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IDENTIFICATION OF ARSENIC COMPOUNDS IN MUSHROOMS, AND EVIDENCE FOR MYCELIAL METHYLATION

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Since Edmonds et al. (1977) identified non-toxic 'fish arsenic' present in crustacea and fish as arsenobetaine (carboxymethyl trimethylarsonium zwitterion, $(\text{CH}_3)_3\text{As}^+\text{CH}_2\text{COO}^-$), and subsequently a variety of dimethyltribosylarsine oxides in marine algae, (Edmonds et al. 1981), about a dozen arsenic compounds are now known to occur in the marine environment (Cullen & Reimer 1989). In contrast, information on the compounds present in the terrestrial environment is scarce. We now wish to report progress in identification of arsenic compounds in higher fungi, and some evidence of methylation processes occurring in the mycelium. A recent study (Byrne et al. 1995) on some arsenic-accumulating mushrooms in which we found not only methylated arsenic species, but also—for the first time in the terrestrial environment—arsenobetaine (AB) prompted further investigation of the distribution of arsenic metabolites in the Fungal Kingdom. Analysis of about fifty species of basidiomycetes (_lejkovec et al. 1997) showed AB to be the most common arsenic compound present, and also suggest its preponderance in evolutionarily more advanced fungal groups, i.e. Gastrales and Agaricaceae.

Interest in higher fungi in relation to arsenic (and other trace element) cycling in the terrestrial environment hardly needs justification in view of the dominant role of fungi in decomposition, in transfer and in transformation of nutrients and trace elements. Our earlier finding of arsenic accumulation by *Laccaria amethystina* (Byrne et al. 1979, Byrne & Tu_ek-Znidaric 1983) led to identification of the compound present as dimethylarsinic acid (DMA) (Byrne et al. 1991), and this stimulated us to identify the arsenic species present in a number of other arsenic-accumulating mushrooms, namely methylarsonic acid (MA) in *Sarcosphaera coronaria*, inorganic arsenic in *Entoloma lividum*, a mixture of inorganic arsenic, MA, DMA and AB in *Sarcodon imbricatus*, and AB in two *Agaricus* species (*A. placomyces* and *A. haemorrhoidarius*) (Byrne et al. 1995). Analyses of different collections showed the pattern to be consistently related to the mushroom species. Hence our recent survey (_lejkovec et al. 1997) covering the main orders and genera of basidiomycetes was intended to identify the arsenic compounds present, and perhaps relate them to taxonomic position.

In these studies arsenic compounds were determined in methanol extracts from the mushrooms by high performance liquid chromatography (HPLC) with an inductively coupled plasma-mass spectrometer (ICP-MS) system as an arsenic specific detector. Arsenite, DMA, MA, arsenate and AB were separated on a Supelcosil LC-SAX anion exchange column, and trimethylarsine oxide (TMAO), arsenocholine (AC) and the tetraarsonium cation (TETRA) on a Supelcosil LC-SCX column, the concentrations being obtained from comparison of peak areas with the calibration responses for the authentic compounds. Radiochemical neutron activation analysis (RNAA) was used for total arsenic determination, and to lesser extent, hydride generation—atomic absorption spectroscopy (HG-AAS).

The mushrooms examined in this survey (_lejkovec et al. 1997) were not accumulators, but had normal arsenic concentrations. As stated above, the most frequently encountered metabolite was AB. As well as the other compounds mentioned above, some mushrooms have small amounts of unknown compounds, but these were generally insignificant. Although our survey is still very incomplete, it is already possible to report some interesting observations.

There was apparently no relation between the occurrence of AB, or we might say, the ability to metabolise it (this is discussed below) and the biology of fungi, i.e. saprophytic species were as apt to contain it as mycorrhizal ones. Although the number of mushrooms analysed is too small to permit firm conclusions on a relationship between arsenic metabolism and taxonomy, there is little doubt that the ability to synthesize AB was acquired only during the higher stages of fungal evolution. We find AB most expressed in the Gastrales (puffballs, and the related earth star, *Geastrum*), Table 1, which are at the top of the evolutionary ladder (Courtecuisse 1994). AB is also dominant in the more advanced gilled fungi, notably the Agaricaceae and Lepiotaceae (Table 2). The Agaricaceae have many features in common with the Lycoperdaceae, e.g. formation

Table 1: Arsenic compounds in some mushrooms from the order Gastrales ($\mu\text{g g}^{-1}$ dry weight); individual metabolites are given as percentage of sum of species in methanol extract.

| Mushroom | Total | Sum of species | As(III) | DMA | AB | MA | As(V) |
|--------------------------------|-----------|----------------|---------|-------|-----|-------|-------|
| <i>Calvatia excipuliformis</i> | 0.72±0.06 | 0.8 | 4% | 20% | 72% | – | 4% |
| <i>Calvatia utriformis</i> | 0.79±0.07 | 0.5 | trace | 9% | 85% | – | 6% |
| <i>Lycoperdon echinatum</i> | 1.23±0.10 | 0.3 | trace | 12% | 78% | – | 10% |
| <i>Lycoperdon perlatum</i> | 2.81±0.24 | 3.6 | trace | 5% | 88% | 7% | trace |
| <i>Lycoperdon piriforme</i> | 0.46±0.09 | 0.5 | 8% | trace | 62% | trace | 30% |
| <i>Geastrum</i> sp. | 3.12±0.20 | 2.9 | 2% | 2% | 94% | trace | 2% |

(Total arsenic in Tables 1 and 2 was determined in 2 or 3 replicate determination using RNAA.)

of urea (Stijve & Diserens 1988), accumulation of silver (Schmitt *et al.* 1978, Byrne *et al.* 1979) of selenium (Stijve 1977) and mercury, and biosynthesis of methylmercury (Stegnar *et al.* 1973, Stijve & Roschnik 1974).

Other evolutionarily advanced gilled fungi are the Cortinariaceae, a few genera of which are clearly linked to Gasteromycetes. Two arsenic-rich *Cortinarius* species, *Telamonia bivela* and *Phlegmacium melleolens*, collected in a former mining area, were found to contain virtually only AB (Gössler *et al.* unpublished). Such links are less evident in the Amanitaceae, but at least in the Fly Agaric (*A. muscaria*), AB is the predominant metabolite, with the unusual precursor arsenocholine (AC). This was also the finding of Kühnelt *et al.* (1997) in some Austrian specimens.

The Aphyllophorales are a most heterogeneous group, especially from the morphological point of view. *Sarcodon imbricatus*, a representative analysed earlier (Byrne *et al.* 1995) contains AB, but apparently biosynthesis is not very efficient, since many intermediates are present at significant levels. The genus *Albatrellus* (which are terrestrial polypores), *Ramaria pallida* and *Sparassis crispa*, contain AB and several other organic and inorganic arsenic compounds. In *Sparassis crispa* arsenocholine is the predominant metabolite. In *Gomphus clavatus*, an intermediary species between the Clavariales and the Cantharellaceae (the latter having rudimentary gills), AB predominates to the extent of 90 per cent, while in *Thelephora terrestris*, belonging to the more primitive crust and parchment fungi, AB is absent.

In the Tricholomataceae, another diverse group, AB occurred less frequently (only in 3 of 6 genera investigated). But in the more developed genus *Collybia*, in two species collected near an old smelter site in southern Austria and containing much arsenic, AB was the main compound (Kühnelt *et al.* 1997). We also analysed a few reddish spored fungi belonging to the Plutaceae and the Entolomataceae, which are rather difficult to classify. It is generally recognized that Plutaceae are the more evolved ones and indeed in *Volvariella volvacea* arsenic is largely present as DMA with a trace of AB, whereas in *Entoloma rhodopolium* there is mainly inorganic arsenic, just like in *Entoloma lividum* analysed earlier (Byrne *et al.* 1995).

The question of course arises whether biosynthesis, *i.e.* methylation, is involved, and this presumably in the mycelium which is very long-lived, rather than in the ephemeral fruitbodies themselves (where the compounds were detected). The alternative hypothesis is selective uptake and transport of compounds from soil or pore water, synthesised by other means, *e.g.* microorganisms (Cullen *et al.* 1995). The fact that mushroom species from different sites and countries display a consistent arsenic compound pattern supports the idea of direct