obtain fluorescence spectra of the algal products. Fractions were collected in the time intervals which corresponded to the elution times for the standards. Fluorescence spectra were recorded on collected fractions with an Aminco-Bowman spectrophotofluorometer that corrects for instrumentation artifacts including lamp output, photomultiplier response and monochromator grating. Collected fractions which corresponded to peak retention times of the *trans*-4,5- and *trans*-7,8-dihydrodiol standards had no detectable fluorescence. Fractions corresponding to peak retention times of the *cis*-4,5- and *cis*-7,8-dihydrodiol standards had fluorescence spectra with identical excitation and emission maxima to the synthetic 4,5- and 7,8-dihydrodiol standards, respectively (fig. 2). It should be noted that while fluorescence spectroscopy can distinguish positional BaP isomers, this method cannot distinguish between the *cis* and *trans* isomers of these dihydrodiols. However, the combination of cochromatography and fluorescence spectroscopy has allowed the identification of these metabolites as *cis*-dihydrodiols.

The metabolism of BaP to *cis*-dihydrodiols by a green alga suggests that this eukaryotic microorganism metabolizes PAHs *via* a dioxygenase system similar to that observed in prokaryotes. However, formation of *cis*-dihydrodiols by *cis*-hydration of epoxides is a feasible alternative. Such a mechanism has been reported for hydration of an aliphatic epoxide in a fungus, *Fusarium solani pisi*[17]. The stereospecificity reported here for a green alga is significant from an evolutionary standpoint in that the green algae are generally regarded as being the progenitors of the higher plants. Studies are ongoing in our laboratory to determine whether the higher orders of green plants metabolize PAHs to *cis*-dihydrodiols, as does the green alga *S. capricornutum*, or whether they have acquired a monooxygenase enzyme system similar to that of fungi and mammals.

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Photodecomposition of orellanine and orellinine, the fungal toxins of *Cortinarius orellanus* Fries and *Cortinarius speciossimus*

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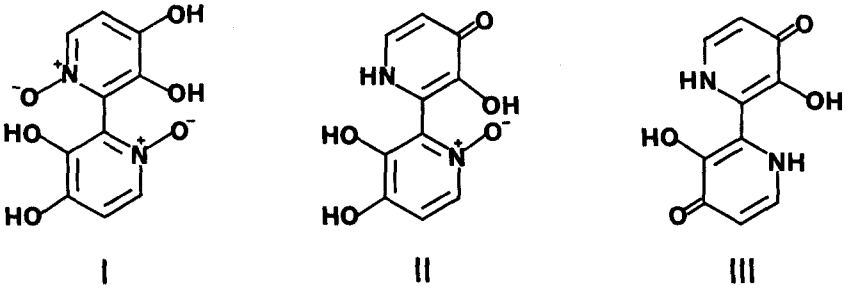
Summary. The photosensitivity of orellanine, the main toxin of *Cortinarius orellanus* Fries mushrooms, and its transformation to orelline via orellinine is discussed. All three substances were found in methanolic extracts of *Cortinarius orellanus* and *Cortinarius speciossimus* mushrooms. The problem of homogeneity of orellanine is also discussed.
Key words. Orellanine; orellinine; orelline; *Cortinarius orellanus, Cortinarius speciossimus.*

Research on the toxins present in *Cortinarius orellanus* Fries was initiated by Grzymala[1], who isolated a crystalline, colorless substance, and called it orellanine. Orellanine had toxic effects in humans, and also in cats, mice and guinea pigs, and caused histopathological changes in kidneys, liver and spleen identical with those caused by the feeding of intact fungi or their methanolic extract. Grzymala found that orellanine underwent rapid explosive decomposition when heated to about 270 °C, yielding a yellow, sublimable product which was nontoxic. Years later, research on toxins of *Cortinarius orellanus* was undertaken by other authors[2-10].

Paper chromatography of the ethanol extract of the mushrooms, performed by Gruber[2], showed the presence of some undefined, fluorescing substances. Testa[3], using preparative TLC on silica gel and a weakly polar developing system (cyclohexane-ethyl acetate 3:1), isolated four substances from the methanol extract of the fungus, and named them: grzymaline, benzonine 'a' and 'b', and cortinarine, respectively. Testa suggested that these isolated substances were the components of Grzymala's orellanine, which, according to him, was a heterogenous material. Gramper[4] and Moser[5,6] came to similar conclusions. Moser and Kürnsteiner[6], using gel filtration on Sephadex LH20, isolated a substance which showed great similarity in spectral data, identical biological activity, and sensitivity to UV radiation, with the orellanine isolated by Grzymala and by us. The substance isolated by these authors differed from orellanine in its solubility in water; orellanine was soluble only in aqueous alkali, and not in neutral and weakly acidic solutions. Besides this 'slow-action toxin', the presence of another toxin in the methanol extract of the fungus was discovered[6]. The second toxin, in contrast to the first one, showed rapid (observable) onset of biological activity. Using the improved Grzymala's procedure we isolated[7,9] from *Cortinarius orellanus* mushrooms the toxic substance, which showed after purification chromatographic homogeneity and



I II III

Figure 1. Structures of orellanine (I), orellinine (II), and orelline (III).

which was identical in physicochemical properties and biological activity with Grzymala's orellanine. On the basis of a thorough structure examination we proposed[8,9] for this substance the structure of 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide (I).

In this paper, we now show that the N-oxide and phenolic functions in orellanine are responsible for two types of transformations. A gradual change of color from cream to pink of lyophilized (from diluted ammonia solution) samples of orellanine, when exposed to atmospheric oxygen[7] is caused by oxidation of the phenolic groups. This transformation caused changes in its chromatographic and spectroscopic properties[7,9]. Thin-layer chromatograms, stored in the open air, were seen to have a pink color where orellanine was present.

The other type of chemical transformation of orellanine consists of a two-stage successive loss of both oxygen atoms in the N-oxide functions, leading to the formation of two new compounds, which we named orellinine (II) and orelline (III), respectively. The latter substance, orelline, is the sublimable, nontoxic product of the thermal pyrolysis of orellanine. Orellinine was found to be a toxic substance, with toxicity similar to orellanine[9]. UV radiation, similarly to thermal decomposition, can cause the transformation of orellanine into orelline via orellinine. The sensitivity of orellanine to UV radiation explains our observation[7,9], and the observation of Moser and Kürnsteiner[6], that the orellanine spot on TLC chromatograms shows no fluorescence, but after exposure to UV light for a few sec it exhibits the light blue fluorescence characteristic of orelline. A hypothesis that orellanine decomposes under UV light yielding orellinine and orelline was supported by the following experiment. A mixture of orellanine, orellinine and orelline was resolved on a cellulose plate by a mixture of isopropanol-conc. HCl-water (85:22:18) as three spots of R_f 0.78 (navy-blue to UV light: orellanine), R_f 0.66 (dark-blue fluorescence: orellinine), and R_f 0.48 (light-blue fluorescence: orelline)[10]. The dried chromatogram was exposed to UV radiation, $\lambda = 254$ nm, for several min, and then developed with the same solvent system in a direction perpendicular to the first one. The upper spot once more split into three spots analogous to those obtained as the result of the first development, and the middle spot into two, whereas the lower spot remained unchanged.

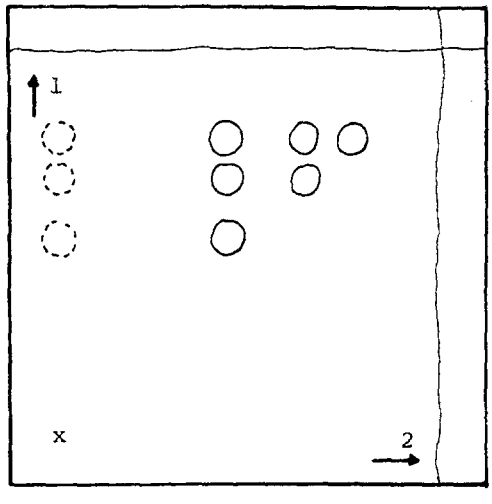


Figure 2. Photodecomposition of orellanine and orellinine under influence of short-wave UV light. The dotted line indicates the positions of UV light absorption after the first development of the chromatogram, the solid line after the second development. x = starting point; 1 = direction of the first development; 2 = direction of the second development.

In a control experiment, the develop chromatogram of these three compounds was kept in darkness accessible to air for several h, and then developed in a perpendicular direction giving an unchanged pattern, i.e., each spot obtained after the first development gave only one analogous spot after the second development.

The above observations enabled us to present the following equation: orellanine $\xrightarrow{x}$ orellinine $\xrightarrow{x}$ orelline where x may denote: 1) heating above 180 °C, 2) H_2 in the presence of Pt, 3) UV radiation.

All three substances gave a positive color-reaction with $FeCl_3$ solution (orellanine: dark-violet, orellinine: violet-red, orelline: blue), which indicated the presence of phenolic groups in the molecule. The substances reacted positively also with $FeSO_4$ solution (orellanine, and orellinine: dark brick-red coloring, orelline: violet-red color), which confirms the presence of the 2,2'-bipyridyl skeleton.

We were also able to show the presence of the three substances in a cold methanol extract of *Cortinarius orellanus* Fries. Orellinine and orelline, however, occurred in rather small quantity compared to that of orellanine.

Orellinine and orelline as well as some unidentified decomposition products may appear in samples of orellanine stored for a long time or insufficiently purified, which could give rise to the suggestion that Grzymala's orellanine is not a homogeneous substance, but a mixture containing numerous toxic and nontoxic components. In our hands, however, the original sample of Grzymala's orellanine as well as the substance isolated by us showed homogeneity in their chromatographic behavior. Both substances showed identical physiochemical properties (solubility, TLC-R_f values, MS, UV and IR spectra) as well as identical toxicity in mice, and both of them caused identical histopathological changes of mouse tissue[9]. The homogeneity of the substance isolated by us is supported by elemental analysis, and spectroscopic data (MS, ^{13}C-NMR, and ^{1}H-NMR) are compatible with the molecular formula $C_{10}H_8O_6N_2$[8,9].

It appears to us that orellanine and the 'slow-acting' toxin isolated by Moser and Kürnsteiner might in fact be the same toxin that is responsible for the toxic properties of *Cortinarius orellanus* Fries. This is based on the similar and, in many cases, identical properties of these substances (with the exception of the increased solubility in water of the product isolated by Moser and Kürnsteiner; however, we cannot exclude the possibility that they could have been dealing with an impure sample of orellanine or its salt).

We have also found orellanine, orellinine and orelline in methanol extracts of another species – the *Cortinarius speciossimus* mushroom. Using the procedure described for orellanine we isolated from this fungus a substance with chromatographic and spectroscopic properties were identical with those of orellanine from *Cortinarius orellanus*.

Recently, Caddy et al. have reported[11,12] the isolation from *Cortinarius speciossimus* of a fluorescent toxin cortinarine A and its derivative, probably nontoxic substance, cortinarine C for which they suggested a polypeptide structure. It is interesting that cortinarine A, like orellanine, underwent decomposition at 270 °C, yielding a yellow product.

Although the total synthesis of orellanine has not been achieved, some model compounds prepared in our laboratory with structural features resembling those of orellanine and orelline (4,4′-dihydroxy-2,2′-bipyridyl and its bis N-oxide, 3,3′-dihydroxy-2,2′-bipyridyl, 3,4-dihydroxypyridine and 2-(2′-hydroxyphenyl)-pyridine-N-oxide) showed many similarities in chemical and spectroscopic properties[9,13] to the natural products.

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The role of the substantia nigra on the rage reaction elicited by hypothalamic stimulation, in the cat[1]

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Summary. The effects of substantia nigra stimulation on the rage reaction evoked by hypothalamic activation were studied. The reference value of the rage reaction was the latency of the hissing, which was constant in all animals when hypothalamic stimulation was performed with the same parameters. Simultaneous activation of substantia nigra and hypothalamus determined a significant decrease in hissing latency. The influence of the substantia nigra on the affective components of the aggressive behavior is underlined.
Key words. Striatal system; aggressive behavior; ventromedial hypothalamus; rage reaction; hissing.

Electrical stimulation of specific points within the hypothalamus of the cat may induce two forms of aggressive behavior: a quiet biting, predatory type of attack, which is preferentially directed at a rat, with no signs of autonomic participation[3] and a typical rage reaction with mydriasis, piloerection, snarling and showing of the teeth, which culminates in hissing[4–6]. It has been shown that the striatal system participates in the control of aggressive behavior: On the one hand, caudate nucleus stimulation inhibits both rage and attack display[7,8]; on the other hand, lesions of the striatal pathways to and from the substantia nigra at the level of the medial forebrain bundle result in a contralateral loss of patterned reflexes, which mediate attack behavior in the cat[9]. In this paper the effects of substantia nigra stimulation on the rage reaction elicited by hypothalamic activation will be considered.

Material and methods. The experiments were performed on five cats with chronically implanted electrodes. After general anesthesia (pentobarbital 50 mg/kg i.p.) the animals were placed on a stereotaxic apparatus (DKI 1404) and pairs of stainless steel wires (1–1.5 interelectrode distance, 25–50 μm tip) were implanted bilaterally in the ventromedial hypothalamic nucleus (A 9–12, L 1–2, H 4–5) and in the substantia nigra (pars compacta) (A 3–4, L 4–5, H 5–6)[10]. The animals, 6–8 days after surgery were subjected to experimental sessions in a behavioral cage (70 × 70 × 100 cm, inside dimensions). Both hypothalamus and substantia nigra were stimulated unilaterally and the side of the hypothalamic stimulation was chosen in relation to the lower threshold for the appearance of the behavioral response. The hypothalamus was stimulated with trains varying between 15–20 sec, 30–60 c/sec, 0.1–1 msec and with progressively higher current values (0.2–0.6 mA) until the complete affective display appeared and culminated in the hissing. The interval between the beginning of the hypothalamic stimu-

Effects of substantia nigra stimulation on hissing latency

Cat No.	Control sec[a]	Substantia nigra sec[a]	%[b]	t
1	8.16 ± 0.93	5.87 ± 0.57	28.06	3.17*
2	11.05 ± 1.29	6.92 ± 0.45	37.37	4.36*
3	10.07 ± 1.36	6.47 ± 0.33	35.75	4.04*
4	9.35 ± 0.92	4.55 ± 0.20	51.33	7.16**
5	11.08 ± 1.01	5.58 ± 0.30	49.64	6.29**

[a] Average ± SD latencies (n = 8) of the hissing in single (hypothalamic alone) and dual (hypothalamic and substantia nigra) stimulations.
[b] Percentage of nigral facilitation.
* $p \leq 0.01$; ** $p \leq 0.001$, Student's t-test.