galactose, run under the same conditions.

Analysis of fraction 3 showed only traces of nitrogenous substances. Thus, the identity of the dideoxy sugar present in soil is definitely 2-amino-2,6-dideoxygalactose (fucosamine). The fact that this compound had not been identified before is not surprising because of the hydrolytic conditions used previously. As reported by Wheat (1966), fucosamine is converted to oxonorleucine in 6 M hydrochloric acid in the overnight hydrolysis of a fucosamine-containing polysaccharide. A recent study of a pneumococcal polysaccharide (Benzing-Purdie and Perry, 1980) showed that the conditions used here are appropriate for the determination of dideoxyamino sugars in polysaccharides.

The internal standard myoinositol was used in order to obtain a quantitative estimation of the dideoxyamino sugar in the soil studied. The value obtained was 8 $\mu g/g$ of soil. However, the actual amount in soil is probably higher because, as in the case of glucosamine and galactosamine, there is considerable loss during cation-exchange chromatography and also all the fucosamine may not have been released upon acid hydrolysis (Bondietti et al., 1972; Nelson et al., 1979). On comparison of the amount of fucosamine with that of another amino sugar recently identified in the soil, muramic acid (Millar and Casida, 1970), the value is approximately 1 order of magnitude lower. It should be noted, however, that while the latter is a component of nearly all bacterial cell walls, fucosamine is not so widespread. It has been found in the lipopolysaccharides of seven strains of *Pseudomonas aeruginosa*, a potentially dangerous bacterial pathogen (Horton et al., 1977) and plant pathogen (Cother et al., 1966), and in a specific lipopolysaccharide of a *Chromobacterium violaceum* strain (Hepper, 1975). In one instance, it has been reported that *Achromobacter georgiopolitanum*, another soil bacterium, synthesized a fucosamine-rich polysaccharide (Hepper, 1975). The contribution of fucosamine to the total N in the soil is very small, but its identification is of considerable importance. The origin of soil polysaccharides cannot yet be assigned with confidence (Hayes and Swift, 1978). The presence of fucosamine in soil supports the hypothesis that they are of microbial origin. As stated by Finch et al. in 1971, "The evidence for cell wall origin would be far greater if some soil polysaccharides were known to contain muramic acid, fucosamine, viosamine, and possibly neuraminic acid". Two of these compounds have now been identified. Although it is difficult to assess whether all fucosamine originates from the living biomass or whether some is associated with the nonliving humus fraction, it seems more likely that the latter is the case. As more work is done on the structural determination of bacterial polysaccharides, the amounts of fucosamine in soil may be correlated with the presence in soil of certain bacterial genera.

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Differential Identification of Toxic Mushrooms by Pyrolysis–GLC Coupling

The authors propose the use of alcoholic extracts analyzed by pyrolysis–GLC for the identification of mushrooms. The "fingerprint" pyrogram should be compared with a preestablished catalog of pyrograms. The method is rapid, easy, and may be used with as little as 15 mg (dry weight) of material. It is thought that the method can be used to detect mushroom poisoning. Sixteen mushrooms of various species have been analyzed to illustrate the possibilities of the method.

In cases of intoxication by toxic mushrooms, symptomatology is not enough for identification. Gastroenteritis, even appearing after eating a dish of mushrooms, is not always related to the mushrooms. Identification needs to be ascertained by other means. Sometimes it is possible to find mushrooms which have not been cooked and identification might then proceed from determination of botanical characteristics. In other cases the only material

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available will be from gastric washing or from the feces and this will require difficult microscopic search for spores by a fully trained mycologist. This sort of specialist is usually not present in the hospital though identification should be done as soon as possible. A series of colored reactions have been proposed (Girre and Gerault, 1978). These might be criticized since they cannot be extended to every toxic mushroom, as each species of mushroom requires a particular testing procedure.

The method is based upon the property of high weight molecules being decomposed by pyrolysis (i.e., heating at high temperature) into complex mixtures of volatile molecules. Such mixtures are characteristic of each substance and can be analyzed by gas–liquid chromatography (GLC); the pyrogram thus obtained is the fingerprint of the substance (Reiner and Hicks, 1972). This method has been used to identify various *Penicillium* (Thorburn Burns et al., 1976) and *Bacillus* (Oxborrow et al., 1977) species. We chose to use ethanol extracts of 16 uncooked mushrooms to examplify the possibilities of the method in the field of toxicology.

In a previous work (Benkheder et al., 1979) we tested the validity of the method on amino acids and polypeptides and showed that subsequent analysis by mass spectrometry (MS) allowed identification of fragments and enabled us to detect the presence or the absence of a specific amino acid in a polypeptide. In the same way, use of GLC–MS on mushroom pyrolysate will also allow identification of amino acids or other components, as has been done for biological macromolecules (Stack, 1968), chitin (Schlotzhauer et al., 1976), microfungi (Weijman, 1976, 1977), and various biological material [reviewed by May et al. (1977)].

MATERIALS AND METHODS

Mushrooms. Mushrooms tested were as follows: *Amanita vaginata* Fr. ex Bul.; *Amanita muscaria* Fr. ex L.; *Amanita solitaria* Bul.; *Amanita phalloides* Fr.; *Amanita citrina* Fr. ex Schaef.; *Lepiota procera* Fr. ex Scop.; *Lepiota excoriata* Fr. ex Schaef.; *Psalliota bispora* Lange; *Galera marginata* (Fr. ex Batsch) Kuhn; *Cortinarius cinnamoneus* Fr. ex L.; *Cortinarius praestans* Cordier; *Entoloma lividum* (Fr. ex Bul.); *Tricholoma terreum* Fr. ex Schaef.; *Tricholoma pardinum* Q.; *Lactarius piperatus* Fr. ex Scop.; *Russula olivacea* Fr.

All the mushrooms were collected around Grenoble (French Alps) and dried to avoid any flexibility in the extraction due to variability of water content. Dry mushrooms (1 g) were ground and boiled for 15 min in 10 cm^3 of absolute ethanol. Pyrolysis was performed on the alcoholic extracts.

Pyrolysis. The pyrolysor with a regulated temperature filament was built in the laboratory of one of us and has been fully described in a previous paper (Waysman et al., 1976). The temperatures were calibrated on benzil and potassium bromide. The alcoholic extract (50 μL) was deposited on the spires of the pyrolysor and evaporated to dryness by means of a jet of warm air. The sample deposition was repeated 3 times. Pyrolysis was conducted at 500 °C for 10 s in a nitrogen atmosphere. After pyrolysis, the pyrolysates were carried into the chromatograph by the vector gas (nitrogen).

Chromatography. The gas chromatograph was a Carlo Erba 2400 V Fractovap fitted with a flame ionization detector. The conditions were the following: column length, 2 m; 3% OV 17 on Chromosorb W (80–100 mesh); vector gas, nitrogen (50 cm^3/min), hydrogen (50 cm^3/min), oxygen (300 cm^3/min). Temperature was programmed from 70 to 300 °C at 10 °C/min; paper, 1 cm/min. Attenuation was usually 80.

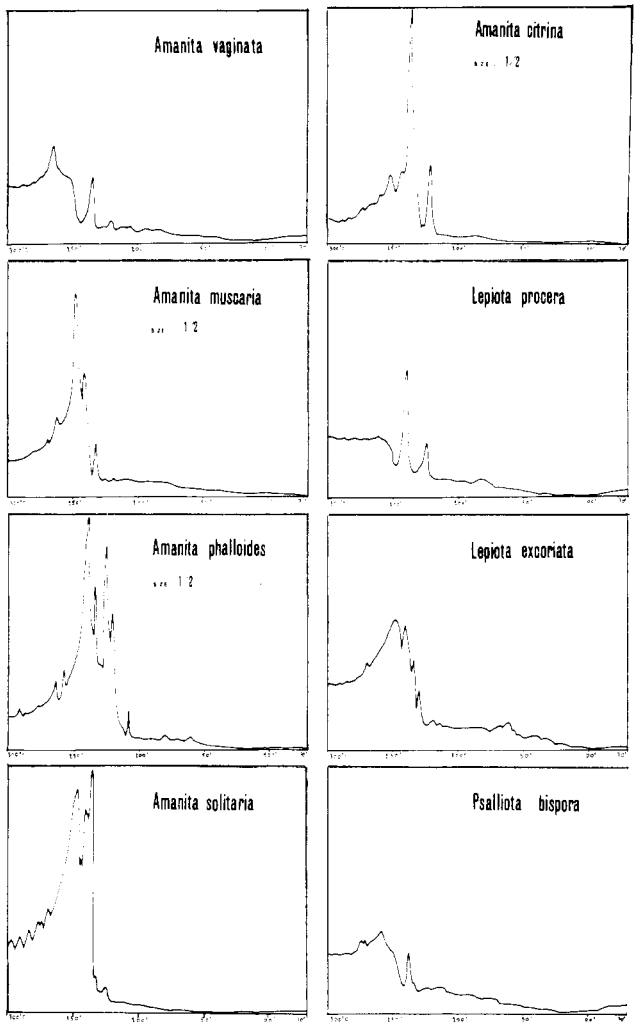

Figure 1. Pyrograms of absolute alcohol extracts of eight extracts of mushrooms, T = 500 °C, 10 s.

RESULTS AND DISCUSSION

The pyrograms of the 16 mushrooms analyzed are given in Figures 1 and 2. The height of the pyrogram is proportional to the response of the detector (attenuation 80) unless stated "size $^1/_2$" (attenuation 160) or "size $^1/_3$" (attenuation 240).

Absolute ethanol was chosen as the solvent since it is a good solvent for both hydrophilic and hydrophobic substances, it is not too volatile and allows easy extraction on boiling, and it is volatile enough to allow easy evaporation to dryness on the pyrolysor's filament. Most of the toxins of toadstools are soluble in alcohol, but this is not a reason for our choice since toxins are usually present in fungus in far from 1%. This is why for a rapid method it seems wiser not to try to identify the toxins themselves; using a standard method of extraction by ethanol, there is no need to select the best solvent for every single fungus. We do not claim that this process permits a good extraction of the mushrooms nor that it will extract the toxic principles of every species of mushroom. We tried to demonstrate that each extract was reproducible and characteristic of a single mushroom and when analyzed by pyrolysis–GLC coupling will give a characteristic pyrogram. The finding that fungi giving analogous intoxications do not yield the same pyrogram (*A. phalloides*, *E. lividum*, and *G. marginata*) is not unusual as we identify the fungus by its fingerprint and not by its toxins; the pyrogram may be representative as well of the inert components but will allow the identification of the mushroom.

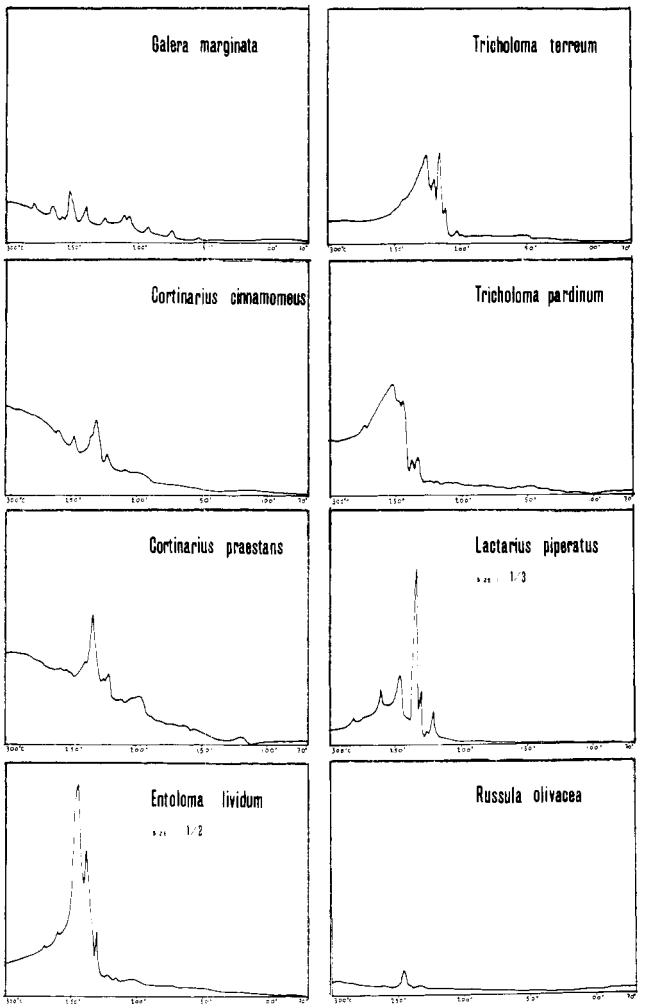

Figure 2. Pyrograms of absolute alcohol extracts of eight extracts of mushrooms, T = 500 °C, 10 s.

The pyrograms have been obtained with a good reproducibility, and the fingerprint of each of them is quite different from the others. There is only one exception: the pyrogram from *E. lividum* is similar to the pyrogram of *A. muscaria*. Though intoxication by *E. lividum* is similar to intoxication by *A. phalloides*, the analogy with *A. muscaria* is far from evident. Though all extractions were carried out under the same conditions, there are great differences in the intensity of the peaks, indicating that the ethanol-soluble substances do not exist in the same proportion in each mushroom. *R. olivacea* exhibits only a tiny little peak with attenuation 80. On the other hand, several *Amanita species*, with attenuation 160 (i.e., size $^1/_2$) or even 240 (i.e., size $^1/_3$), give numerous large peaks. In the *Amanita* genus a major peak always appears at 240–245 °C. The edible *A. vaginata* is easily distinguished by its wide peak at 270 °C, not evident in other species.

The two neighboring species of *Lepiota* tested, *L. procera* and *L. excoriata*, seem to yield very different pyrograms. A closer examination shows that the tall peak at 245 °C of *L. procera* can also be found in *L. excoriata*, but is hidden by a new wide peak at 255 °C.

The pyrogram from *G. marginata* is not similar to the one from *A. phalloides* although the toxin content is very similar. *A. phalloides* yields about 10-fold more ethanol-soluble substances than *G. marginata*. Mass spectrometric analysis of every peak would be necessary to determine if some of them (210, 235, and 270 °C) are the same and are the toxins. The difference between toxic *C. cinnamoneus* and edible *C. praestans* is small. The intensity of *C. praestans* is higher, and the pyrogram of *C. cinnamoneus* bears an additional peak at 250 °C. Out of the *Tricholoma* genus, we tested edible *T. terreum* and *T. pardinum*. They give two pyrograms which were much alike, but shifted by 30 °C. The pyrogram of *T. terreum* forms a thick mass with two main peaks at 200 and 250 °C, while the main peaks of *T. pardinum* appear at 230 and 280 °C.

CONCLUSION

We propose a simple and rapid method to identify dangerous mushrooms by pyrolysis–GLC. It is possible to make a catalog of the fingerprints of dangerous and doubtful mushrooms that might be mistaken for them. It cannot be excluded that pyrograms of cooked mushrooms might be different from uncooked ones. If so, it will be necessary to give two fingerprints for such mushrooms. This method is easy to extend to every mushroom. It does not require much material. We used 1 g of mushroom for 10 cm^3 of extract while we needed only 150 μL of extract. When necessary, the quantity of mushrooms can be lowered to 15 mg and even less. The method is easy, inexpensive, and may reduce time in the treatment of poisoned people. Once the method is established, it does not require a specially trained technician. The method is inexpensive: a gas chromatograph can be found in every hospital laboratory and a pyrolysor is an unsophisticated and rather inexpensive unit, easily connected to the GLC. The method is rapid, being completed in less than 1 h.

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