Cellular toxicity of orellanine: a short review*

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Every year poisonings with *Cortinarius* species subgenus of the section *Orellani* are reported and often result in acute renal insufficiency. In this report results of investigations carried out mainly in the last decade on the toxicity of these fungi are reviewed. During this time numerous data of enzymatical, morphological and chemical investigations accumulated, leading to a new hypothesis on the mode of action of orellanine.

Keywords: Agaricales, basidiomycetes, mycotoxins, toxicity.

In 1952 a remarkable mass poisoning (102 persons) affected a number of families in Poland. Interviews of the victims showed that all of them had consumed meals prepared with the same mushrooms. Therefore, the suspected mushrooms were collected and tested experimentally on animals. The toxicity tests resulted in comparable and similar histopathological manifestation to those seen in humans (Gryzmala, 1957). The fungus responsible for the poisoning was identified as *Cortinarius orellanus* Fr. and its toxin was designated as orellanine. Chemotaxonomic studies on additional *Cortinarii* of the subgenus *Leprocybe* section *Orellani* (Gruber, 1969) led to the conviction that *C. orellanoides* Hry., also containing orellanine, is nearly related to *C. orellanus* (Moser, 1969). These findings prompted to an extensive screening of different *Leprocybes* and showed that a North American species, *C. raineriensis* Smith & Stuntz, also contained orellanine. In another study on „eight" *Cortinarii* with regard to their orellanine content a further species, *C. fluorescens* Horak, was shown to contain orellanine (Rapior & al., 1988). These results were confirmed by Pöder & Moser (1989), who described a rapid and simple method to detect orellanine. Summing up to the present, orellanine could be unequivocally detected in four species of the genus *Cortinarius*.

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* This paper is dedicated of Professor M. Moser on the occasion of his seventieth birthday.

It has been shown that *C. orellanoides*, *C. speciosissimus* Kühn. & Romagn., *C. brunneofulvus* Fr. ss. Bres. and *C. henrici* Remaux are synonymous (Pöder & Pipitz, 1986; Høiland, 1985). The habitats of the two European species *C. orellanus* and *C. orellanoides* differ in the following characteristics: *C. orellanus* is mainly found in deciduous and mixed forest, especially in close vicinity of pine-trees of temperate warm areas (Moser, 1969), whereas *C. orellanoides* is found on acidic soil in moorlands and coniferous wood. The basidiomata are medium-sized and show a typical orange-brown color. In general, the pileus of *C. orellanoides* is distinctly umbonate and possesses a distinct, yellowish velum partiale, in contrast to the more or less plano-convex pileus of *C. orellanus* without remnants of veil. A characteristic feature of both species are the thickish and distant lamellae. The species forms ectomycorrhizae with various trees.

Basic studies on orellanine poisoning

Gryzmala (1957) first documented the toxicity of orellanine, soon reported by contemporary european mycologists (Moser, 1969; 1971). Case reports are summarized in detail elsewhere (Schumacher & Høiland, 1983; Michelot & Tebbett, 1990). A connection between symptomatology and severity of intoxication and the latent period and the amount of consumed mushrooms could be demonstrated. The weakest poisoning was characterized by a delayed onset of 10-17 days, with an increase in urine output and recovery within days. More serious intoxications with a latent peroid of 2-5 days developed loin pain with oliguria, proteinuria and the excretion of blood cells, cell debris and other urine precipitates. However, no serious dysfunction of the kidneys could be observed. The fatal cases are characterised, additionally to the symptoms mentioned above, by uraemic symptoms and an acute renal failure 3-20 days after ingestion. The severity of the impairment seems to be contingent upon the constitution of the injured person and the time needed to concentrate the toxin until a distinct threshold value has been reached. The long period between ingestion and the onset of symptoms is the most striking feature of an intoxication with orellanine. Therefore, a correlation of symptoms with a preceding mushroom ingestion could be missed. The kidneys proved to be the primary target organ as histopathological studies on intoxicated rats corroborated the occurence of a tubular interstitial nephritis (Prast & al., 1988).

Physiological and ultrastructural studies revealed the onset of a pronounced nephrotoxicity with epithelial cells of the proximal tubules affected at first. A strong degradation of the brush border in this area, a swelling of mitochondria and vacuolization of cells could

be observed already 24–36 h after application of the toxin. In rats a dose of 2 g dried fungus per kg body weight caused a decrease of the glomerular filtration rate and a strong reduction of the reabsorption of H_2O, Na^+, K^+ and glucose. In vitro studies on respiration of isolated rat kidney mitochondria showed an inhibition of cytochrom C oxidase and consequently a decreased oxidative phosphorylation. There were first indications that orellanine affects the activity of several renal brush border enzymes but mainly the alkaline phosphatase seems to be reduced directly (Prast, 1982; Prast & Pfaller, 1988).

Basic chemistry

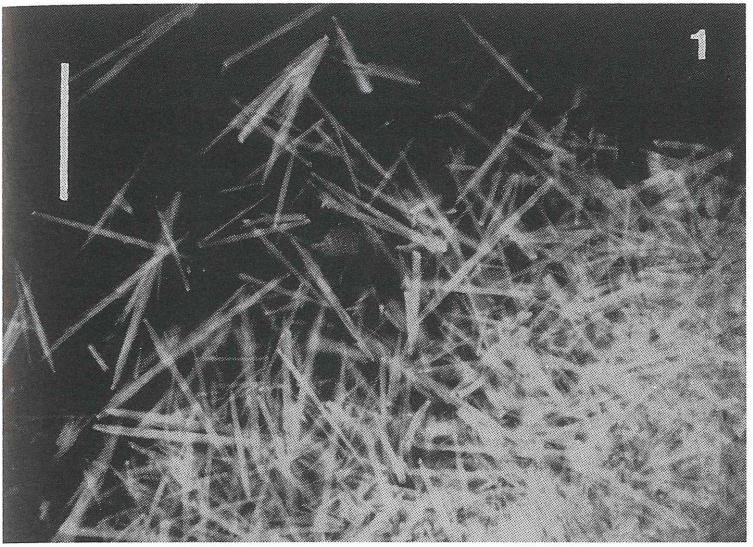
First attempts to isolate the toxin(s) from *C. orellanus* were performed by Gryzmala (1962). This author prepared an impure crude extract that, tested on animals, produced comparable effects as ingested mushrooms. The purification of this crude extract included evaporation and acidification steps and yielded light beige, 95% pure crystals, resistant against chemicals and heat (Figs. 1–3). Above 150°C orellanine decomposed to a non-toxic yellowish substance termed orelline. Spectrometric data supported a bipyridilic structure, 3,3´,4,4´tetrahydroxy-2,2´dipyridyl-bis-N-oxide (Antkowiak & Gessner, 1975) later confirmed by synthetic production of orellanine (Dehmlow & Schulz, 1985). This synthetic orellanine exhibited the same biological effects as the one extracted from mushrooms. Moreover, a high susceptibility to UV-light was found, and during exposure to UV-light orellanine degraded to orelline, thereby losing its toxicity (Kürnsteiner & Moser, 1981). Meanwhile, a further but still toxic intermediate during photodecomposition was detected and termed orellinine (Antkowiak & Gessner, 1985; Rapior & al., 1986).

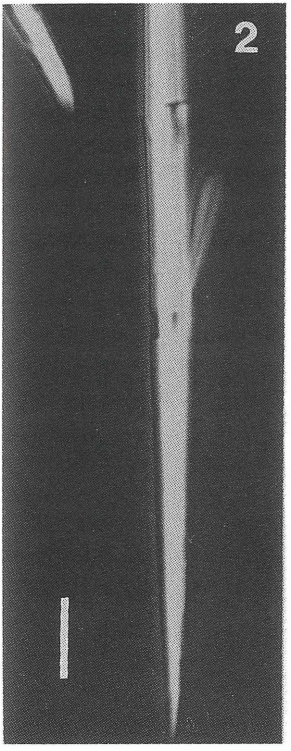
Two herbicides, paraquat and diquat, also bipyridiles, were tested as comparable compounds concerning chemistry and their mode of action. A mechanism that included oxidation/reduction chain reactions with ultimate formation of radicals and thus the lowering of NADPH was found to underlie the destructive action of paraquat, diquat and extracts of *C. orellanoides* on the choroplasts of *Lemna minor* (Schumacher & Høiland, 1983).

Mode of action of orellanine

Enzymatic investigations

To avoid animal experiments, specific research aimed at establishing a suitable alternative system. Renal epithelial cell cultures were





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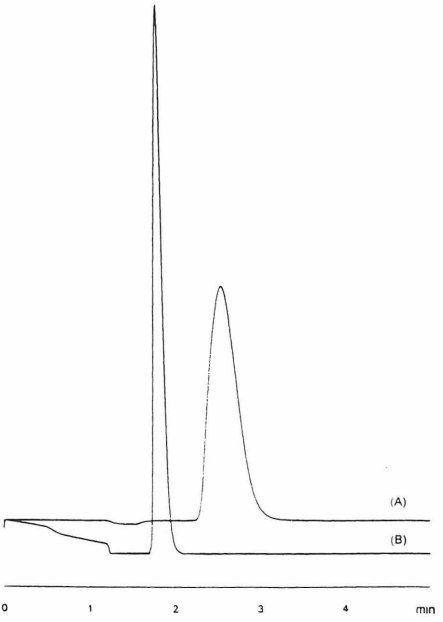

Fig. 1–3. – 1. Crystalls of purified orellanine. Bar: 100 μm (photograph by R. Pöder). – 2. One crystall of purified orellanine. Bar: 10 μm (photograph by R. Pöder). – 3. Signals of the HPLC-analyses show pure orellanine (A) and the artificial orellanine-glycosid (B) distinguishable by different retention times.

found suitable to test orellanine toxicity (Gstraunthaler & Prast, 1983; Pfaller & al., 1989). The results of several enzyme activity measurements using rat kidney and liver homogenate showed an impairment in enzyme activities upon orellanine exposure (Prast, 1982) and led to further assays of bacterial and fungal enzymes. In the course of these studies paraquat and diquat, because of their chemical structure similar to that of orellanine, were thought to be suitable to compare the mode of action. Alkaline phosphatase (AP) was apparently the most affected enzyme. Thus, the isoenzymes of AP were tested and different modes of inhibition were detected. The renal isoenzyme was studied in LLC-PK_1 cells (originated from proximal tubules), whereas for the intestinal type the Caco-2 cells (originated from a colon carcinoma) were used. Commercially available enzymes served as controls as well as to represent the placental isoform of AP Orellanine acted as a noncompetitive inhibitor on renal AP and on the intestinal as well as on the placental isoform as a competitve inhibitor. Additionally, the toxin exhibited an extraordinarily stronger effect on these enzymes in comparison to paraquat and diquat (Ruedl & al., 1989; Ruedl & al., 1990). Further studies using epithelial cell lines from other origins (OK and NRK both derived from proximal tubule; MDCK from distal tubule of the kidney: Meißlitzer, 1991), *E. coli* AP, *Staphylococcus aureus* AP (Teissl, 1988), as well as AP of *Physarum polycephalum* (Mellauner, 1991) confirmed the direct and primary effect of orellanine on AP, and a concentration-dependent influence on the protein synthesis (Mellauner, 1991). OK cells were impaired in comparable dimensions, although they do not express any AP activity. Thus, AP cannot be assumed to be the most important target in cell injury. Inhibition of AP activity and overall cells integrity could be positively influenced by application of metal ions like Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+} (Teissl, 1988; Sigl-Michelitsch, 1990), possibly because of complex formation. However, a preincubation of the cells in order to gain a sufficient intracellular ion level seemed to be necessary.

Furthermore, an increase in lactate-dehydrogenase release indicated an impaired plasma membrane integrity and a decrease in the ATP level in LLC-PK_1 cells (Fischer, 1991). Forty-eight hours after application of the toxin a slight reduction of lactate uptake could be observed, but with little influence on transport and uptake of glucose (Grasser, 1989). Subsequently, additional key enzymes were tested with regard to their susceptibility to orellanine. Based on the in vitro studies of Prast (1982) concerning the cytrochrome C oxidase activity in isolated rat kidney mitochondria, investigations using OK cells homogenate indicated a strong and direct inhibition of this respiratory chain enzyme (Kampfer, 1993). Such inhibition was conclusively demonstrated in time course studies, during which first

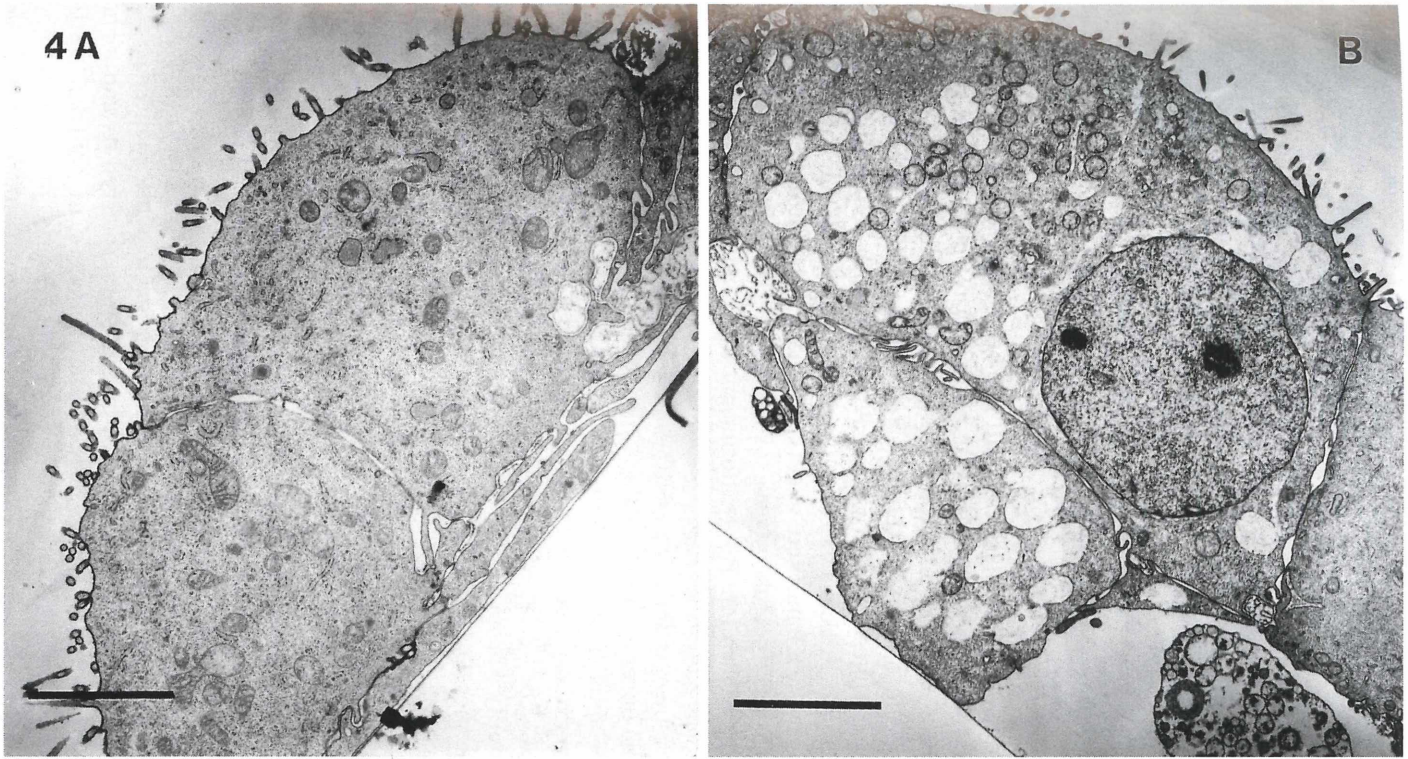

Fig. 4. – TEM-micrographs of LLC-PK monolayer sectioned perpendicularly to the growth aerea. – A, Control cells. B, Orellanine treated cells with vacuolization proved to be swollen ER. Bar: 5 μm (photographs from Burger, 1992).

visible cell injuries occured much later than inhibition of cytochrome C oxidase. In contrast to cytochrome C oxidase, phosphate-dependent glutaminase as well as malate-dehydrogenase increased, the latter being responsible for producing $NADH+H^+$ needed in case of oxidative phosphorylation (Kampfer, 1993). All these data were indicative of a direct influence on the cell respiration capacity and energy production.

Histology

Morphological changes could not be recognized earlier than 24 h after application of 10^{-3} M orellanine in *in vitro* studies on renal cell cultures. First signs of impairment were the collapsing of domes and the floating of cells in the culture medium. All four cell lines used showed changes of the brush border region with deformation and loss of microvilli. All cells exhibited a more (OK and MDCK) or less (LLC-PK_1 and NRK) marked vacuolization, with a pronounced vacuole close to the nucleus in case of MDCK cells. Vacuolization mainly referred to swollen ER (endoplasmatic reticulum)-cisternae (Rupp, 1989). The rough ER was diminished and ribosomes appear to be reduced in number. Forty-eight h after intoxication injured nucleoli could be observed in three cell lines in the form of karyopyknosis and karyorrhexis (Burger, 1992). Mitochondria in OK and MDCK displayed disrupted cristae membranes, which were less pronounced in LLC-PK_1 cells (Rupp, 1989). Two cell lines developed an abnormally large number of lysosomal structures and autophagosomes. OK cells, although expressing virtually no AP activity at their apical membrane, exhibited similar morphological alterations. The observations made on cultured epithelial cells in vitro confirmed the manifestation of orellanine intoxication seen in rat kidneys in vivo (Prast, 1982; Prast & Pfaller, 1988) (Fig. 4 and Fig. 5).

Studies using the water lentil *Lemna minor* as a test object resulted in different effects caused by orellanine, depending on the exposure to light. Incubation of the intoxicated sprouts under daylight conditions resulted in the development of chlorotical spots and the sprouts appeared to be light-green to white, whereas cells growing in the dark developed brown clusters, referred to as bunched chloroplasts (Eder, 1992). These findings were in contrast to the results of Høiland (1983), who could not observe any effects when orellanine treatment of *Lemna minor* was carried out in the dark.

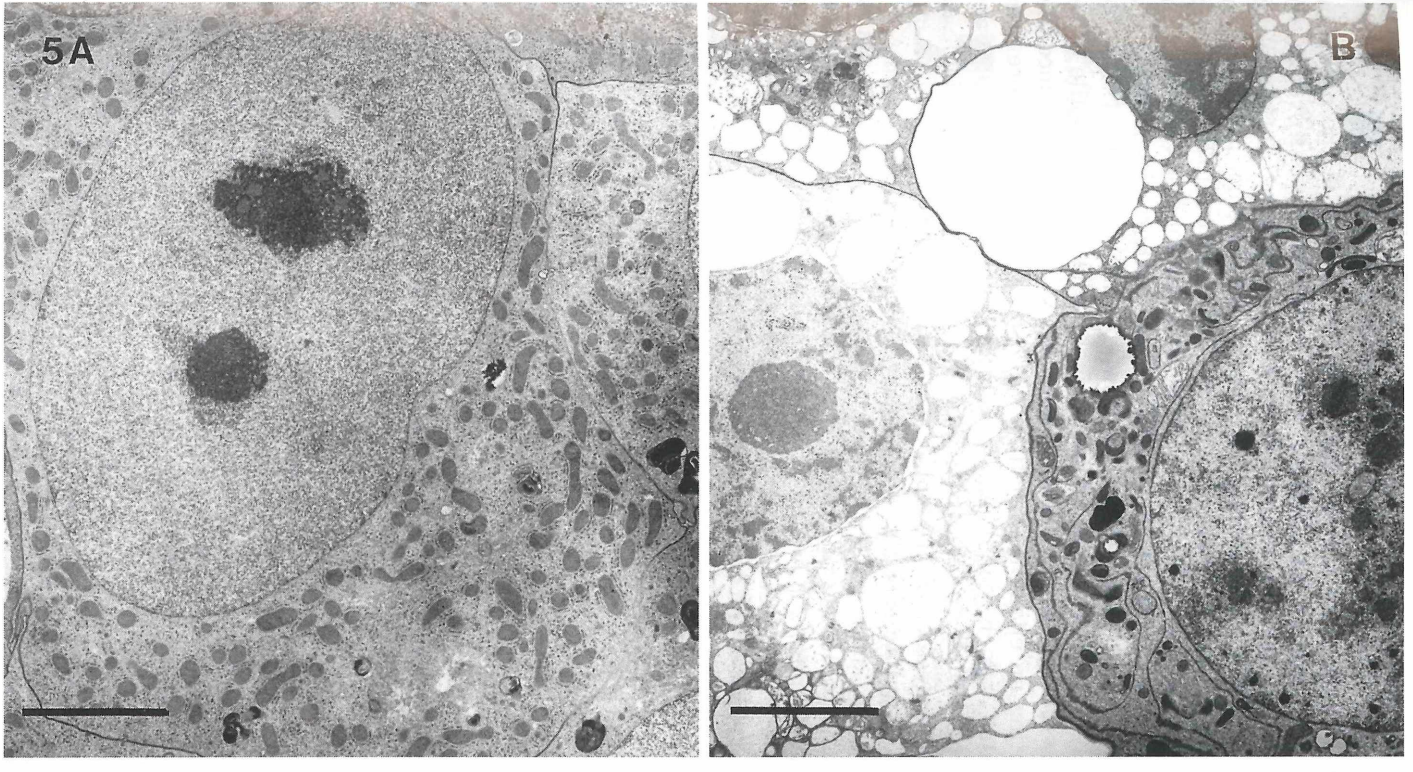

Fig. 5. – TEM-micrographs of OK monolayer sectioned parallel to the growth aerea. A: Control cells. B: Orellanine treated cells with remarkable vacuolization proved to be swollen ER and karyopyknosis. Bar indicates 5 μm (photographs from Burger, 1992).

Chemical studies

Although there are no doubts on the toxicity of the orellanine molecule, data on its chemical structure remain controversial. In contrast to the postulated bipyridilic structure (Antkowiak & Gessner, 1979; Kürnsteiner & Moser, 1981) some investigations proposed a peptide character for the toxin (Testa, 1970; Tebbett & Caddy, 1984; Caddy & al., 1982), but this was conclusively ruled out by Laatsch & Matthies (1991). Other authors favored a new intermediate of hepatic metabolism (Nieminen & al., 1976) and recently the existence of a hypothetical orellanine metabolite of yet unknown origin was postulated (Richard & al., 1991). Intermediates presenting an epoxidic structure with an isooxazolinium core were suggested which could emerge during photodecomposition and covalently bind to numerous proteins (Andary & al., 1986). An epoxide character of orellanine could not be confirmed since X-ray crystal structure excluded a coplanary molecule (Cohen-Addad & al., 1987) again supporting the structure postulated by Antkowiak & Gessner (Kubicki & al., 1990). Earlier investigations described two toxic principles, a slow and a fast acting component (Gamper, 1977; Kürnsteiner & Moser, 1981; Aberham, 1981). The slow acting toxin corresponded to the orellanine as postulated by Antkowiak & Gessner (1979), although it showed a different behaviour concerning its solubility, and led to the assumption that this toxin component is part of a larger molecule (Kürnsteiner, 1978). Studies on the effects of other bipyridiles such as paraquat and diquat on enzyme activities indicated that both substances are acting in a similar manner as orellanine, but a direct comparison revealed the remarkably higher toxicity of orellanine (Gstraunthaler & Prast, 1983; Ruedl & al., 1990). Moreover, electrochemical studies (Cantin & al., 1988) accentuated the dissimilarity of the substances. Paraquat and diquat are considerably easier to reduce, thus the bipyridilic structure is decisive for toxic effects, whereas in case of orellanine different functional groups (–OH, –NO) may play the more important role in its mode of action.

The contradictory findings reported above indicated that other molecules or substances may be involved in the mode of action of the toxin and started a new evaluation of different isolation methods and chemical analyses of the extraction products by ^{1}H-NMR and HPLC (Rohrmoser & al., 1993). All extraction procedures to yield pure orellanine included an acidification step to precipitate the toxin, whereby the native toxin character could be destroyed. On this account crude extract was prepared under buffered conditions to avoid breakdown of the native toxin molecule present within the basidiomata. ^{1}H-NMR data revealed that the native toxin consists of

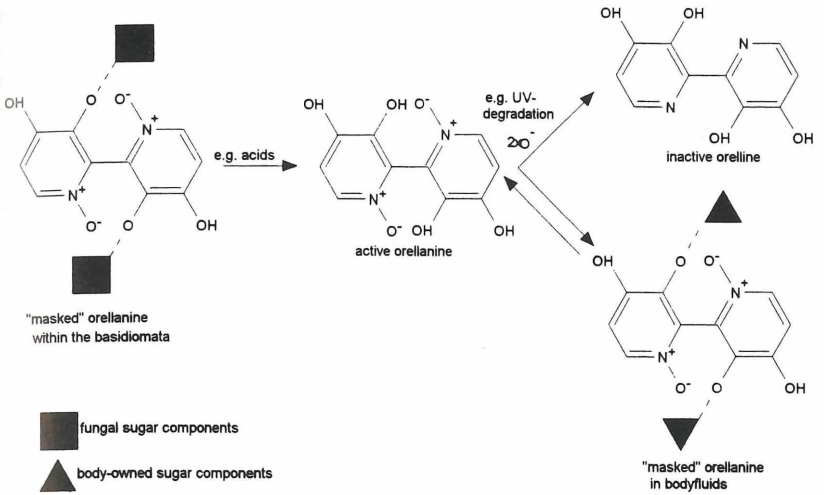

Fig. 6. – Hypothetical model of the mode of action of orellanine.

orellanine and a glycosidic component, whereas no sugar was detectable when the toxin was derived from extraction followed by a crystallisation step using acids. Mixing up pure crystallised orellanine according to Andary & al. (1986) and glucose resulted in a newly formed compound of orellanine and glucose, as shown by the delayed retention time of pure orellanine in comparison to the orellanine glycoside (see also Fig. 3). In addition, this *in vitro* formed molecule was more resistent against acids, because HPLC was performed with 1M H_3PO_4 at pH 1.0.

Conclusions and outlook

During the last decade the orellanine molecule occupied a special position within the bipyridiles with respect to its chemical properties. The definite mode of action remains to be clearified. The kidney is apparently the primary target organ and the liver, although being the most important detoxification organ, seems to be unimpaired. Thus, there is evidence for an intermediate produced during passage through the body and a masked toxin molecule, respectively. The ability of orellanine to form stable compounds with sugars indicates new biochemical properties which might be important in the poisoning process.

The native orellanine within the basidiomata is composed of an orellanine core (active orellanine) and sugar, possibly an aminosugar. This might explain the differences in solubility between the orellanine extracted by Antkowiak & Gessner (1979) and that extracted by

Kürnsteiner (1978). In addition, the orellanine glycoside is very sensitive to acids decomposing or splitting off the linked sugar. Thus, within the gastrointestinal tract the aminosugar could be cleaved by the action of gastric acids to release the active orellanine core. This free orellanine core would now be able to bind sugars as they occur in body fluids and could form more stable and acid insensitive compounds, leading to a new, masked orellanine in the body, which reaches the kidney via bloodstream. The glucosidases abundant in the kidneys could cleave the glycosidic binding, thus giving raise again to an instable core orellanine. This active orellanine, released after cleavage of linked sugars, could free oxygen radicals from the –NO group and cause severe cell injuries (Fig 6). Moreover, the sugars, both within the fungus and in body fluids, might exert a preserving and stabilizing effect on the entire molecule as orellanine seems to be resistant against irridiation with UV-light within the basidiomata. The presence of an orellanine-glycoside in the basidioma may explain the non-toxicity of the orellanine for the fungus itself, which contains the toxin in a concentration of 1-2% of dry weight. Although orellanine-sugar compounds could be found both in fungus and *in vitro*, the biochemical mode of action still remains hypothetical and will have to be elucidated in further investigations.

Additional detailed knowledge is necessary to evaluate the biochemical effects of fungal toxins to take measures suitable to avoid fatal consequences like chronical kidney injury or kidney transplantation. In this connection it should be mentioned that *Amanita smithiana* Smith & Stuntz, a North American species suspected to contain orellanine (Tulloss & Lindgren, 1992), was shown in our laboratory to contain a substance distinct from orellanine which was responsible for toxic effects on cultured cells. AP was not impaired and the toxin had no effect on two (OK, NRK) of the four cell lines tested. Moreover, the toxin had an amino acid character comparable to an allenic norleucine found in related *Amanita* species (Pelizzari & al., 1993).

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References

Aberham, R. (1981). Untersuchungen über Giftstoffe von *C. orellanus*. – Ph. D. thesis, University of Innsbruck.

Andary, C., S. Rapior, A. Fruchier & G. Privat (1986). *Cortinaries* de la section *Orellani*: Photodecompostion et hypothèse de la phototoxicité de l'orellanine. – Cryptogamie Mycol. 7 (3): 189–200.

Antkowiak, W. Z. & W. P. Gessner (1975). Isolation and characteristics of toxic components of *C. orellanus* Fr. – Bull. Acad. Polon. Sci. 23 (9): 729–733.

— & — (1979). The structures of orellanine and orelline. – Tetrahedron Lett. 21: 1931–1934.

— & — (1985). Photodecompostion of orellanine and orellinine, the fungal toxins of *C. orellanus* Fr. and *C. speciosissimus*. – Experientia 41 (6): 769–771.

Burger, A. (1992). Lichtmikroskopische und elektronenoptische Darstellung der morphologischen Veränderungen von Nierenepithelzellkulturen (LLC–PK_1, MDCK, OK, NRK) durch das Pilzgift Orellanin. – Diplomarbeit, University of Innsbruck.

Caddy, B., C. B. M. Kidd, J. Robertson, I. R. Tebbett, W J. Tilstone & R. Watling (1982). *C. speciosissimus* toxins – a preliminary report. – Experientia 38: 1439–1440.

Cantin, D., J. – M. Richard, J. Alary & D. Servé (1988). Electrochemical study of the mushroom toxin orellanine and of related pyridine-1-oxides. I. Reduction. – Electrochem. Acta 8: 1047–1059.

Cohen-Addad, C., J.-M. Richard & J.-C. Guitel (1987). Structure of an orellanine-trifluroacetic complex: evidence of a very short OH–O hydrogen bond. – Acta Crystallograph. C 43: 504–507.

Dehmlow, E. V & H. Schulz (1985). Synthesis of orellanine, the letal poison of toadstool. – Tetrahedron Lett. 26 (40): 4903–4906.

Eder, I. (1992): Wirkung von Orellanin auf Chlorophyll und Photosyntheseaktivität. – Diplomarbeit, University of Innsbruck.

Fischer, H. (1991). In vitro Untersuchungen zu akuter Nierenepithelzellschädigung durch das Pilzgift Orellanin. – Diplomarbeit, University of Innsbruck.

Gamper, R. (1977). Untersuchungen über die Giftstoffe der Pilze *C. orellanus* Fr und *C. speciosissimus*. – Ph. D. thesis, University of Innsbruck.

Grasser, H. P. (1989). Metabolische Wirkung von Orellanin auf kultivierte Nierenepithelzellen (LLC–PK_1 und OK). – Diplomarbeit, University of Innsbruck.

Gruber, I. (1969). Fluoreszierende Stoffe der *Cortinarius*-Untergattung *Leprocybe*. – Z. Pilzkunde 35: 249–261.

Grzymala, S. (1957). Erfahrungen mit *Dermocybe orellana* (Fr) in Polen. B. Massenvergiftungen durch den orangefuchsigen Hautkopf. – Z. Pilzkunde 23: 139–142.

— (1962). L'isolement de l'orellanine poison de *C. orellanus* Fries et l'étude de ses effets anatomo-pathologiques. – Bull. Soc. Mycol. Fr. 78: 394–404.

Gstraunthaler, G. & H. Prast (1983). Studies on the nephrotoxicity of *C. orellanus* Fr.: The effect of dipyridyles on renal epithelial cell cultures. – Sydowia 36: 53–58.

Høiland, K. (1983). Extracts of *C. speciosissimus* affecting the photosynthetic appatatus of *Lemna minor*. – Trans. Br. mycol. Soc. 81: 633–635.

— (1985). Is *C. orellanus* an earlier name for *C. speciosissimus*? – Nord. J. Bot. 5: 489–492.

Kampfer, S. (1993). Wirkung von Orellanin auf Mitochondrien und mitochondriale Enzyme bei der Nierenepithelzellkulturlinie OK (opossum kidney). – Diplomarbeit, University of Innsbruck.

Kubicki, M. T. Borowiak & W. Z. Antkowiak (1990). Crystal structure of orellanine hydrate. – J. Crystallogr. Spectrosc. Res. 21 (4): 401–405.

Kürnsteiner, H. (1978). Untersuchungen über Giftstoffe von *C. orellanus* Fr. – Ph. D. thesis, University of Innsbruck.

— & M. Moser (1981). Isolation of a letal toxin from *C. orellanus* Fr. – Mycopathol. 74: 65–72.

Laatsch, H. & L. Matthies (1991). Fluorescent compounds in *C. speciosissimus*: Investigations for the presence of Cortinarins. – Mycologia 83(4): 492–500.

Meißlitzer, C. (1991). Wirkung des Orellanin auf die Aktivitäten von Nierenepithelenzymen (alkalische Phosphatase, Leucin-Aminopeptidase und -Glutamyltransferase) der Zellkulturlinien LLC-PK1, MDCK, OK und NRK. – Diplomarbeit, University of Innsbruck.

Mellauner, M. M. (1991). Hemmende Wirkungen von Orellanin auf den Myxomyceten *Physarum polycephalum*. – Diplomarbeit, University of Innsbruck.

Michelot, D. & I. Tebbett (1990). Poisoning by members of the genus *Cortinarius* – a review. – Mycol. Res. 94 (3): 289–298.

Moser, M. (1969). Gibt es neben dem orange-fuchsigen Schleierling (*C. orellanus*) weitere giftige Schleierlinge?– Z. Pilzkunde 35: 29–34.

— (1971). Neuere Erkenntnisse über Pilzgifte und Giftpilze. – Z. Pilzkunde 37: 41–56.

Nieminen, L., K. Pyy & Y. Hirsimäki (1976). The effect of furesamid on the renal damage induced by toxic mushroom *C. speciosissimus* in the rat. – Br. J. Exp. Pathol. 57: 400–403.

Pelizzari, V., E. Feifel, M. Rohrmoser, G. Gstraunthaler & M. Moser (1994). Isolation and characterisation of toxic compounds of *Amanita smithiana*. – Mycologia, in press.

Pfaller, W., G. Gstraunthaler, H. Prast, L. Rupp, Ch. Ruedl, S. Michelitsch & M. Moser (1989). Effects of the fungal toxin orellanine on the renal epithelium. – In: Nephrotoxicity: Mechanisms, Early Diagnosis and Therapeutic Management (Bach, P. H., N. J. Gregg, M. F Wilks & Delacruz, eds.). Marcel Dekker, New York 59–66.

Pöder, R. & M. Moser (1989). Eine einfache empfindliche Methode zum makrochemischen Nachweis von Orellanin. – Mycol. Helvet. 3 (3): 283–290.

— & E. Pipitz (1986). Cos'è il *Cortinarius brunneofulvus* Fr. ss. Bresadola? – Boll. Gruppo Micol. Bresadola 29 (5–6): 221–228.

Prast, H. (1982). Nephrotoxizität von *C. orellanus*. – Ph. D. thesis, University of Innsbruck.

— & W Pfaller (1988). Toxic properties of the mushroom *C. orellanus* Fr.: II. Impairment of renal function in rats. – Toxicol. 62: 89–96.

— E. R. Werner, W Pfaller & M. Moser (1988). Toxic properties of the mushroom *C. orellanus*. I.Chemical characterisation of the main toxin of *C. orellanus* (Fries) and *C. speciosissimus* (Kühn. & Romagn.) and acute toxicity in mice. – Arch. Toxicol. 62: 81–88.

Rapior, S., C. Andary & A. Fruchier (1986). Orellanine, toxine „phenolique" isolée de *C. orellanus* Fr. – Bull. Liaison – Groupe Polyphenols 13: 608–610.

— C. Andary & G. Privat (1988). Chemotaxonomic study of orellanine in species of *Cortinarius* and *Dermocybe*. – Mycologia 80: 741–747

Richard, J.–M., E. E. Creppy, J.–L. Benoit-Guyod & G. Dirkheimer (1991). Orellanine inhibits protein synthesis in Madin-Darby canine kidney cells, in rat liver mitochondria and in vitro: indication for activation prior to in vitro inhibition. – Toxicol. 67: 53–62.

Rohrmoser, M. M., E. Feifel, G. Laus, Ch. Scheffknecht, G. Gstraunthaler & M. Moser (1993). Strong affinity of orellanine, a fungal nephrotoxin to sugars. – Arch. Toxicol, subm.

Ruedl, Ch., G. Gstraunthaler & M. Moser (1989). Differential inhibitory action of the fungal toxin orellanine on alkaline phosphatase isoenzymes. – Biochim. Biophys. Acta 991: 280–283.

— M. Moser & G. Gstraunthaler (1990). The toxic action of orellanine and other dipyridyles on different epithelial cell cultures (LLC-PK_1, Caco-2 and OK). – Mycol. Helvet. 4: 99–109.

Rupp, L. (1989). Morphologische Veränderungen von Nierenepithelzellkulturen durch das Pilzgift Orellanin. – Diplomarbeit, University of Innsbruck.

Schumacher, T. & K. Høiland (1983). Mushroom poisoning caused by species of the genus *Cortinarius* Fries. – Arch. Toxicol. 53: 87–106.

Sigl-Michelitsch, S. (1990). Untersuchungen zu Hemmechanismen für Orellanin. – Diplomarbeit, University of Innsbruck.

Tebbett, I. R. & B. Caddy (1984). Mushroom toxins of the genus *Cortinarius*. – Experientia 40: 441–446.

Teissl, I. (1988). Hemmende Wirkung von Orellanin auf Bakterien. – Diplomarbeit, University of Innsbruck.

Testa, E. (1970). Indagini sulla tossicità dei funghi del genere *Cortinarius*. Nota 1: *C. orellanus*. – Rassegna micologica ticinese no. 2 (Soc. micol. Carbo Benzoni, Chiasso).

Tulloss, R. E. & J. E. Lindgren (1992). *Amanita smithiana* taxonomy, distribution and poisonings. – Mycotaxon 45: 373–387.

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