stated in the literature[2] and to identify retinol in the extracts of corpora lutea.

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Amaninamide, a new toxin of *Amanita virosa* mushrooms[1]

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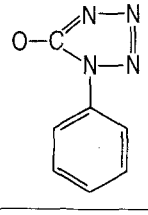
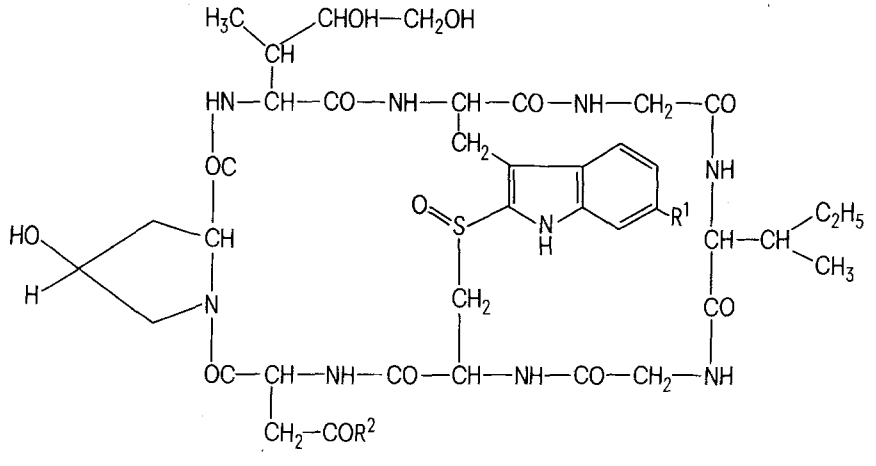
Summary. Amaninamide, a toxin closely related to the family of amatoxins, was found exclusively in *Amanita virosa* mushrooms. It differs from the well known toxin α-amanitin in that it lacks the 6′-hydroxyl group of the tryptophan unit, and from the toxin amanin found in *Amanita phalloides* by the presence of a carboxamide group instead of a carboxylic acid group.

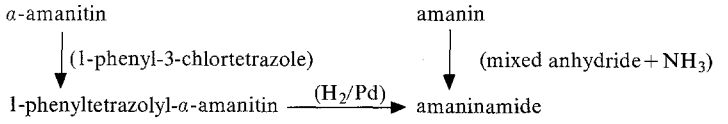
When methanolic extracts of *Amanita virosa* (FR.) mushrooms were analysed by chromatographic procedures[2], some specimens contained a toxin absent in analogous separation procedures of *A. phalloides* mushrooms. This toxin shows the typical UV-absorption spectrum of α-indolylsulfoxides, as known from the semisynthetic phalloidinsulfoxides[3] as well as from the naturally occurring amanin[4]. However, in chromatographic behaviour it proved dissimilar to either of these compounds. We succeeded in identifying the toxin as amaninamide, the amide of the naturally occurring acidic peptide amanin. Comparison of the toxin with authentic semisynthetic samples of amaninamide provided a direct structural proof.

Materials and methods. Amanita virosa mushrooms were obtained either from Austria (a generous gift of M. Moser, Innsbruck) or from North America (collected by B. Witkop and one of us, Th.W.). Extraction of the mushrooms, column chromatography and further purification on thin layers were as described previously[5]. Pure amaninamide was obtained by a final column chromatography on LH 20/0.004 M NH_4OH. Analysis of amino acids, including the lactone of hydroxylated isoleucine, was performed according to reference[5]. For TLC on silica (Merck, 60F-254) 3 different solvents were used.: I: chloroform-methanol-water (65:25:4, vol/vol); II: sec.butanol-ethylacetate-water (14:12:5, vol/vol); III: sec.butanol-ammonia (2N) (100:44, vol/vol). High voltage paper electrophoresis, according to Wieland and Pfleiderer, was performed using a buffer pH 6.5 containing acetic acid-pyridin-water (2:20:178, vol/vol). Preparation of amaninamide was either from α-amanitin as previously described[6] or from amanin as follows: 9 mg amanin (ca. 10 μmoles) were dissolved in 5 ml of dry dimethylformamide. After addition of 10 μmoles N-methylmorpholine, the mixture was cooled to −15 °C and 10 μmoles isobutylchloroformate were added. After 20 min,

	R^1	R^2
Amaninamide	H	NH_2
Amanin	H	OH
α-Amanitin	OH	NH_2
O-(1-phenyltetrazolyl)-α-Amanitin		NH_2







Scheme 1.

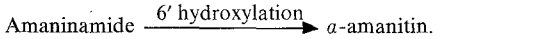
ammonia gas was passed in and allowed to react over 2 h. After evaporation, the residue was applied to a small DEAE Sephadex column (OH^- form) and developed with water; 5 mg (56% yield) of amaninamide was eluted as a single component, while the starting material and byproducts remained bound.

Results and discussion. The new toxin was found in extracts of *A. virosa* both from Europe and Northern America in amounts of 0.5–0.9 mg/g dry weight. During the adsorption chromatography on Sephadex LH 20 the toxin eluted midway between the newly detected group of virotoxins[2] and the group of neutral phallotoxins. In many of its properties, the toxin is indistinguishable from amanin; this accounts for the typical UV-spectrum of α-indolylsulfoxides as well as its reaction with cinnamic aldehyde/hydrochloric acid, which yields a characteristic blue-gray color. Also, the amino acid analysis displayed the pattern typical for amanin including aspartic acid, glycine, hydroxyproline and isoleucine in a ratio, 1:2:1:1. In addition, the lactone formed from the hydroxylated amino acid was that of γ,δ-dihydroxyisoleucine as is found in amanin. However, on high voltage paper electrophoresis, the toxin proved to be dissimilar to amanin, in that it was a neutral cyclic peptide rather than an acidic one.

Most of the amatoxins thus far isolated from *A. phalloides*, and whose structures have been elucidated by Th. Wieland and coworkers[7], exist in both acidic and neutral forms. In all cases, this is the result of the substitution of asparagine for aspartic acid. The pairs of neutral and acidic amatoxins include β-amanitin and α-amanitin, ε-amanitin and γ-amanitin, amanullin and amanullinic acid. Correspondingly, we were compelled to expect the new toxin from *A. virosa* to be amaninamide, the partner of the acidic toxin amanin. Amaninamide has not yet been detected in *A. phalloides*.

The simplest way to confirm the structure was by semisynthesis of amaninamide. This has recently been achieved by Buku et al.[6] by hydrogenation of 1-phenyltetrazolylether (formula) of α-amanitin. We have now also prepared amaninamide via amanin by reaction of amanin-mixed carbonic anhydride with ammonia.

Both preparations of amaninamide yielded identical products, which in turn proved to be identical with the newly isolated unknown toxin. This was confirmed by silica TLC, using different solvent mixtures: (R_f = 0.24 in I; R_f = 0.24 in II; R_f = 0.32 in III), as well as by the identity of the UV-spectra, electrophoretic behaviour and amino acid analysis. The *A. virosa* sample from Europe contained a considerable amount of α-amanitin in addition to amaninamide. This was in contrast to the sample from North America, which contained amaninamide exclusively. β-Amanitin was absent in both samples. Therefore, it seems reasonable to suggest that in *A. virosa* amaninamide is the precursor of α-amanitin, the hydroxylation reaction of the indole ring being incomplete in the case of the European sample and totally absent in the case of the North American sample.



Scheme 2.

To some extent this is supported by the results of Yocum and Simons[8], who recently reported the analysis of various *Amanita* species of North America, including 4 samples of *A. virosa* from different areas in the U.S. These authors found α-amanitin to be present in 2 samples while absent in the other 2. However, in the latter 2 samples amanin was detected. Their identification of amanin was by UV-spectrum only and, consequently, had to be a tentative one, because an authentic sample of amanin was not available. Since amanin has been detected in none of our samples of *A. virosa*[2,9] we believe that the toxin found by Yocum and Simons was, in fact, the new toxin amaninamide.

1 Communication No. 57 of the series: Components of the green deathcap toadstool Amanita phalloides. No. 56: E. Munekata, H. Faulstich and Th. Wieland, Liebigs Ann. Chem., in press.
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Diurnal fluctuation in the rate of synthesis of a specific protein fraction in the rat brain

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Summary. A protein fraction has been identified in microsomes prepared from the rat hypothalamus whose rate of synthesis fluctuates diurnally in ovariectomized animals.

The mammalian brain controls many functions that follow tightly controlled circadian rhythms. The suprachiasmatic nucleus, a small area of the hypothalamus positioned just above the optic chiasma, has recently been shown to play an important role in generating such rhythms. Rats, in which this nucleus has been surgically destroyed, show randomized drinking and locomotor activity[1], loss of adrenal and pineal rhythmicity[2,3] and disrupted reproductive cyclicity[4]. It has also been demonstrated that the rate of uptake of [^{14}C] deoxyglucose (a measure of functional activity) into the suprachiasmatic nucleus fluctuates diurnally, although it was not reported whether this fluctuation occurred only in this part of the hypothalamus[5].

The findings presented here were made during the course