Mushroom Toxins - The Meixner Test

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Abstract

Mushrooms can be found extensively in a variety of natural environments and visual identification of mushroom species is well established. However, from a scientific point of view there are still key areas of mushroom research that warrant investigation.

This dissertation concentrated on two areas of mushroom science. The first area investigated was the chemistry of the Meixner test, a spot test widely used in the field by mycologists to facilitate the identification of Death Cap mushrooms (amatoxin). It is performed by applying small quantity of mushroom juice onto low quality lignin paper then adding concentrated hydrochloric acid. A positive spot test results in a blue colouration. The detailed chemistry of the test had not been elucidated to date. However, experiments and conditions investigated in this research suggest that a possible reaction in the Meixner test is substitution on the indole ring, present in all amatoxins. The research demonstrated the complexity of the underlying chemistry of the Meixner test. It was found that the type of paper used could affect the results, as the lignin structure is not consistent between different paper manufacturers. The complexity of the lignin structure makes it difficult to define the exact monolignol(s) which undergo the reaction yielding the blue colour of the spot.

The second area of this dissertation was to identify and collect local mushrooms. A mushroom stumpery was set up at the Food and Environment Research Agency. A protocol for mushroom identification, using up to date and currently available databases was also developed as part of this dissertation. The protocol can be used to help others identify mushrooms and ascertain if they are safe to eat. 156 different species of mushrooms were collected and many were reported with the assistance of The British Mycological Society.

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Declaration

The research described in this thesis was carried out at The University of York and The Food and Environment Research Agency (Fera) between October 2011 and December 2012 and is original, expect where specific acknowledgements has been made.

Marcin Fiedziukiewicz

1 Chapter 1 – Introduction

1.1 Introduction

Toxins occur naturally in many different species of plants and animals. Mushrooms are of particular interest owing to the limited knowledge of mushroom toxins at the present time giving opportunities for further investigative studies.

Mushrooms play a great part in numerous cuisines across the world. All mushrooms that are considered poisonous have been classified as such; the classification is generally done following hospital admissions of individuals who, after consuming mushrooms, have suffered symptoms of poisoning. However, it is possible that many of mushrooms will contain toxins which have not yet been scientifically analysed and identified. Previously, when a mushroom was labelled as poisonous, scientists were rarely interested in identifying the hazardous chemical constituents. The lack of interest in the fungi kingdom caused many gaps in our current knowledge about mushrooms[I], fungi[II] and their toxins.

In most cases, identification of a mushroom causing poisoning symptoms is a result of a patient being admitted to a hospital Accident & Emergency unit after consumption of a mushroom. Good practice when cooking wild mushrooms is at least one specimen should be left uncooked in case it is poisonous and required by medical staff as a reference. Not many people follow the advice so in situations when a patient is unconscious and cannot provide any information on the mushroom consumed, the choice of the treatment is a decision made medical staff with little information on the poisoning incident. Numerous incidents involving mushroom poisoning have created a need to review the knowledge and develop a simple, cheap and quick method to identify the mushroom and toxin.

Currently, most reported cases of fatal mushroom poisoning are caused by *Amanita phalloides* (commonly known as The Death Cap)[VI]. There could be a few reasons why *A. phalloides* is at the top of the list. Death Cap mushrooms are quite common and they can grow among the edible species. The other reason could be its high death rate (between 50 – 90% of people who consume Death Cap mushrooms die [1]). The poisoning symptoms of the Death Cap become less intense after a few hours (which is often taken as a sign of

[I] Mushrooms are fruiting bodies of fungi. They generally grow over ground and are part of the fungi, which are collected as a hobby or for culinary value.

[II] Fungus is a whole body including the mycena growing underground and the fleshy fruiting body.

improvement) only to relapse again. It is common to use a Meixner test in order to aid identification of a key mushroom toxin – the amatoxins. In some reported cases of mushroom poisoning, the test has been used to help identify the toxin causing the poisoning of an admitted hospital patient (as long as a sample of the ingested mushroom is still available)[2]. The Meixner test needs to be interpreted with caution as there are other mushroom constituents that result in the same colour in the Meixner test as amatoxins e.g. psilocin found in some of the hallucinogenic fungi.

1.2 British and European poisonous mushrooms

All fungi contain a set of definable physical features and using individual characteristics will allow correct identification of most fungal species, certainly to the genus level. Details at the macroscopic level[III] (e.g. stem and cap) and microscopic level[IV] (e.g. spores) are the two fungal features that help to distinguish between species. However, when the mushroom is in its young stage or damaged, the chances of confident identification are significantly reduced. In addition, the mushrooms may be distinguishable only by small details in which case there is a greater chance of misidentification.

1.3 *Paxillus involutus*

Until the mid-twentieth century, *Paxillus involutus* mushrooms were considered an edible species in Eastern Europe. After the death of a German mycologist, who ate the *P. involutus* mushrooms, it is now considered poisonous[3]. The delay in the onset of *Paxillus* syndrome (the poisoning effect associated with accidental consumption) makes the mushroom particularly dangerous. After some years, it was discovered that the syndrome is due to a compound called involutin (*Compound 1.1*). Now *Paxillus involutus* is amongst the most dangerous known mushrooms in UK and Europe.

[III] Features that are visible to the naked eye

[IV] Features that need a microscope to be examined

1.3.1 Symptoms

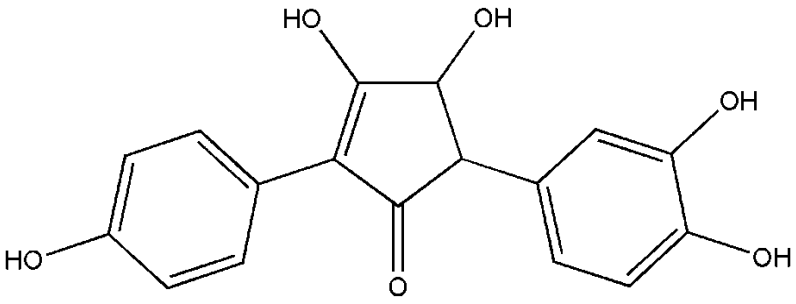
The greatest threat of the *Paxillus involutus* mushroom is that its toxins accumulate in the human body and therefore symptoms of poisoning will not appear immediately. Symptoms often appear many years after ingestion; the delay in onset is caused by the effect of bioaccumulation within the body. Such delay in occurrence of the symptoms means that a lethal amount of the toxin can be consumed before any signs of poisoning appear[4]. *P. involutus* mushrooms contain antigens that will trigger an autoimmune disease causing haemolysis leading to renal failure[5,6]. Other symptoms of the Paxillus syndrome include vomiting, diarrhoea and abdominal pain. The cumulative effect tends to occur with people who do not develop any sorts of illness or poisoning symptoms immediately and consume the mushroom for a prolonged period. A group from Finland reported no change to liver, kidney or heart of a rat upon exposure to the toxin[7]. The rat exposure results imply that short-term consumption in low levels will not trigger the development of symptoms. However, quoted work reported significant changes in the liver when the rat was exposed to the toxin at high levels over a longer period. Those results fall within the observed effects and assumptions on humans who are poisoned by *P. involutus*.

1.3.2 Chemicals in *Paxillus involutus*

The mushroom contains thermo labile toxins[V] which can result in gastroenteritis if the mushrooms are consumed without adequate cooking beforehand. There are some inconsistencies in the reported chemical and physical features of the toxin(s) in *P. involutus*, as one study reported the mushroom poison to be a non–polar, temperature and a low pH stable compound[5]. Those conclusions were drawn from existing toxicological effects from exposing the mushroom extract to variety of environments. Quoted observation contradicts the fact that the mushroom can be eaten if cooked (as the compound(s) supposedly decompose in high temperatures) as was commonly accepted.

During research undertaken in 2001, scientists discovered that compound causing the *Paxillus* syndrome is involutin (*Compound 1.1*)[8]. Due to the phenol functionality, there is a probability that involutin will react with the Meixner test and, as a result, give a discolouration. The experiment will be described in the third chapter (section 3.1.3).

[V] Toxins that decompose when exposed to heat.



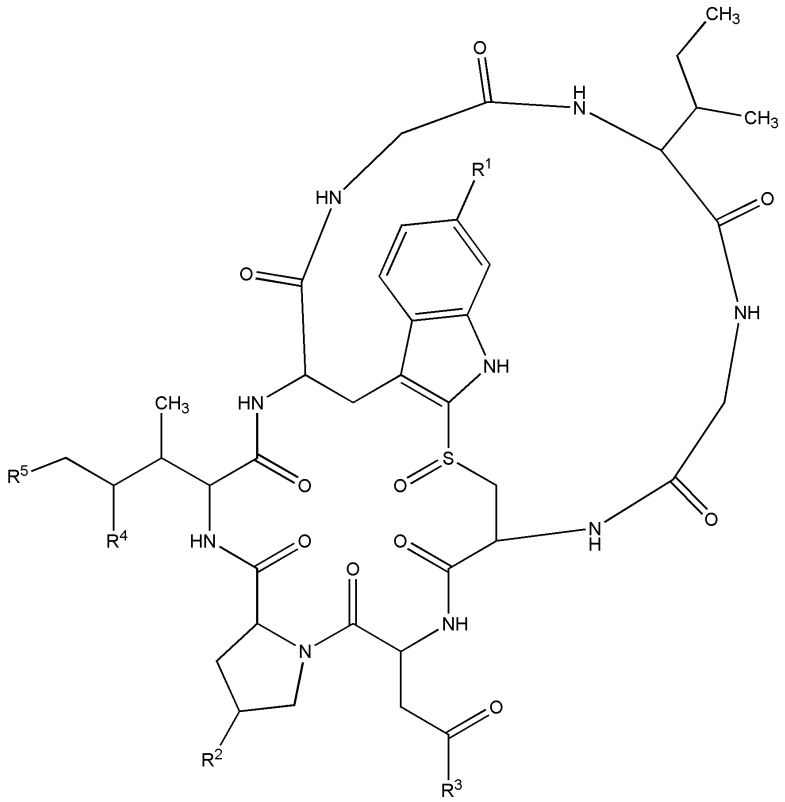
Compound 1.1 – Involutin

1.4 Other mushroom species

1.4.1 Death Cap – *Amanita phalloides*

The Death Cap is a common and poisonous[VI] mushroom species. Misidentification of the Death Cap mushroom as well as *Amanita virosa*, causes approximately 75% of total fatal poisonings in Europe[9]. Juvenile Death Caps resemble certain species from *Russula* genus and puffballs. Many of the poisonous *Amanita* mushrooms produce at least one of the three main families of toxins: amatoxins, phallotoxins and virotoxins. All of the poisonous compounds are small, bicyclic peptides, containing seven to nine amino acids (*Compound 1.2*).

[VI] Compounds are poisonous when any administered amount will react on a cellular level. The amount might not be enough to cause serious symptoms but cellular change will occur.



Compound 1.2 – General structure of amatoxins: R^1, R^2, R^3, R^4 and R^5 are one of three functional groups: -H, -OH and –NH_2. R^1 position, is usually substituted by OH.

The distribution of the amatoxins within the Death Cap mushroom fruiting body is not even. The concentration reported were in following descending order: gills, cap, stipe, bulb, the smallest concentration was reported to be in the ring of the fungi fruiting body. The same study provided evidence of the variation in the amatoxins concentration depending on the collection site[10].

To treat patients from amatoxin poisoning the hospital first treats patients with penicillin-G sodium or activated charcoal[11]. However, a significant majority of patients require a liver transplant to achieve full recovery[12].

1.5 Other poisoning modes[VII]

There are five types of poisoning modes. Two have been discussed - cytotoxic effects of the Death Cap mushroom and the Paxillus syndrome caused by the *P. involutus* mushroom.

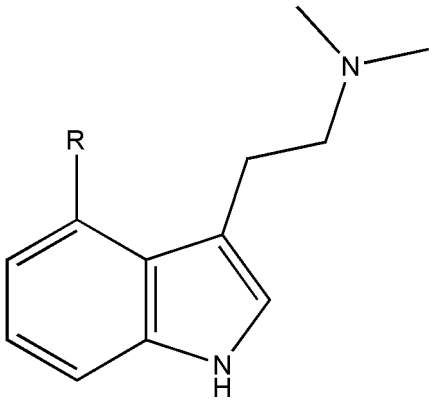
[VII] The compounds mentioned in this section are presented in the Appendix 6.3.

1.5.1 Antabuse - *Coprinus sp.*

The most common mushrooms causing the antabuse symptoms are species in the *Coprinus* genus. They contain a compound (coprine) which blocks the ability of the body to metabolise alcohol and consequently causes the body to accumulate and magnify normal alcohol intoxication symptoms[13].The symptoms will not occur if the alcohol was consumed two days after a *Coprinus* mushroom was ingested, however some reports say the effects of coprine can last for as long as a week[6]. The consumption of the mushroom alone will not cause any symptoms, only ingestion of alcohol will trigger poisoning symptoms.

1.5.2 Neurotoxic poisoning

Neurotoxic poisoning causes mainly hallucinations but there are other symptoms like nausea, bronchospasm, confusion, anxiety and altered time and space sense. Psilocybin (*Compound 1.3* **B**) and its derivatives are the most common compounds that cause neurotoxic poisoning. Psilocybin is most commonly found in *Psilocybe semilanceata* (the Liberty Cap).



*Compound 1.3 – **A** – Psilocin, R – OH; **B** – Psilocybin, R – dihydrogen phosphate*

Other mushroom neurotoxins are isoxazoles, for example, ibotenic acid, muscazone, and muscarine, which are found in the common Fly Agaric (*Amanita muscaria*) and Panther Cap (*Amanita pantherina*). These mushrooms are often deliberately ingested because of their hallucinogenic properties[6].

1.5.3 Gastrointestinal poisoning

The type of poisoning described in this section is the least severe and displays symptoms such as nausea, vomiting, diarrhoea and abdominal pain. Many mushrooms can cause these symptoms, such as *Agarics* and *Boletus*. Occurrence and intensity of symptoms is dependent on the individual. Sometimes the same type of mushroom will trigger symptoms with one group of people but not others, with no obvious pattern. The symptoms are rarely severe and tend to stop within a few hours[6].

1.6 Chemistry of the Meixner test

Cyclopeptide toxins (e.g. amatoxins or virotoxins) and other tryptamines (e.g. psilocybin) can be detected by their reaction with wood lignin when catalysed by concentrated hydrochloric acid[26,23]. Wieland altered the original phenol and wood test and used high lignin paper and amatoxin-containing mushroom extract. The result was a blue colouration of the paper. Meixner later observed that newsprint also gives the same colour change. It was also noted that paper from poor quality paper production processes (such as newspaper) does not have lignin removed during the manufacturing process and therefore such paper can be used successfully in the test. The test is known as the Meixner test (as it will be referred to in this thesis but it can also be known as the Wieland or Wieland/Meixner test). The Meixner test now used for the detection of most the commonly occurring poisonous compounds in mushrooms – the amatoxins. Reaction is observed when an amatoxin – containing mushroom extract is applied to low quality paper and a drop of concentrated hydrochloric acid is applied on top. The result of the Meixner test is a blue to green/blue colour change (positive result).

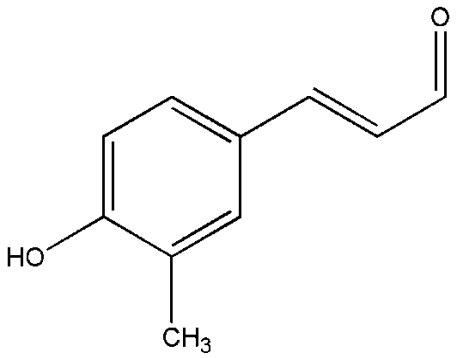
1.6.1 *Stropharia* species

One study claimed that *Stropharia aeruginosa* mushrooms contain the hallucinogenic compound psilocybin[14]. Two of the mushrooms in the *Stropharia* genus (*S. aeruginosa* and *S. cearullea*) were said to be hallucinogenic by the British Mycological Society[15] but no conclusive studies were found. *S. aeruginosa* and *S. cearullea* have similar evolutionary history and scientists only recently classified them as two separate species by comparing the genomes. Therefore, it is likely that they share similar chemical content (hallucinogens). Testing *Stropharia aeruginosa* using the Meixner test would give an

indication if the mushroom samples were truly hallucinogenic. The test should result in a blue colour (the same as in the presence of amatoxins), colour change would indicate a positive result. Analysis of *S. aeruginosa* mushroom using such a rapid test could potentially be used within police forces to test for illegal mushrooms. The Meixner test would give grounds for further investigation when stopping people suspected of possession of 'class A' hallucinogenic drugs (*Psilocybe semilanceata* – Magic Mushroom[16]).

1.6.2 Lignin structure in paper

Lignin is a complex natural product, a constituent of cell walls in plants. To date, no research has resulted in a definitive structure of lignin as isolation from the natural source results in modification of the natural structure. Lignin content of paper varies depending on the manufacturing process used. It has been suggested that the reaction occurs between aromatic coniferyl aldehyde in lignin and indole ring present in the toxin[20]. Coniferyl aldehyde – 4-hydroxy-3-methoxycinnamaldehyde (*Compound 1.4*) may be a key component in colour formation[17,20].



Compound 1.4 – 4-hydroxy-3-methoxycinnamaldehyde – Coniferyl aldehyde

One report stated that the Meixner test turned blue without actually applying any mushroom extract. The list of lignin compounds listed phenol as one of the elements[17]. If the phenol levels are high enough within the lignin, the paper theoretically can turn blue just by adding HCl. A potential reaction might occur between phenol and coniferyl aldehyde[17].

1.6.3 Ehrlich reaction

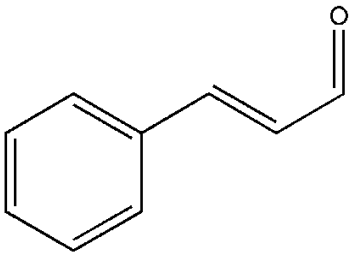
The Ehrlich reaction detects aromatic compounds containing a nitrogen atom within the aromatic system for example, indole and pyrrole containing compounds. The colour change in the Ehrlich test occurs by reaction with *p*-dimethyl-aminobenzaldehyde (Ehrlich reagent) and HCl. A study showed that cinnamaldehyde could be a substitute for Ehrlich reagent. All compounds that gave a positive test with Ehrlich reagent also reacted with cinnamaldehyde[18].

There are two suggested reaction pathways of an aromatic aldehyde with tryptophan and tryptophan derivatives; the first pathway is by formation of a Schiff base, the second pathway is reaction of C-2 or C-3 indole ring with *p*-dimethyl-aminobenzaldehyde. The second reaction pathway occurs at the C-3 position on the indole ring, unless already substituted, in which case, C-2 position in the ring will become occupied[19].
Because of the striking similarities of the Meixner test and the Ehrlich reagent, it is possible that the Meixner test occurs in a similar manner to the Ehrlich reaction (an aromatic aldehyde reacting with an indole ring, catalysed by strong acid).

To date literature on the chemistry involved in the Meixner test is limited. Papers from 1986 and 2004, which was one of the last to review the chemistry of the Meixner test, says that the reaction mechanism remains unclear[11],[26]. The biggest effort to understand chemical tests with lignin was made in 1921 by E.C. Crocker. Crocker's study was first to suggest that the constituent in lignin which reacts with phenols was an aromatic aldehyde[20]. Later research also quoted the same information[21]. Historically, it was believed that lignin oxidises to vanillin and it is the compound which undergoes the colour reaction[25].

In investigations into the chemistry of the Meixner test lignin was substituted with an aldehyde found in the bark of the cinnamon tree – cinnamaldehyde (*Compound 1.5*). Scientists used a freshly prepared 1% cinnamaldehyde solution in MeOH to visualise amatoxins on a TLC plate after chromatographic separation of an *Amanita phalloides* extract[23]. The reactant turned red when applied onto the TLC plate. Using cinnamaldehyde the reaction product turns a red / violet colour[22]. Using cinnamaldehyde the detection limit

was between 1 – 2 μg[23] but the product was not isolated and identified and as the Meixner test turns blue it is unlikely that the same reaction is involved in the Meixner test.



Compound 1.5 - Cinnamaldehyde

Studies have shown that a variety of compounds can give a colour reaction with lignin. Colour changing compounds included; pyrrole (red), indole (cherry red), resorcinol (blue) and phenol (blue)[24]. Phallotoxins, the second most dangerous family of poisons after amatoxins, give a negative result[25]. The negative result of phallotoxin implies that unsubstituted tryptamine does not give a colour change on reaction with lignin. Tryptamines with oxygenation around the aromatic ring of the indole do give a colour change. A grey to pale blue colour is observed for 4-substituted tryptamines, 5–substituted tryptamine ring will yield a reddish reaction with lignin and a light greenish blue for 6-substituted tryptamines under the Meixner test conditions [25, 26].

1.7 Importance of the Meixner test

1.7.1 Test reproducibility and false positives

In the Meixner test there are factors limiting the reproducibility of the test; the first is the paper used, different papers can have various levels of lignin, which will cause variation in colour intensity. The second factor is the volume/concentration of the reagents used; if not used accurately, defined variations in colour will be observed. The level of toxins in the mushroom tested can vary greatly between individual samples; differences in toxin levels can be another source of result variation.

Psilocybin and psilocin (present in 'Magic mushrooms') give the same colour change in the Meixner test as amatoxins, therefore the Meixner test cannot be used to distinguish between amanitins and *Psilocybe* mushroom's hallucinogens. In addition to the Magic mushrooms giving false positive result, Beutler together with Vergeer published

mushrooms which also give false positive results[25]. The absence of amatoxins in studied mushrooms was confirmed by TLC.

Applications of the Meixner test

The Meixner test has been applied in two areas; mycology and medicine. Mycologists use the test to identify mushrooms containing amatoxins in their fruiting bodies (e.g. *Amanita* species). The advantages of the test (cheap, easy, relatively quick and simple) have made it popular amongst mushroom pickers. In hospitals, a clinician may perform the Meixner test if a person presents with mushroom poisoning symptoms and a sample of the consumed mushroom. As the *Amanita* genus is the main source of lethal cases of mushroom poisoning, the test is used to crudely identify of the toxin and thus identify appropriate treatment. Caution needs to be taken in attempting to apply the Meixner test using a urine sample from a suspected mushroom poisoning case, as some metabolites present can react under the Meixner test conditions e.g. 5-hydroxytryptamine (serotonin). The levels of indole derivatives in urine can be linked to some medical conditions like acute appendicitis or melanoma[27 28].

As stated previously the Meixner test gives a positive results for 'Magic mushrooms'. A positive result in the presence of hallucinogenic tryptamines has its potential application with authorities. Police can gain an indication on the street of the possession of hallucinogenic mushrooms. The positive result would give "grounds to believe" that the person in question is in possession of hallucinogenic type, class A drugs. The current state of our knowledge regarding the mechanisms of the Meixner test prevents its use in official proceedings.

Aims and objectives

This project had two aims. The first was to assist The Food and Environment Research Agency (FERA) to create a bank of dried mushrooms that can be accessed for further analysis. The mushrooms have to be collected, identified and dried. For practical reasons the mushrooms were to be collected locally. The second aim was to investigate the reaction occurring in the Meixner test. The reaction pathway was not described in any of the available articles.

2 Chapter 2 – Sample collection and identification

2.1 Sample collection

During two field trips to Castle Howard and to Benninborough in North Yorkshire 50 different species of mushrooms were collected. Multiple fungal forays around the Food and Environment Research Agency`s (Fera) site resulted in 26 other mushroom species. Altogether, including mushroom collected by Fera colleagues, 163 individual mushroom species have been found in total (see appendix 6.2). Included in the total number are mushrooms species growing on tree stumps. All the stumps were collected and stored in a greenhouse at Fera. During those activities, mushrooms were being identified and as a result a standard step-by-step guide for mushroom identification was created (see appendix 6.1).

2.2 Recording mushrooms with BMS

Mushrooms that had sufficient data (Latin name of the mushroom, date of collection, GPS location, OS grid location and Latin name of associated organism) were registered with the British Mycology Society and mushroom entered into the national database[15].

2.3 Stumpery

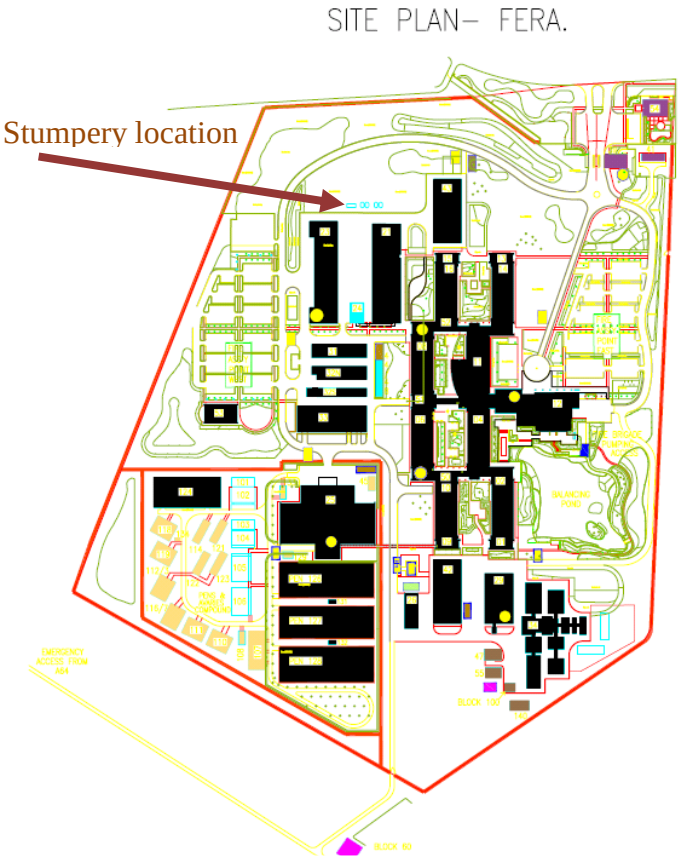


Figure 2.2 – Location of the stumpery on Fera`s site.

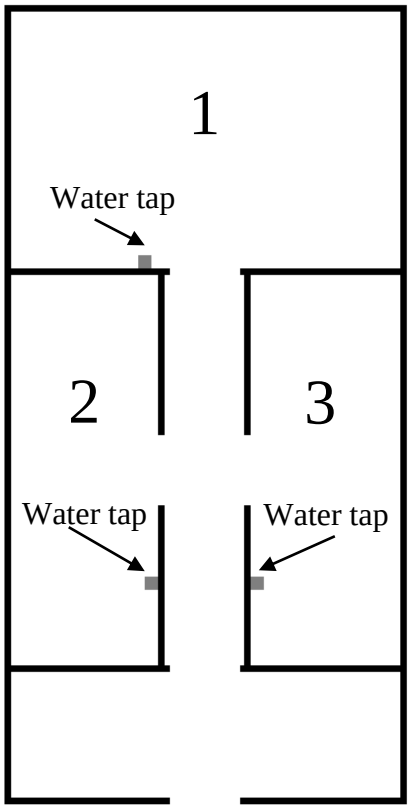


Figure 2.1 – Room layout within the stumpery.

As part of this MSc research a stumpery was set up on north side of Fera`s site in Sand Hutton, North Yorkshire, the (GPS location: 54.0185, -0.9715). The stumpery is a glasshouse (9 m x 4.5 m), the basic layout of the facility is shown in *Figure 2.1*. The facility was fitted with an automated watering system. Watering times were set to six hour intervals: 12 am, 6 am, 12 pm and 6 pm. Such regular breaks allowed sufficient watering system was fitted within each room in the stumpery.

The stumps with mushrooms growing are stored in the areas labelled with numbers 1 – 3. A list of the fungi collection in the stumpery is presented in the *Table 2.1*(for pictures see section 6.6).

Picture 2.1 – An overview of the stumpery – Area 3

The table below gives list of the mushrooms in the stumpery.

Genus	Species	Common name	Picture number [VIII]	Stored in area
Auricularia	*auricula-judae*	Jelly ears	Picture 14	1
Bjerkandera	*adusta*	Smoky bracket	Picture 11	1
Chondrostereum	*purpureum*	Silverleaf fungus	Picture 10	3
Clitocybe	*dealbata*	Ivory Funnel	Picture 13	1
Coprinellus	*flocculosus*	-	Picture 2	3
Crepidotus	*cesati*	-	Picture 8	1
Crucibulum	*laeve*	Common bird's nest	Picture 1	2
Fomes	*fomentarius*	Horse hoof	Picture 5	1
Hypholoma	*fasciculare*	Sulphur tufts	Picture 12	1

[VIII] For pictures refer to the appendix – section 6.6

Nectria	*cinnabarina*	Coral spot	Picture 7	1
Peziza	*vesiculosa*	Blistered cup	Picture 2	3
Phlebia	*radiata*	Wrinkled crust	Picture 6	1
Polyporus	*Squamosus*	Dryad`s Saddle	Picture 17	3
Schizophyllum	*commune*	Splitgill	Picture 3	3
Stropharia	*aeruginosa*	Verdidris agaric	Picture 9	2
Thelophora	*terrestris*	Earth fan	Picture 4	1
Trametes	*versicolor*	Turkeytail	Picture 16	1
Xylaria	*hypoxylon*	Candlesnuff	Picture 15	1

Table 2.1 – A list of the mushroom in the stumpery

2.4 Mushroom identification

A method to identify mushrooms in 12 steps was developed during sample collection (see section 6.3). All data was added to a Microsoft Excel spreadsheet and a Microsoft Word document and included any photographs (examples are in section 6.5)

Sampling precision

In order to illustrate the difficulty that can be encountered in identification of some fungi, the table below presents a comparison of the microscopic features of six samples, which initially were undistinguishable. After comparing the microscopic features, the five samples were identified as two species: *Agrocybe praecox* (Spring Field Cap Mushroom) and *Agrocybe dura*. The last sample, sixth, could not have been identified across four specimens but from the same species

.

Table 2.2 – Spore print colour and spore size measurements in comparison with the reference sizes.

Sample	Spore print colour	Spore size µm	Reference species	max-min Spore size µm	
		Length - Width		Length	Width
1	Chestnut	11.3 - 8.6	*Agrocybe dura*	10 - 14	6.5 - 7.5
2	Milky coffee	8.3 - 5.3	*Agrocybe praecox*	8.5 - 10	5 - 6
3	Chestnut	8.1 - 5.2			
4	Chestnut	8.7 - 5.3			
5	Rusty tawny	9 - 5.2			
6	Fuscous black	7.8 - 4.7	Unknown	-	-

2.5 Assessment report of safety of the sport`s day in a primary school

On the Thursday 14 June 2012 Emergency Response and Recovery team at Fera received an e-mail from Matthew Prince [the head teacher of Newtown C of E (Voluntary Controlled) Primary School in Gosport in Hampshire]. The email was a request to assess the safety of mushrooms growing on the school fields[29]. The concern was brought to attention due to the upcoming sport event that was to be held on the school fields on week commencing Monday 18 June 2012.

Identification of the mushroom species was part of the MSc research undertaken at Fera. The mushrooms were identified by telepresence (not having the actual mushroom samples).

Mushrooms

The pictures below are the mushrooms *in situ* but descriptions come from mycology book[36].

Agrocybe praecox


Cap size: 3-9 cm. **Cap colour:** cream sometimes-brownish ochre. **Cap shape:** convex. **Edible**

Coprinus micaceus


Cap size: 1-4 cm tall but various diameters. **Cap colour:** cinnamon in the centre turning ochre brown. **Cap shape:** conical. **Very common**, Edible.

Marasimus oreades

Cap size: 2-5 cm. **Cap colour:** tan. **Cap shape:** convex, flattening with age and broadly umbonate. **Edible**

Panaeolina foenisecii

Cap size: 1-2 cm. **Cap colour:** dull brown when wet more reddish when dry. **Cap shape:** hemispherical to expanded convex. **Gills:** pallid brown darkening with age to chocolate brown. **Inedible** but no known poisonings reported.

Panaeolus fimicola

Cap size: 1.5-3.5 cm. **Cap colour:** greyish with sepia or hazel-brown colourings. **Cap shape:** hemispherical. **Inedible**

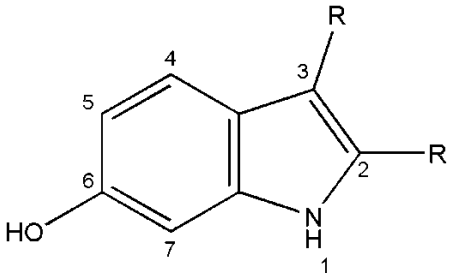
Assessment

The situation was assessed to be safe, as the identified mushrooms were not deemed to be hazardous. None of the fungi listed above was of concern to organisers or parents. As a precaution it was suggested to mow the lawn before holding the sport`s week. Outdoor events are almost impossible to hold without the occurrence of common mushroom species, especially after rain.

3 Chapter 3- Results and discussion Meixner test

3.1 The Meixner test

As discussed in the introduction, the Meixner test is a colour-based chemical test developed to detect the presence of toxins in mushrooms[30]. The test detects toxins containing a hydroxy-substituted indole residue such as amanitins (*Compound 3.1***Error! Reference source not found.**), cyclic polypeptides with a hydroxylated indole group bridging the peptide ring[25].



Compound 3.1 – Amanitin general structure; R - cyclopeptide

The test involves spotting an extract of the mushroom onto poor quality (high lignin) paper then subsequently treating the spot with concentrated hydrochloric acid. The acid-catalysed reaction of the tryptamine bridge (presumed to be with the oxygenated indole ring) and lignin gives the observed colour. The Meixner test gives a range of colours depending on the structure with which it reacts. A grey to pale blue colour is observed for 4-hydroxy substituted indole ring, a reddish-brown colour is seen for 5-hydroxy substituted indole and a light greenish blue colour is seen for 6-hydroxy substituted indole[25]. Tryptamine that has no oxygenation on the indole ring (e.g. phallotoxins), do not show a colour change[25].

The exact structure of the observed chromophores in the Meixner test has not been elucidated and the aim of the work described in this chapter is to:

1) Reproduce the Meixner spot test for amatoxins and psilocin.
2) Investigate the chemistry of the Meixner test using compounds that mimic the structure of both the toxins and lignin.

Amatoxins are the toxic components of the A*manita* mushroom species. The toxins all contain a 6-hydroxy-subsituted indole moiety within a more complex cyclopeptide structure (*Compound 3.1*). All mushroom extracts used in this section were prepared according to method described in section 5.3.1. 0.5 g of mushroom was extracted with MeOH (3 mL) (0.1667 g/mL, concentration = mushroom weight/volume of MeOH). Experiments were performed to develop an optimised method which would give a reproducible colour change. Four variables were investigated: temperature, volume of

mushroom extract used, volume of HCl added and concentration of mushroom extract. In addition, the method of applying liquid extract and reagents onto the paper was also investigated.

3.1.1.1 Temperature

Methanolic extract of the Death Cap mushroom (50 µL) was spotted on high lignin paper (telephone book) using a micropipette. The spot was allowed to dry at ambient temperature for five minutes. The same spotting procedure was repeated twice (150 µL total), before 50 µL HCl (37%) was added (spots 1a and 1b). As controls, an extract of commercial Button Mushroom- *Agaricus bisporus* (spots 2a and 2b) and a MeOH blank (spots 3a and 3b) were used.

A grid of six spots was produced. Spots 1a-3a were heated to 60 ^{o}C for five minutes and spots 1b-3b allowed to develop at ambient temperature for five minutes. It can be seen from Figure 3.1 that the expected green/blue colour was observed in spot 1b only.

Figure 3.1 - **Key:** 1 = Death Cap mushroom (positive), 2 = Button Mushroom (negative control), and 3 = MeOH (blank), a = dried at 60 ^{o}C, b = dried at ambient temperature.

As the spots in this experiment were very diffuse, the amount spotted was reduced to 10 µL in total (2 x 5 µL). Five µL of Death Cap extract was pipetted onto paper. The spots were allowed to dry at ambient temperature and the application process was repeated. 5 µL of 37% HCl was added to the dried spots. Spots 1a – 3a, were heated to 40 ^{o}C for five minutes. Spots 1 – 3 b were allowed to develop at ambient temperature for five minutes

As observed in the previous experiment, heating the spots speeded up the test but perturbed the colour change by introducing more orange/yellow colour. The reduction of the temperature of heating (60 ^{o}C to 40 ^{o}C) still resulted in the orange/yellow colour developing. It appears that the optimal colour change is observed when the spots are allowed to develop an ambient temperature. The reduction in volume of mushroom extract used in the test produced significantly less diffuse spots (Figure 3.3).

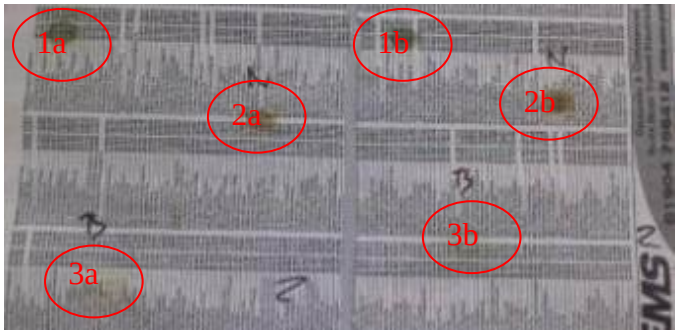


Figure 3.2 - ***Key:*** *1 = Death Cap mushroom (positive), 2 = Button Mushroom (negative control), and 3 = MeOH (blank), a = dried at 40 ^{o}C, b = dried at ambient temperature.*

3.1.1.2 Mushroom extract

The amount of mushroom extract was varied to investigate volume and optimal observed colour change. 5 to 25 µL of extract was added to the paper in aliquots of 5 µL. 37% HCl (5 µL) was added and the spots developed at ambient temperature for five minutes (*Table 3.1*).

Table 3.1 Volume of mushroom extract and observed colour change

Death cap extract/µL	**Colour observed by visual inspection**
5	Pale blue/green with yellow
10	Blue with greenish/yellow
15	Light blue with greenish/orange
20	Green with yellow/orange
25	Green with orange

On visual analysis the optimal blue colour was detected using 10-15 µL of extract.

Figure 3.3 - ***Key:*** *N = negative control (2 x 5 µL - Button Mushroom extract), B = blank (2 x 5 µL MeOH); x 1 = 5 µL; x 2 = 2x 5 µL; x 3 = 3x5 µL; x 4 = 4x5 µL; x 5 = 5x5 µL.*

Spots: x 2, x 3 and x 4 developed over 5 min. Spot x 5 needed more time for HCl to soak through the spot to the paper. Spot x 1 showed the slowest colour change, taking approximately seven minutes to develop and giving a less defined blue colour.

The reaction was repeated in order to investigate the reproducibility of the method. The reaction was performed in quadruplicate (2x5 µL of extract) and the resultant spots are shown in *Figure 3.4* below.

Figure 3.4 - ***Key:*** *N = negative control (2 x 5 µL - Button Mushroom extract), B = blank (2 x 5 µL MeOH); 1 - 4 = repetitions*

The reaction showed good reproducibility using 2x5 µL of mushroom extract.

3.1.1.3 Hydrochloric acid

To date, 5 µL of 37% HCl had been used to develop the Meixner test. In order to ascertain if the volume gave the optimal results, the volume of HCl was varied. 37% HCl was added in 5 µL aliquots to 2 x 5 µL of mushroom extract and the colour was noted after five minutes (*Table 3.2*).

Table 3.2 - Volume of 37% hydrochloric acid and observed colour change.

Hydrochloric acid/µL	**Colour observed by visual inspection**
5	Dark blue mixed with orange
10	Dark blue mixed with yellow
15	Blue mixed with yellow

It was observed that an optimal observable blue colour was obtained with 10 µL (2 x 5 µL) of 37% HCl.

*Figure 3.5 - **Key:** N = negative control (2 x 5 µL - Button Mushroom extract), B = blank (2 x 5 µL MeOH): 1 = 5 µL 37% HCl; 2 = 2 x 5 µL 37% HCl; x 3 = 3 x 5 µL 37% HCl on 10 µL (2 x 5 µL) of methanolic Death Cap extract.*

Reproducibility of the optimal reagents and conditions were investigated. The test was performed in quadruplicate using 2 x 5 µL of extract and 2 x 5 µL 37% HCl, the colour was allowed to develop over 5 min at ambient temperature. On visual inspection, all spots developed a blue colour with approximately the same colour intensity (*Figure 3.6*).

Figure 3.6 - ***Key:*** *N = negative control (2 x 5 µL - Button Mushroom extract), B = blank (2 x 5 µL MeOH); 1 - 4 = repetition*

The result shows little variation between the observed colour of the spots if developed under the same conditions.

3.1.1.4 Mushroom extract concentration

To date all experiments used a methanolic extract of 0.5 g of mushroom. Three mushroom extracts were prepared with significantly higher concentrations [concentration is defined here as mushroom weight/volume of MeOH]. The extracts were five (0.834 g/mL, extract A), ten (1.667 g/mL, extract B) and twenty (3.334 g/mL, extract C) times more concentrated relative to the original extract (0.167 g/mL).

Table 3.3 - Mushroom extracts and observed colour change

Extract	Volume of extract/µL	Colour observed by visual inspection
A	1x5	Pale blue
B	1x5	Pale blue
C	1x5	Blue
A	3x5	Blue with orange/yellow spot
B	3x5	Pronounced blue with orange spot
C	3x5	Orange with some blue

Figure 3.7 - ***Key:*** *A 5 fold concentration increase, B 10 fold concentration increase, C 20 fold concentration increase; a = 5 µL of mushroom extract; b = 15 µL (3 x 5 µL) mushroom extract.*

The reactions were repeated in triplicate to test for variations within the same conditions (*Figure 3.8*).

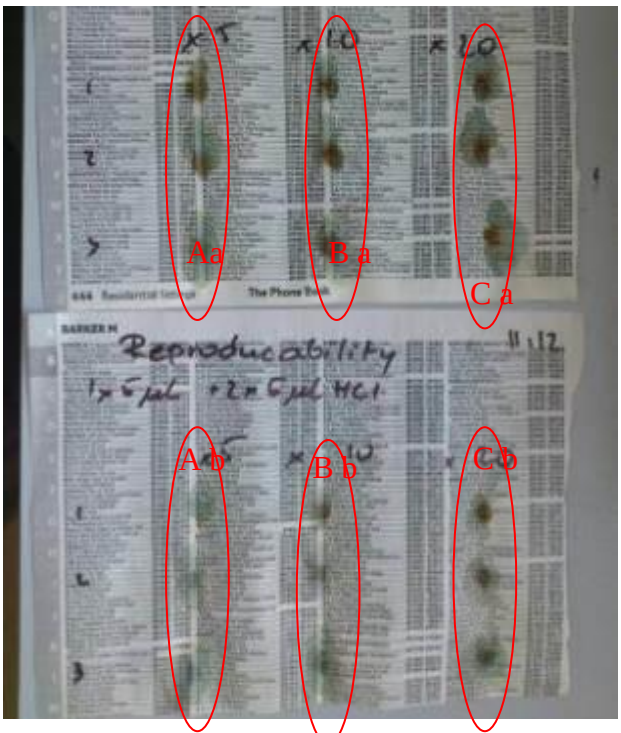


Figure 3.8 - ***Key:****A = five times original concentration, B, 10 = ten times original concentration, C = twenty times original concentration; a = 5 µL of mushroom extract; b = 15 µL (3 x 5 µL) mushroom extract.*

From the results shown, it can be seen that increasing the concentration of the mushroom extract does not aid colour detection with increasing concentration leading to a much darker colour, not immediately recognisable as blue.

3.1.1.5 Application method

A micropipette and a TLC spotter were used to apply the methanolic Death Cap extract A (0.834 g/mL). A TLC spotter was employed to apply 37% HCl onto the extract spots (Figure 3.10).

Figure 3.9 - ***Key:*** *A = 5 µL spotted using a micropipette; B = triplicate spotting performed using TLC spotter.*

TLC spotter allows better control over the spotting area but it is difficult to accurately determine if five µL is delivered to the spot.

In conclusion, the Meixner test works best for Death cap mushroom extract when using a TLC spotter to apply liquids onto the paper. MeOH extracts are absorbed by paper very quickly so to achieve a small spot area it is important to apply small volumes of extract. Extra layers of mushroom extract can be added to dried spots to increase the amount of extract. Application of volumes of HCl onto the paper causes the spot to spread. Paper does not absorb the HCl easily, a hemispherical drop forms on paper which spreads over a couple of minutes. Smallest possible volumes are easier to control on the surface. TLC spotters allow application of the acid onto controlled area (dried spot size). The drawback of using a TLC spotter is that accurate volume delivery cannot be controlled.

Concentrating analysed extracts will potentially increase the amount of toxin; theoretically, increased concentration will result in decreased sample volumes required for spotting. It

was found that increasing the concentration of the mushroom extract resulted in an introduction of a yellow/orange colour to the spot area so giving a perturbed colour.

3.1.1.6 Optimised method for Death Cap mushrooms

The optimised method for the Meixner test is described in the section 5.2.1 and 5.2.2. In summary:

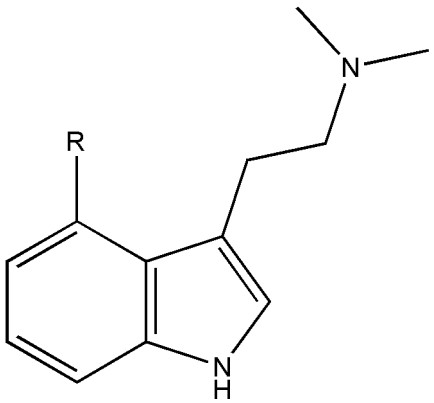
Table 3.4 – Method developed for the Meixner test.

Step number	Solution added to paper	Drying time at ambient temperature (min)
1	2 or 3 x 5μL mushroom extract (0.167g/mL)	5
2	5 μL HCl (37%)	≤ 20

The optimised method was used to perform the tests described in section 3.1. Exceptions are noted in the text.

3.1.2 Meixner test on psilocin and psilocybin containing mushroom species

Psilocin (*Compound 3.*2 **A**) and psilocybin (*Compound 3.*2 **B**) are also reported as giving positive Meixner test[26]. The two compounds are considered 'false positives' as these are not toxic compounds. *Stropharia caerullea* is thought to contain both psilocin and psilocybin components[15].



*Compound 3.2 – **A** – Psilocin, R – OH; **B** – Psilocybin, R – dihydrogen phosphate*

3.1.2.1 Stropharia caerullea

All mushroom extracts used in this section were prepared according to method described in section 5.2.1. The mushroom was extracted using MeOH - 0.5 g of mushroom in 3 mL of methanol.

The extracts were applied on to the paper using a micropipette (in order to accurately control the volume used). 15 µL were dispensed onto paper (3x5 µL) and allowed to dry between applications. A larger volume was used than for Death Cap mushrooms in the previous section. After drying, 10 µL HCl was added (2x5 µL) and was the colour allowed to develop at ambient temperature for 10 min. The detected colour was pale pink and it faded over 18 h.

Figure 3.10 - S.A. = Stropharia caerullea, D.C. = Death Cap, B.M. = Button Mushroom, Bl = Blank (MeOH).

The reaction with *Stropharia* was repeated in quadruplicate (*Figure 3.11*). The pink/red/brown colour was observed in all four spots. In our hands, a distinct colour difference was observed between a Death Cap extract (blue/green) and a *Stropharia* extract (pink/red/brown). The observed colour change would suggest the presence of 5-oxygenated tryptamines (e.g. 5-hydroxy-3,3-dimethyltryptamine - *Compound 3*.3) rather than the 4-oxygenation found in psilocybin and psilocin.

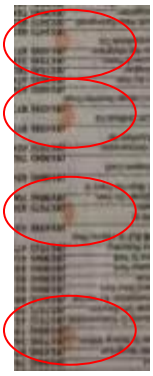
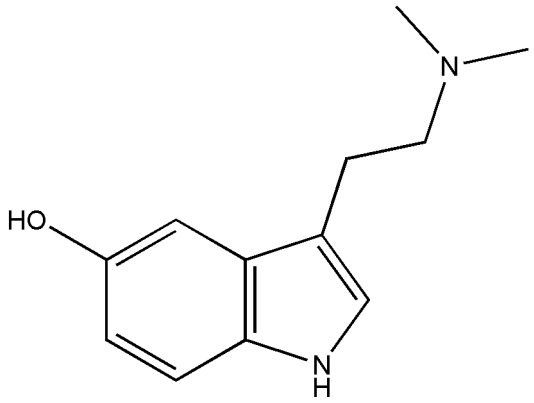


*Figure 3.11 - **Key:** Spots of Stropharia caerullea repeated four times*



Compound 3.3 – 5-hydroxy-3,3-dimethyltryptamine

The presented result was not conclusive. However, it gives some grounds to believe that there could be other indole containing compounds present in the *Stropharia caerullea* mushroom. If psilocin and psilocybin were present, a pale blue colour would be expected. Therefore, the result of the performed Meixner test contradicts with the recent studies published, which state that *Stropharia caerullea* mushrooms contains psilocybin and psilocin[31].

3.1.2.2 Psilocybe semilanceata

Four unidentified mushrooms were suspected to be hallucinogenic members of the *Psilocybe* genus. The Meixner test was performed in order to support the identification of the dried specimens. A blue colour change in the Meixner test would support the presence of the psilocin and psilocybin.

The mushrooms (0.34 g) were soaked for an hour in MeOH (2.04 mL). 5 μL of the extract was applied onto commercial newsprint and developed with the same volume of 37% HCl. The spot was developed for 5 min.

The spot with mushroom extract turned pale blue colour, the colour change was significant compared to the blank spot (MeOH).

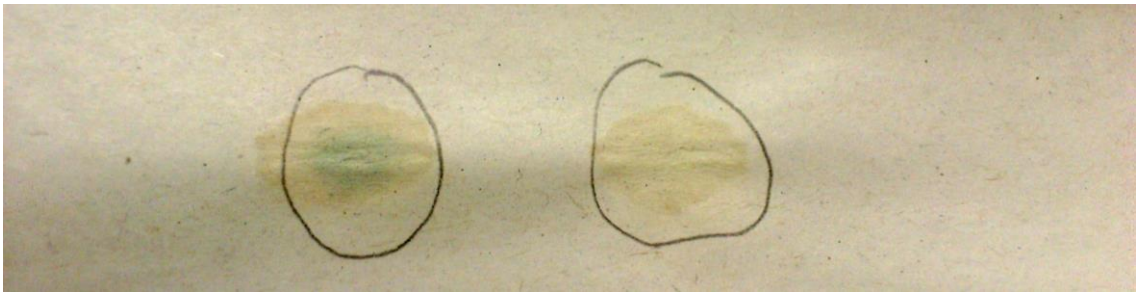


Figure 3.12 – Left: Psilocybe mushroom; Right: blank (pure MeOH).

The blue colour indicated that the mushrooms provided were in fact members of *Psilocybe* genus. Closer visual identification of the mushroom samples was impossible due to the dry and deformed nature of the samples.

3.1.3 Meixner test on *Paxillus involutus*

Panaeolus ater, is also known as Lawn Mower`s Mushroom. A very common lawn mushroom, it grows after mowing the lawn especially in wet conditions[12]. To date there is no published research on using the Meixner test on *P. ater* mushrooms. It is proposed that the *P. ater* mushrooms contain psilocybin or psilocin[15] therefore should result in blue colour under Meixner test conditions.

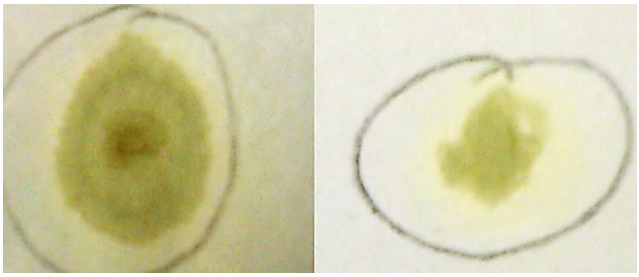


*Figure 3.13 – Meixner test performed on Paxillus involutus. On the left - P. involutus (*brown colour is visible in the middle of the left-hand side spot, it is the colour of the mushroom extract itself); on the right – blank (37% HCl).*

3.1.4 Meixner test on *Panaeolus ater*

The brown colour observed, is from the mushroom extract itself, no blue colour was detected. Lack of blue discolouration means that the available specimen of *P. ater* does not contain psilocybin or psilocin. The negative test does not mean that no specimens of *P. ater* will contain the hallucinogenic compounds. It could depend on the environment and growing conditions of the individual mushroom.

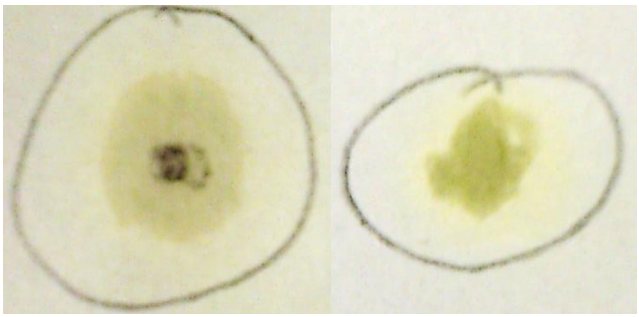


Figure 3.14 – Left: Meixner test on Panaeolus ater; Right: blank

3.1.5 Model compounds for Meixner test

The Meixner test currently consists of a crude spot test on high lignin newsprint. The colour change if it occurs, is qualified subjectively by visual inspection. The Meixner test is rarely used in clinical settings, mainly due to the lack of confidence, which physicians have in interpreting the results. A recent study has shown that the process of clinicians' interpretation of a colour change can be prone to error [13] as the Meixner test does not adequately distinguish between lethal amanitin compounds and other non-lethal mushroom indole compounds.

3.1.5.1 Indole derivatives

In order to work towards a robust test, which can be used reliably by non-chemists, the underlying chemistry of the test needs to be defined.

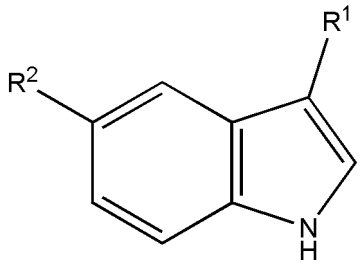
The Meixner test gives a colour change with oxygenated indoles, found in alkaloid compounds of mushroom origin. The position of the oxygen around the aromatic ring defines the colour observed in the Meixner test (*Table 3.5*).

Table 3.5 –Position of indole oxygenation and colour detected in the Meixner test.

Position of oxygen	**Colour observed**
7	Unknown
6	Light green-blue (Amatoxins)[26]

5	Red/brown [26]
4	Grey-pale blue (a false Meixner test) [26]
No oxygenation	Red [IX]

In order to explore the chemistry of the Meixner test, non-toxic, non-hallucinogenic compounds needed to be identified as suitable model compounds. As 5-oxygenation around indole gives a red/brown colour in the Meixner test, a potential model compound, 5-hydroxyindole acetic acid – 5-HIAA (*Compound 3.4*), was used in a newsprint based Meixner test.



Compound 3.4 – 5-Hydroxyindole-3-acetic acid (5HIAA): R^1 – acetic acid, R^2 – OH;
Indole acetic acid (IAA): R^1 – acetic acid, R^2 – H;
Indole: R^1, R^2 - H

In order to avoid the use of pre-printed newsprint (telephone book) a selection of unprinted high lignin paper was purchased from *www.candigifts.co.uk*. A methanolic solution of 5-HIAA (2 mg/mL) was spotted onto commercial high lignin paper, the Meixner test was performed using the optimal conditions previously identified on both high lignin paper and paper that contains no lignin (filter paper). 5-HIAA gave a distinct colour when compared to the blanks (*Figure 3.15*).

[IX] The colour has been tested using indole compound (described in the section 3.1.5.1)

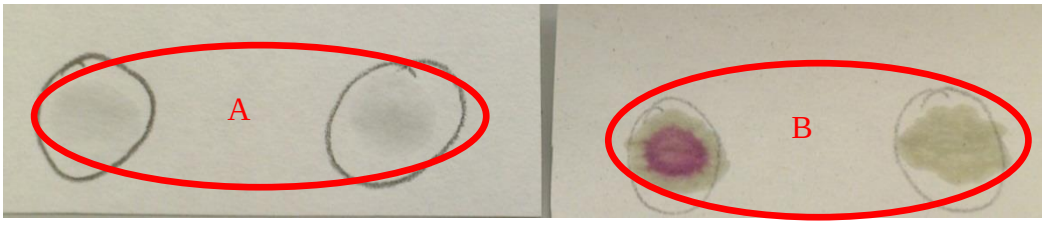


*Figure 3.15 – **A** – Filter paper, from left:5HIAA, blank (MeOH); **B** – Lignin paper, from left: 5HIAA, blank (MeOH)*

37% HCl is a strong acid with a pKa of - 8 in water[32]. Therefore, it was asked whether a strong acid was necessary to obtain a colour change in the Meixner test. Acetic acid with a pKa of ~4.7[32], was used under standard conditions, developed in the process of this project, on high lignin paper.

Acetic acid resulted in a very pale reddish tan spot with the 5-HIAA. Spot developed with HCl yielded intense purple colouration. As expected, blank spots did not develop any colour. From these results, it appears that strong acid is necessary for a successful colour change.

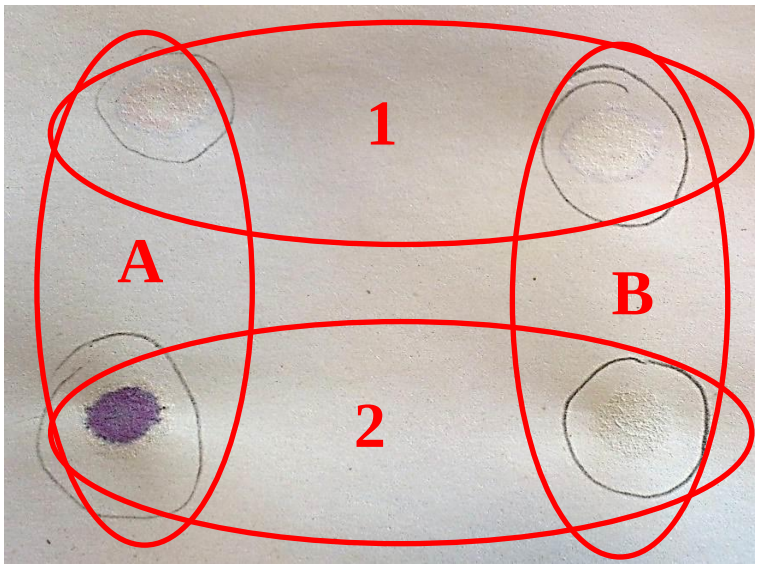
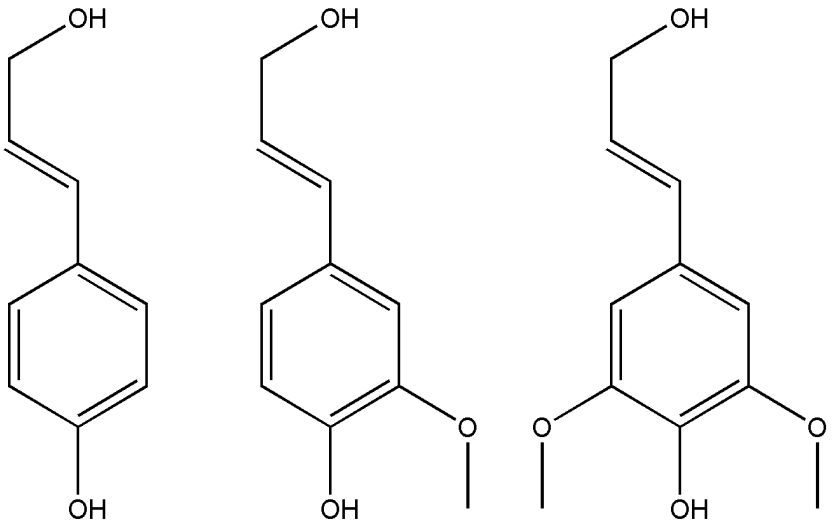


*Figure 3.16 – Key: **A** - spots with 5HIAA; **B** - blank reference spots (MeOH); **1** - spots developed with acetic acid; **2** - spots developed with 37% HCl.*

Attention was then turned to the lignin component of the test. Lignin is a complex, high molecular weight, plant polymer. The details of the reacting species in the Meixner test have remained elusive. Lignin is a highly complex molecule proposed to be a random

polymer of coumaryl, coniferyl and sinapyl alcohols (*Compound 3*.5)[33]. Determination of structure in the natural state is still an active area of research[33].



*Compound 3.5 – **A**: coumaryl alcohol; **B**: coniferyl alcohol; **C**: sinapyl alcohol*

Low quality newsprint has grey/brown colour because of the presence of aromatic rings within the lignin structure. High quality paper is delignified in the production process, resulting in a white paper. Delignification can include bleaching in an acidic solution, which dissolves the lignin structure which then can be extracted[33].

In a paper by Moerke[34], looking at colour tests of amino compounds with newsprint, the paper was extracted with hot HCl and a colour test with sulfonamides performed in solution rather than on the paper. The research states that the reactive lignin component was "*freed the reactive group of lignin, previously fixed*". To explore if a significant lignin component was extracted from commercial newsprint under acidic conditions, high lignin paper was extracted with 37% HCl over 1h and 3.5h. 100 μL of the acidic extract was added to methanol solution of 5-HIAA (2 mg/mL). Both 5-HIAA solutions gave slight pinkish colouration using lignin extracts (see *Figure 3.17*). The intensity of the colour was very faint, possibly caused by high dilution of the lignin extract.

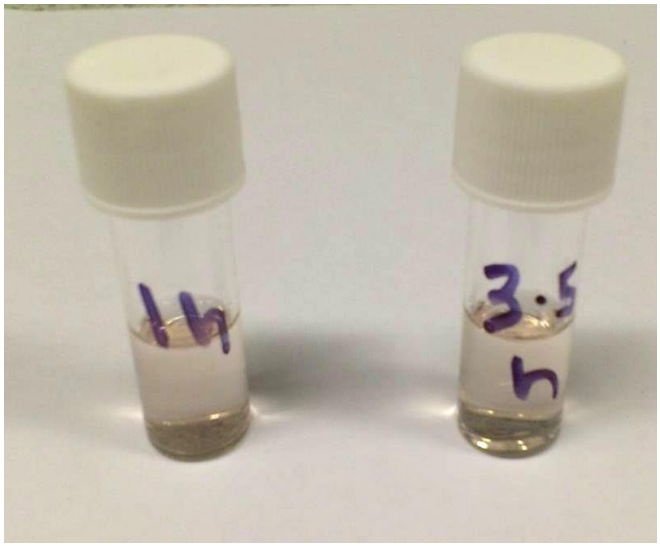


Figure 3.17 – Colour change with lignin extracted for 1 hour (left), for 3.5 hours (right)

The test was repeated using an increased amount (300 µL) of the acidic lignin extract (*Figure 3.18*). In the repeated test, colours in both vials have changed. As expected, the colour in the vial where 300 µL of the acidic lignin extract was added turned darker giving a more intense colour compared to the vial with only 100 µL of the lignin extract (*Figure 3.18*).

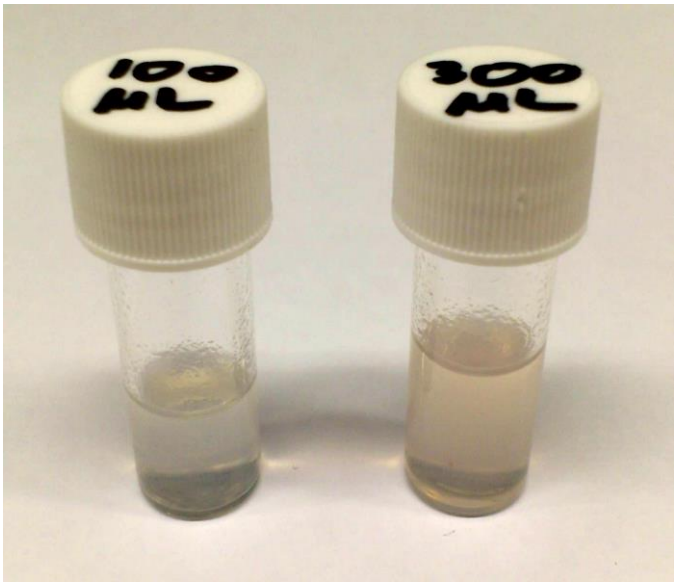


Figure 3.18 – Left: vial with 1 mL of 5-hydroxyindole-3-acetic acid and with 100 µL of lignin extract; Right: vial with 1 mL of 5-hydroxyindole-3-acetic acid and with 300 µL of lignin extract.

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In both experiments, only a faint colour change was observed when acidic lignin extract was added to methanolic 5-HIAA. If the reaction was reversed, methanol 5-HIAA added to neat acid extract of newsprint, the colour change could be more significant.

Newsprint was extracted with 37% HCl for 1 hour (2mg/mL) added. A green-brown colour was observed not the expected pink colour. The colour became stronger with increased amount of 5-HIAA solution (*Figure 3.19*). Any colour change could be masked by the original yellow/green newsprint extract when using 37% HCl as the extraction media.

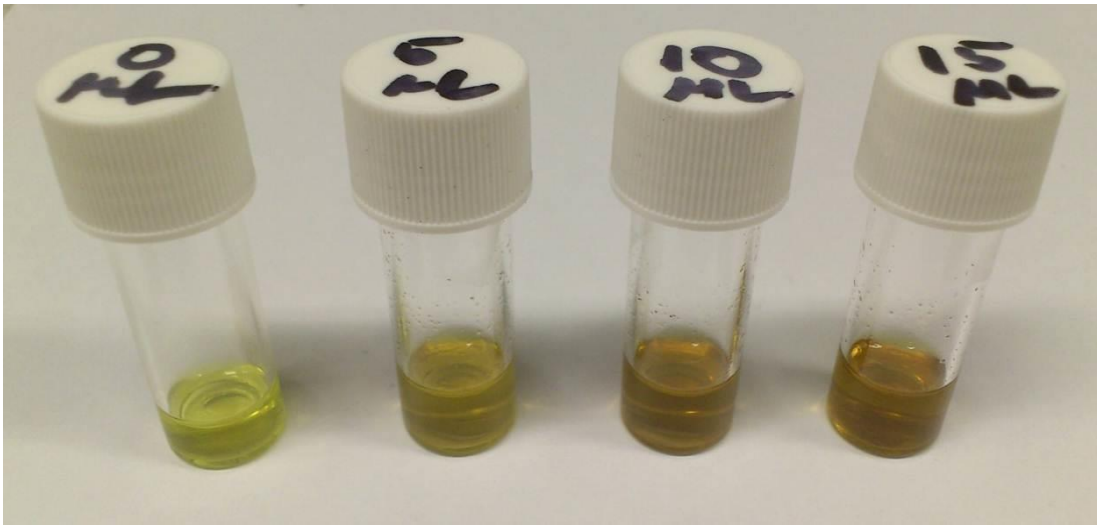


Figure 3.19 – Colour change by addition of (from left: 0 µL, 5µL, 10 µL, 15µL) 2mg/mL of 5-hydroxyindole-3-acetic acid solution in MeOH.

In attempt to reduce the yellow colouration and find an optimal concentration of HCl to extract the paper the Meixner spot test was performed using four different concentrations of HCl. 5 µL of methanolic 5-HIAA was spotted onto newsprint. Various concentrations of hydrochloric acid were applied to the spots (5 µL).

In the positive tests a purple colour was observed with a lessening in intensity of colour as the concentration of acid decreased (*Figure 3.20*, *Table 3.5*). The colour change varied when the acid concentration was increase from 3M to 6M. The spots developed with 6M, 9M and 12M HCl were very similar in colour intensity.

Table 3.6 – Table presents the effect of four different concentrations of hydrochloric acid on the resulting colour of the Meixner test using the 5-hydroxyindole-3-acetic acid.

Acid strength/M HCl	**Colour observed**
3 (9.25%)	pink / pale purple
6 (18.5%)	dark purple
9 (27.75%)	dark purple
12 (37%)	dark purple

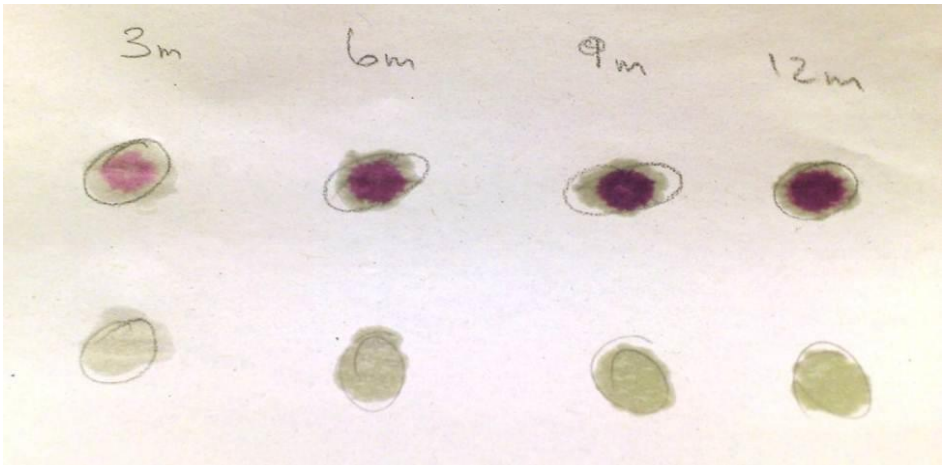


Figure 3.20 - Meixner spot test of 5-hydroxy-3-indole acetic acid (top row) using varying concentrations of HCl –from left: 3, 6, 9 and 12 M. Bottom row blanks HCl 12M).

The newsprint was extracted with the two lowest concentrations of HCl (3M and 6M for 1 hour). The paper was removed and 5, 10 and 15 μL of methanolic 5-HIAA (2 mg/mL) added. With the lowest acid concentration, no colour was observed (*Figure 3.24*). When the concentration of HCl was increased to 6 M, a noticeable colour change occurred when 10 and 15 μL of 5-HIAA solution were used (*Figure 3.22*).

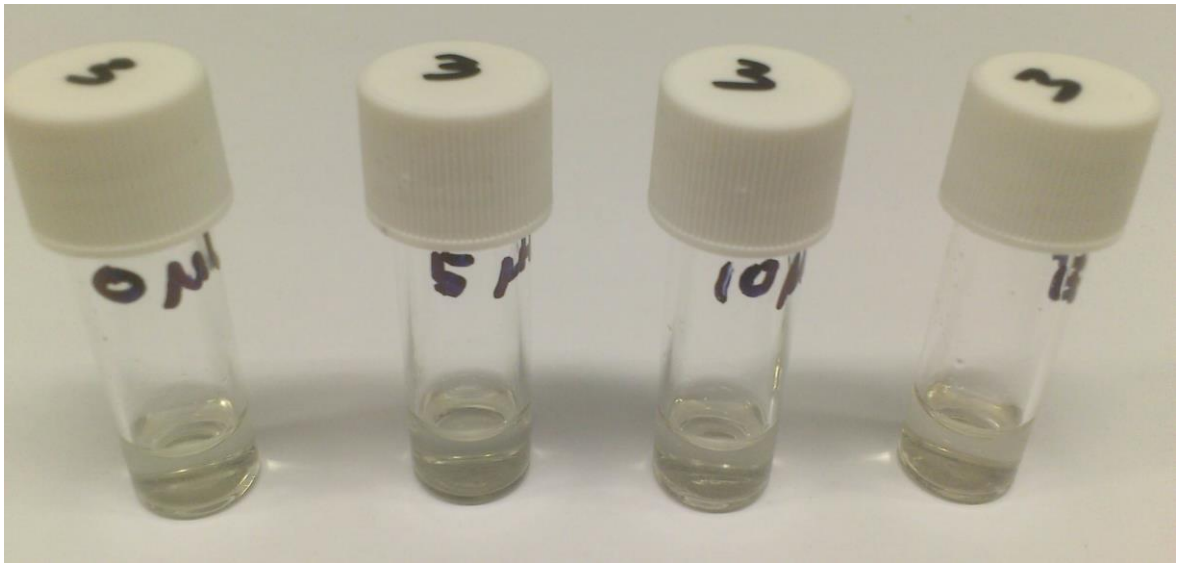


Figure 3.21 – Colour changes in reaction between 5-HIAA and lignin extracted in 3 M HCl.

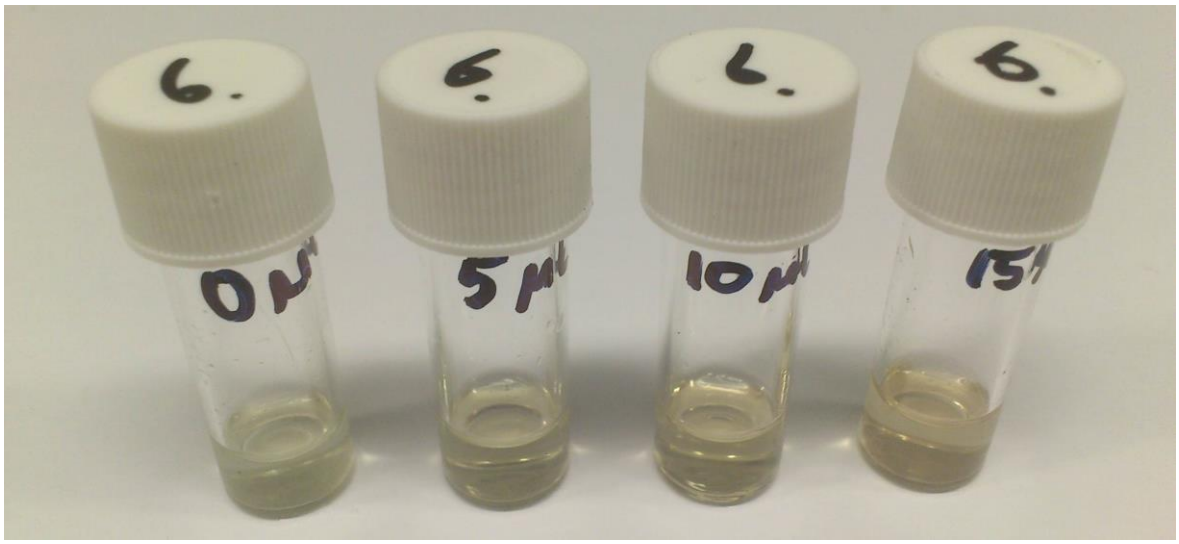


Figure 3.22 – Colour changes in reaction between 5-HIAA and lignin extracted in 6 M HCl.

The Meixner test with acetic acid (*Figure 3.16*) gave a faint colour change. Acetic acid was also used to extract lignin from the paper. The paper was soaked for an hour in acetic acid (3 mL) then 5, 10 and 15 µL of methanolic 5-HIAA (2 mg/mL) was added . It was noted that the paper extract was purple without addition of any 5-HIAA. The colour did not change significantly when 5 µL of the 5-HIAA solution was added. The colour intensity increased when 10 µL of 5-HIAA was added but the addition of 15 µL of 5-HIAA did not result in an increase in the colour intensity (*Figure 3.23*).

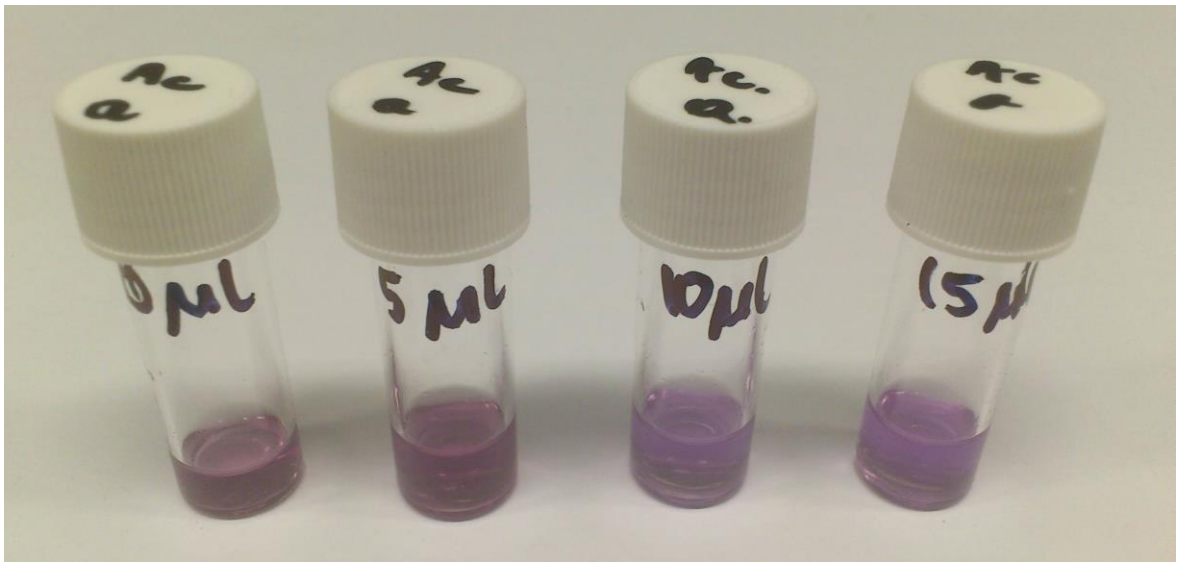


Figure 3.23 – Colour changes in reaction between 5-HIAA and lignin extracted in acetic acid.

In order to investigate if the observed colour change is reliant on the presence of the OH functionality on the aromatic ring, the focus was turned to indole acetic acid (IAA). The phallotoxins do not have the hydroxyl group on the indole ring and they do not undergo a colour change in the Meixner test[2].

To test the colour changes observed with IAA, 5 µL methanol solution of IAA (2 mg/mL) was applied onto newsprint paper followed by 5 µL of 37% HCl. Unexpectedly, IAA turned pink/red. The same test with 5-HIAA gave a much stronger colour (*Table 3.7* and *Figure 3.24*).

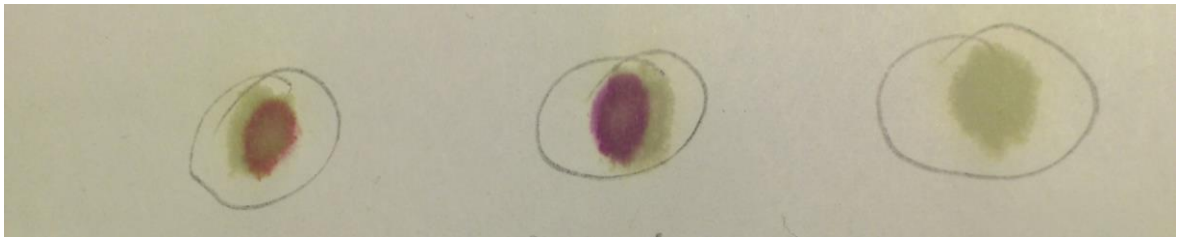


Figure 3.24 – From left: Indole-3-acetic acid (IAA), 5-hydroxyindole-3-acetic acid (5-HIAA), blank - methanol

Additionally, the 15 µL of 2 mg/mL of IAA were pipetted into 0.5 mL of lignin extract to compare with the same amounts of the 5-HIAA. Lignin extract was prepared by soaking 0.5 g of lignin paper in 3 mL of 37% HCl. The result was, both IAA and 5-HIAA changed the colour but IAA had slightly different colour hue (*Figure 3.25*).

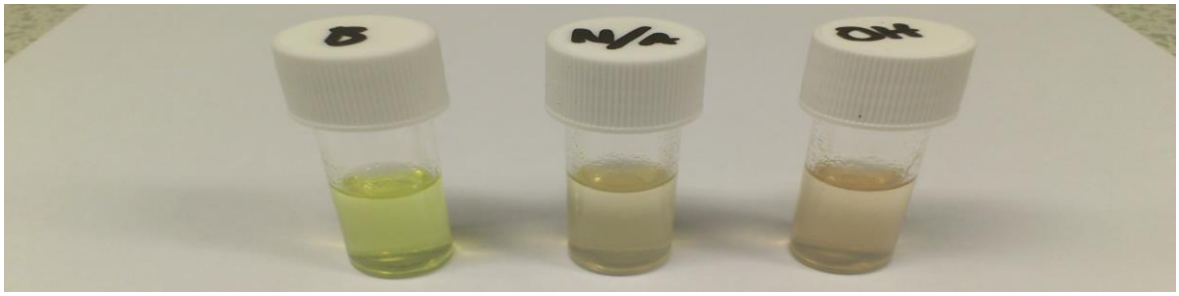


Figure 3.25 – From the left: blank (lignin extract), IAA in lignin extract, 5HIAA in lignin extract.

Table 3.7 – Colour changes and comparison of the indole acetic acid and 5-hydroxyindole-3-acetic acid developed with 37% HCl

Compound	Colour on paper	Colour in lignin extract
Indole-3-acetic acid	Red	Yellow/orange
5-Hydroxyindole-3-acetic acid	Purple	Orange/red
Blank (MeOH)	-	Yellow/green

There is a great variety of different types of paper on the market. It was necessary to examine the effects of available types of newsprint on the results of the Meixner test. Two of the available unprinted newsprints were compared to see how they affect the test results. There is an indication that position of the hydroxyl group on the ring is responsible for the colour change in the reaction. As neither indole nor IAA have any –OH groups on the ring they could be expected to yield the same colour. All solutions were made up to the 2 mg/mL in MeOH. Each spot was 5 µL and the same amount of 37% HCl was applied on top of each one of them. An extra blank spot was prepared with 5 µL of MeOH.

Indole and IAA gave different colours (dark purple and red respectively). In fact, the indole colour change was closer to the 5-HIAA, which was purple. The blank spot did not change in colour as expected. The difference between the two types of paper was very

small but significant. Paper supplied by "Seawhite of Brighton" resulted in clearer colours than one purchased on following links: http://www.candigifts.co.uk/items/PRODUCT-RANGE/WRAPPING~FILLING/PACKING-PAPER/list.htm and http://www.preservationequipment.com/Store/Products/Disaster-$4-Cleaning/Materials/Newsprint-Paper. The results can be seen in the *Figure 3.26* below.

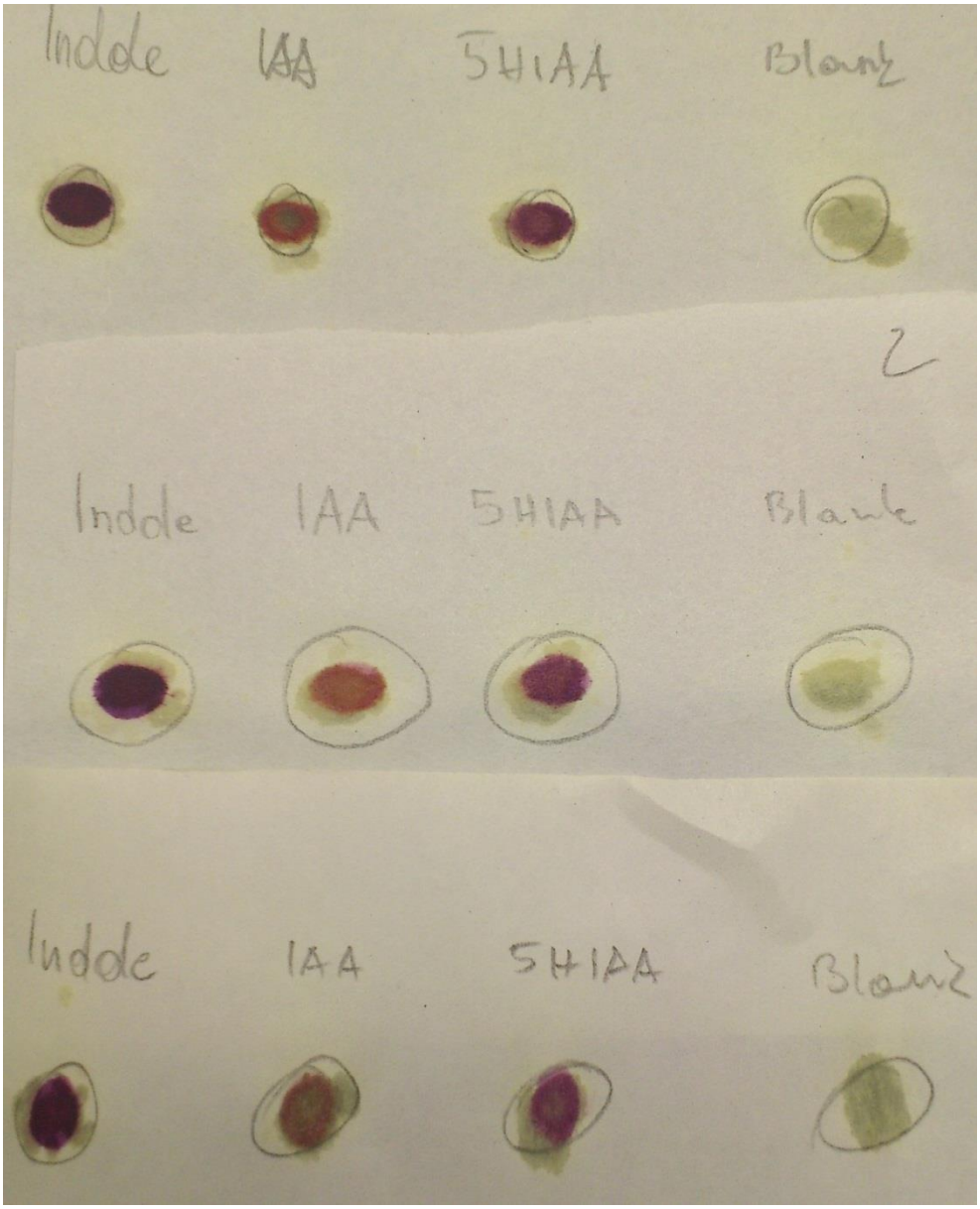


Figure 3.26 – Top row: paper purchased from www.candigifts.co.uk; Middle row: purchased from "Seawhite of Brighton" paper; Bottom row: purchased from http://www.preservationequipment.com; From the left: Indole, indole-3-acetic acid, 5-hydroxyindole-3-acetic acid and blank (MeOH).

Paper purchased from different sources gave a different colour change of all the indole and indole derived compounds. The paper obtained from "Seawhite of Brighton" have very similar colours to one from www.candigifts.co.uk website, despite this fact there was a difference in colours and colours were more intense and more saturated on the paper obtained from http://www.candigifts.co.uk website.

3.1.5.1.1 Meixner test IR spectroscopy

The Meixner test using 5-hydroxyindole-3-acetic acid (5-HIAA) was analysed using IR spectroscopy in order to see if any structural information from the coloured product of the Meixner test could be obtained.

The spot was prepared applying 5µL of 2 mg/mL of methanolic 5-HIAA onto newsprint paper. The spot was left to dry and then 5 µL of 37% HCl applied on top. The measurement was taken on the spot (black line) and on the clean area (blank – blue line) of the lignin paper (A4 Newsprint from Seawhite of Brighton).

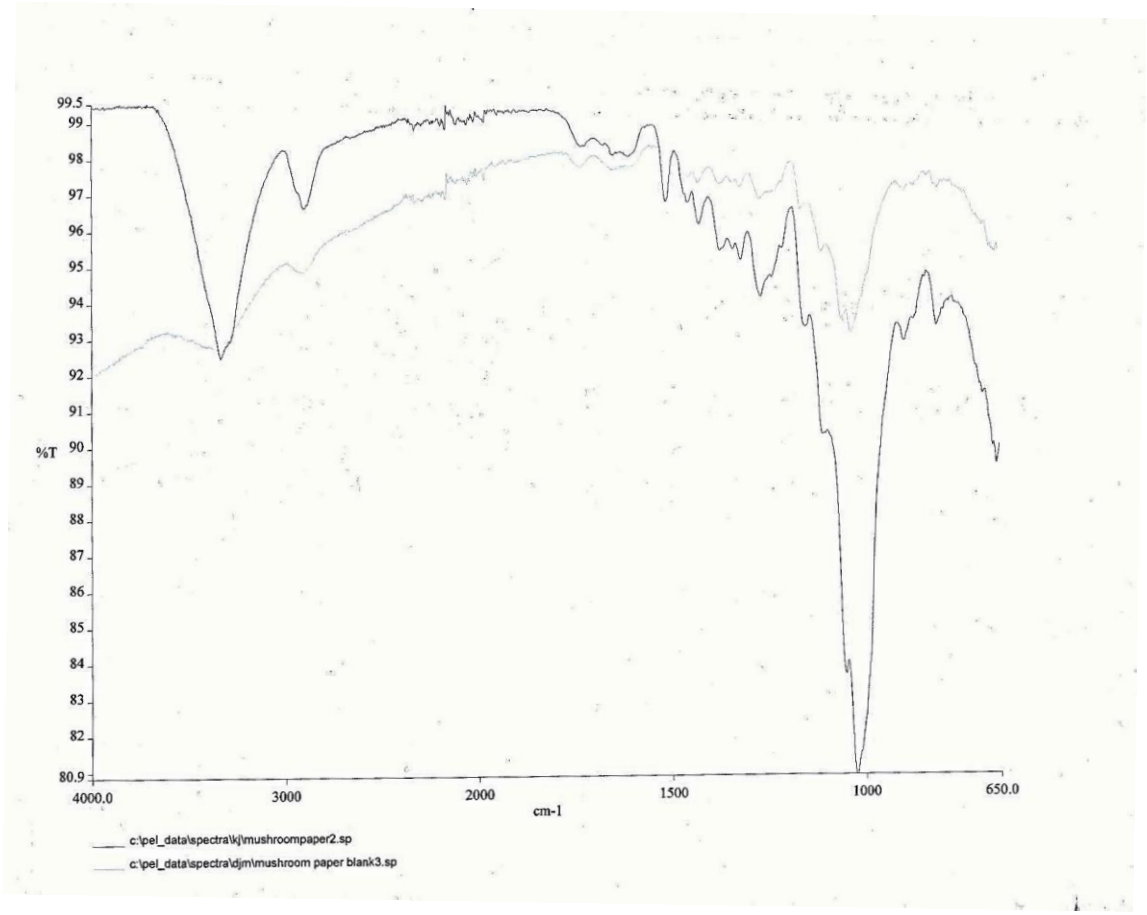
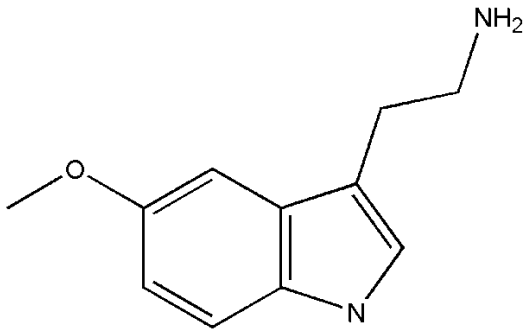


Figure 3.27 – Meixner test IR spectrum. Pale line – blank paper; dark line – Meixner test.

There were no obvious differences between spectra.

3.1.5.2 Tryptamine

5-Methoxytryptamine (5-MT - *Compound 3*.6) was has been reported to yield a blue colour in the Meixner test [25] so it was chosen as an alternative model compound.



Compound 3.6 – 5-methoxytryptamine (5-MT). Tryptamine has similar substituents on the indole core structure as amatoxins.

2 mg/mL and 0.2 mg/mL methanolic stock solutions were prepared. Both solutions were spotted (5 µL) onto lignin paper together with 5-HIAA at two concentrations 2 mg/mL and 0.2 mg/mL creating a four spot grid. 5µL of 37% HCl was added each of the four spots.
The difference between the 5-HIAA and 5-MT at 2 mg/mL was small; there was no observable change over the time.

Figure 3.28 – The difference in colour between the two tested compounds after 10 minutes at 2 mg/mL. Left: 5-HIAA; right: 5-MT.

The diluted solutions gave more information. Within the first minute after the test the colour of the diluted spots were similar. After 10 minutes, colour difference was more significant between the 5-HIAA and 5-MT, reddish and purple/blue respectively (*Figure 3.29*). After the initial period, the colour remained over time (3 hours). The blue colour reported in the paper started developing between one to two minutes. By the fifth minute of the observing time, it got more intense and remained consistent (see *Figure 3.30*).

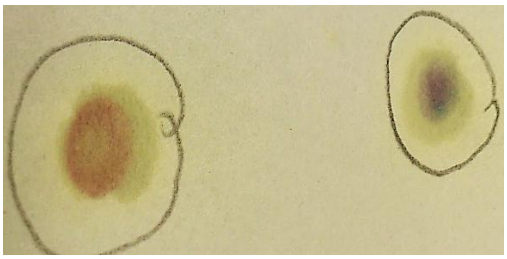


Figure 3.29 – The difference in colour between the two tested compounds after 10 minutes at 0.2 mg/mL. Left: 5-HIAA; right: 5-MT.

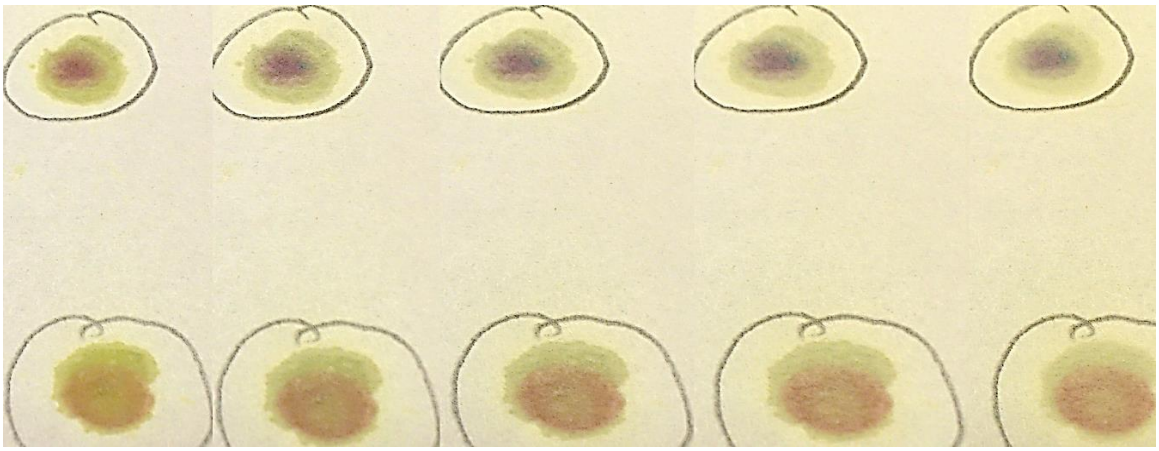


Figure 3.30 – Time lapse of the colour change. The colour change after x minute;, from the left: 1 min, 3 min, 5 min, 7 min and 10 min. Top 5-MT, bottom 5-HIAA

The 0.2 mg/mL 5-MT gave the blue discolouration but it is possible that the reaction is a mixture product as there is a blue colour hotspot visible on the purple background (*Figure 3.31*).

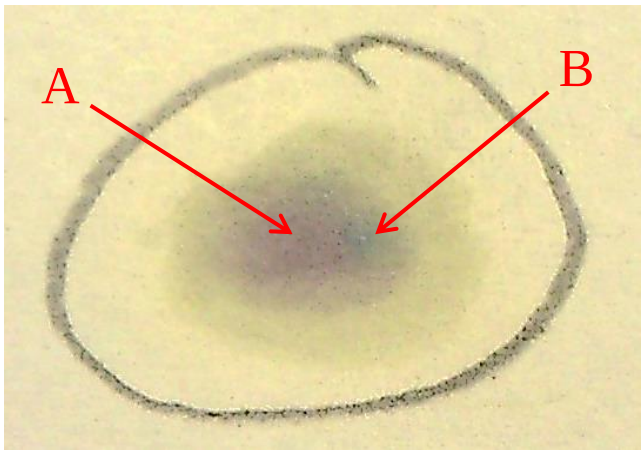


*Figure 3.31 – Hotspots of purple - **A**; blue - **B** colour.*

Table 3.8 – Table of model compounds colours

Compound	Spot colour
Indole	Purple
Indole acetic acid	Red
5-Hydroxyindole acetic acid	Purple/pink
5-Methoxytryptamine	Purple/blue

3.1.5.3 Lignin model compound

Coniferyl aldehyde is a potential monolignol in the Meixner test. A solution of coniferyl aldehyde was substituted for high lignin paper. 0.5 mL of a 20 mg/mL methanolic coniferyl aldehyde solution was pipetted into four separate vials. 15 µL of 2 mg/mL of three mushroom model compounds (indole, indole acetic acid and 5-hydroxyindole-3-acetic acid) were pipetted into three of the vials (one vial is left as a control sample). 15 µL of HCl (37%) was added.

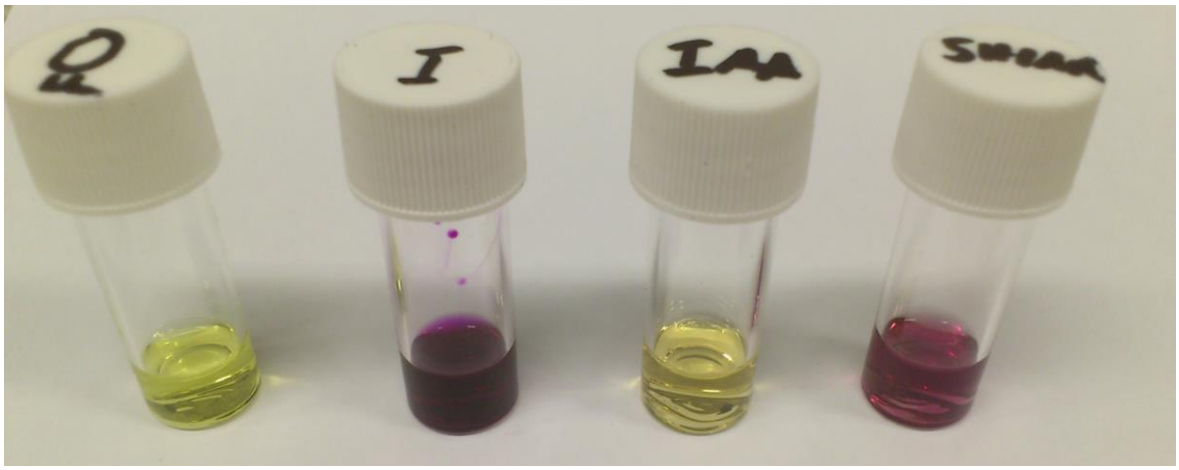


Figure 3.32 – From the left: blank vial with coniferyl aldehyde (yellow/green); indole added to the coniferyl aldehyde (violet); indole acetic acid added to coniferyl aldehyde (orange/green); 5-hydroxyindole acetic acid added to coniferyl aldehyde (purple).

All three coniferyl aldehyde solutions changed colour when reacted with indole, IAA and 5-HIAA to violet, orange and purple respectively. The colour changed immediately after hydrochloric acid was added to the solutions. Colours obtained from the solution of the

coniferyl aldehyde could support the theory that particular monolignol present in wood material, reacts with indole ring as a substrate of the reaction.

3.1.6 Indole oligomerisation

Indoles can undergo a reaction where two or more heterocyclic rings can react together forming one structure. Those compounds can be coloured and could be the colour changes observed in previous sections especially where a pink to red colour is observed as it has been reported that indole dimers display colour change - brown[35]. Deducing from colour difference by using different indole derivatives in the Meixner test, using variety of using indole derivatives to dimerise should give other colours as well. 15 μL of 37% HCl was added to 0.5 mL of 2 mg/mL of the 5-HIAA in MeOH No apparent signs of a visible colour change were observed. The solution was dried on a hot plate at 35 °C for four hours. When dried, the residue developed blue discolouration and was re-suspended in 0.5 mL of MeOH. The re-suspended solution became less intense, but did remain blue colour.

One microliter of the 5-HIAA stock solution and the reacted solution were spotted onto a silica TLC plate. The plate was developed in ethyl acetate:acetic acid 95:5. The stock solution resulted with one spot with an R_f = 0.59. The reacted sample showed three spots with R_f values of 0.37, 0.63 and, 0.85.

Due to the potential oligomerisation of the indole ring, a colour change can be observed on the non-lignin paper. The reaction is observed when a 5 μ of 2 mg/mL of indole is applied onto filter paper and developed with HCl.

The presented result suggests that a second reaction between small indole derivatives could be occurring alongside the Meixner test reaction. The larger, sterically hindered amatins are unlikely to dimerise.

3.2 Mushrooms and their identification

A list of poisonous mushrooms was prepared as the part of this research. The information was based on the allocated categories described by two mycology books[36],[37]: edible, inedible and poisonous (deadly or lethal were included into the poisonous category; mushroom species labelled as suspect were not included). The search returned 106 different species categorised as being poisonous.

The website: rogersmushrooms.com lists 180 species as poisonous, in addition, there are 91 mushrooms which are suspected to be poisonous but were not included in the above count.

The list of the 106 poisonous mushrooms is presented in the section 6.4.

Out of the 106 mushrooms on the prepared list, there are 62 mushrooms classed as poisonous but no poisonous compounds have been identified in a survey of the literature. The number means that more than 58% of the mushrooms have an unidentified compound causing poisoning symptoms. Compounds which have been identified, are presented in the appendix in the section 6.3.

The performed survey has demonstrated how little is actually known about mushroom poisoning.

3.2.1 *Agrocybe praecox* – microscopic features

To show how misleading macroscopic features for mushroom identification can be, six mushrooms were collected under the name *Agrocybe praecox*. They were visually the same and found in the same area. Spore size of the *A. praecox* is 5 – 5.5 µm wide and 9 – 10 µm long[1].

Spore prints of all six specimens were taken and the colour codes together with the spore size are presented in the table (*Table 3.9*).

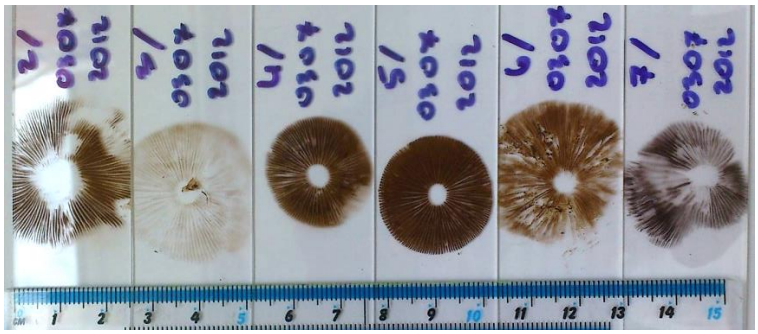


Figure 3.33 – Spore prints of six mushroom specimens named Agrocybe praecox after macroscopic investigation. Slide`s reference numbers from the left: 2/03072012, 3/03072012, 4/03072012, 5/03072012, 6/03072012, 7/03072012.

Sample No.	Spore size (µm)		Colour
	Length	Width	
2/03072012	11.3	8.6	Chestnut
3/03072012	8.3	5.3	Milky Coffee
4/03072012	8.1	5.2	Chestnut
5/03072012	8.7	5.3	Chestnut
6/03072012	9	5.2	Rusty Tawny
7/03072012	7.8	4.7	Fuscous Black

Table 3.9 – The sample number is the reference number figuring on the microscope slide above; all spore size values are presented in µm; Colour codes are given in accordance to the colour chart in the "Encyclopedia of Fungi of Britain and Europe" by Michael Jordan

Out of the six specimens, only four fall within ±1 µm of the referenced spore size. If the spore size measurements are measured against the given reference values, only one of the collected mushrooms can be identified to be *A. praecox* with high confidence.

For mushrooms with no unique visible features, it is very important to take and measure the spores. It often is the key information necessary for the identification of the mushroom, but despite having the measurements, identification still can be prone to error.

4 Chapter 4- Conclusions and future work

4.1 Conclusions

As part of the mushroom collection process, a list of 105 common poisonous mushrooms from Great Britain and Europe has been collated. The list has been done based on the classifications of poisonous mushrooms in two mycology guides[36],[37].

Creation of a bank of mushrooms was accomplished in close cooperation with the recorder of the Mid Yorkshire Fungal Group (MYFG) – Malcolm Greaves. Over the period of this study (between October 2011 – July 2012), 156 different species were collected in total. About 80, out of the samples collected, were recorded with the British Mycological Society (BMS) in the official database of British mushrooms.

To identify the mushrooms a guide to maximize the chance of correct identification has been developed. The guide takes a user step-by-step through the data collection points which are important to positively identify any mushroom.

To obtain the best conditions for the Meixner test a set of experiments were performed to explore five variables. The experiments analysed drying conditions, different volumes of mushroom extract, and different volumes of 37% hydrochloric. Methods of application of the liquids onto the paper and the concentration of the mushroom extract were also explored. The optimised method can be used to detect or help identify poisonous *Amanita* genus.

There are many mushrooms which have unknown compounds in their bodies and newly discovered chemicals have the potential to react with the Meixner test.

Knowing the toxin distribution is changing depending on the part of the fruiting body of the mushroom it can be expected that the spot will be most intense if the extract was used from the gills of the mushroom. Considering practicality, the tissue which is the easiest to use, is from the cap of the mushroom as it is the second part of the body to have most concentrated level of toxin[10].

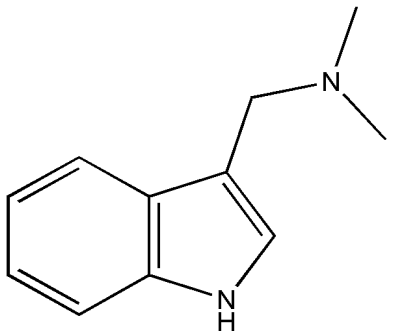
Additional tests

During the process of this project IR spectrum was obtained on the selected areas of the paper using an IR spectroscopy which gave limited information.

4.2 Future work

The whole area of science described in this thesis is still greatly under-investigated. Therefore, some areas should be examined. Future work should concentrate on yet untested mushrooms, using a combination of the Meixner test with more instrument-based analysis such as LC-MS.

Some species of grasses (family *Polleacae*) contain an indole derivative, which is very close in structure to tryptamine – gramine (see *Compound 4.1*)[38]. Having an indole as the main element of structure suggests it may react with newsprint the same way as mushrooms.



Compound 4.1 - Gramine

Understanding the key reaction(s) occurring in the Meixner test will help to understand what type of mushrooms it is possible to test and what results are to be expected. The different colours could be an indication of the type of compound present in the mushroom.

4.2.1 Application of the Meixner test to other plants

In 1963, a group of scientists published their study in which they performed Ehrlich reaction on active compounds of plants from *Cannabis* genus. The active compounds were cannabidiol, cannabinol and tetrahydrocannabinol. Since both the Ehrlich test and the Meixner test involve similar reagents, it is possible that a colour change could be observed under Meixner test conditions[39].

5 Chapter 5 - Experimental methods

5.1 Spore size measurement

5.1.1 Taking a spore print

To take a spore print, separate the cap from the mushroom stem. A thin or delicate specimen might require using a knife or scissors to prevent damage to the cap. Place the cap on a sheet of white or coloured paper to complement gill colour. If the mushroom feels dry, use a pipette to add a drop of water to the cap. Cover the cap with a glass dish or plastic cup. Leave the mushroom cap undisturbed for a few hours, preferably overnight so that it has enough time to release sufficient number of spores. Finally, take a photograph of the spore print alongside a ruler and colour chart.

5.1.2 Mushroom drying

Place the mushroom in large container with airflow (e.g. 500 mL beaker, a large plastic cup or a cardboard tray). Put mushrooms into a fume cupboard. Turn over every 8 hours for even drying. After 2-3 days, depending on the size of the mushroom (large mushrooms can take even up to 4 days to dry), put the samples into plastic pots with screw caps. If a new pot is being set up write the name on the side and on the screw cap put the first letter of the genus and first three letter of the mushroom`s species. Such practice allows for quick and efficient identification.

5.1.3 Spore size measurement

Actions in this section are performed after taking the spore print as described in section 5.1.1. Scrape the spores from the spore print to the edge of the paper. Next, transfer the spores into a small tube. Pipette small amount of HPLC grade water (0.5 mL) into the tube. Place a drop (3 μL) of the suspended spores onto a microscope slide. Put a cover slip onto the top of the microscope slide. Put the slide into a ringing table[X], Spin the plate and as it spins freely use a see-through nail hardener make an undisturbed seal around the cover slip.

[X] Ringing table – is an instrument that is fitted with a pair of clips, which hold down the slide on spinning plate that allows the placement of putting a neat and even seal around the cover slip.

The microscope:

Model: Zeiss Axiovision A1 Imager

Camera: Axiocam

Software: Axiovision 4.8

Magnification (x 1600 or x 2500):

Place the sample onto the slide table. Locate the area to be analysed using low magnification (x450 – lens x 45 and x 10 magnification binoculars). Change the lens to a higher magnification (x 100). Adjust the focus on the area which has to be analysed. If necessary, use extra magnifications (x 1, x 1.6 or x 2.5). Binocular has fixed ten times magnification.

Mode of operation:

The microscope was operating in differential interference contrast (DIC) phase. To stop the refraction of light a single drop of oil was used at magnifications x 1600 and x 2500.

Calculations:

Use the formula for average to get a spore size and standard deviation to calculate its spread.

5.2 Meixner test

5.2.1 Performing the Meixner test

To perform the Meixner test first weigh Death Cap mushroom and a Button Mushroom (0.5 g dry weight - DW) into separate 50 mL polypropylene centrifuge tubes. Add methanol (20 mL) into an additional 50 mL polypropylene centrifuge tube, as a pipetting reservoir. Pipette MeOH (3 mL) into each of the tubes containing the mushrooms. Shake by hand for one to two minutes. Leave the extracts in the tube with the cap screwed on (to prevent MeOH evaporation) for a few hours or overnight, at room temperature. Remove the mushrooms from the MeOH extracts using tweezers. Place the mushrooms into a garlic press and squeeze the liquid extract from the mushrooms back into the individual tubes. Dispose of the mushroom parts from the garlic press (to waste) and rinse the press and the tweezers with MeOH before handling the next one of the samples. Apply two spots of the mushroom extract (5 μL) to lignin paper. Allow each spot to dry in between spotting and keep the area of the spotting down to 3 mm in diameter. To make the process reproducible, make another three spots of each extract following exactly the same procedure. Perform the process with control samples (negative – Button Mushroom extract and blank - MeOH). Develop the spots by applying 5 μL of 37% aqueous hydrochloric acid (HCl) to each spot using a clean TLC capillary tube (tap the tube with HCl to cover the spot area with acid). Leave the test in the fume cupboard to develop for 10 to 20 minutes. Take a picture of the spot with a copy of a colour chart ("The Encyclopedia of Fungi of Britain and Europe by Michael Jordan, Revised edition 2004, p.16) next to it (keep the two as close as possible). Record the relevant details (see section 5.2.3) of the spot and matching colour from the colour chart.

5.2.2 Performing the Meixner test with concentrated extract

Weigh out a Death Cap mushroom and a Button Mushroom (0.5 g dry weight - DW) into separate 50 mL polypropylene centrifuge tubes. Next, add methanol (MeOH) into an additional 50 mL polypropylene centrifuge tube. Pipette MeOH (3 mL) into each of the tubes containing the mushrooms. Shake by hand for one to two minutes. Leave the extracts in the tube with the cap screwed on (to prevent MeOH evaporation) for a few hours or overnight at room temperature. Now the extract is in concentration of 0.1667 g/mL (grams of dried weight of mushroom / volume of alcohol). Remove the mushroom from the MeOH extract using tweezers. Place the mushroom into a garlic press and squeeze the liquid extract from the mushroom back into the individual tubes. Dispose of the mushroom parts from the garlic press and rinse the press and the tweezers with MeOH after handling each one of the samples. Use sample concentrator to dry the extract. Pipette 0.6 mL of MeOH into the tubes and shake well. After pipetting extract is 0.0333 g/mL (grams of dried weight of mushroom / volume of alcohol). Apply two spots of the mushroom extract to the same place on the lignin paper using the end of TLC capillary tubes. Use clean TLC tubes to apply different extracts. Allow each spot to dry in between spotting and keep it down to three millimetres in diameter or less. Make four separate spots in the same way (apply the same amount of extract). Repeat the process with control samples (negative – Button Mushroom and blank - MeOH). Develop the spots by applying 5 μL HCl (37%) to each spot using a clean TLC capillary tube (tap the tube with HCl to cover the spot area with acid). Leave the test in the fume cupboard to develop for 10 to 20 min. Take a picture of the spot with a colour chart from the Encyclopedia of Fungi[36] next to it (keep the two as close as possible). Put the paper with the test on it next to the colour closest to the colour of the colour developed on the spot.

5.2.3 Meixner test using model compounds

5.2.3.1 Mushroom model compounds

The model compounds used were 5-hydroxyindole-3-acetic acid – 5-HIAA, indole-3-acetic acid - IAA and indole. Each was prepared into MeOH solution by dissolving 10 mg of the individual compound in MeOH (5 mL), apply 5 μL of the solution(s) onto the paper keeping the spot(s) size to the absolute minimum. Dry the spot(s) in ambient temperature. Using a clean fixed-volume TLC capillary, apply 5 μL of 37% HCl onto the dried spot(s).

5.2.3.2 Lignin model compound

100 mg of coniferyl aldehyde was weight out into 5 mL glass vial. Five millilitres of MeOH were pipetted into the vial to prepare a 20 mg/mL stock solution.

5.2.4 Lignin extraction from paper

Weight out 0.5 g of lignin paper. Cut the paper to ~1mm^2 pieces add to beaker. Add 37% HCl (3 mL). Allow the paper to absorb the acid and leave the extract to soak for one hour. Using a spatula put the wet paper into a garlic press. Squeeze the extract back into the beaker and dispose of the paper. Set a hot plate to 35°C. Put the beaker with the acid extract onto the hotplate. Leave the extract until the acid evaporates. Re-suspend the lignin residue in MeOH (3 mL). To 0.5 mL of methanol lignin solution pipette 15 μL of 2 mg/mL of 5-HIAA, IAA or indole.

5.2.5 Chemicals supplier details

5-Hydroxyindole-3-acetic acid, indole acetic acid and indole were supplied by Sigma Aldrich, 37% hydrochloric acid, methanol ethanol, acetic acid, dichloromethane, diethyl ether were supplied by Fulka.

5.3 Literature review search terms

The Literature review has been carried out using the scientific databases: Science Direct (SD), Web of Knowledge (WoK) and ChemSpider. Below is presented a list of mushrooms searched for:

Agaricus dulcidulus, Agaricus moelleri, Agaricus pilatianus, Agaricus xanthodermus, Amanita echinocephala, Amanita gemmate, Amanita muscaria, Amanita phalloides, Amanita phanterina, Amanita rubescens, Amanita virosa, Armillaria cepistipes, Armillaria ostoyae, Boletus legaliae, Boletus rhodopurpureus, Boletus rhodoxanthus, Boletus satanas, Boletus satanoides, Boletus torosus, Ciprinus picaceus, Claviceps purpurea, Clitocybe delbata, Clitocybe dilatata, Clitocybe rivulosa, Cortinarius bolaris, Cortinarius gentilis, Cortinarius limonius, Cortinarius orellanus, Cortinarius speciosissimus, Cystoderma cinnabarium, Cystoderma granulosum, Cystolepiota bucknallii, Cystolepiota hetieri, Disciotis venosa, Entoloma chalybaeum, Entoloma incanum, Entoloma rhodopolium, Entoloma serrulatum, Entoloma sinuatum, Gyromitra esculenta, Gyromitra infula, Hebeloma crustuliniforme, Hebeloma longicaudum, Hebeloma mesophaeum, Hebeloma pseudoamarescens, Hebeloma radicosum, Hebeloma sinapizans, Helvella acetabulum, Helvella leucomelaena, Hygrocybe calyptriformis, Hygrocybe citrina, Hygrophoropsis aurantiaca, Inocybe bongardii, Inocybe calospora, Inocybe cincinnata, Inocybe cookie, Inocybe erubescens, Inocybe flocculosa, Inocybe fuscidula, Inocybe geophylla, Inocybe godeyi, Inocybe hystrix, Inocybe lilacina, Inocybe maculate, Inocybe napipes, Inocybe rimosa, Inocybe sindonia, Lactarius chrysorrheus, Lactarius cilicioides, Lactarius helvus, Lactarius pubescens, Lactarius tabidus, Lactarius torminosus, Lactarius necator, Lepiota aspera, Lepiota brunneoincarnata, Lepiota clypeolaria, Lepiota cristata, Lepiota fuscovinacea, Lepiota grangei, Lepiota hystrix, Lepiota subincarnata, Leucoagaricus croceovelutinus, Leucoagaricus marriage, Leucoagaricus pilatianus, Melanophyllum haematospermum, Omphallotus illudens, Paxillus involutus, Peziza badia, Peziza vesiculosa, Psilocybe crobula, Psilocybe cyanescens, Psilocybe semilanceata,
Ramaria formosa, Russula betularum, Russula emetic, Russula luteotacta, Russula nobilis, Russula solaris, Sarcosphaera coronaria, Stropharia aeruginosa, Tricholoma album, Tricholoma lascivum, Tricholoma pessundatum, Tricholoma scalpturatum, Tricoloma ustale.

6 Chapter 6 – Appendix

6.1 Mushroom collection protocol

First, photograph the mushroom in situ before picking.
Note its location and the organism the mushroom is associated with.

Prepare a macroscopic description of the mushroom.
Describe: the habitat, cap, stem,
gills and colours of the individual parts.
Use the chart in the bottom right corner
to aid description of shape.

Enter the avaliable collected macroscopic details on the website
www.rogersmushrooms.com/gallery/chooser.asp.
When submitted the website will list
out number of probable indentifications.
If only one hit the identification can be verified
by comparing to referance :
1) "Mushrooms" by Roger Philips
2)"Encyclopaeida of Fungi of Britain and Europe"
by Michael Jordan
or the computer database
3)MycoKey v4.0 from www.mycokey.com.

Compare the macroscopic features of the mushrooms
listed by the website to the mushroom collected.

If it is not possible to identify following steps till now:
take a spore print (see section 5.1.1) and narrow down the hits
by spore print colour if only one hit verify with the
referance materials.

Prepare the microscope slide with spores following the
procedures in the section 5.1.1 and then section 5.1.3
and calculate the average size.

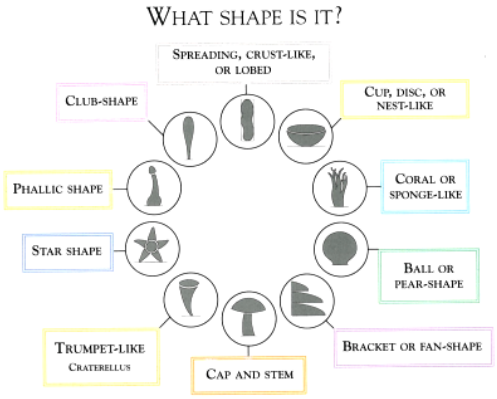
Using the reference materials
compare the size and shape of the spores
of the mushroom to the spore in the references
to excude species that do not fit the description.

If it does not fit, repeat all the steps and follow the
procedure with 1 full grown, 1 part grown and 1 not grown.

Dry the sample following the method described in section 5.1.2.

E-mail the details together with the pictures and
suggested ID for Malcolm Greaves to approve.

Enter the sample into a database, giving the details:
genus, species, common name, collection place, date collected,
person collecting, comment (e.g. edible, inedible, poisonous),
pots (full or not), Top 100 (yes/no).



6.2 Collection of mushrooms

No	Genus	Species	Common Name	Place	Date collected	Person collecting	Comment	Pots	Top 100
1	*Agaricus*	*arvensis*	Horse Mushroom	FERA Sand Hutton	26-10-2011	D Clarke	Edible		No
2	*Agaricus*	*augustus*	The prince	Victoria`s lawn	13-11-2011	V Bailey	Edible	FULL	No
3	*Agaricus*	*campestris*	Field mushroom	Ryton	19-11-2011	D Clarke	Edible	FULL	No
4	*Agaricus*	*silvaticus*	Blushing wood mushroom	Benningborough	06-11-2011	D Clarke	Edible	FULL	No
5	*Agrocybe*	*praecox*	Spring fieldcap	Kew at Castle Howard	18-06-2012	D Clarke	Edible		No
6	*Amanita*	*citrina*	False feath cap	Castle Howard	18-10-2010	D Clarke	Poison		Yes
7	*Amanita*	*excelsa/spissa*	Grey Spotted Amanita	Kew@Castle Howard	15-10-2011	D Clarke	Edible	FULL	No
8	*Amanita*	*fulva*	Tawny grisette	Bishops Wood	15-10-2011	D Clarke	Edible		Yes
9	*Amanita*	*muscaria*	Fly agaric	Bishops Wood	15-10-2011	D Clarke	Poison	FULL	Yes
10	*Amanita*	*pantherina*	Panther cap	Scotland	16-09-2010	M Walls	Poison		No
11	*Amanita*	*phalloides*	Death cap	FERA Sand Hutton	01-09-2011	D Clarke	Lethal	FULL	No
12	*Amanita*	*rubescences*	The blusher	Bishops Wood	25-09-2011	D Clarke	Edible	FULL	Yes
13	*Amirillaria*	*ostoyae*	Dark honey fungus	Victoria`s lawn	16-11-2011	V Bailey	Poisonous		No
14	*Armillaria*	*gallica*	Bulbous honey fungus	Kew@Castle Howard	15-10-2011	D Clarke	Poisonous	FULL	No
15	*Armillaria*	*mellea*	Honey fungus	Kew@Castle Howard	15-10-2011	D Clarke	Edible		Yes
16	*Auricularia*	*auricula-judae*	Jelly ears	Kew@Castle Howard	15-10-2011	D Clarke	Edible	FULL	Yes
17	*Bjerkandera*	*adusta*	Smoky bracket	FERA Sand Hutton	24-11-2011	D Clarke	Inedible	FULL	Yes
18	*Bolbitus*	*titubans/ vitellinus*	Yellow fieldcap	Kew@Castle Howard	15-10-2011	D Clarke	Inedible	FULL	Yes
19	*Boletus*	*luridiformis*	Scarletina bolete	Selby	01-09-2011	AS Lloyd	Inedible	FULL	No
20	*Bovista*	*plumbea*	Grey Puffball	FERA Sand Hutton	18-10-2011	D Clarke	Edible young		No
21	*Calocybe*	*carnes*	Pink domecap	Fera front lawn	14-05-2012	D Clarke	Edible		No

22	*Calocybe*	*gambosa*	St George's mushroom	Fera front lawn	15-05-2012	D Clarke	Edible		No
23	*Chlorophyllum*	*rhacodes*	Shaggy parasol	Benningborough	06-11-2011	D Clarke	Edible	FULL	No
24	*Chondrostereum*	*purpureum*	Silverleaf fungus	FERA Stumpery	24-11-2011	D Clarke	Inedible		No
25	*Chroogomphus*	*rutilus*	Copper spike	Bishops Wood	15-10-2011	D Clarke	Edible		No
26	*Clavulina*	*acuta*	Pointed club	Ryton	19-11-2011	D Clarke	Inedible		No
27	*Clavulina*	*cristata*	White coral fungus	FERA Sand Hutton	27-11-2011	D Clarke	Inedible		No
28	*Clitocybe*	*ditopa*	Mealy frosted funnel	FERA Sand Hutton	24-11-2011	D Clarke	Unknown		No
29	*Clitocybe*	*fragrans*	Fragrant funnel	Kew at Castle Howard	04-05-2012	D Clarke	Edible		No
30	*Clitocybe*	*geotropa*	Trooping Funnel	Kew at Castle Howard	26-10-2011	D Clarke	Edible	FULL	No
31	*Clitocybe*	*nebularis*	Clouded agaric	Kew@Castle Howard	15-10-2011	D Clarke	Edible	FULL	Yes
32	*Clitocybe*	*dealbata*	Ivory Funnel	Benningborough	06-11-2011	D Clarke	Lethal		Yes
33	*Collybia*	*butyracea*	Greasy tough-shank	Kew@Castle Howard	15-10-2011	D Clarke	Inedible	FULL	Yes
34	*Collybia*	*confluens*	Clustered Toughshank	Kew@Castle Howard	26-10-2011	D Clarke	Edible poor		Yes
35	*Collybia*	*peronata*	Wood wollyfoot	Kew@Castle Howard	15-10-2011	D Clarke	Edible		Yes
36	*Conocybe*	*apala*	Milky conecap	Fera front lawn	05-06-2012	D Clarke	Inedible		No
37	*Conocybe*	*velata/ appendiculata*		Ryton	07-05-2012	D Clarke	Unknown		No
38	*Conocybe*	*aporus*		Ryton	02-05-2012	D Clarke	Inedible		No
39	*Coprinellus*	*disseminatus*	Fairy inkcap	Ryton	19-10-2011	D Clarke	Edible		No
40	*Coprinellus*	*micaceus*	Glistening inkcap	Ryton	26-10-2011	D Clarke	Edible	FULL	Yes
41	*Coprinellus*	*flocculosus*		Ryton	27-11-2011	D Clarke	Unknown		No
42	*Coprinus*	*atramentarius*	Common inkcap	Ryton	15-09-2011	D Clarke	Antabuse	FULL	Yes
43	*Coprinus*	*comatus*	Shaggy inkcap	FERA Sand Hutton	18-10-2011	D Clarke	Edible	FULL	Yes
44	*Coprinus*	*impatiens*		Ryton	27-04-2012	D Clarke	Unknown		No
45	*Coprinus*	*lagopus*	Hares foot inkcap	Ryton	20-05-2012	D Clarke	Edible		No
46	*Coprinus*	*picaceus*	Magpie inkcap	Kew@Castle Howard	26-10-2011	D Clarke	Unknown	FULL	No

47	*Coprinus*	*plicatilis*	Pleated inkcap	Ryton	02-05-2012	D Clarke	Inedible		Yes
48	*Coprinus*	*romagnesianus*		Ryton	29-10-2011	D Clarke	Unknown	FULL	No
49	*Cortinarius*	*semisanguineus*	Surprise webcap	FERA Sand Hutton	24-11-2011	D Clarke	Inedible		No
50	*Crepidotus*	*cesatii*		FERA Sand Hutton	22-12-2011	M Greaves	Inedible		No
51	*Crucibulum*	*laeve*	Common bird's nest	FERA Sand Hutton	24-11-2011	D Clarke	Inedible		No
52	*Cystoderma*	*amianthium*	Earthly Powdercap	Kew at Castle Howard	26-10-2011	D Clarke	Edible		No
53	*Daedaleopsis*	*confragosa*	Blushing Bracket	Kew@Castle Howard	15-10-2011	D Clarke	Inedible	FULL	Yes
54	*Daldinia*	*concentrica*	King Alfred`s Cakes	Kew@Castle Howard	26-10-2011	M Greaves	Inedible		Yes
55	*Entoloma*	*conferendrum*	Star pinkgill	Ryton-meadow	24-06-2012	D Clarke	Unknown		Yes
56	*Exida*	*glandulosa*	Witches butter	Ryton -Riggs Rd#1	19-05-2012	D Clarke	Inedible		No
57	*Flammulina*	*velutipes*	Velvet shank	Ryton	13-02-2012	D Clarke	Edible	FULL	Yes
58	*Fomes*	*fomentarius*	Horse hoof	Unknown	15-10-2011	M Greaves	Inedible	FULL	No
59	*Galerina*	*hyporum*	Moss bell	Fera front lawn	15-05-2012	D Clarke	Inedible		No
60	*Galerina*	*phillipsi*		Unknown	11-2011	D Clarke	Inedible		No
61	*Ganoderma*	*australe*	Southern bracket	Benningborough	06-11-2011	D Clarke	Inedible	FULL	Yes
62	*Geastrum*	*striatum*	Striated earthstar	Ryton -George's	12-05-2012	D Clarke	Inedible		No
63	*Geastrum*	*triplex*	Collared Earthstar	Kew at Castle Howard	26-10-2011	M Greaves	Inedible		No
64	*Grifola*	*frondosa*	Hen of the wood	Benningborough	06-11-2011	D Clarke	Inedible	FULL	No
65	*Handkea*	*exipuliforme*	Pestle puffball	Sand Hutton	20-10-2011	D Clarke	Edible	FULL	No
66	*Hebeloma*	*sinapizans*	Poisonpie	Ryton	15-10-2011	D Clarke	Inedible	FULL	No
67	*Hellvela*	*acetabulum*	Vinegar cup	Ryton	07-05-2012	D Clarke	Inedible		No
68	*Hellvela*	*corium*		Fera gravel back	07-05-2012	D Clarke	Inedible		No
69	*Hellvela*	*lacunosda*	Elfin saddle	Brotherton	04-06-2012	A Lloyd	Inedible		No
70	*Hydnum*	*repandum*	Hedgehog fungus	Unknown	15-10-2011	M Greaves	Edible		No
71	*Hygrocybe*	*coccinea*	Scarlet waxcap	Ryton	27-11-2011	D Clarke	Edible		Yes
72	*Hygrocybe*	*nigrescens/*	Blackening waxcap	FERA Sand Hutton	12-10-2011	D Clarke	Edible		Yes

		conica							
73	*Hygrocybe*	*pratensis*	Buff waxcap	Ryton	24-09-2011	D Clarke	Edible		Yes
74	*Hygrocybe*	*psittacina*	Parrot waxcap	Ryton	15-10-2011	D Clarke	Edible		Yes
75	*Hygrocybe*	*virginea*	Snowy waxcap	Ryton	15-10-2011	D Clarke	Edible		Yes
76	*Hypholoma*	*fasciculare*	Sulphur tufts	Ryton	15-09-2011	D Clarke	Inedible	FULL	Yes
77	*Hypoxylon*	*fragiforme*	Beech woodwart	Malton Orchard fields	29-10-2011	D Clarke	Edible		Yes
78	*Hypoxylon*	*multiforme*	Birch woodwart	Fera oak woods	11-2011	D Clarke	Inedible		Yes
79	*Ionocybe*	*erubescens*	Deadly fibrecap	Ryton-Eden camp	23-06-2012	D Clarke	Lethal		No
80	*Ionocybe*	*lacera*	Torn fibrecap	Brotherton	2011/2012	A Lloyd	Poisonous		No
81	*Ionocybe*	*rimosa*	Torn fibrecap	Ryton-Eden camp	21-06-2012	D Clarke	Poisonous		No
82	*Laccaria*	*laccata*	The deceiver	FERA Sand Hutton	24-11-2011	D Clarke	Edible		Yes
83	*Lacrymaria*	*lacrymabunda*	Weeping widow	Fera front lawn	18-06-2012	M Greaves	Inedible		Yes
84	*Lactarius*	*deterrimus*	False Saffron Milkcap	Kew@Castle Howard	26-09-2011	D Clarke	Edible		No
85	*Lactarius*	*pubescens*	Bearded milkcap	Kew@Castle Howard	15-10-2011	D Clarke	Poisonous		No
86	*Lactarius*	*subdulcis*	Mild Milkcap	Benningborough	06-11-2011	D Clarke	Edible		Yes
87	*Lactarius*	*torminosus*	Woolly Milkcap	Ryton	26-10-2011	D Clarke	Poisonous		No
88	*Laetiporus*	*sulphureus*	Chicken of the woods		26-11-2011	M Walls	Edible		No
89	*Leccinum*	*scabrum*	Brown birch bolete	Ryton	10-09-2011	D Clarke	Edible	FULL	Yes
90	*Lepiota*	*cristata*	Stinking Dapperling	FERA Sand Hutton	29-10-2011	D Clarke	Poisonous		Yes
91	*Lepiota*	*ventriospora/ magnispora*		Washburn	26-09-2011	J Powell	Inedible		No
92	*Lepista*	*flaccida*	Tawny funnel	Benningborough	06-11-2011	D Clarke	Edible	FULL	Yes
93	*Lepista*	*nuda*	Wood blewit	FERA Sand Hutton	01-09-2011	D Clarke	Edible	FULL	Yes
94	*Lepista*	*saeva*	Field blewit	FERA Sand Hutton	26-10-2011	D Clarke	Edible	FULL	No
95	*Leratiomyces*	*ceres*	Redlead roundhead	FERA Sand Hutton	24-11-2011	D Clarke	Inedible		No
96	*Leucocoprinus*	*brebissonii*	Skullcap dapperling	Bishops Wood	07-09-2011	D Clarke	Inedible		No

97	*Lycoperdon*	*nigrescens*	Dusky puffball	Sand Hutton	01-09-2011	D Clarke	Edible	FULL	Yes
98	*Lycoperdon*	*pyriforme*	Stump puffball	Kew@Castle Howard	15-10-2011	D Clarke	Edible	FULL	Yes
99	*Lyophyllum*	*decastes*	Clustered dome cap	Ryton	15-10-2011	D Clarke	Edible		No
100	*Marasmius*	*oreades*	Fairy ring champignon	Fera front lawn	15-05-2012	D Clarke	Edible		Yes
101	*Melanoleuca*	*cognata*	Spring cavalier	Ryton	02-05-2012	D Clarke	Edible		No
102	*Melanoleuca*	*polioleuca*	Common cavalier	Ryton	29-06-2012	D Clarke	Edible		No
103	*Morchella*	*esculenta*	Common morel	Brotherton	27-04-2012	A Lloyd	Edible		No
104	*Mucilago*	*crustacea*	Slime mould	Benningborough	06-11-2011	D Clarke	Inedible		No
105	*Mycena*	*aetites*	Drab Bonnet	Victoria`s lawn	06-11-2011	V Bailey	Inedible		No
106	*Mycena*	*arcangeliana*	Angel`s Bonet	Kew at Castle Howard	26-10-2011	D Clarke	Inedible		No
107	*Mycena*	*galericulata*	Common bonnet	Kew@Castle Howard	26-09-2011	D Clarke	Edible		Yes
108	*Mycena*	*inclanata*	Clustered Bonnet	Kew at Castle Howard	26-10-2011	D Clarke	Inedible	FULL	Yes
109	*Mycena*	*leptocephala*	Nitrous bonnet	FERA Sand Hutton	24-11-2011	D Clarke	Inedible		No
110	*Mycena*	*pura*	Lilac Bonnet	Kew@Castle Howard	26-10-2011	D Clarke	Inedible		Yes
111	*Mycena*	*silvaernigrae*		Kew at Castle Howard	26-10-2011	D Clarke	Unknown	FULL	No
112	*Panaeolina*	*foenisecii*	Brown hay cap	Ryton -Home	14-06-2012	D Clarke	Inedible		No
113	*Panaeolus*	*ater*	Turf mottlegill	Ryton	07-05-2012	D Clarke	Inedible	FULL	No
114	*Panaeolus*	*semiovatus*	Egghead mottlegill	Ryton -Michael's	17-06-2012	D Clarke	Inedible		No
115	*Paxillus*	*Involutus*	Brown roll rim	Ryton	15-09-2011	D Clarke	Poisonous	FULL	Yes
116	*Peziza*	*vesiculosa*	Blistered cup	Ryton	27-11-2011	D Clarke	poisonous		No
117	*Phallus*	*impudicus*	Stinkhorn	Kew@Castle Howard	15-10-2011	D Clarke	Edible		Yes
118	*Phlebia*	*radiata*	Wrinkled crust	FERA Sand Hutton	22-12-2011	M Greaves	Inedible		No
119	*Pholiota*	*aurivella*	Golden scalycap	Benningborough	06-11-2011	D Clarke	Inedible	FULL	No
120	*Pholotia*	*gummosa*	Sticky cap	Dave,s willow Ryton	30-10-2011	D Clarke		FULL	No
121	*Pholotia*	*squarrosa*	Shaggy pholita	Ryton	10-09-2011	D Clarke	Inedible	FULL	No
122	*Piptoporous*	*betulinus*	Birch polypore	Kew at Castle Howard	26-10-2011	D Clarke	Inedible		Yes

123	*Pleurotus*	*cornucopiae*	Branching oyster	Benningborough	06-11-2011	D Clarke	Edible	FULL	No
124	*Polyporus*	*squamosus*	Dryad's saddle	Ryton	13-05-2012	D Clarke	Edible	FULL	Yes
125	*Psathyrella*	*corrugis*	Red-edge Brittlestem	Kew at Castle Howard	26-10-2011	D Clarke	Inedible		No
126	*Psathyrella*	*multipedata*	Clustered Brittlestem	Kew at Castle Howard	26-10-2011	D Clarke	Inedible	FULL	No
127	*Psathyrella*	*spadiceogrisea*		Kew at Castle Howard	26-10-2011	D Clarke	Inedible		No
128	*Psilocybe*	*semilanceteatu*	Liberty cap	Kew@Castle Howard	15-10-2011	D Clarke	Hallucinogenic		No
129	*Reticularia*	*lycoperdon*		Ryton -Rye bridge	2011/2012	D Clarke	Inedible		No
130	*Rhytsima*	*acerinum*	Tar spot	Heslington - York	31-10-2012	M Fiedziukiewicz	Inedible		Yes
131	*Russula*	*atropurpea*	Blue/Black Russula	Kew at Castle Howard	26-10-2011	D Clarke	Edible	FULL	Yes
132	*Russula*	*betularum*	Birch brittlegill	Kew@Castle Howard	15-10-2011	D Clarke	Inedible		No
133	*Russula*	*ioconochlora*	Oilslick brittlegill	Fera-gym hill	18-06-2012	D Clarke	Edible		No
134	*Russula*	*ochroleuca*	Common yellow russula	Kew@Castle Howard	15-10-2011	D Clarke	Edible	FULL	Yes
135	*Schizophyllum*	*commune*	Splitgill	FERA Sand Hutton	22-12-2011	M Greaves	Inedible		No
136	*Scutellinia*	*scutellata*	Common eyelash	Ryton-home	2012	D Clarke	Inedible		No
137	*Stereum*	*hirsutum*	Hairy curtain crust	Fera forestry comm	04-05-2012	D Clarke	Inedible	FULL	Yes
138	*Strobilurus*	*tenacellus*	Pinecone cap	Ryton	03-05-2012	D Clarke	Inedible		No
139	*Stropharia*	*aeruginosa*	Verdidris agaric	Kew@Castle Howard	15-10-2011	D Clarke	Poisonous		No
140	*Stropharia*	*aurantica*		FERA Sand Hutton	17-11-2011	D Clarke	Poisonous		No
141	*Stropharia*	*caerulla*	Blue roundhed	Benningborough	06-11-2011	D Clarke	Inedible	FULL	No
142	*Suillius*	*granulatus*	Granulated bolete	Kew@Castle Howard	15-10-2011	D Clarke	Edible		No
143	*Suillius*	*grevillei*	Larch bolete	Kew@Castle Howard	15-10-2011	D Clarke	Edible		No
144	*Suillus*	*luteus*	Slippery Jack	Kew@Castle Howard	26-10-2011	D Clarke	Edible		No
145	*Thelophora*	*terrestris*	Earth fan	Roger's rose Pickering	30-10-2011	D Clarke	Inedible		No
146	*Trametes*	*versicolor*	Turkeytail	Kew@Castle Howard	26-10-2011	D Clarke	Inedible	FULL	Yes

147	*Tricholoma*	*cingulatum*	Girdled knight	FERA Sand Hutton	24-11-2011	D Clarke	Edible		No
148	*Tricholoma*	*fulvum*	Birch Knight	Kew@Castle Howard	26-10-2011	D Clarke	Edible		No
149	*Tricholoma*	*scalpturatum*	Yellowing knight	Covance Harrogate	27-06-2012	D Clarke	Edible		No
150	*Tricholoma*	*terrum*	Grey knight	Malton A64	24-11-2011	D Clarke	Edible		No
151	*Tricholoma*	*utsale*	Burnt Knight	Kew@Castle Howard	26-10-2011	D Clarke	Poison	FULL	No
152	*Tricholomopsis*	*rutilans*	Plums & custard	Unknown	15-10-2011	M Greaves	Inedible		Yes
153	*Tubaria*	*hiemalis*		FERA Sand Hutton	24-11-2011	D Clarke	Inedible		No
154	*Volvariella*	*gloiocephala/ speciosa*	Stubble rosegill/volvar	Great Habton - Ryton	30-10-2011	D Clarke	Edible	FULL	No
155	*Xerula*	*radicata*	Rooting Shank	Kew at Castle Howard	26-10-2011	D Clarke	Edible		Yes
156	*Xylaria*	*hypoxylon*	Candlesnuff	Ryton	01-11-2011	D Clarke	Inedible		Yes
157	*Xylaria*	*longipes*	Dead Molls Fingers	Ryton -Rye bridge	19-06-2012	D Clarke	Inedible		No

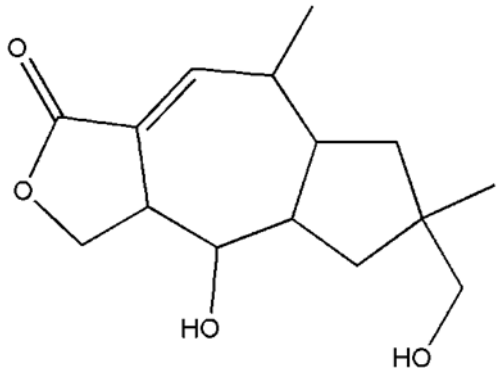
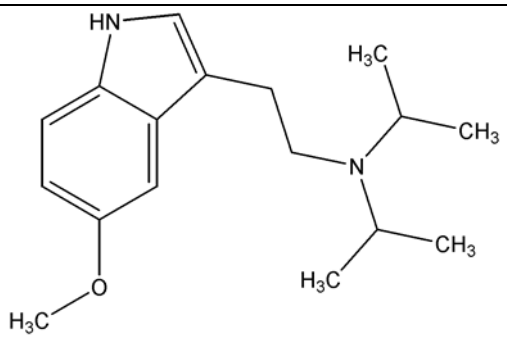
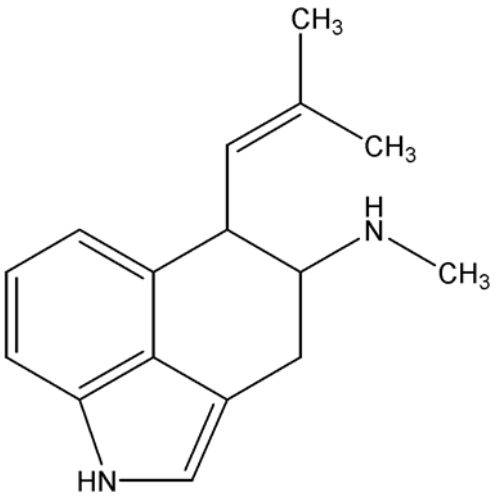
Those identified but not stored.							**Cultured *in situ***		
157	*Calocera*	*cornera*	Small stagshorn	Ryton	13-05-2012	D Clarke	Fera stumpery		Yes
158	*Dacrymyces*	*stillatus*		Ryton	13-05-2012	D Clarke	Fera stumpery		Yes
159	*Diatrype*	*disciformis*	Beech barkspot	Fera oak woods	2012	D Clarke	Fera stumpery		Yes
160	*Exidia*	*thuretiana*		Ryton	13-05-2012	D Clarke	Fera stumpery		No
161	*Lycogala*	*terrestre*	Wolf's milk	Ryton	07-05-2012	D Clarke	Fera stumpery		No
162	*Lycogala*	*epidendrum*	Toothpaste slime	Fera stumpery	13-05-2012	D Clarke	Fera stumpery		No
163	*Nectorinia*	*cinnabarina*	Coral spot	Ryton	2012	D Clarke	Fera stumpery		Yes

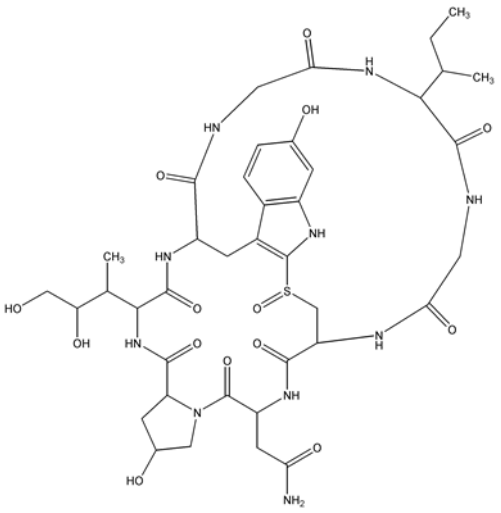
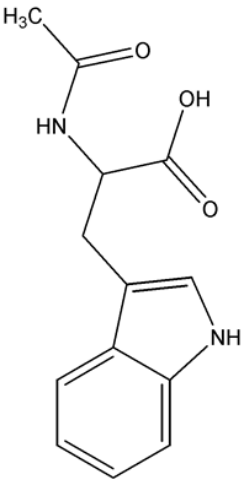
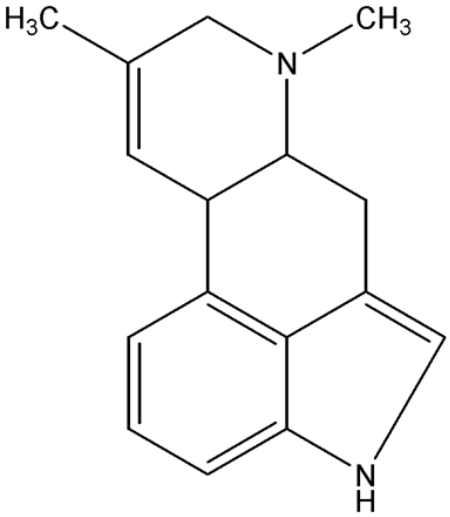
Table 6.1: **No -** is a sequential number of the mushroom in the table. **Genus** – genus of the mushroom collected. **Species** – species of the mushroom collected. **Common name** – gives the common name of mushroom (if available if not left blank). **Date collected** – presents the date when the mushroom

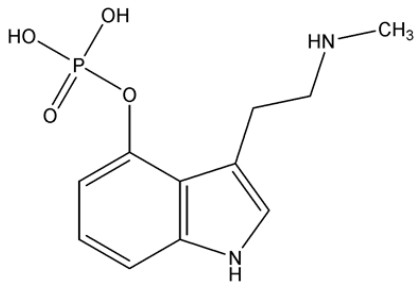
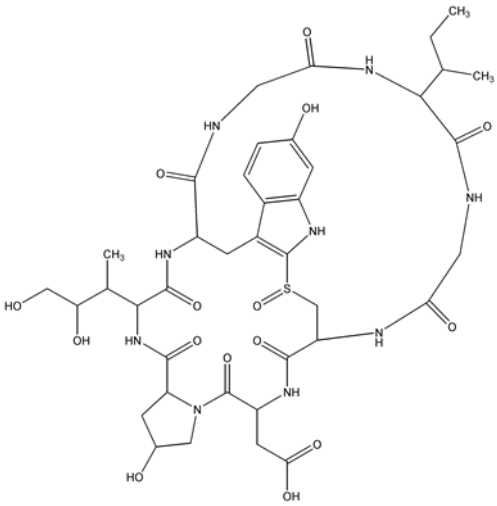
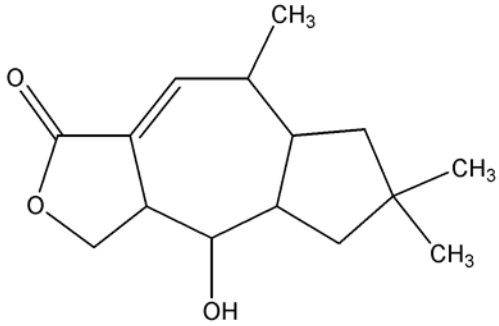
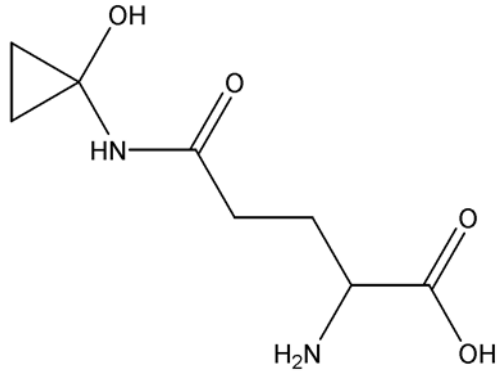
was collected. **Person collected** – first initial and a surname of the person who collected the mushroom. **Comments** – information on edibility of mushroom. **Pots –** label "Full" indicates if the particular dried mushroom filled up the 150mL plastic pot (positions were left blank if the container was not filled up). **Top 100** – The term adopted from the mycology book informing of the general value of the mushroom. **N/A –** information not available.

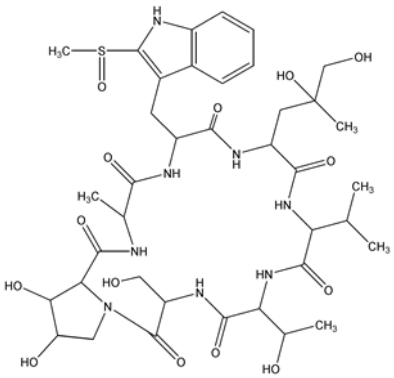
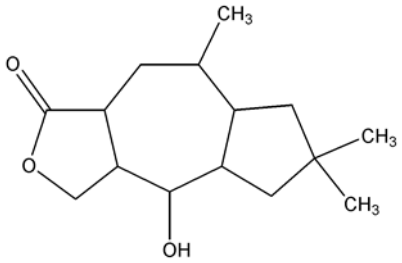
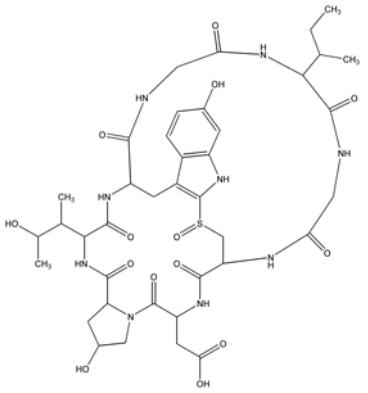
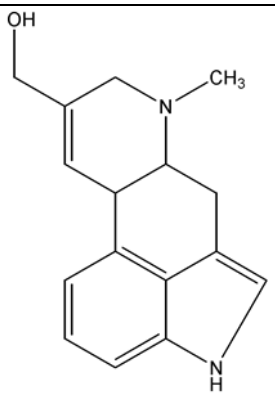
6.3 Poisonous compounds in mushroom

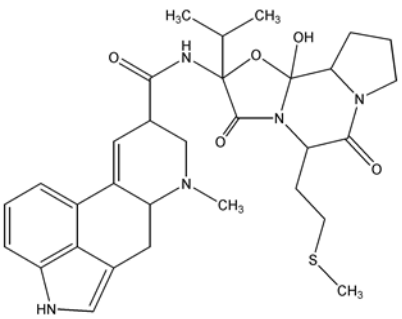
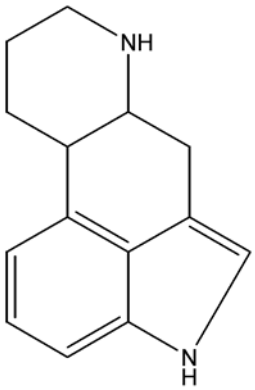
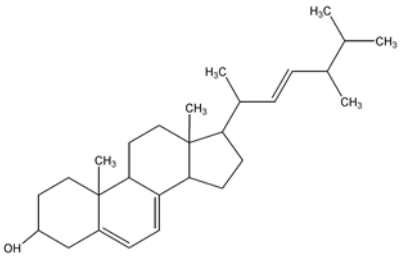
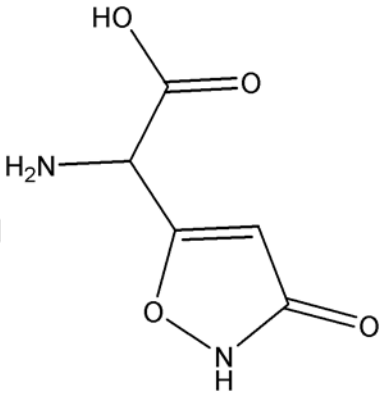
Table 6.2 – A list of known poisonous compounds

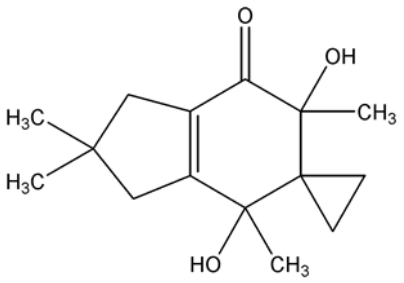
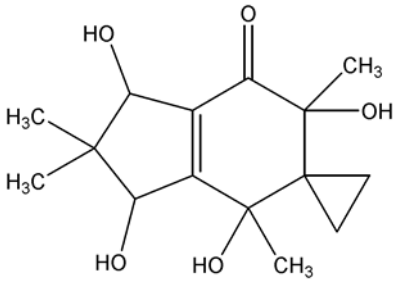
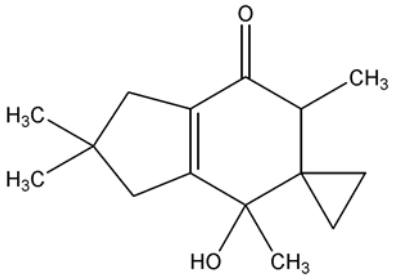
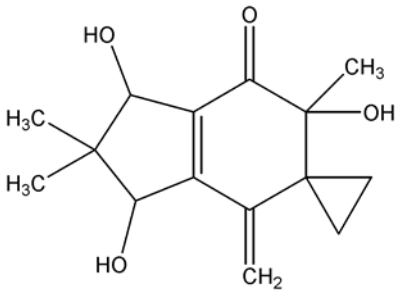
No	Structure	Name	Molecular formula
1		15-hydroxyblennin	$C_{15}H_{23}O_4$
2		5-methoxy-N,N-diisopropyltryptamine	$C_{17}H_{26}N_2O$
3		6,7-seco-agroclavine	$C_{16}H_{20}N_2$

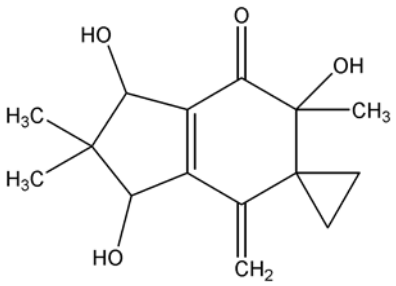
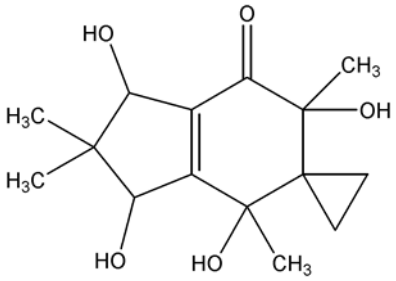
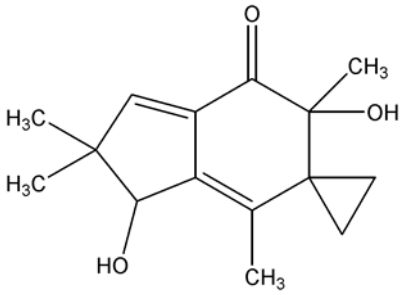
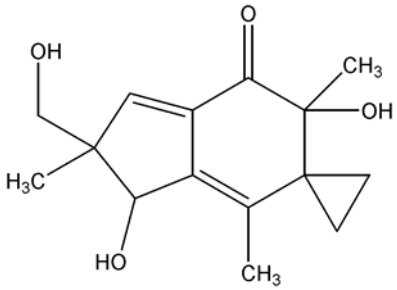
4		A-amanitin	$C_{36}H_{54}N_{10}O_{14}S$
5		Acetylacetyl-L-tryptophan	$C_{13}H_{14}N_2O_3$
6		Agroclavine	$C_{16}H_{18}N_2$

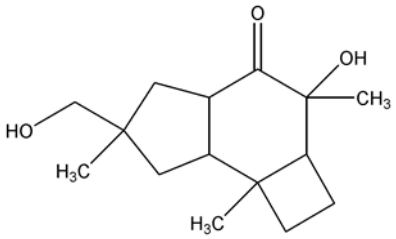
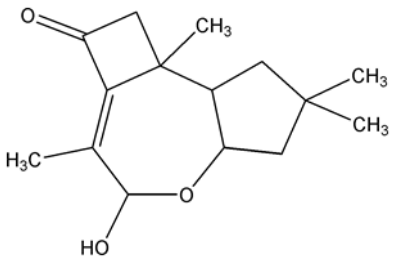
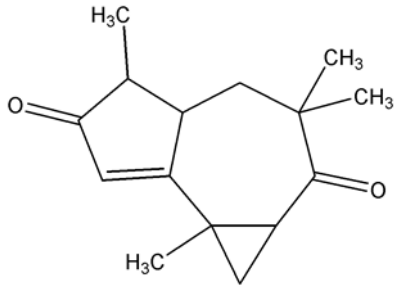
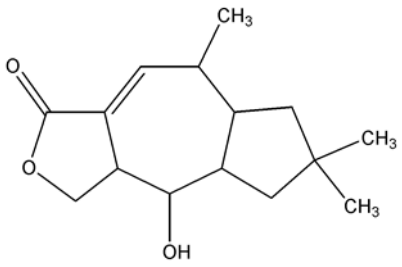
9		Baeocystin	$C_{11}H_{15}N_2O_4P$
10		B-amanitin	$C_{39}H_{53}N_9O_{15}S$
11		Blenin A	$C_{15}H_{23}O_3$
12		Coprine	$C_8H_{14}N_2O_4$

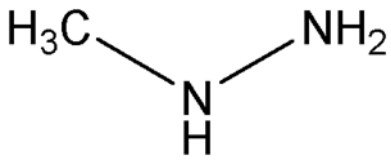
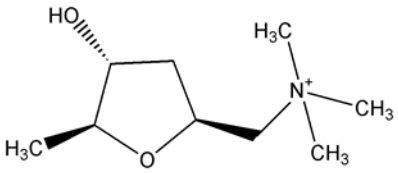
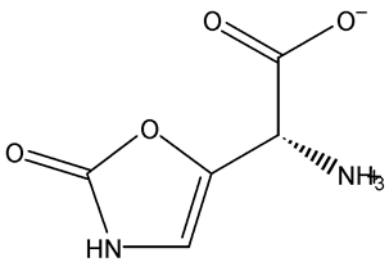
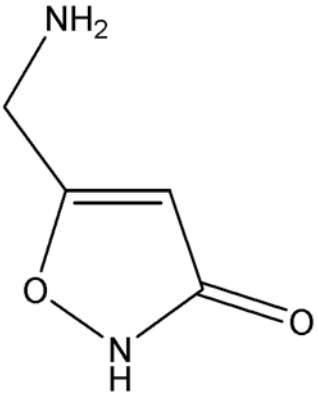
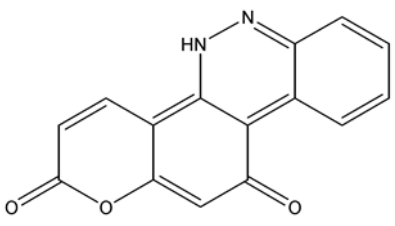
13		Deoxoviroidin	$C_{38}H_{56}N_8O_{14}S$
14		Deoxydihydroketolactarorufin	$C_{15}H_{22}O_3$
15		e-Amanitin	$C_{39}H_{53}N_9O_{14}S$
16		Elymoclavine	$C_{16}H_{18}N_2O$

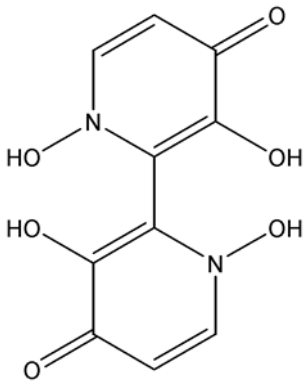
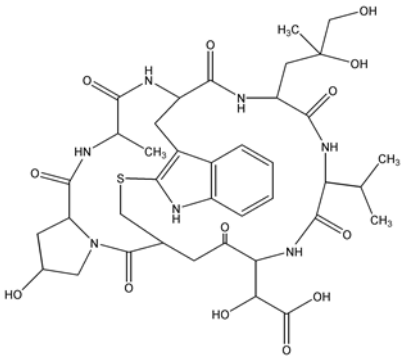
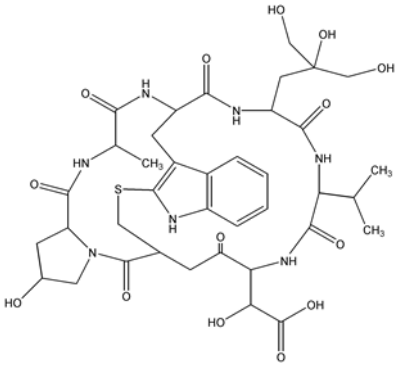
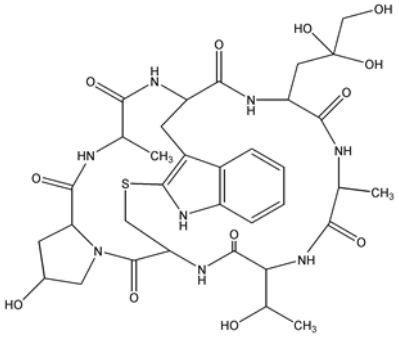
17		Ergoladinine	$C_{31}H_{39}N_5SO_5$
19		Ergoline	$C_{14}H_{16}N_2$
20		Ergosterol	$C_{28}H_{44}N$
21		Ibotenic acid	$C_5H_6N_2O_4$

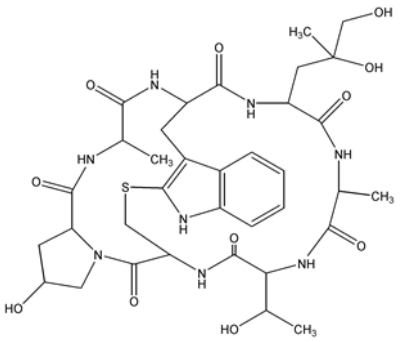
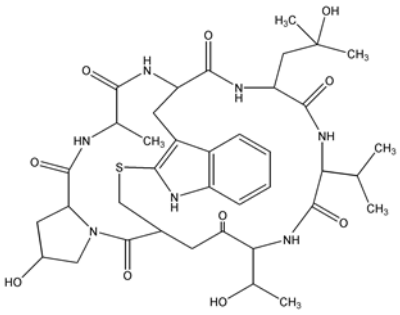
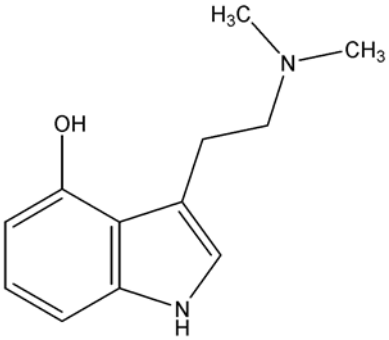
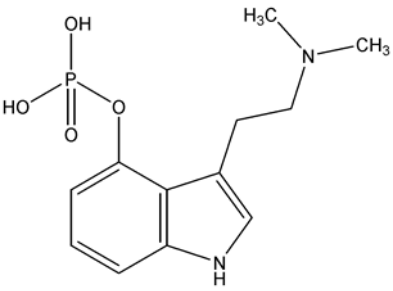
22		Illudin A	$C_{15}H_{22}O_3$
23		Illudin B	$C_{15}H_{22}O_5$
24		Illudin D	$C_{15}H_{25}O_2$
25		Illudin F	$C_{15}H_{20}O_4$

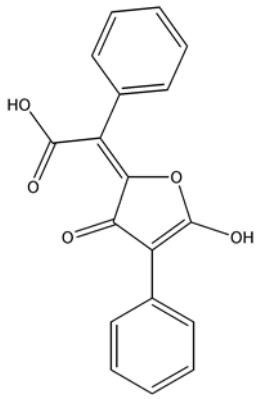
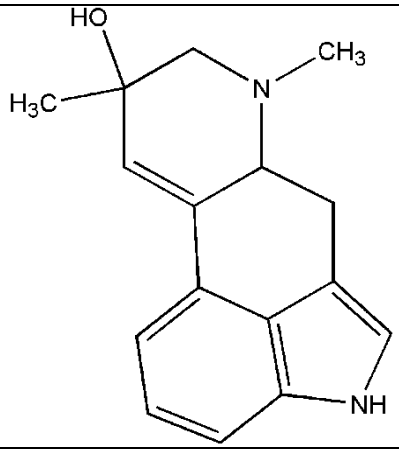
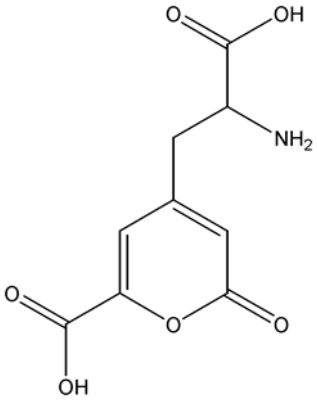
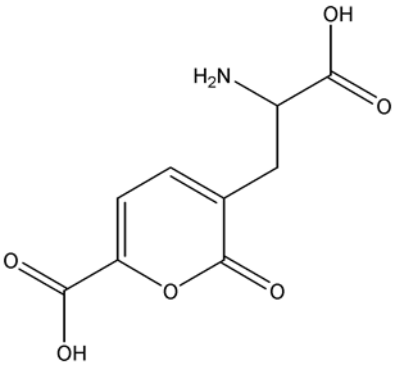
26		Illudin G	$C_{15}H_{20}O_4$
27		Illudin H	$C_{15}H_{22}O_5$
28		Illudin M	$C_{15}H_{20}O_3$
29		Illudin S	$C_{15}H_{19}O_4$

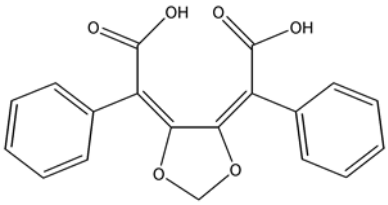
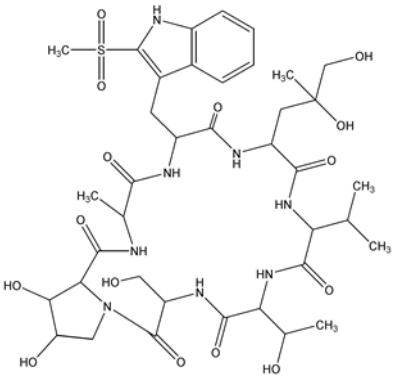
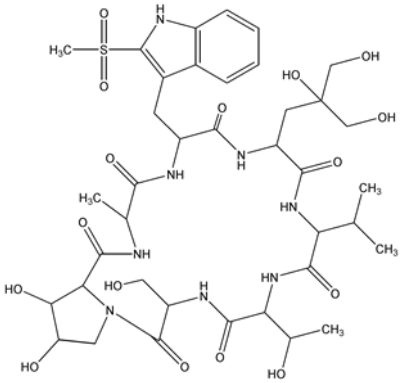
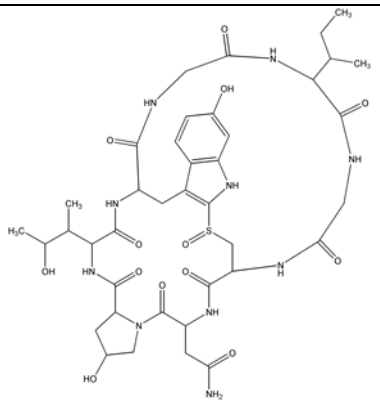
30		Illudiolone	$C_{15}H_{24}O_3$
31		Illudosone hemiacetal	$C_{15}H_{22}O_2$
32		Isolludin M	$C_{15}H_{20}O_3$
33		Lactarorufin	$C_{15}H_{22}O_3$

34		Methylhydrazine	CH_6N_2
35		Muscarine	$C_9H_{20}NO_2$
36		Muscazone	$C_5H_6N_2O_4$
37		Muscimol	$C_4H_6N_2O_2$
38		Necatorin	$C_{15}H_8N_2O_3$

39		Orellanine	$C_{10}H_8N_2O_6$
40		Phallacidin	$C_{37}H_{50}N_8O_{13}S$
41		Phallisacin	$C_{37}H_{50}N_8O_{14}S$
42		Phallisin	$C_{35}H_{48}N_8O_{12}S$

43		Phalloidin	$C_{35}H_{48}N_8O_{11}S$
45		Phalloin	$C_{35}H_{48}N_8O_{10}S$
46		Psilocin	$C_{12}H_{16}N_2O$
47		Psilocybin	$C_{12}H_{17}N_2O_4P$

48		Pulvinic acid	$C_{18}H_{12}O_5$
49		Setoclavine	$C_{16}H_{20}N_2O$
50		Stizolobic acid	$C_9H_9NO_6$
51		Stizolobinic acid	$C_9H_9NO_6$
52		Toxophallin	large protein

53		Ustalic Acid	$C_{19}H_{14}O_6$
54		Viroidin	$C_{38}H_{56}N_8O_{15}S$
55		Viroisin	$C_{38}H_{56}N_8O_{16}S$
56		Y-amanitin	$C_{39}H_{54}N_{10}O_{12}S$

6.4 List of poisonous mushroom

Table 6.3 – The list of the most common poisonous mushrooms and their poisonous compounds.

No.	Genus	Species	Toxins
1	*Agaricus*	*dulcidulus*	
2	*Agaricus*	*moelleri*	Amatoxins
3	*Agaricus*	*pilatianus*	
4	*Agaricus*	*xanthodermus*	
5	*Amanita*	*echinocephala*	
6	*Amanita*	*gemmata*	Stizolobinic acid
			Stilozobic acid
			Ibotenic acid
			Muscimol
7	*Amanita*	*muscaria*	Ibotenic acid
			Muscimol
			Muscazone
			5-Methoxy-N,N-diisopropyltryptamine
			Dimethylarsinic acid
			Arsenobetaine
			Arsenocoline
			Tetramethyl arsonium
8	*Amanita*	*phalloides*	A-amanitin
			B-amanitin
			Y-amanitin
			Phallacidin
			Phallisacin
			Phalloidin
			Phallisin
			Phalloin
			Toxophallin
			Dimethylarsinic acid
			Arsenobetaine
			Methylarsonic acid
9	*Amanita*	*phanterina*	Ibotenic acid
			Muscimol
			Stizolobic acid
			Stilozobinic acid
10	*Amanita*	*rubescens*	Amatoxins
			Dimethylarsnic acid
11	*Amanita*	*virosa*	Phallotoxins
			Amatoxins

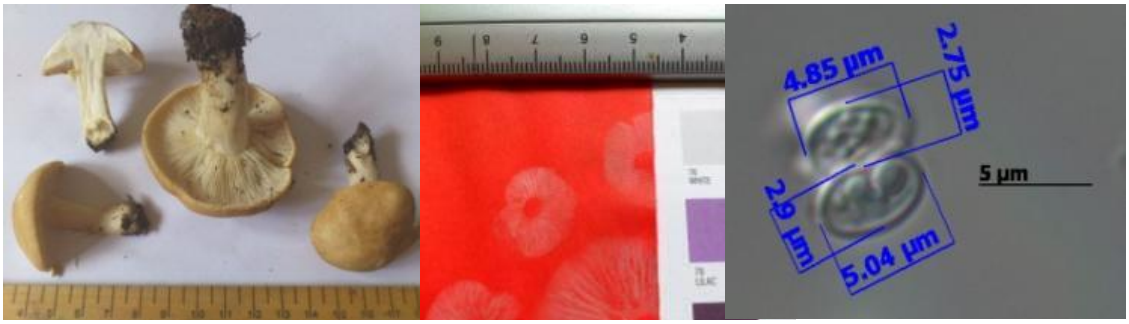
			Phallotoxins
			Virotoxins
12	*Armillaria*	*cepistipes*	
13	*Armillaria*	*ostoyae*	
14	*Boletus*	*legaliae*	
15	*Boletus*	*rhodopurpureus*	
16	*Boletus*	*rhodoxanthus*	
17	*Boletus*	*satanas*	Lectin
			Bolesatine
18	*Boletus*	*satanoides*	
19	*Boletus*	*torosus*	
20	*Ciprinus*	*picaceus*	
21	*Claviceps*	*purpurea*	Ergopeptines
			N-acetyl-L-tryptophan
			Agroclavine
			Elymoclavine
			Setoclavine
			6,7-Seco-agroclavine
			Ergoladinine
22	*Clitocybe*	*delbata*	Muscarine
23	*Clitocybe*	*dilatata*	
24	*Clitocybe*	*rivulosa*	Muscarine
25	*Cortinarius*	*bolaris*	
26	*Cortinarius*	*gentilis*	
27	*Cortinarius*	*limonius*	
28	*Cortinarius*	*orellanus*	Orellanine
			Cortinarin
29	*Cortinarius*	*speciosissimus*	Cortinarin
30	*Cystoderma*	*cinnabarium*	
31	*Cystoderma*	*granulosum*	
32	*Cystolepiota*	*bucknallii*	
33	*Cystolepiota*	*hetieri*	
34	*Disciotis*	*venosa*	
35	*Entoloma*	*chalybaeum*	
36	*Entoloma*	*incanum*	Viroidin
			Viroisin
			Deoxoviroisin
			Ala-viroidin
			Ala- deoxoviroidin
			Deoxoviroidin
37	*Entoloma*	*rhodopolium*	Dimethylarsinic acid
38	*Entoloma*	*serrulatum*	
39	*Entoloma*	*sinuatum*	
40	*Gyromitra*	*esculenta*	Gyromitrin

			Monomethylhydrazine
41	*Gyromitra*	*infula*	
42	*Hebeloma*	*crustuliniforme*	
43	*Hebeloma*	*longicaudum*	
44	*Hebeloma*	*mesophaeum*	
45	*Hebeloma*	*pseudoamarescens*	
46	*Hebeloma*	*radicosum*	
47	*Hebeloma*	*sinapizans*	
48	*Helvella*	*acetabulum*	Gyromitrin
49	*Helvella*	*leucomelaena*	Gyromitrin
50	*Hygrocybe*	*calyptriformis*	
51	*Hygrocybe*	*citrina*	
52	*Hygrophoropsis*	*aurantiaca*	Pulvinic acid
53	*Inocybe*	*bongardii*	Cortinarin
54	*Inocybe*	*calospora*	Muscarine
55	*Inocybe*	*cincinnata*	Muscarine
56	*Inocybe*	*cookei*	Muscarine
57	*Inocybe*	*erubescens*	Muscarine
58	*Inocybe*	*flocculosa*	
59	*Inocybe*	*fuscidula*	Muscarine
60	*Inocybe*	*geophylla*	Muscarine
61	*Inocybe*	*godeyi*	Muscarine
62	*Inocybe*	*hystrix*	Muscarine
63	*Inocybe*	*lilacina*	Muscarine
64	*Inocybe*	*maculata*	Muscarine
65	*Inocybe*	*napipes*	Muscarine
66	*Inocybe*	*rimosa*	Muscarine
67	*Inocybe*	*sindonia*	Muscarine
68	*Lactarius*	*chrysorrheus*	
69	*Lactarius*	*cilicioides*	
70	*Lactarius*	*helvus*	
71	*Lactarius*	*pubescens*	
72	*Lactarius*	*tabidus*	
73	*Lactarius*	*torminosus*	Lacarorufin N
			Deoxydihydroketolactarorufin
			Blenin A
			15-Hydroxyblenin
74	*Lactarius*	*necator*	Necatorin
75	*Lepiota*	*aspera*	
76	*Lepiota*	*brunneoincarnata*	Amatoxins
77	*Lepiota*	*clypeolaria*	
78	*Lepiota*	*cristata*	Amatoxins
79	*Lepiota*	*fuscovinacea*	
80	*Lepiota*	*grangei*	

81	*Lepiota*	*hystrix*	
82	*Lepiota*	*subincarnata*	
83	*Leucoagaricus*	*croceovelutinus*	
84	*Leucoagaricus*	*marriagei*	
85	*Leucoagaricus*	*pilatianus*	
86	*Melanophyllum*	*haematospermum*	
87	*Omphallotus*	*illudens*	Illudosone hemiacetal
			Isoomphadione
			Illudiolone
			Illudin S
			Illudin M
			Isolludin S
			IIsolludin M
			Illudin A
			Illudin B
			Illudin D
			Illudin H
			Illudin G
			Illudin F
			Muscarine
88	*Paxillus*	*involutus*	
89	*Peziza*	*badia*	
90	*Peziza*	*vesiculosa*	
91	*Psilocybe*	*crobula*	
92	*Psilocybe*	*cyanescens*	Psilocybin
			Psilocin
93	*Psilocybe*	*semilanceata*	Psilocybin
			Psilocin
94	*Ramaria*	*formosa*	
95	*Russula*	*betularum*	Ergot-7-en-3-ol
			Ergosta-7,22-dien-3-ol
			Ergosta-5,7-dien-3-ol
			Ergosterol
96	*Russula*	*emetica*	
97	*Russula*	*luteotacta*	
98	*Russula*	*nobilis*	
99	*Russula*	*solaris*	
100	*Sarcosphaera*	*coronaria*	Monomethylhydrazine
101	*Stropharia*	*aeruginosa*	Baeocystin
102	*Tricholoma*	*album*	
103	*Tricholoma*	*lascivum*	
104	*Tricholoma*	*pessundatum*	
105	*Tricholoma*	*scalpturatum*	
106	*Tricoloma*	*ustale*	Ustalic acid

6.5 Mushroom ID cards - examples

6.5.1 Mushroom ID – *Calocybe gambosa* – St. George`s Day Mushroom



Description of the fungus:

Location: University of York, Heslington, York, North Yorkshire, UK.

GPS location: 53.95005, -1.05468

OS grid reference: SE 62138 50852

Ecosystem: deciduous woodland

NCC Ecosystem code: A 111

Substrate: soil

Collected on: 11 April 2012

Collected by: Marcin Fiedziukiewicz

Identifier: Marcin Fiedziukiewicz

Confirmer: Malcolm Greaves

Associated organism: *Aesculus hippocastanum*

Macroscopic features:

Cap – white / cream; 2 - 5 cm; convex / hemispherical

Stem – cream; 2 - 5 cm; brittle; simple

Gills – white; adnate; crowded; of various lengths

Microscopic features:

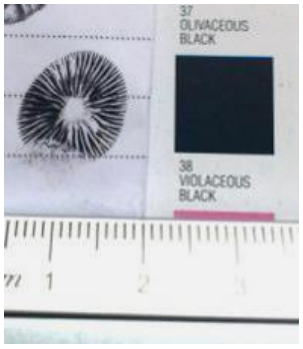
Spore:

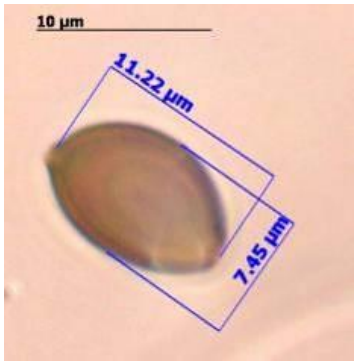
Size: Average **3.01 ± 0.23 µm x 4.84 ± 0.48 µm**; reference size (R. Philips, *Mushrooms*, 2006, Macmillan: London. 5 – 6 x 3 – 4 µm) are within **1 σ** (standard deviation) from the average measurement of the mushroom`s spores **Spore shape:** ovoid and smooth

Colour: 78 - white

Note: *www.rogersmushrooms.com* website`s "easy key" implies that *Calocybe gambosa* occurs only in North America

6.5.2 Mushroom ID - *Panaeolus ater*





Description of the fungus:

Location: Fera, Sand Hutton, Yorkshire, England, UK.

GPS location: 54.01721 -0.96983

OS grid reference: SE6760 5840

Ecosystem: Grassland domestic lawn

NCC Ecosystem code: B2

Substrate: soil

Collected on: 23 April 2012

Collected by: Don Clarke

Identifier: Marcin Fiedziukiewicz

Confirmer: Malcolm Greaves

Associated organism: *Poaceae*

Macroscopic features

Cap: dark brown with brighter patches; hemispherical / bun shape

Stem: ~ 4 cm; pale brown at the top darkening towards the bottom; simple

Gills: dark brown / grey; crowded; adnexed; of various lengths

Size: 1 - 2 cm - cap

Microscopic feature

Spores:

Size: Average **11.48 ± 0.53 µm x 7.31 ± 0.53 µm** (number of spores = 15); the reference measurement (www.rogersmushrooms.com) is within **1 σ** (standard deviation) from the average **Spore shape:** ovoid, some have single pore

Colour: violaceus black - 38

6.5.3 Mushroom ID - *Helvella corium*

Description of the fungus:

Location: Fera, Sand Hutton, Yorkshire, England, UK.

OS grid reference: SE 67579 58193

Habitat: gravel

Ecosystem: gravel on urban site

NCC Ecosystem code: I / J 13

Substrate: bare soil (gravel)

Collected on: 30 April 2012

Collected by: Marcin Fiedziukiewicz

Identifier: Don Clarke

Confirmer: Malcolm Greaves

Associated organism: N/A

Macroscopic features:

Cap: black; cup shape; 2 - 4 cm across

Stem: black ~ 3 cm; white inside and black outside; rudimentary

Gills: absent

Microscopic feature:

Size: Average **16.83 ± 3.35 µm x 10.69 ± 2.23 µm** (number of spores = 11); the reference measurement of both dimensions: 20 - 22 µm x 12 - 14 µm (published on www.rogersmushrooms.com) is within **1 σ** (standard deviation)from the average

Shape: ovoid and smooth **Spore print:** absent **Spore release type:** shooter

www.rogersmushrooms.com website`s easy key came up with only **two** hits:

- *Helvella corium* syn. *Cyathipodia corium*

- *Peziza praetervisa* – wrong colour black on the inside but purple black / grey on the outside, no stem

6.5.4 *Mushroom ID - Coprinus comatus* – Shaggy Inkcap


Description of the fungus:

Location: Fera, Sand Hutton, Yorkshire, England, UK.

Ecosystem: broad - leaved plantation

NCC Ecosystem code: A 112

Substrate: soil

GPS location: N 54º 1.118 W 0º 58.223

OS Grid Ref: SE 6755 5855

Collected on: Monday 16th April 2012

Collected by: Don Clarke

Identifier: M. Fiedziukiewicz

Confirmer: D. Clarke

Associated organism: *Betula pendula [Birch]* and *Fagus sylvatica[Beech]*

Macroscopic features

Cap: white; brown scales; conical shape ~ 10 cm long **Stem:** with a ring; the same as colour as the cap **Gill:** dark purple/black

6.5.5 Mushroom ID – *Helvella lacunose* – Elfin Saddle

Description of the fungus:

Location: Brotherton, Selby, North Yorkshire, UK.

GPS location: 53.73138 - 1.28072

OS grid reference: SE 475 263

Ecosystem: Broadleaf woodland

NCC Ecosystem code: A 111

Substrate: leaf litter

Collected on: 5 June 2012

Collected by: Antony Lloyd

Identifier: Don Clarke

Confirmer: Malcolm Greaves

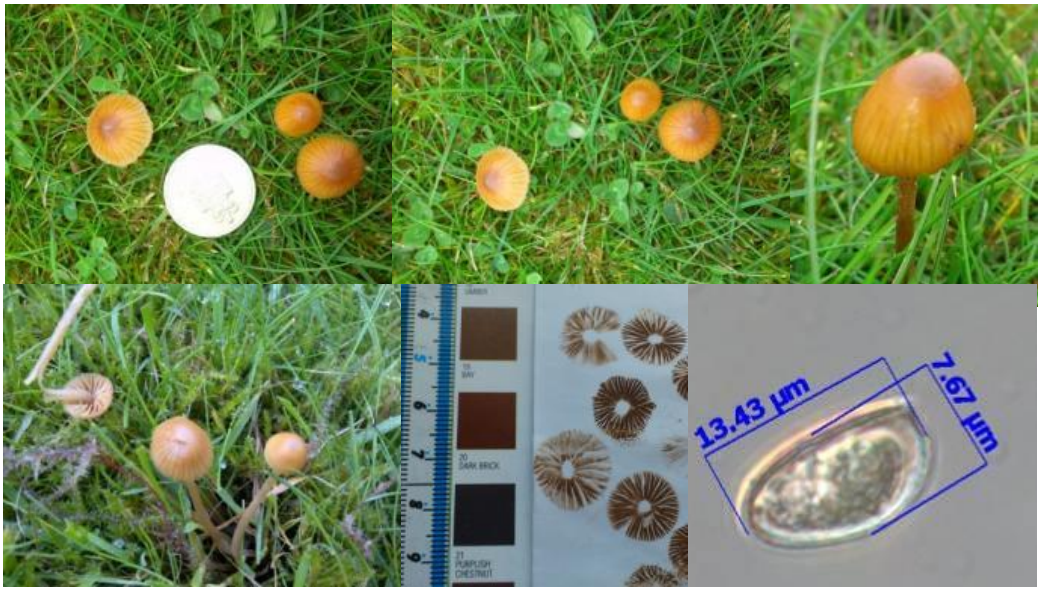
Associated organism: *Betula* (birch) *Quercus* (oak)

Macroscopic features:

Cap: pringle / saddle shaped, uneven; black; ~ 5 cm in diameter

Stem: grey; hollow; deep cuts/lined **Gills:** absent

6.5.6 Mushroom ID – *Galerina hypnorum* – Moss Bell



Description of the fungus:

Location: Fera, Sand Hutton, North Yorkshire, UK.

GPS location: 54.01802 -0.96941

OS grid reference: SE 6752 5851

Ecosystem: garden mossy lawn

NCC Ecosystem code: B2

Substrate: mossy soil

Collected on: 3 May 2012

Collected by: Don Clarke

Identifier: Don Clarke

Confirmer: Malcolm Greaves

Associated organism: *Poaceae*

Macroscopic features:

Cap: bun - shaped; light brown / orange; 1 - 2 cm in diameter; radial dark coloured striations

Stem: simple; brown; thin white hair like fibres

Gills: brown / orange; of various lengths; adnexed

Microscopic feature:

Size: Average **13.17 ± 0.78 µm x 7.36 ± 0.59 µm** (number of spores = 19); the reference measurement of both dimensions: 9 – 12 (14) µm x 5.3 - 7 µm (published on www.rogersmushrooms.com) is within **1 σ** (standard deviation)from the average size

Spore shape: ovoid **Colour:** "dark brick 20"

6.5.7 Mushroom ID – *Polyporus squamosus* – Dryed`s Saddle

Description of the fungus:

Location: University of York, Heslington, York, North Yorkshire, UK.

GPS location: 53.95005, -1.05468

OS grid reference: SE 62138 50852

Ecosystem: deciduous wood stump covered with moss in a deciduous woodland

NCC Ecosystem code: A111

Substrate: Broad leaf stump

Collected on: 09 June 2012

Collected by: Marcin Fiedziukiewicz

Identifier: Marcin Fiedziukiewicz

Confirmer: Malcolm Greaves

Associated organism: *Aesculus hippocastanum*

Macroscopic features:

Cap: yellow with brown scales; ~20 cm; eccentric

Stem: cream, turning brown towards the base; ~5 cm

Gills: Absent

6.5.8 Mushroom ID – Morchella esculenta

Description of the fungus:

Location: Brotherton, Selby, North Yorkshire, UK.

GPS location: 53.73094 -1.28149

OS grid reference: SE 475 263

Ecosystem: ashy soil on the edge of woodland

NCC Ecosystem code: A111

Substrate: ashy soil

Collected on: 25 April 2012

Collected by: Anthony Lloyd

Identifier: Marcin Fiedziukiewicz

Confirmer: Malcolm Greaves

Associated organism: *Quercus* (Oak)

Macroscopic features:

Cap – brown; prolate spheroid shape in honeycomb – like pattern; hollow inside

Stem – cream; hollow inside; brittle; clavate

Gills – absent; no spore print

Size: ~ 9 cm full fruiting body

www.rogersmushrooms.com website`s easy key came up with only **18** hits but only **five** match the cap type:

- *Morchella esculenta*
- *M. vulgaris* – stem not hollow; dark brown cap
- *Gyromitra esculenta* – brain like shape
- *G. gigas* – brain like shape
- *G. infula* – brain like shape

6.6 Stumpery collection pictures

	**Picture 1** ***Crucibulum laeve* – Common Bird`s Nest**
	**Picture 2** ***Coprinellus flocculosus***
	**Picture 3** ***Schizophyllum commune***
	**Picture 4** ***Thelophora terrestris***

	**Picture 5** ***Fomes fomentarius***
	**Picture 6** ***Phlebia radiata***
	**Picture 7** ***Nectria cinnabarina***
	**Picture 8** ***Trametes versicolor***

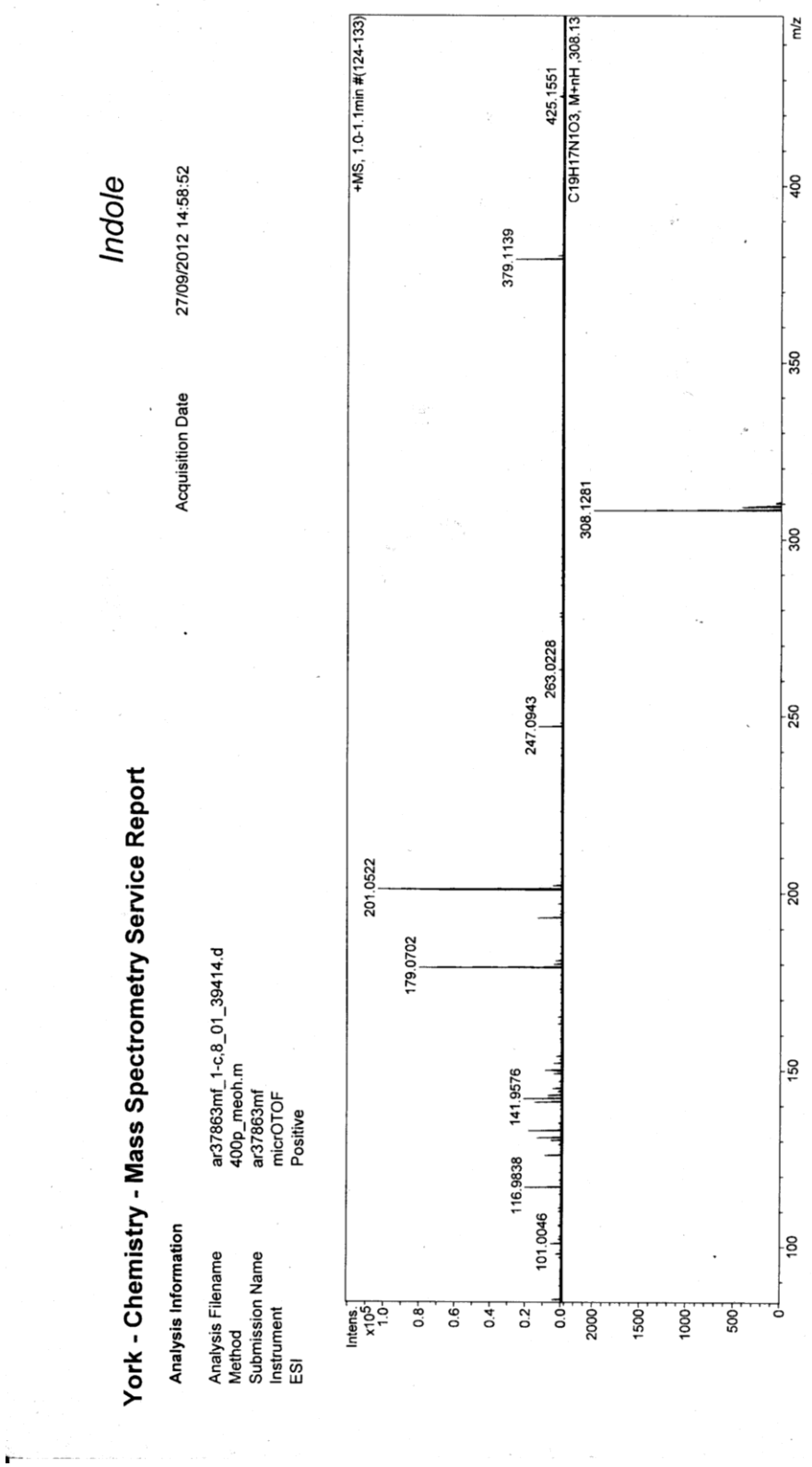
	**Picture 9** ***Stropharia aeruginosa***
	**Picture 10** ***Chondrostereum purpureum* – Turkey Tale**
	**Picture 11** ***Bjerkandera adusta***
	**Picture 12** ***Hypholoma fasciculare***

	**Picture 13** ***Clitocybe dealbata***
	**Picture 14** ***Auricularia auricula-judae* – Jew`s Ear**
	**Picture 15** ***Xylaria hypoxylon* – Candle Snuff**
	**Picture 16** ***Trametes versicolor***

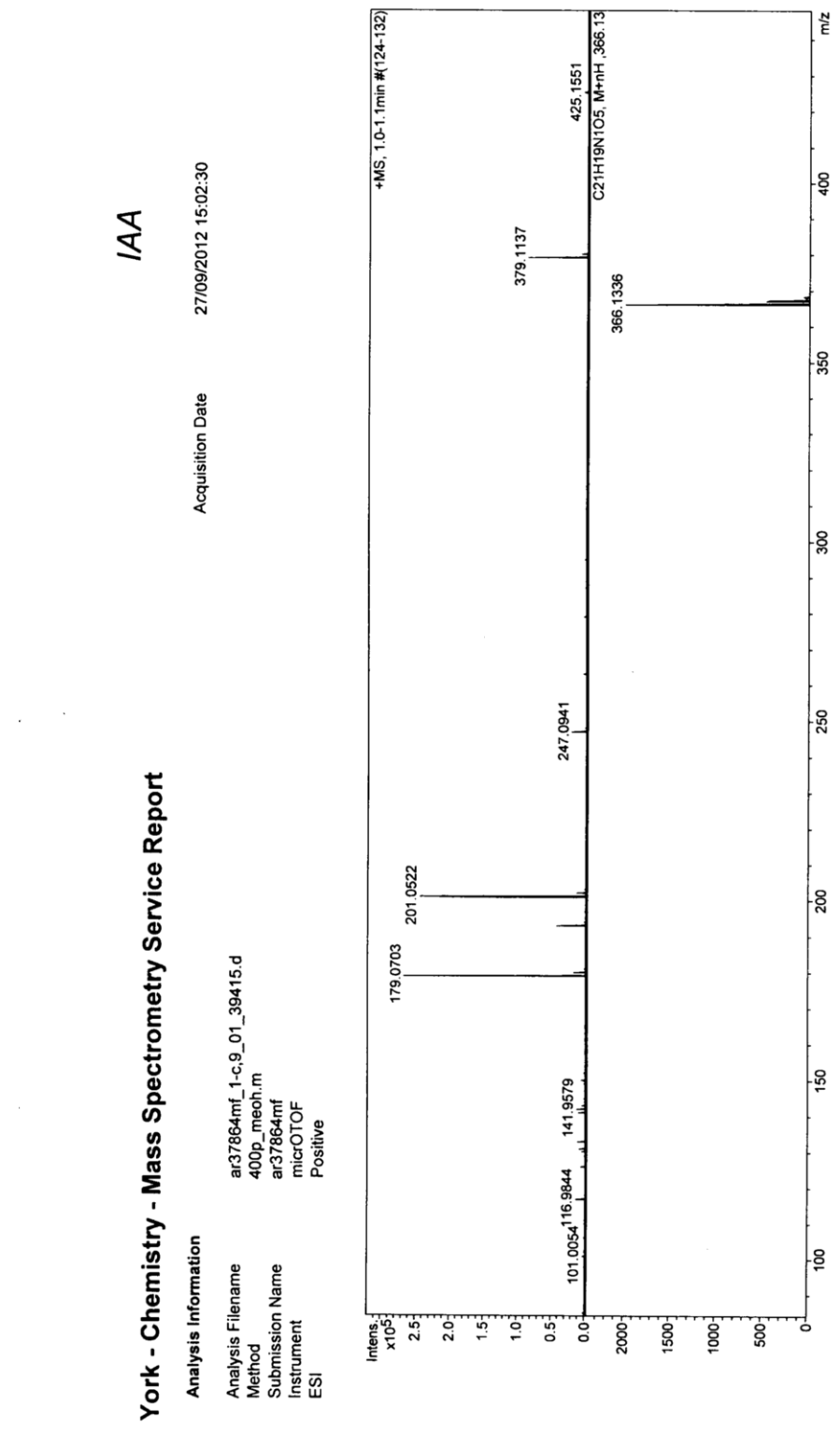
	**Picture 17** ***Polyporuc squamosus***
	**Picture 18** ***Lycogata terrestrae***

6.7 Mass spectrometry

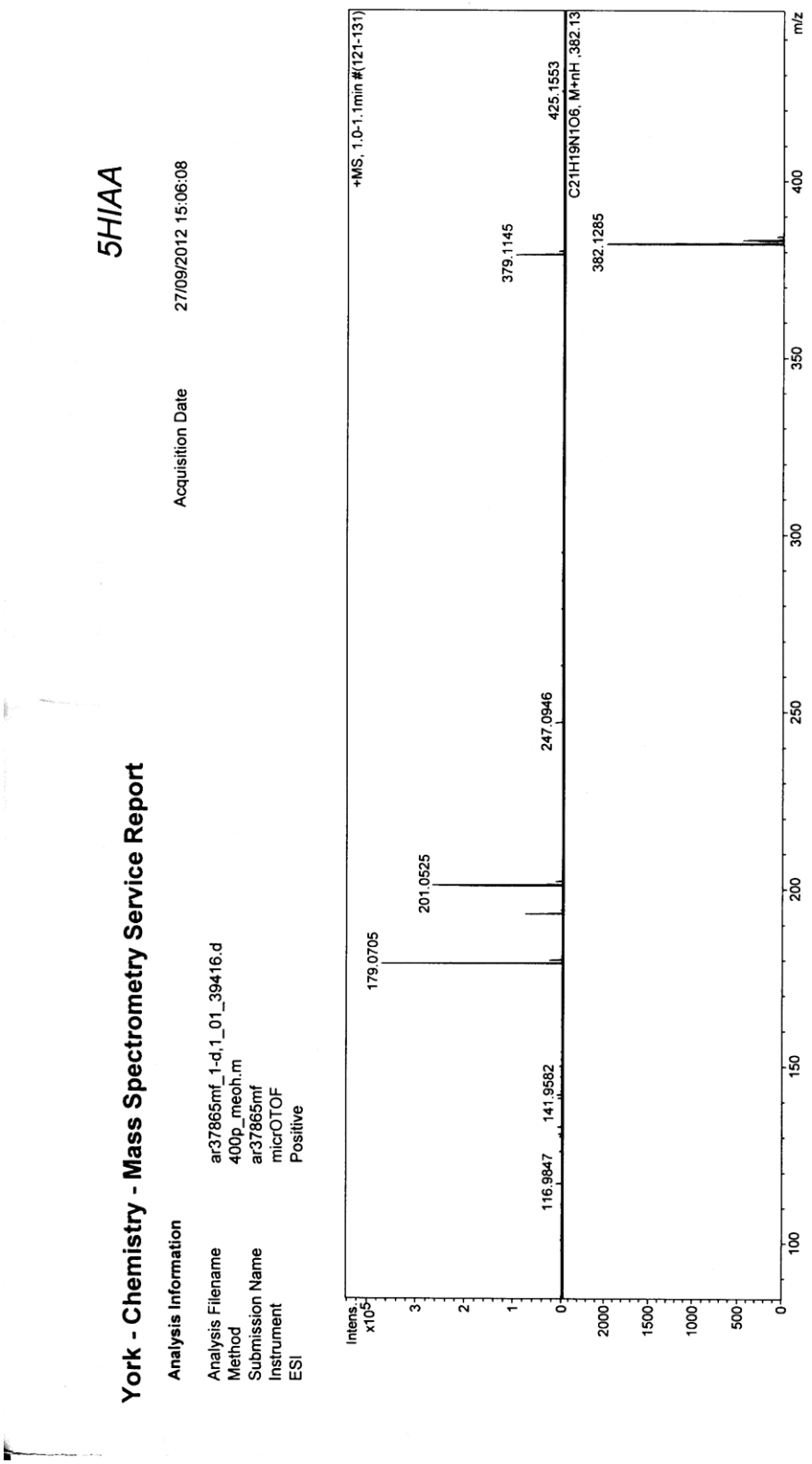
6.7.1 Indole with coniferyl aldehyde



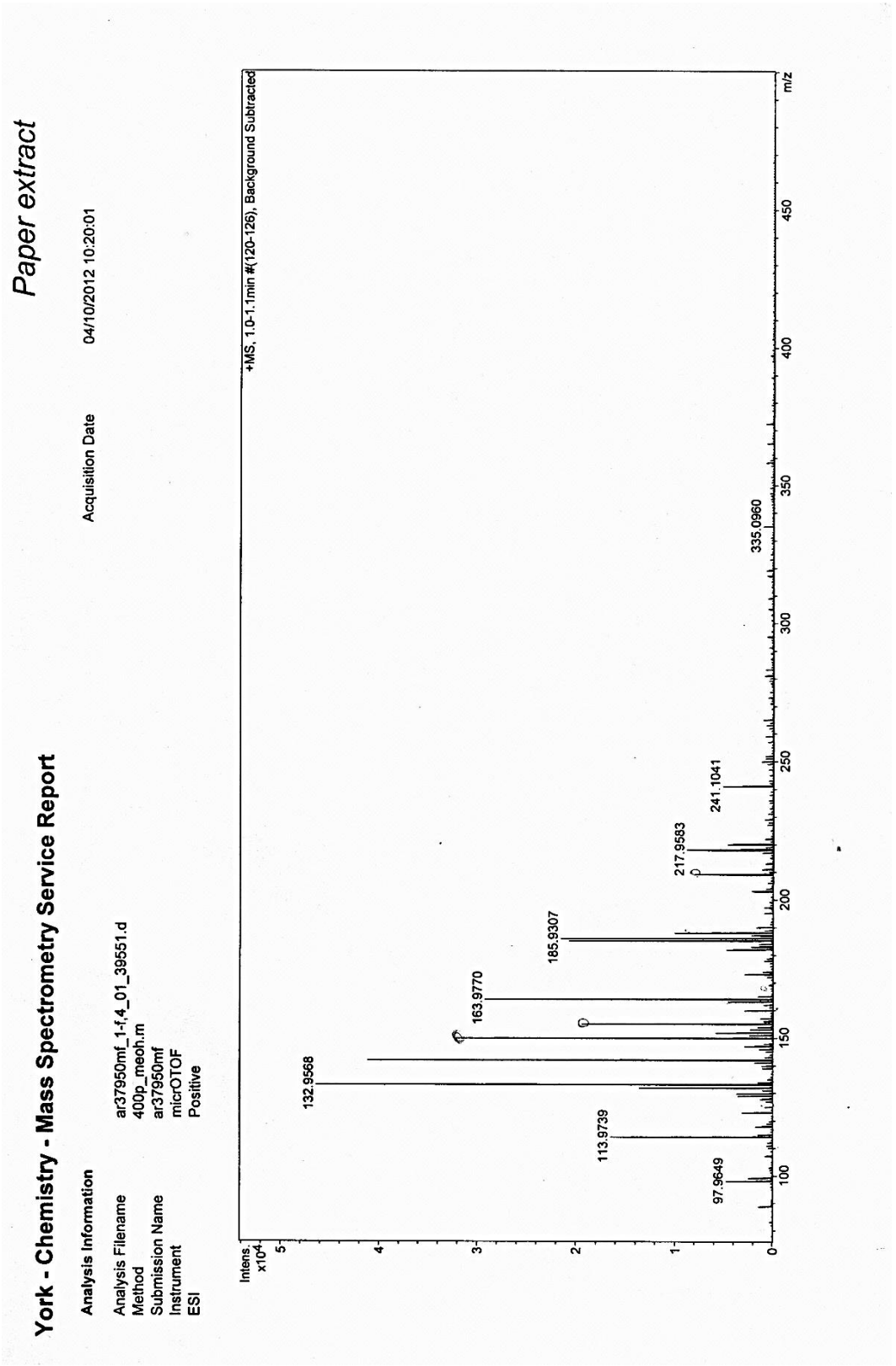
6.7.2 Indole acetic acid with coniferyl aldehyde



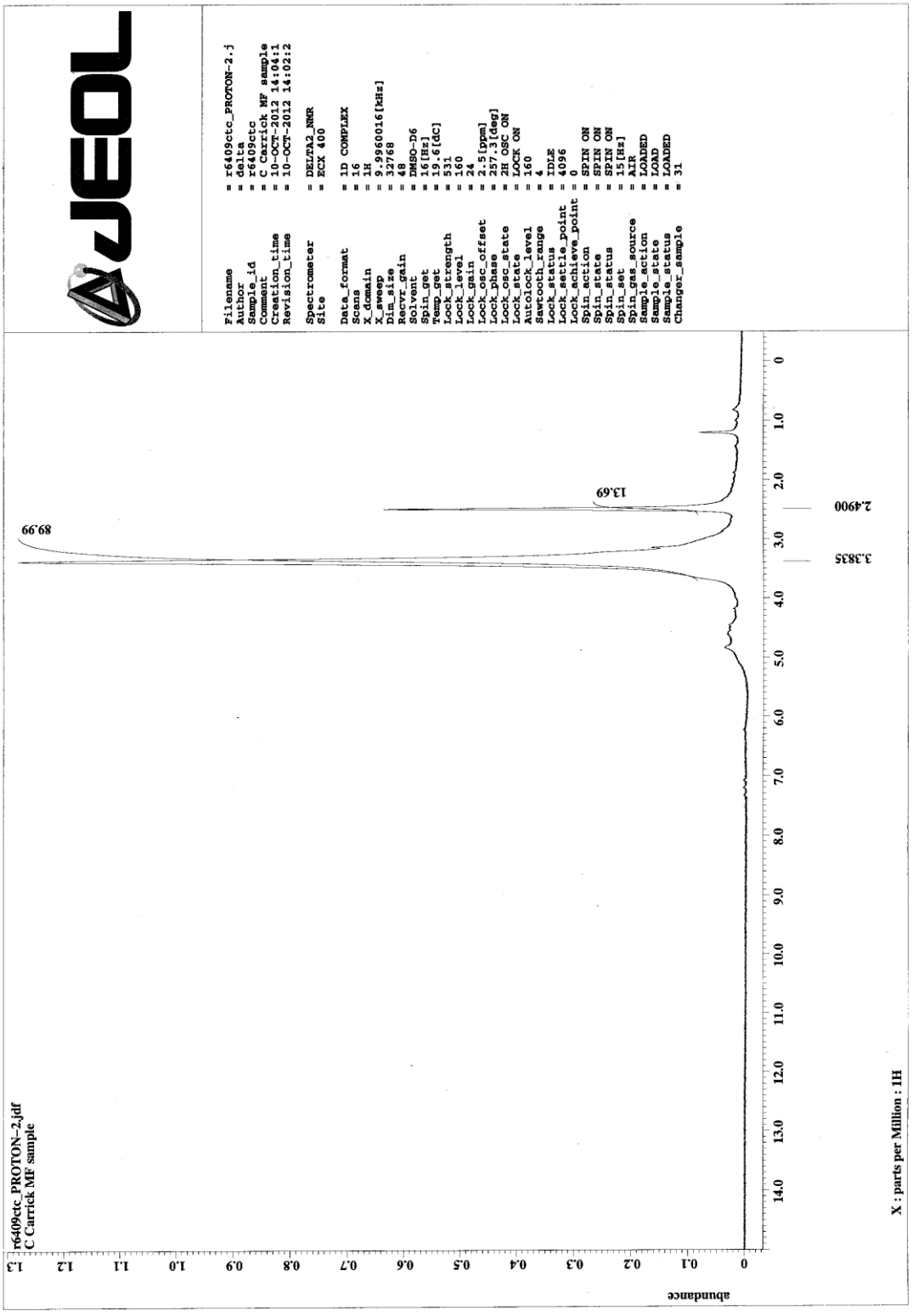
6.7.3 5-Hydroxyindole-3-acetic acid with coniferyl aldehyde



6.7.4 Paper extract



6.8 Lignin extract NMR



7 Chapter 7 - Abbreviations

5-Hydroxyindole-3-acetic acid	(5-HIAA)
Button Mushroom	(BM)
Death Cap	(DC)
Differential Interference Contrast	(DIC)
Dry Weight	(DW)
Food and Environment Research Agency	(Fera)
Hydrochloric acid	(HCl)
Hydrophilic Interaction Chromatography	(HILIC)
High Performance Liquid Chromatography	(HPLC)
Indole-3-acetic acid	(IAA)
Identification	(ID)
Liquid Chromatography	(LC)
Methanol	(MeOH)
Mass Spectrometry	(MS)
Science Direct	(SD)
Thin Layer Chromatography	(TLC)
Time of Flight	(ToF)
United Kingdom	(UK)
Web of Knowledge	(WoK)

8 Chapter 8 - References

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