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High Performance Thin-layer Chromatographic Determination of the Toxic Principles of some Poisonous Mushrooms

Introduction

Every year, through ignorance or imprudence, several people are poisoned by eating toxic mushrooms.

Identifying the species responsible for such an intoxication is very important because it will enable the physician to choose the appropriate treatment. The identification should be rapid and can be performed according to several methods.

The physician usually makes his diagnosis of the type of intoxication by carefully considering the symptoms. For example, a short incubation time and profuse salivation, perspiration and lacrimation, accompanied by a slow pulse and small pupils immediately suggests the muscarinic syndrome which is characteristic for fungi containing muscarine, such as Inocybe and some Clitocybe species.

On the other hand, a long latency time, followed by vomiting, diarrhea, jaundice and coma are indicative of a poisoning by the deadly Amanitae, such as A. phalloides.

Unfortunately, the symptoms are not always so typical and the experience of discomfort after eating a dish of mushrooms may be due to other things than fungal poisons.

It is also possible to identify the toxic mushroom species by examining the leftovers from the preparation of the dish. In some cases one can even examine the fragments present in the vomitus of the patient. This type of identification is based on the botanical characteristics and should always be completed by a microscopic examination, notably of the spores.

Such an analysis requires the knowledge and experience of a skilled mycologist who is not always available. For this reason, several authors have proposed a system of identification based on the chemical analysis of the mushroom toxins.

Most of the published methods deal with paper- or thin-layer chromatographic identification of the notoriously toxic cyclopeptides present in the deadly Amanitae (1—5).

Recently, *Girre* and *Gérault* (6) have proposed a series of tests for the detection of four classes of toxic mushrooms: the Amanitae containing cyclopeptides, the hallu-

cinogenic fungi belonging to the genus Psilocybe, the Gyromitras containing derivatives of methyl hydrazine, and the fungi having muscarine as the toxic principle.

The system consist of two thin-layer chromatographic examinations, one simple colour reaction in a test tube, and of a biological assay (on a guinea pig) for the detection of muscarine.

Manufacturers of soups and sauces containing edible mushrooms as an essential ingredient, are also interested in analytical methods for the detection of fungal toxins.

During the last few years the suppliers of raw materials for culinary products are increasingly offering powdered and granulated mushrooms. Formerly, those mushrooms were sold as dried fragments permitting easy determination of the botanical characteristics of the species which is virtually impossible to do in the powdered or granulated fungi.

For this reason, this paper describes an improved system for the rapid identification of the principal mushroom toxins based on a simple extraction procedure followed by high performance thin-layer chromatography (HPTLC) of the crude extract.

Method

Field of application

The method can be used for the determination of the following toxins:

- Amanitins and phalloidin in Amanita phalloides, A. verna, A. virosa and in other fungi reported to contain amatoxins such as Galerina marginata and Lepiota brunneoincarnata.
- Muscarine in Inocybe species and in Clitocybe dealbata and Cl. rivulosa.
- Muscimol c. q. ibotenic acid in Amanita muscaria and A. pantherina.
- Ethylidene gyromitrine and N-methyl-N-formylhydrazine (MFH) in Gyromitra esculenta.

Reagents and chemicals

- Methanol, n-butanol, 2-butanol, ethanol 95%, chloroform, methylene chloride, ethyl acetate, acetic acid, formic acid, light petroleum, all of a quality suitable for chromatography.
- Alpha-amanitin lyophilised, Serva no. 12 300 (beta- and gamma-amanitin were generously supplied by Dr. H. Faulstich, Max-Planck-Institut für Medizinische Forschung, Heidelberg, GFR).
- DL-muscarine (chloride salt) Serva no. 29 851.
- Muscimol, Fluka no. 70 015
- Ethylidene gyromitrine and N-methyl N-formylhydrazine (MFH) were synthesized as described by *List* and *Luft* (7).

- Reference solutions for HPTLC analyses:
  Prepare solutions containing 1.0 mg of the individual compounds in 2.0 ml of methanol. For ethylidene gyromitrine and MFH, prepare these solutions in chloroform. Keep in the refrigerator at 4 °C. Prepare fresh every 2 months.
- HPTLC plates Silica gel 60 F254, 10 x 10 cm for nano-TLC, Merck no. 5628.
- ditto coated with cellulose, without fluorescent indicator, Merck no. 5787.
- Mobile phases for HPTLC
  of *amanitins:* chloroform / methanol / acetic acid 100% / water 75 : 33 : 5 : 7.5 v/v (5)
  of *muscarine:* n-butanol / ethanol 95% / acetic acid 100% / water 80 : 20 : 10 : 30 v/v
  of *muscimol:* 2-butanol / ethanol 95% / acetic acid 100% / water 75 : 25 : 5 : 25 v/v (8)
  of *ethylidene gyromitrine and MFH:* Methylene chloride / methanol 9 : 1 v/v (9).
- Chromogenic reagents for HPTLC
  of *amanitins:* Dissolve 1 ml of cinnamaldehyde, trans-3-phenyl-propenal, Fluka no. 96 320, in 100 ml of methanol. Prepare fresh every day.
  of *muscarine:* Use the Dragendorff reagent as modified by *Thies* and *Reuther* (10). Dry bismuth subcarbonate over concentrated sulfuric acid during 24 hours. Bring 2.6 g of the dried salt in 25 ml 100% acetic acid, add 7 g sodium iodide and bring to boil under reflux. Allow to boil gently during 5 minutes and allow to cool overnight. Eliminate the precipitated sodium acetate crystals by filtering over a glass filter. Mix 20 ml of the filtrate with 80 ml ethyl acetate. This stock solution is stable during at least 6 months. For the preparation of the spraying reagent, mix 10 ml of the stock solution with 25 ml glacial acetic acid, 60 ml ethyl acetate and add dropwise 5 ml of water. Prepare fresh every week.
  of *muscimol:* Dissolve 0.5 g ninhydrine in 93 ml n-butanol, saturated with water. Add 7 ml acetic acid.
  of *ethylidene gyromitrine and MFH:* Dissolve 0.3 ml cinnamaldehyde in a freshly prepared mixture of 15 ml methanol and 5 ml concentrated hydrochloric acid. Prepare fresh every day.
- Aluminium oxide, Woelm, acid (anionotropic) activity II: Remove possible present impurities by heating at 500 °C overnight. After cooling, standardize adsorbent by adding 3 % by weight of distilled water. Mix well and allow to equilibrate before use during at least 6 hours. Keep in a tightly stoppered flask and prepare fresh every week.

Apparatus

- Nanoplicator of Camag (Homburgerstr. 24, CH-4132 Muttenz, Switzerland), permitting application of volumes ranging from 10-230 nanoliters.

– Chromatography chambers for HPTLC, for example those of Camag 10 x 10 cm with stainless steel lid, no. 25 155 or 20 x 10 cm with glass lid, no. 25 253.

Preparation of the test portion

Clean the mushrooms by removing adhering soil, leaves, etc. If the material should be kept for reference purposes: lyophilize the fruit bodies and grind them to a fine powder. Store in a glass stoppered bottle.

If lyophilizing is not desirable or possible, keep the mushrooms in the freezing compartment of the refrigerator.

Cut the material in very small pieces, for example with a meat chopper.

If the material under examination are mushroom preserves or meals suspected to contain poisonous fungi, try to sort out as many mushroom fragments as possible.

Remove possible present fat by refluxing the material twice with light petroleum, decant and discard the extracts.

Detection of amanita toxins

Bring 0.5 g of lyophilised or dried mushroom powder into a 100 ml round bottomed flask and add 50 ml of methanol. From fresh material or defatted preparations weigh 10 g of the finely chopped sample into a 250 ml flask and add 100 ml of methanol.

Heat the flasks under reflux on a water-bath during 1 hour. Allow to cool and cautiously decant the extract through a plug of glass wool into a 250 ml flask. Add the glass wool plus the adhering material to the remaining solids, and re-extract during 1 hour with 50, respectively 100 ml of methanol.

Add the second extract to the first, connect the flask to a rotavapor apparatus and evaporate until dry at 40 °C, using slight vacuum.

With a pipette, add 2.00 ml of methanol to the residue. Bring the toxins into solution by intermittently swirling the flask and letting it stand for a few minutes. Decant the extract, thus obtained, into a small vial and keep well closed until HPTLC analysis.

N. B. The more elaborate procedure outlined above aims at the quantitative extraction of the toxins. For diagnostic purposes, when speed is all important, it is sufficient to extract the material only once during 15—30 minutes. The extract will contain enough amatoxins for their HPTLC detection.

On a silica gel 60 nanoplate, at 1 cm from bottom edge, apply with the nanoplicator 100, 200, 300 and 400 nanoliters of the methanol extract. These volumes correspond with 25, 50, 75 and 100 μg of lyophilized, or 0.5, 1, 1.5 and 2 mg of fresh material. Leave 0.5 cm distance between each application. For comparison apply 100, 200, 300 and 400 nanoliters of a mixed amanitin standard solution, representing 0.05, 0.1, 0.15, and 0.2 μg of each toxin.

Develop the plate by ascending migration in the mobile phase for amanitins over a distance of 8 cm. The migration time is approximately 45 minutes under conditions of saturation.

Remove the plate from the chamber and let adherent solvent evaporate *completely* under a hood. (Residual solvent in the layer will lower the sensitivity of the amanitin detection.)

Spray the chromatogram with the 1 % methanolic cinnamaldehyde solution until the layer is just moistened, but not dripping wet.

Place the chromatogram into a large glass tank containing a 100 ml flask filled with freshly poured out fuming hydrochloric acid. Allow to bubble air through the acid in order to saturate the tank with vapours. Amanitins appear as brightly red spots on a yellow background. After 5—10 minutes exposure 0.05 μg of the amanitins should be clearly visible. Phalloidin turns up first orange and finally yields a bright blue spot just above gamma-amanitin.

The order of migration is beta-amanitin < alpha-amanitin < gamma-amanitin < phalloidin.

Remove the plate from the chamber and place it in a stream of air. The yellow background will dissappear, thus augmenting visibility of the spots.

If quantitative determination is required, estimate concentration of the amanitins in sample aliquots by comparison with series of standards, either visually or by direct spectrophotometric measurement (densitometry). Perform this estimation without delay, because the colour of the amanitins fades already within 20 minutes (14).

Detection of muscarine

Perform extraction as described for amanita toxins. Bring suitable aliquots of the concentrated extract on cellulose nanoplates, applying 0.1, 0.2, 0.3, and 0.5 μg muscarine as a reference compound. Develop by ascending migration in a saturated tank containing the prescribed mobile phase.

Dry the developed plate in an air stream and spray with the modified Dragendorff reagent.

Muscarine will turn up as an orange spot at Rf 0.55. If necessary, improve visibility of the spots by lightly spraying 0.05 N sulfuric acid on the plate. This treatment yields a more lightly coloured background and the spots stand out more clearly: 0.1 μg of muscarine can be detected (11). Most mushroom extracts will show a bright red spot at Rf 0.40. This is choline, a naturally occurring non toxic quaternary base.

Detection of muscimol

Allow dry material to rehydrate in a tenfold weight of water (for example 1 g in 10 ml) during at least 2 hours. Take 10 g of fresh material. Add 100 ml of methanol and 1 ml of formic acid and extract by boiling under reflux for about 1 hour.

Filter over a folded paper filter and re-extract solids during 1 hour with 100 ml of methanol-water 4 : 1 v/v. Filter again. Combine extracts in a 500 ml round bottomed flask and evaporate to dryness in the rotavapor apparatus at 40 °C using slight vacuum.

Take up residue in 2 ml 50% aqueous methanol and subject suitable aliquots, for example 200, 500 and 1000 nanoliters to HPTLC on a cellulose nanoplate, using muscimol as reference compound in concentrations from 0.1—0.5 μg. Develop the plate in the prescribed mobile phase under conditions of saturation.

Spray the developed and dried plate with ninhydrin reagent and heat the chromatogram during a few minutes at 100 °C. Muscimol turns up as a bright yellow spot at Rf 0.35. Co-extractives usually give bluish violet spots.

The yellow colour of the muscimol spot characteristically turns brownish after a few hours and finally violet overnight.

Crude extracts of Amanita muscaria and A. pantherina generally contain sufficient muscimol for its unequivocal detection, but in presence of many co-extractives the spot may be obscured. In that case, perform clean-up as follows:

Tamp a small plug of glass wool into a 8 x 200 mm glass chromatography tube, fitted with an outlet stopcock and having a 30 ml reservoir at the upper end.

Bring 15 ml of methanol into this tube and add slowly 5 g of standardised aluminium oxide. Ensure tight packing of the adsorbent by gently tapping sides of the column with a glass rod.

When adsorbent has settled, let the solvent run through to about 0.5 cm from the top of the column. Discard the solvent which has run through.

Transfer extract quantitatively to the alumina column, using a few ml portions of 50% aqueous methanol as a rinse. Let run through into a 250 ml round bottomed flask and elute muscimol with 50 ml 50% aqueous methanol. Evaporate eluate to dryness in the Rotavapor apparatus and take up residue in 2 ml of methanol-water 1 : 1, v/v.

Repeat HPTLC analysis as described above.

Detection of ethylidene gyromitrine and MFH

Perform extraction of free and chemically bound toxicants as described in reference no. 12. Analyze the concentrated chloroform extracts by HPTLC, using SiO_2 nanoplates and dichloromethane-methanol 9 : 1 v/v as a mobile phase.

Use a series of 0.1—0.5 μg of both ethylidene gyromitrine and MFH as reference compounds. Spray the developed chromatogram with the cinnamaldehyde-hydrochloric acid reagent and heat a few minutes at 80 °C.

The toxicants show up as bright yellow spots. Sometimes as little as 0.02 μg can be distinguished.

Results and discussion

The HPTLC method for amanitins described in this paper was successfully used for the determination of these toxins in carpophores of Amanita verna (13) and A. phalloides from various origin (14).

Moreover, the procedure permitted the determination of 2000 mg/kg (0.2 %) alpha-amanitin and of 1000 mg/kg phalloidin in a sample of Amanita virosa, collected near Uppsala in Sweden during August 1979.

The method was also found to be sufficiently sensitive for the quantitation of relatively low levels of alpha-amanitin in Galerina marginata as is shown in table 1.

Table 1 Alpha-amanitin concentrations in Galerina marginata (Fr.) Kühn

Origin	Year of collection	Alpha-amanitin content % dry weight
Würzburg GFR	1974	0.030
Klosterforst GFR	1974	0.030
Weidenhüll GFR	1974	0.022
Châtel-St-Denis CH	1977	0.032
Amsterdam NL	1978	0.044
Lally CH	1979	0.015

It is interesting to note that the amanitin content of this species varies within a factor of less than 3. Similar narrow fluctuations were observed in the amanitin content of the deadly Amanitas (13, 14).

We analysed a number of fungi by the HPTLC method for muscarine and obtained the results listed in table 2 of which several are in agreement with the values reported in the literature (15). Muscarine could be easily determined in crude

Table 2. Muscarine concentrations in different fungi

Species	Origin and year of collection	Muscarine content % dry weight
Inocybe Patouillardi	Steinbachtal GFR 1977	0.33
Inocybe fastigiata	Geesdorf GFR 1977	0.22
I. geophylla	Châtel-St-Denis CH 1978	0.10
I. geophylla var. lilacina	Châtel-St-Denis CH 1978	0.13
I. pudica	Châtel-St-Denis CH 1978	0.07
Clitocybe dealbata	Vevey CH 1977	0.08
Mycena pura	Puidoux CH 1977	< 0.01
Amanita muscaria	Puidoux CH 1975	0.009
Amanita pantherina	Helmstadt GFR 1975	< 0.0005

extracts of Inocybe species and of Clitocybe dealbata, but detection in Mycena pura was hampered by the presence of a large quantity of betaine.

Low levels in Amanita muscaria could only be estimated after separation of muscarin from a more than hundred fold excess of choline by clean-up on a alumina column according to a method used for the determination of the plant growth regulator chlorocholine, a compound which has similar solubility characteristics as muscarine (16).

When we subjected a crude extract of Amanita pantherina to this purification procedure, we were unable to detect any muscarine (detection limit 5 mg/kg on dry weight) in this species, in spite of the fact that in parallel experiments added muscarine was recovered for 75% at the 0.05% level.

The real toxic principle of A. muscaria and A. pantherina, ibotenic acid was found in the same samples at levels of respectively 0.19 and 0.30%. It should be pointed out that these concentrations represent ibotenic acid and muscimol, expressed as the latter compound, since we decarboxylated all ibotenic acid by performing the extraction in presence of formic acid (8).

The HPTLC method used for quantitation of muscimol is a direct adaptation from the paper chromatography procedure used by *Müller, Good* and *Eugster* in 1965 (8, 17).

HPTLC detection of gyromitrine and its degradation product MFH could be used for demonstrating the fraudulent use of Gyromitra esculenta in a sauce morilles as served by an unscrupulous restaurant-keeper. For this purpose, the mushroom fragments were picked out with a pincet, washed with water and extracted with chloroform.

Both compounds were readily detected. The HPTLC analysis may serve as corroborating evidence, since Morchella and Gyromitra can also be distinguished by the botanical characteristics of the sorted out fragments.

In order to prove the value of the method for the detection of possible present amanitins in commercially available powdered mushrooms, we prepared a series of samples of dried Boletus edulis, Suillus luteus and Agaricus bisporus (the cultivated champignon de Paris) which we fortified with 1, 2, 5 and 10% of powdered A. phalloides.

The samples were given to laboratory technicians together with unfortified mushroom powders and they were asked to screen out the samples containing A. phalloides.

The mushroom powders spiked with 2, 5 and 10% were unequivocally identified as containing deadly amanitins by three independently working analysts. The results became somewhat doubtful at the 1% level, because of the predominance of co-extractives. At this concentration, however, the toxins are so diluted that they hardly represent a health hazard anymore.

It is important to note that the analysts did not report any false positives.

We have not yet fully explored all the possibilities of the analysis of fungal toxins by HPTLC, but the results obtained so far suggest that the method has considerable potential for forensic and clinical analysis in cases of mushroom poisoning.

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Summary

A sensitive and rapid method is proposed for the determination of the toxic principles in the following mushrooms: Amanita phalloides and other deadly Amanitae, Amanita muscaria and A. pantherina, Gyromitra esculenta and some species belonging to the genera Inocybe and Clitocybe.

A suitable aliquot of a crude extract of the material under examination is subjected to high performance thin-layer chromatography (HPTLC).

The separated compounds are detected by spraying with selective chromogenic reagents. The detection limit for most of the toxins is about 50 ng.

The method can be used for diagnostic purposes in cases of mushroom poisoning. Furthermore, it can be applied to check the powdered edible mushrooms which are used as raw materials in the food industry.

Zusammenfassung

Es wird eine rasche und empfindliche Methode für den Nachweis der Toxine in folgenden Giftpilzen vorgeschlagen: Amanita phalloides und andere tödlich giftige Knollenblätterpilze, Amanita muscaria und A. pantherina, Gyromitra esculenta und weiter einige Pilzarten, die zu den Gattungen Inocybe und Clitocybe gehören.

Ein bestimmtes Volumen eines Rohextraktes des zu untersuchenden Materials wird der Hochleistungs-Dünnschichtchromatographie unterworfen. Die aufgetrennten Giftstoffe werden mit selektiven Sprühreagenzien sichtbar gemacht. Die Nachweisgrenze für die Mehrheit der Toxine beträgt etwa 50 ng.

Die Methode kann für diagnostische Zwecke in Fällen von Pilzvergiftungen benützt werden.

Sie ist auch geeignet zur Untersuchung von Speisepilzpulvern, die in der Nahrungsmittelindustrie verwendet werden.

Résumé

L'auteur propose une méthode sensible et rapide pour la détermination des principes toxiques contenus dans les champignons suivants: Amanita phalloides et autres Amanita mortelles, Amanita muscaria et A. pantherina, Gyromitra esculenta, ainsi que dans quelques espèces appartenant aux genres Inocybe et Clitocybe.

Une partie aliquote d'un extrait brut du matériel à analyser est soumise à la chromatographie sur couche mince à haute performance.

Les composés recherchés sont décelés par des réactifs sélectifs. La limite de détection pour la plupart des toxines est d'environ 50 ng.

La méthode peut être utilisée à des fins diagnostiques lors des empoisonnements par des champignons.

On peut aussi l'employer pour contrôler les poudres de champignons comestibles, utilisées dans l'industrie alimentaire.

Literature

1. *Block, S. S., Stephens, R. L.* and *Murril W. A.:* The Amanita toxins in mushrooms. J. Agric. Food Chem. **3**, 584–587 (1955).
2. *Kamp, P. E.* and *Wit, W. M.:* Het aantonen van de giftige bestanddelen van Amanita phalloides. Pharm. Weekblad **103**, 813–822 (1968).
3. *Palyza, V.:* Schnelle Identifizierung von Amanitinen in Pilzgeweben. Arch. Toxicol. **32**, 109–114 (1974).
4. *Faulstich, H., Georgopoulos, D.* and *Bloching, M.:* Quantitative chromatographic analysis of toxins in single mushrooms of Amanita phalloides. J. Chromatogr. **79**, 257–265 (1973).
5. *Andary, C., Enjalbert, F.* et *Privat, G.:* Dosage des amatoxines par spectrophotométrie directe sur chromatogramme chez Amanita phalloides Fries (Basidiomycetes). J. Chromatogr. **132**, 525–532 (1977).
6. *Girre, L.* et *Gérault, A.:* Identification des espèces ayant provoqué une intoxication. Aspect médico-légal. Champignons toxiques. Compte rendu de la réunion du Groupement français des centres anti-poisons. Pont-A-Mousson, 26 mai 1977, p. p. 167–175. Ed. Masson, Paris 1978.
7. *List, P. H.* und *Luft, P.:* Gyromitrin, das Gift der Frühjahrslorchel. Arch. Pharm. **301**, 294–305 (1968).
8. *Müller, G. F. R.* und *Eugster, C. H.:* Muscimol, ein pharmakodynamisch wirksamer Stoff aus Amanita muscaria. Helv. **48**, 910–926 (1965).
9. *Luft, P.:* Das Gift der Frühjahrslorchel, Helvella (Gyromitra) esculenta Fr. ex Pers. Inaugural Dissertation, Marburg 1967.
10. Anfärbereagenzien für Dünnschicht- und Papier-Chromatographie no 95 E. Merck AG, Darmstadt.
11. *Hughes, D. W., Genest, K.* and *Rice, W. B.:* The occurrence of muscarine in Clitocybe dealbata. Lloydia **29**, 4, 328–332 (1966).
12. *Stijve, T.:* Ethylidene gyromitrine and N-methyl-N-formylhydrazine in commercially available dried false morels, Gyromitra esculenta Fr. ex Pers. Trav. chim. aliment. hyg. **69**, 492–504 (1978).
13. *Seeger, R.* and *Stijve, T.:* Amanitin content and toxicity of Amanita verna Bull. Z. Naturforsch. **34c**, 330–333 (1979).
14. *Stijve, T.* and *Seeger, R.:* Determination of alpha-, beta-, and gamma-amanitin by high performance thin-layer chromatography in Amanita phalloides (Vaill. ex Fr.) Secr. from various origin. Z. Naturforsch. **34c**, 1133–1138 (1979).
15. *Rumack, B. H.* and *Salzman, E.:* Mushroom poisoning: diagnosis and treatment, page 110. CRC Press, Inc. West Palm Beach, Florida 1978.
16. *Stijve, T.:* Thin-layer chromatographic determination of chlormequat residues in various substrates. Deut. Lebensm. Rundschau **76**, 234–237 (1980).

17. *Good, R., Müller, G. F. R.* und *Eugster, C. H.:* Isolierung und Charakterisierung von Prämuscimol und Muscazon aus Amanita muscaria (L. ex Fr.) Hooker. Helv. **48**, 927–930 (1965).

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