Zeitschrift: Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 70 (1979)

Heft: 2

Artikel: Bufotenine concentrations in carpophores of Amanita citrina (Schff) S.F. Gray

Autor: Stijve, T. / Diserens, H. / Diserens, J.M.

DOI: https://doi.org/10.5169/seals-983721

Trav. chim. aliment. hyg. **70**, 246—253 (1979)
Received 3 April 1979. Accepted 27 April 1979

Bufotenine Concentrations in Carpophores of Amanita citrina (Schff) S. F. Gray

T. Stijve
Control Laboratory of Nestlé Products Technical Assistance Co Ltd,
La Tour-de-Peilz
Technical assistance: *H. Diserens* and *J. M. Diserens*

Introduction

Amanita citrina (Schff) S. F. Gray, formerly known as A. mappa (Batsch ex. Fr.) Quel. has long been considered a deadly poisonous fungus. Most mycological handbooks from before the second World War list it as such, but there is little doubt that the warnings against this species are simply due to misidentification of the notorious death cup, Amanita phalloides.

In fact, the innocuousness of A. citrina was already demonstrated at the 1925 banquet of the Mycological Society of France, when it was served and eaten without ill effect (1).

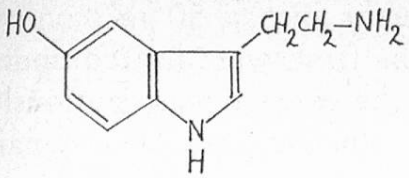
It has occasionally been eaten in Europe (2, 3), but most authors regard it as unfit for consumption, because of its nasty taste and offending odour of raw potatoes (4, 5, 6).

In 1953, *Wieland* and *Motzel* discovered the indole alkaloid bufotenine in A. citrina (7). So far, bufotenine, 5-hydroxy-N,N-dimethyltryptamine, had only been found in the secretions of toadskins, and it was also known as a major constituent of Cohoba, the narcotic snuff of ancient Haiti, prepared from the seeds of the shrub Piptadenia peregrina (8).

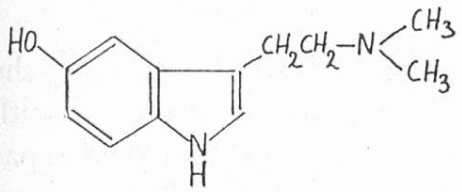
Although bufotenine is a powerful inhibitor of cholinesterase, it is quite harmless when taken orally. Only when injected in large amounts, it provokes some rather transient psychotropic effects (9).

In 1961, *Catafolmo* and *Tyler* confirmed the presence of bufotenine in A. citrina and they also reported the occurrence of this indole base in the related species A. porphyria (10) and A. tomentella (11). A more thorough analysis of A. citrina and A. porphyria was carried out a few years later by *Tyler* and *Gröger* who identified the following 5-hydroxytryptamine derivatives in both mushrooms: bufotenine, serotonine, N-methylserotonine, bufotenine-N-oxide and 5-methoxy-N,N-dimethyltryptamine (12).

In addition, they also found a trace of N,N-dimethyl-tryptamine in A. citrina, and a relatively large number of unidentified tryptamine derivative. Considering



Serotonine = 5-hydroxytryptamine



Bufotenine = N,N-dimethyl 5-hydroxytryptamine

that these compounds could well possess far stronger psychotomimetic properties than the major constituent bufotenine, the authors recommended that both Amanita species should remain classified as potentially dangerous.

It is unfortunate that neither *Wieland,* nor *Tyler* and co-workers have quantitatively determined the concentrations of bufotenine and related compounds in A. citrina.

Wieland and *Motzel* obtained from 100 kg of fresh carpophores 15 g of cristallised bufotenine picrate, corresponding to 45 mg of the free base per kg of mushroom (7). This is really not much, but it should be remarked that the isolation procedure used by the authors was not very efficient. The real bufotenine content could easily be 10 times higher.

The purpose of this paper is to report an investigation with regard to the amounts of bufotenine and of related compounds in carpophores of A. citrina from different origin.

Experimental

Sampling

A collection of 11 pooled samples of A. citrina, collected between 1970 and 1975 was kindly supplied by Ruth Seeger of the Pharmacological and Toxicological Institute of the Würzburg University in the GFR. These samples consisted of unequivocally identified carpophores, gathered at different sites in Unterfranken (South Western Germany), that had been lyophilized and kept in tightly closed glass bottles until analysis.

In addition, one sample from the Netherlands and two from Switzerland were also included in the investigation.

Extraction of bufotenine and other tryptamine derivatives

Initially, the extraction procedure described by *Tyler* and *Gröger* (12) was used. This method gave very clean extracts, but it was a rather time consuming operation and the yield for bufotenine was definitely not higher than that obtained by simple methanol extraction:

0.25—0.50 g of the lyophilized material was brought into a 50 ml conical flask. After adding 8 ml of methanol, the contents of the flask were heated under reflux on a water bath during 1 hour. After cooling, the extract was cautiously decanted through a plug of glass wool into a 25 ml volumetric flask. The remaining solids were re-extracted twice under the same conditions and the combined extracts were brought to volume and mixed by shaking.

In order to check if bufotenine had passed completely into the extract, the solids of the first samples under investigation were extracted a 4th time with 10 ml of methanol and this extract was concentrated to 1 ml and analysed separately. As it was found to contain less than 4% of the initially extracted bufotenine, it was considered not worth our while to perform more than three extractions.

Thin-layer chromatography (TLC)

The TLC systems used for the determination of the tryptamine derivatives in the mushroom extracts are listed in table 1.

Table 1. Rf-values of bufotenine and related compounds in four TLC systems

	Cellulose Propanol-ammonia 5:1 v/v	Al_2O_3E Butanol-ethylacetate water 70:60:25 v/v	SiO_2/Kieselgur Butanol saturated with 3% ammonia	Al_2O_3E Methanol-methyl-ethylketone 1:1
Bufotenine	0.90	0.76	0.65	0.50
N-methylserotonine	0.80	—	0.50	—
Serotonine	0.60	0.15	0.30	0,02
Bufotenine-N-oxide	0.45	0,25	0.46	0.05

Chromogenic reagents

— Pauly's reagent = diazotised sulphanilic acid (13)
This reagent gave dark reddish violet spots.
The limit of detection of all compounds ranged from 0.2—0.6 µg.

— Trans cinnamaldehyde
A 1% freshly prepared ethanolic solution of this chemical (Fluka No 96320) was sprayed on the chromatogram. Subsequently, the plate was placed in a glass tank filled with hydrochloric acid vapours generated by bubbling air through 38% HCl. Cherry red spots were obtained against a yellow background and sensitivity was often as good as 0.1 µg.

— Fast Blue salt B (di-o-anisidine tetrazolium chloride)
We used a freshly prepared 0.5% solution in 50% aqueous ethanol that gave reddish purple spots. Visibility could sometimes be improved by exposure to ammonia vapour. Limit of detection was 0.2 µg.

— Human serum/indoxyl acetate — reagent for inhibitors of cholinesterase (14)
A freshly prepared 1% aequeous solution of lyophilized human serum was

sprayed on the chromatogram until the layer was well moistened, but not dripping wet. The plate was incubated in a stove at about 37 °C during 20—30 minutes. The substrate solution was prepared by dissolving 10 mg indoxyl acetate (Sigma Chemical Company) in 1 ml of ethanol 96% and mixing with 9 ml of distilled water of 40 °C.

After spraying and incubating again, bufotenine appeared as a white spot against a turquoise (light blue) background. The limit of detection was about 0.1 µg.

Gas chromatography

We experimented first with different non polar silicone columns, among which 3% OV-17, coated on Chromosorb W 100—120 mesh, HP, was found to be suitable for the GLC of bufotenine as the free base. At a column temperature of 210 °C and a carrier gas flow of 35 ml/min, the compound was eluted in about 8 min. Detection was by flame ionisation at 240 °C. The chromatograms of the crude A. citrina extracts were remarkably clean. Apparently, co-extracted impurities did not chromatograph on OV-17, because the samples showed the same picture as the standard solution.

However, irregular losses in peak height response occurred already during 10 consecutive extract injections. The bufotenine peak broadened and showed tailing and quantitation was found to be impossible.

Better results were obtained on a 3% Versamid 900 column heated at 250 °C. Bufotenine eluted in 7 minutes as a sharp, but slightly tailing peak. The performance of this column remained constant and quantitation was not hampered by absorption phenomena.

Results and discussion

The results of our comparative bufotenine analyses as performed by gas- and thin-layer chromatography are listed in table 2.

It is remarkable that the concentrations of this indole alkaloid fluctuate only within a factor of two, in spite of the widely different origin of the mushrooms. Although quantitation by TLC was simply done by visual estimation of spot dimensions, the results are in fair agreement with those obtained by GLC.

According to *Tyler* and *Gröger* (12) A. citrina contains appreciable amounts of other tryptamine derivatives. These autors reported the following compounds and their relative concentrations (bufotenine = 10): bufotenine-N-oxide 4, serotonine 2, N-methylserotonine 2, 5-methoxy-N,N-dimethyltryptamine 0.25 and N,N-dimethyltryptamine 0.25.

However, on our chromatograms, the large bufotenine spot was only accompanied by two very minor spots which were unequivocally identified as serotonine and bufotenine-N-oxide in 4 different TLC systems.

In our samples serotonine concentrations ranged from 100—200 mg/kg, whereas the bufotenine-N-oxide content was estimated at 300—600 mg/kg.

Table 2

Origin of sample	Bufotenine content in % as determined by	
	GLC	TLC
Germany		
Rüdern 6. 9. 70	0.91	0.75
Geesdorf 13. 9. 70	0.89	0.95
Wiesentheid 14. 9. 70	0.70	0.40
Kirchschönbach 13. 9. 70	0.70	0.50
Kirchschönbach 20. 9. 70	0.90	0.70
Kreuzwiese 20. 9. 70	0.90	0.80
Gamburg Distelherd 15. 9. 74	1.30	1.0
Klosterforst 6. 11. 74	0.76	0.60
Geesdorf 23. 9. 75	0.94	0.80
Gamburg 24. 9. 75	0.95	1.10
Gamburg Hochhausen 28. 9. 75	1.30	1.20
Netherlands		
Hollandse Rading August 74	0.80	0.65
Switzerland		
Mont Pélerin August 75	1.50	1.30
Bern September 77	1.10	1.0

These compounds were only detected by the chemical chromogenic reagents: the spots also turned reddish with cinnamaldehyde and purple violet with Fast Blue salt B. When using the enzymatic (human serum/indoxyl acetate) detection system, the mushroom extracts, even in aliquots representing as much as 5 mg lyophilized tissue, were found to contain only bufotenine as a cholinesterase inhibitor.

The difference between Tyler's results and ours is hard to explain. It is unlikely that the indole alkaloids other than bufotenine would have degraded during storage of the lyophilized mushrooms. We were, indeed, also unable to find more than trace amounts of serotonine and bufotenine-N-oxide in fresh carpophores.

However, in an autolysate of A. citrina we found a high concentration of bufotenine-N-oxide and several other cinnamaldehyde positive spots.

Unfortunately, there was not sufficient material left for the identification of these compounds, but the experiment will be repeated at some later date.

Meanwhile, the question remains open whether *Tyler* and *Gröger's* observation of multiple tryptamine derivatives is due to the fact that they analysed A. citrina specimens that were in an advanced state of decomposition.

Distribution of bufotenine in different parts of the fruit body

From 5 adult A. citrina specimens the bulbs, stalks and caps were separated, lyophilized and subjected to bufotenine analysis.

The following results were obtained:

Cap	0.8%
Stalk	1.5%
Bulb	0.065%

The near absence of bufotenine in the bulb is somewhat surprising. Interestingly, on the chromatogram of this part of the mushroom a compound was observed that yielded a bright orange spot with Fast Blue Salt. It was identified as 5-hydroxy-tryptophan and it may well be the precursor of serotonine and bufotenine.

Its presence in the bulb suggests that biosynthesis of the two compounds occurs not only in the mycelium, but even during the growth of the carpophore.

This possibility is under investigation in this laboratory.

Non-occurrence of bufotenine in five other Amanita species

We analysed the following Amanita species for bufotenine: Amanita phalloides, A. verna, A. muscaria, A. rubescens and A. pantherina, gathered in Germany, the Netherlands and Switzerland.

In all samples the results were negative, i. e. the fungi contained less than 0.005% (50 mg/kg) on dry weight of this indole alkaloid.

Acknowledgement

The author wishes to thank Dr. *Ruth Seeger* of the Institut für Pharmakologie und Toxikologie der Universität Würzburg, FRG, for her generous gift of A. citrina samples and for her many helpful suggestions.

Summary

The bufotenine contents of 14 pooled samples of Amanita citrina mushrooms from different origin were determined by gas chromatography of crude methanolic extracts. Confirmatory analyses were carried out by thin-layer chromatography, using either chemical or enzymatic methods of detection.

Bufotenine concentrations ranged from 0.70—1.50% expressed on dry weight. In addition, all samples were found to contain 100—200 mg/kg of serotonine and 300—600 mg/kg bufotenine-N-oxide.

Analyses of different parts of the carpophore indicated that bufotenine was nearly absent in the bulb, whereas the stalk contained about twice as much as the cap (1.5 compared to 0.8%). However, the bulb contained an appreciable amount of 5-hydroxytryptophane, a precursor of bufotenine. Five other Amanita species: A. phalloides, A. verna, A. muscaria, A. rubescens and A. pantherina were analysed for bufotenine with negative results.

Zusammenfassung

Der Gehalt an Bufotenin von 14 Sammelproben gelber Knollenblätterpilze (Amanita citrina) verschiedener Herkunft wurde gaschromatographisch in den methanolischen Rohextrakten bestimmt. Die erhaltenen Befunde wurden dünnschichtchromatographisch bestätigt, wobei das Bufotenin mittels chemischer oder enzymatischer Methoden angefärbt wurde.

Die Bufoteningehalte lagen zwischen 0,70 und 1,50% der Trockenmasse. Zusätzlich enthielten alle Proben 100—200 mg/kg Serotonin und 300—600 mg/kg Bufotenin-N-oxid.

Analysen der verschiedenen Teile des Fruchtkörpers zeigten, daß Bufotenin in der Knolle praktisch abwesend war, während der Stiel ungefähr doppelt so viel enthielt wie der Hut (1,5 gegenüber 0,8%). Dagegen wies die Knolle einen beträchtlichen Gehalt an 5-Hydroxytryptophan, einer Vorstufe des Bufotenins, auf. Mit negativem Resultat untersuchte man fünf weitere Amanita-Arten auf Bufotenin: A. phalloides, A. verna, A. muscaria, A. rubescens und A. pantherina.

Résumé

La bufoténine a été dosée par chromatographie gaz-liquide dans les extraits méthanoliques de 14 échantillons cumulatifs d'Amanite citrine (Amanita citrina) d'origines différentes. Les résultats obtenus ont été confirmés par chromatographie sur couche mince où la bufoténine est révélée par voie chimique ou enzymatique.

Les teneurs en bufoténine trouvées sont comprises entre 0,70 et 1,50% sur matière sèche. Les mêmes dosages ont montré que tous les échantillons contenaient 100 à 200 mg/kg de sérotonine et 300 à 600 mg/kg de bufoténine-N-oxyde.

Les analyses des différentes parties du carpophore ont indiqué que la bufoténine était pratiquement absente dans le bulbe, tandis que le pied en contenait environ deux fois plus que le chapeau (1,5 contre 0,8%). En revanche, le bulbe contenait une teneur appréciable en 5-hydroxytryptophane, un précurseur de la bufoténine. Dans cinq autres espèces d'Amanites: A. phalloïdes, A. verna, A. muscaria, A. rubescens et A. pantherina il n'a pas éte décelé de bufoténine.

Literature

1. *Litten, W.:* The most poisonous mushrooms. Scientific American, 91—101, March 1975.
2. *Heim, R.:* Les champignons toxiques et hallucinogènes. N. Boubée, Paris 1963.
3. *Michael, E.* und *Hennig, B.:* Handbuch für Pilzfreunde. I. Die wichtigsten und häufigsten Pilze. G. Fischer, Jena 1968.
4. *Monceaux, R. H.:* La vie mystérieuse des champignons sauvages. Editions Stock, Paris 1966.
5. *Mauch, H.* et *Lauber, K.:* Champignons. Petit Atlas Payot, Lausanne 1975.
6. *Savonius, M.:* All colour book of mushrooms and fungi. Octopus Books Ltd, London 1973.
7. *Wieland, T.* und *Motzel, W.:* Ueber das Vorkommen von Bufotenin im gelben Knollenblätterpilz. Ann. Chem. Liebigs **581** ,10—16 (1953).
8. *Hoffer, A.* and *Osmond, H.:* The hallucinogens, chapter IV, p. 443—463. Academic Press, New York and London 1967.
9. *Turner, W. J.* and *Merlis, S.:* Effect of some indole alkylamines on man. A. M. A. Arch. Neurol. Psychiat. **81**, 121—129 (1959).

10. *Catalfomo, P.* and *Tyler Jr., V. E.:* Investigation of the free amino acids and amanita toxins in Amanita species. J. Pharm. Sci. **50**, 689—692 (1961).
11. *Tyler Jr., V. E.:* Indole derivatives in certain North American mushrooms. Lloydia **24**, 71—74 (1961).
12. *Tyler Jr., V. E.* and *Gröger, D.:* Investigation of the alkaloids of Amanita species. II. Amanita citrina and Amanita porphyria. Planta Med. **12**, 397—402 (1964).
13. *Merck, E.:* Anfärbereagenzien für Dünnschicht- und Papierchromatographie. No 302, Seite 100, Darmstadt 1970.
14. *Leegwater, D. C.* et al.: Detectie van residuen van organofosfor-pesticiden met behulp van dunne laagchromatographie. Report No 2790 of the Central Institute of Nutrition and Food Research. Zeist. Netherlands, December 1968.

T. Stijve
Nestlé Products Technical
Assistance Co. Ltd
Control Laboratory
P. O. Box 88
CH-1814 La Tour-de-Peilz