## **B021** Isolation and characterization of an alliinase of the basidiomycete Marasmius alliaceus B. Schmitt and M. Keusgen

Insitut für Pharmazeutische Biologie der Universität Bonn, Nussallee 6, 53115 Bonn, Germany.

Species of the genus Marasmius are saprophytic basidiomycetes typically growing in woody areas of Europe. Marasmius alliaceus (Jacq. ex Fr.) Fr., Tricholomataceae, as well as some

other members of the genus *Marasmius*, is reported to have a characteristic smell reminding to garlic. Volatile sulphur-containing compounds, particularly dimethyl-polysulphides according to those found in various *Allium* species, had already been described (1). These compounds are typically formed by the contact of cysteine sulphoxides with the enzyme alliinase followed by secondary chemical reactions (Figure 1). This formation was already investigated in detail for many Alliaceae, but not for Tricholomataceae. Sulphur compounds resulting from this enzymatic digestion are reported to have antimicrobial and various therapeutical effects.

Alliinase activity of *M. alliaceus* was investigated in the recent study. The enzyme was isolated from dried *Marasmius* material. Alliinase activity was found as high as this had been reported for wild *Allium* species (2). Several cysteine sulphoxides were tested as substrates. In contrast to alliinase from garlic (*Allium sativum* L.), the now described enzyme showed highest activity towards isoalliin.

The strong garlic-like smell leads to the assumption, that also high levels of cysteine sulphoxides are present in cells. In order to carry out further analytical studies, *M. alliaceus* was transferred to agar plates and cultivation was carried on under controlled conditions. Further on, *M. alliaceus* might be tested on biological activity.



Figure 1. Enzymatic cleavage of the cysteine sulphoxide alliin.

References: 1. Rapior, S. et al. (1997) J. Agric. Food Chem. 45: 820-825. 2. Krest, I. et al. (2000) J. Agric. Food Chem. 48: 3753-3760.

## B022 Detection of allergenic urushiols in Ginkgo biloba leaves

K. Schötz

Dr. Willmar Schwabe Pharmaceuticals, Phytochemical Department, Willmar Schwabe Str., 76209 Karlsruhe, Germany. karl.schoetz@schwabe.de

Ginkgo biloba is, like members of the Anacardiaceae family, a plant well known for its content of long chain alkylphenols with allergenic potential. Ginkgo leaves are widely used to prepare extracts for the treatment of peripheral and cerebral circulatory disorders as well as dementia of different aetiology. Since alkylphenols represent a substantial risk factor for adverse drug reactions, suitable techniques for elimination of these compounds from the standardised ginkgo extract EGb 761 have been developed. In order to demonstrate the effectiveness and reliability of this production process, we have now performed a comprehensive qualitative and quantitative analysis of the alkylphenols in ginkgo leaves and followed their fate during manufacturing of EGb 761. For this purpose reference compounds were synthesised or isolated from plant sources. Detection of alkylphenols was performed by silylation GC-MS. Our results confirm that ginkgolic acids constitute the major group of long chain alkylphenols in ginkgo leaves (approx. 2%). Cardanols (3-alkylphenols) were found at a concentration of about 0.1%. Surprisingly, we now observed that ginkgo also contains isourushiols and urushiols (about 20 - 30 ppm), which are by far the most important known contact allergenic compounds from plant sources. Analysis of samples collected at different stages during the patented production process demonstrated that all alkylphenols are removed in parallel. In the final product the content of every alkylphenol was generally below the detection limit of 0.05 ppm. Therefore, it can be concluded that proofing the absence of the predominant and easily quantifiable ginkgolic acids provides a reliable means for control of the pharmaceutical quality of the final product.

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