tissue.

stalk is not homogeneous; the base, which does not display the fibrous structure of the median part, had the lowest toxin content.

The phallotoxin content was higher than the amatoxin content in all tissues investigated (Table 1). However, there was a slight predominance of phallotoxins over the amatoxins in the ring, gills and cap, whereas the stipe and mainly the volva and bulb contained from twofold to fourfold more phallotoxins than amatoxins. Our results are consistent with several reports describing a high amatoxin concentration in the cap and gills (ANDARY *et al.*, 1977; BODENMULLER *et al.*, 1980) as well as a large amount of phallotoxin in the volva (WIELAND, 1986; ENJALBERT *et al.*, 1989). Indeed, the amatoxin content was not equally distributed in the tissues; the levels of α- and β-Ama in the stipe were higher than those in the bulb but lower than those in the cap. A similar distribution of amatoxins has been found in *A. subalpiacea* Murill. specimens; a gradient of amatoxins from the volva to the cap has been reported (PRESTON *et al.*, 1982). Our results also confirm other data reporting a higher toxin content in the gills than in the cap (FAULSTICH *et al.*, 1982; WIELAND, 1986). This distribution pattern is not a specific property of *A. phalloides* toxins; the analysis of different carpophore gills has revealed a high concentration of mercury and cadmium (SEEGER, 1992). Furthermore, the gills of *Cortinarius orellanus* (Fr.) Fr. contained a high level of orellanine (S. RAPIOR, Thesis, University of Montpellier I, 1988).

Table 2 shows the distribution of PCD and PSC (acidic phallotoxins) PHN (neutral phallotoxin), β-Ama (acidic amatoxin) and α-Ama (neutral amatoxin) in the various tissues. These components are the main representatives of each toxin family; the sum of the average values of these five toxins accounted for 91.7–93.4% of the toxin content. Our results point out the compositional differences between the ring and volva which are the remnants of the partial veil and universal veil, respectively. The phallotoxin and amatoxin composition of the ring was similar to that of the cap and gills and very different from that of the volva which displayed a high level of PHD. This study also shows differences between the toxin composition of the stipe and bulb. The neutral phallotoxin (PHD), like the two amatoxins, was not equally distributed between these two tissues; the bulb was richer in PHD than the stipe. The levels of the two acidic phallotoxins (PCD and PSC) were found to be nearly similar in the stipe and the bulb. It is noteworthy that the toxin composition of the bulb was very close to that of the volva. Overall, the results show variations in the tissue distribution of the main amatoxins and phallotoxins in the carpophore.

Table 2 also indicates the relative standard deviation (R.S.D.) of the amounts of toxins

TABLE 2. DISTRIBUTION OF THE MAIN PHALLOTOXINS AND AMATOXINS IN THE VARIOUS TISSUES AND WHOLE CARPOPHORE OF *Amanita phalloides* ($n = 3$)

Tissue	PCD* Mean (%)	PCD* R.S.D.† (%)	PSC Mean (%)	PSC R.S.D. (%)	PHD Mean (%)	PHD R.S.D. (%)	β-Ama Mean (%)	β-Ama R.S.D. (%)	α-Ama Mean (%)	α-Ama R.S.D. (%)
Cap	22.8	19.3	13.0	59.2	17.3	55.5	22.0	10.9	17.0	14.1
Gills	21.6	20.8	12.8	64.8	17.6	60.2	22.8	11.4	17.2	12.8
Ring	23.4	14.9	12.8	67.2	15.1	57.0	23.8	10.1	16.9	8.9
Stipe	29.3	17.7	17.2	66.9	17.8	42.7	15.7	29.3	13.4	11.9
Bulb	32.0	15.6	17.4	62.1	26.9	28.6	8.4	42.9	8.8	10.2
Volva	28.8	13.5	17.0	65.3	27.2	36.0	10.2	24.5	8.5	8.2
Carpophore	24.9	14.9	14.4	61.8	15.1	52.2	19.1	6.3	15.1	12.6
a‡	22.5		4.3		29.8		19.9		13.0	
Carpophore *b*	29.2		17.4		12.2		17.7		16.8	
c	23.1		17.4		13.9		19.7		15.1	

*The toxins are abbreviated as follows: PCD, phallacidin; PSC, phallisacin; PHD, phalloidin; β-Ama, β-amanitin; α-Ama, α-amanitin.

†R.S.D., relative standard deviation.

‡*a*, young specimen; *b* and *c*, two fully developed specimens.

in each tissue and the carpophore showing the variability of the toxin composition. These variations in the distribution of the toxins in the tissues are probably related to the fact that the specimens were collected in the three sites and represented two developmental stages of the carpophore. Comparison of the toxin composition of the three carpophores shows that the PSC and PHD contents of specimen *a* (young carpophore) were different from those of specimens *b* and *c* (fully developed carpophores) (Table 2). These differences might represent developmental variations. On the one hand, with regard to the carpophore, the R.S.D. were distinctly higher for PSC and PHD than for PCD, β- and α-Ama. On the other hand, the R.S.D. of the PCD, PSC and α-Ama concentrations in all the tissues were found to be quite close to those of the carpophore. In contrast, the amounts of β-Ama varied in the stipe, volva and mainly the bulb, while the cap, ring and gills, like the carpophore itself, contained a relatively constant level of this toxin. Furthermore, the levels of PHD displayed less variation in the volva and bulb than in the other tissues. These observations suggest that all the tissues are not affected to the same extent by the developmental change of the carpophore. The most prominent differences in the PHD content were noted for the cap, gills and ring, whereas differences in β-Ama concentration were observed in the volva and bulb. Since our study on the tissue toxin content of the three different specimens showed compositional variations, an investigation should be performed on additional mushrooms collected at the same site and representing different stages of the carpophore development. These assays might shed light on the influence of the degree of maturity of the carpophore on the toxin composition of the *A. phalloides* tissues.

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