

Simple Chemistry

for Better Mushroom ID

Britt A. Bunyard

Besides the many habitat and structural characteristics of a fruit body (like cap color, spore color, gills or pores, presence of a stem or not, presence of a ring on the stem or not, etc.), there are chemical clues that can help you determine your fungus. Odors and color changes can be very helpful. Color changes that occur when the mushroom tissues are handled, bruised, or sliced with a knife are usually discussed in the description found in any guidebook. For example, a yellow or red staining reaction upon handling narrows down species of *Agaricus*. The blue reaction in many boletes is notable, as is the bluing reaction of psilocybin-containing “magic” mushrooms. Many mycophiles keep a few simple chemicals close at hand that, when applied to collected mushrooms, can elicit a color change and assist identification.

Chemical reagents and stains are absolutely crucial in the field of bacteriology and even the most rudimentary lab will have dozens of different ones on hand. For field mycology, there are really only a few that are widely used. If you decide to take the plunge and seek out the chemicals discussed here, there are some things to keep in mind. Most last a very long time as they are stable and each use requires only a drop. Some of these are quite dangerous, so be mindful of this when using them, and when storing them safely. Some ingredients may be controlled substances and require a permit to obtain, (although it seems that many “in the know” among the mycological community have a sort of underground network of sharing small amounts of these compounds). I have never heard



Figure 1. Ehrlich's reagent is used to indicate the presence of indoles like psilocybin in *Psilocybe ovoideocystidiata*. Courtesy J. Plischke III.



Figure 2. A spot of KOH placed on *Hapalopilus rutilans* gives a striking purple color reaction. Photo courtesy J. Thornhill.



Figure 3. Potassium hydroxide (10%) applied to a mixed collection of all-white destroying angels quickly can distinguish between *Amanita suballiacea* (KOH+) and *A. elliptosperma* (KOH-). For the negative reactions, you can see where the dropper tip made a depression on the mushroom cap, but the chemical elicited no color reaction (see arrows).

of anyone from the mycological community ever being injured by any of the reagents discussed below.

Although some other chemicals may be used on occasion, those most commonly used for field mycology are Ehrlich's reagent, potassium hydroxide (or KOH), concentrated hydrochloric acid (or HCl, for doing the Meixner-Wieland Test for amatoxins), and Melzer's reagent. Far and away, Melzer's reagent and KOH are the most important and most used. We will spend the most time discussing it.

Ehrlich's reagent

Ehrlich's reagent is used to indicate the presence of indoles like psilocybin in *Psilocybe* spp. (Fig. 1). This reagent can be useful as *Psilocybes* are very difficult to discern from other little brown mushroom look-alikes, including *Deconicas*, *Stropharias*, *Conocybes*, *Panaeolus*, *Galerinas* and others.

The reagent is prepared by dissolving 0.5–2.0 g of p-dimethylaminobenzalde-

hyde (PDAB) in 50 mL of concentrated hydrochloric acid and 50 mL of 95% ethanol (or some other alcohols).

Ehrlich's reagent is named for Nobel Prize-winner Paul Ehrlich who used it to distinguish typhoid from simple diarrhea. Outside of some mycological uses, Ehrlich tests are quite useful in some medical tests to diagnose various diseases or adverse drug reactions. A very common Ehrlich test is a simple spot test to identify possible psychoactive compounds such as tryptamines (e.g., DMT, psilocybin, and psilocin) and ergoloids (e.g., LSD). Although opiates do not contain the indole functional group, this test will give a positive result for opium because of the natural presence of tryptophan in opium.

Potassium hydroxide

Potassium hydroxide (or KOH) is useful for distinguishing mushroom species within tough groups having many species like *Amanitas*, *Russulas*, boletes, *Cortinarius*, and polypores.

Red, yellow, green, purple, or black color reactions can be expected. Some *Russula* and *Lactarius* species may give a strong olive green reaction. The otherwise dull orange-brown polypore *Hapalopilus rutilans* (= *H. nidulans*) turns a striking purple color (or cherry red, in some regions) (Fig. 2).

For *Amanitas*, KOH mostly is applied to species in Section *Phalloideae* to help distinguish one from another (Fig. 3). In the East this can be very useful as you may encounter two or three different all-white destroying angels in a single outing. One more character, based on a positive or negative reaction to KOH, can be useful. To perform this simple test, a 5–10% solution of fresh KOH is applied to the cap and within a minute a bright lemon yellow color will indicate a positive reaction. The color will fade over time but can be performed again later. A negative reaction will never turn yellow. Caution is needed as potassium hydroxide is caustic and will result in a skin burn.



Figure 4. A positive reaction using the Meixner-Wieland test on specimens of *Amanita phalloides* collected in Santa Cruz, California in 2019 (see arrows).

Meixner-Wieland test

Several disparate groups of mushrooms produce deadly amatoxins including Amanitas, of course, as well as Galerinas and Lepiotas. Amatoxin-containing Amanitas are responsible for some 90% of all mushroom poisoning deaths worldwide.

Some books cite this simple test for the presence of amatoxins in mushrooms but usually omit informing the reader just how tricky it can be to interpret a positive reaction; it is likely that few authors have actually attempted this test firsthand. The Wieland test (also known as the Meixner, Meixner-Wieland, or the “newspaper” test) is famously simple to perform and infamously unreliable.

An expert with amatoxins and their chemistry, Dr. Josep Piqueras of the Hospital Universitario Vall d’Hebron in Barcelona, Spain, is a world authority on the topic and once told me: “As a first point I would like to explain to you an old anecdote. In 1986, I visited Prof. Heinz Faulstich in Heidelberg to learn about his radioimmunoassay (or

RIA, an extremely sensitive test for chemical compounds) for amatoxins. I mentioned the ‘Meixner test.’ He corrected me: ‘It’s better to call this the Wieland test. Theodor Wieland was, in fact, the discoverer of the color reaction of amatoxins.’ In my experience this test was an amazing opportunity to teach something interesting—but perhaps not really very useful—to the young doctors in the emergency room of my hospital.

It never was a main tool to make the diagnosis of amatoxin poisoning, nor has it seemed to me a good idea to divulgate this test among laymen as a surefire test to avoid the ingestion of hepatotoxic mushrooms. Among other reasons, because there exists the possibility of intoxication from other poisons which are not detected using the newspaper test. Or imagine that a special Sunday edition of the local newspaper is made

Part adventure, part science class and totally fungi friendly, these stories take the reader deep into the woods of the Pacific Northwest. *Adult Content.*

Visit ellenkingrice.com for outlets.

Compelling characters and plot with a little fungi throughout! A FINALIST and highly recommended. *The Wishing Shelf Awards.*

ELLEN KING RICE

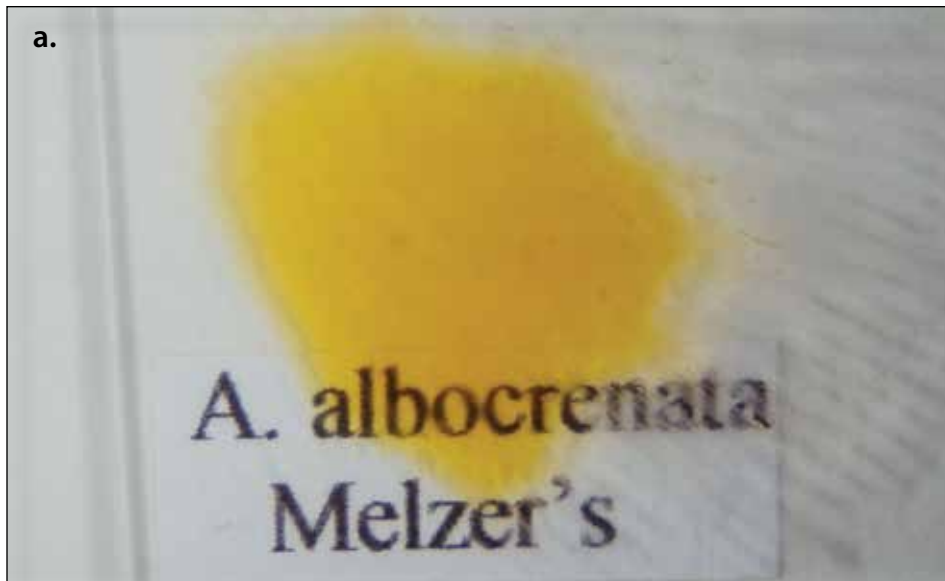


Figure 5. a) *Amanita albocreata* white spore deposit on glass. The drop of Melzer's is yellow and clearly there is no positive black amyloid reaction. b) *Amanita flavorubescens* shows a clear positive black amyloid reaction with Melzer's reagent. Courtesy L. Leonard.



with a good lignin-free paper. There will be no positive reaction from even the most poisonous *Amanita!*"

The so-called newspaper test for amatoxins is performed by pressing fresh sporocarps onto cheap newsprint paper (the paper must have a high lignin content; higher quality papers have very little lignin and will not work; much of the newsprint nowadays won't work either). Circle the mark with a pencil while still wet and visible. Make another circle on the paper—this will be your control for the test. Once the mushroom juice has dried, concentrated

hydrochloric acid (12 N HCl) is spotted on both circled areas of newsprint. Within 15–20 minutes, the presence of amatoxin will be denoted by a greenish-blue color reaction (Fig. 4). If both spots are blue, obviously you are seeing a false positive reaction. As stated, the test can give inconsistent results; sometimes the color is not quite right, false negatives and false positives all can result if the paper has insufficient lignin or if the HCl is not concentrated. Furthermore, false positive results also may result from exposure to sunlight, excessive heat, or several other mushroom

varieties. Reactions with compounds other than amatoxins can result in other colors. Likewise, this test will result in a blue reaction if psilocybin or psilocin is present. And one final note: concentrated HCl is quite destructive to skin, clothing, and many other materials.

Melzer's reagent

Of all the chemicals used in field mycology, Melzer's reagent is the most useful. Melzer's reagent is used to distinguish among white-spored agarics (for example it can be used to distinguish *Lepiota* from *Amanita*, and distinguish between the two subgenera of *Amanita*). This chemical is useful with other groups of fungi, including the study of the asci of many ascomycetes, in visualizing spore ornamentation in the Russulales, etc.

As to comparing Melzer's reagent and other iodine-containing compounds, and where to obtain Melzer's reagent, see the informative report by Lawrence Leonard on page 10.

According to Leonard, the earliest reference to the usage of iodine to identify fungi was by Currey in 1858 on the ascomycete *Amylocarpus encephaloides* and by the Tulasne brothers in 1861 with lichens. Nylander (1865) described iodine bluing in lichens and ascomycetes as did Rolland (1887). It appears that although iodine was used in the mid 1800s in lichen and asci evaluation, it was not widely used on fungal spores. We have Václav Melzer (a Czech teacher and mycologist specializing on Russulas) to thank for that. In 1924, Melzer improved upon a preparation of an iodine potassium iodide solution with chloral hydrate, first developed by botanist Arthur Meyer, and allowed better visualization of spore ornamentation of Russulas.

So how does it work and is Melzer's reagent the same thing as other iodine-based reagents? Besides being very useful as an antiseptic (for many decades), iodine-containing compounds bind to starch and impart a blue-black color. In the case of Russulalean spores, it makes the ornamentations more visible under the microscope. Solutions of iodine potassium iodide (IKI, also called Lugol's solution) can detect starch. Melzer's reagent has added chloral hydrate and is superior to all other iodine reagents

based on very careful tests by Dr. Laurie Leonard. The reason is because chloral hydrate clears the cell contents so the color reaction is more obvious. Because of this, chloral hydrate long has been used in botany to clear cellulose when slide-making, as well as in Hoyer's medium used to prepare slides of animal tissues (including humans).

A word of caution: both iodine and chloral hydrate are poisonous to humans. Indeed, it's interesting to note that a solution of chloral hydrate in ethanol was called "knockout drops" for its ability to incapacitate victims and was used in the concoction of "Mickey Finns." (A few drops were placed in an unsuspecting person's drink and it was lights out—leading to the phrase "slipping someone a Mickey" in old police movies.)

Melzer's reagent is prepared by adding 1.5 g potassium iodide (KI), 0.5 g iodine crystals (I), and 20 g chloral hydrate to 20 mL distilled water. The final product is a yellow liquid. Most sources state that Melzer's reagent will last a very long time; my supply is many years old and still good.

Adding Melzer's reagent to fungal spores and tissues can result in the following: a positive or amyloid reaction, wherein there results a blue or black color; a negative or inamyloid reaction wherein there is no color change (so there may be a yellow color but that's simply the Melzer's reagent); or a dextrinoid reaction wherein there is a red to red-brown color change.

In Laurie Leonard's original paper you can immediately see the usefulness of Melzer's reagent in the analysis of mushroom spores. And, as Leonard points out, all iodine-based reagents are not the same. Without chloral hydrate, you may end up with a misleading reaction. *Amanita* species in subgenus *Amanita*, including *A. albocreata* (misspelled as "albobrenata") have inamyloid spores; the yellow color is from the Melzer's reagent (Fig. 5a). *Amanita* species in subgenus *Lepidella* have amyloid spores and this results in the blue-black color for *A. flavorubescens* (Fig. 5b). In additional tests you can see misleading reactions when using iodine-based reagents without chloral hydrate. Figures 6a and 6b show *Amanita frostiana*, another species in subgenus *Amanita* and

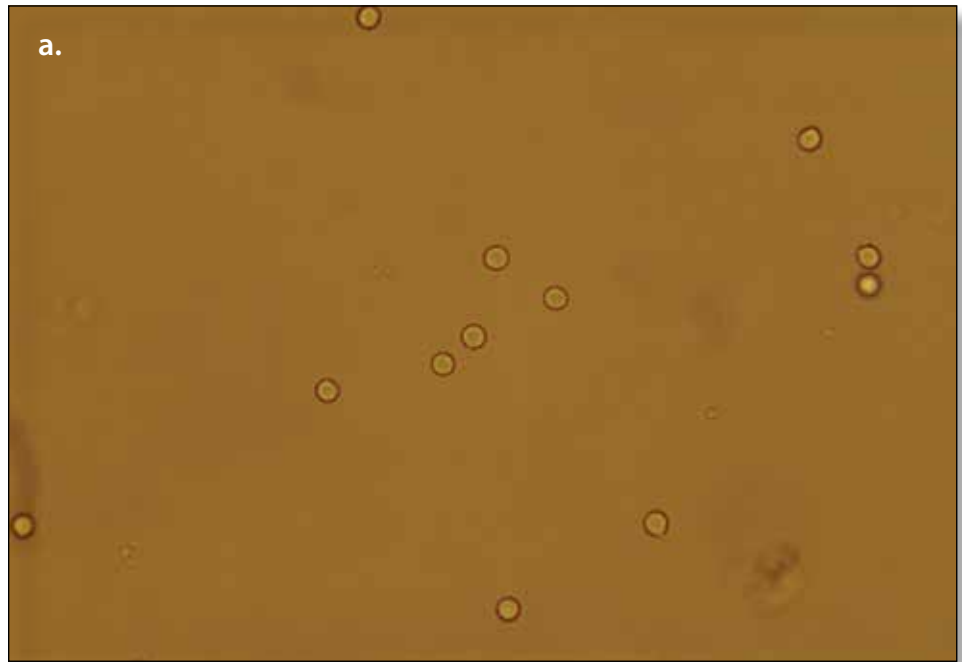
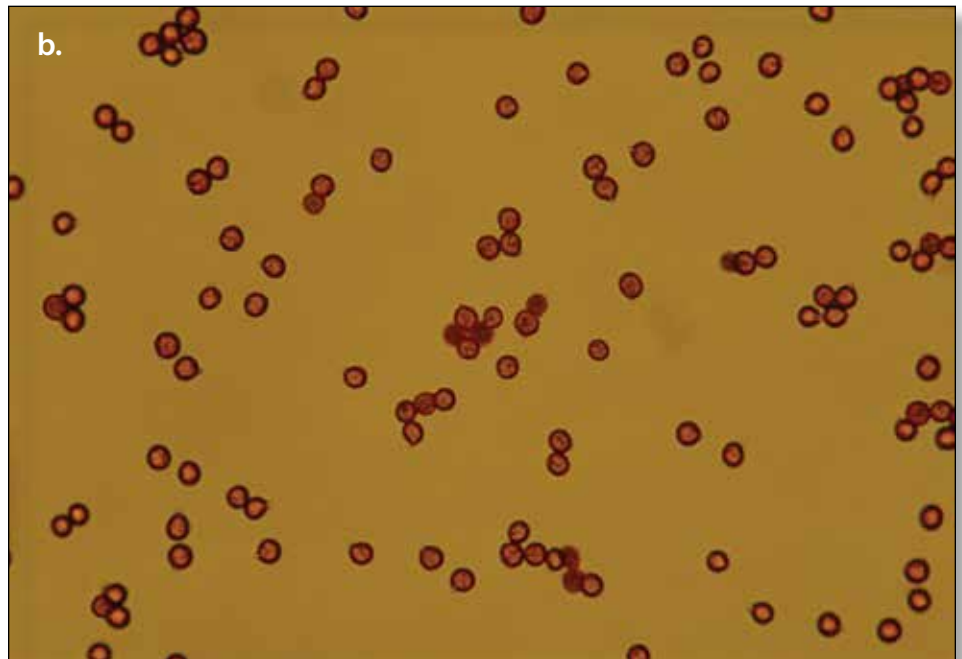


Figure 6. a) *Amanita frostiana* spores shown at 100X microscopy in Melzer's reagent and clearly inamyloid. b) The same mushroom's spores in Lugol's solution appears to be (erroneously) dextrinoid. Courtesy L. Leonard.



definitely known to have inamyloid spores. This is correctly shown under the microscope with Melzer's reagent (Fig. 6a). Using Lugol's solution you get a misleading dextrinoid-looking reaction (Fig. 6b). *Amanita frostiana* and *A. flavoconia* (not shown) are both common species in the East (*A. flavoconia* especially so), and all but indistinguishable to most collectors. They are in different subgenera and thus easily distinguished using Melzer's reagent.

Acknowledgments

Thanks to Jerry Sheine, a long time member of the North American Mycological Association and an expert with microscopy, who first explained to me (decades ago) how and why Melzer's reagent is preferred over other iodine-based reagents without chloral hydrate. Special thanks to Laurie Leonard for much of this information and photographs, as well as enumerable scientific input on mycological matters over the years. †