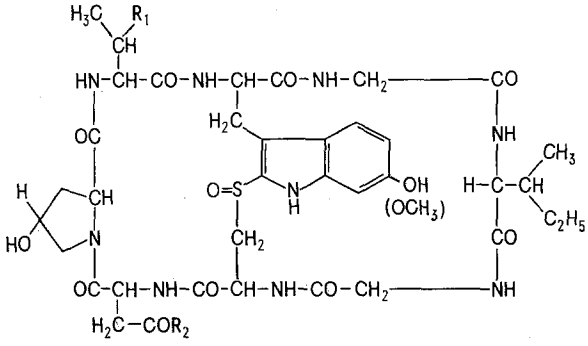
## Conformation and Toxicity of Amanitins*

Recently it was stated that only one conformation of the phallotoxins is responsible for the toxic action of these bicyclic peptides. Most of the chemically modified analogs were less toxic or were nontoxic, due to changes in conformation[1,2]. We investigated the conformation of different amatoxins and some of their derivatives[3] by means of circular dichroism (CD) and optical rotatory dispersion (ORD).

*Methods and materials.* UV-absorption spectra were measured with a spectrophotometer SP 800 (Leitz, Wetzlar), CD-spectra with a Dichrograph II (Roussel-Jouan) equipped with a Xenon lamp. CD-spectra in dimethylsulfoxide were taken in a 0.001 cm cuvette. ORD measurements were performed with a REPM 12 (Zeiss, Oberkochen).

Formula of amatoxins



| | | |
|---|---|---|
| α-Amanitin or Methyl-α-amanitin (I): | $R_1 = CH(OH)-CH_2OH$ | $R_2 = NH_2$ |
| Methyl-α-aldoamanitin (II): | $R_1 = CH=O$ | $R_2 = NH_2$ |
| β-Amanitin: | $R_1 = CH(OH)-CH_2OH$ | $R_2 = OH$ |
| γ-Amanitin or Methyl-γ-amanitin: | $R_1 = CH(OH)-CH_3$ | $R_2 = NH_2$ |
| γ-Desmethyl-methyl-γ-amanitin (III): | $R_1 = CH_2OH$ | $R_2 = NH_2$ |
| Amanullin: | $R_1 = CH_2-CH_3$ | $R_2 = NH_2$ |

*Results and discussion.* In water all biologically active amatoxins, such as α-, β- and γ-amanitin, exhibited almost identical CD-spectra (Figure 1a). At least 3 positive Cotton effects, at 305 nm, 285 nm and 255 nm, and 1 negative Cotton effect at about 232 nm originate from the aromatic part of the molecule, the 2-sulfoxido-6-hydroxyindole moiety. A strongly negative Cotton effect at 220 nm and another positive one at 205 nm are attributed to amide chromophores. That this is correct is shown by the fact that there is no change in the wavelengths for these effects when the CD-spectrum is measured at pH 11 (Figure 1b). All Cotton effects caused by the aromatic part change in alkaline solution due to the formation of the phenolate ion, as can also be observed in the UV-absorption spectrum (Figure 1b'). Amatoxins at pH 11 have 5 Cotton effects for the aromatic part, 3 positive ones at 340 nm, 315 nm and 285 nm, a negative one at 260 nm, and another strong positive one at 237 nm (Figure 1b). In alkaline solution generally, as compared to neutral solution, the dichroism of amanitin is much reduced.

The conformation of the amatoxins cannot be greatly changed in organic solvents such as methanol or acetonitrile (Figure 2b), because only small spectral changes with respect to aqueous solution were observed (Figure 2a). In dimethylsulfoxide, however, the negative Cotton effect of the indole part at 232 nm becomes positive, and some changes are also produced by this solvent in the near UV-range (Figure 2c). The optical density of this solvent did not allow investigation of the 220 nm Cotton effect of amide bonds. This dichroic absorption band, however, appears to be changed also, because the ellipticity at 225 nm, the shortest wavelength accessible, is still zero. So we assume that there are also conformation changes of the peptide backbone in this solvent.

* Part 43 of the series: Components of the green deathcap toadstool *Amanita phalloides*. Part 42: F. FAHRENHOLZ, H. FAULSTICH and TH. WIELAND, Liebigs Annln Chem. *743*, 83 (1971).

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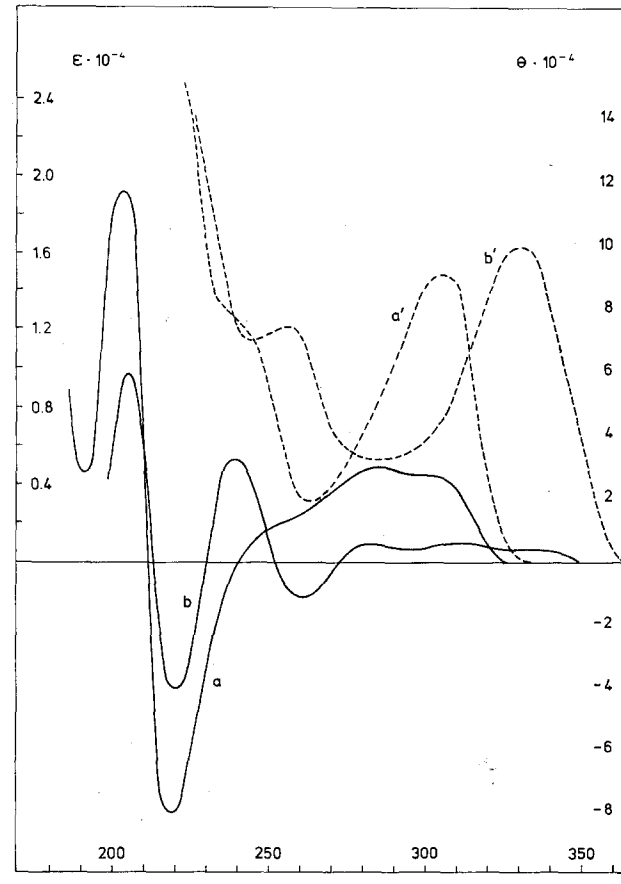
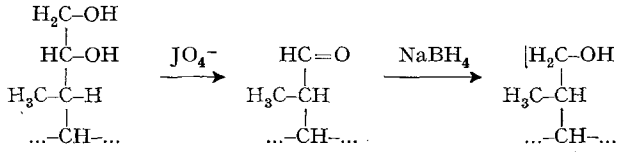



Fig. 1. CD- and UV-spectra of α- (and β-, γ-) amanitin in water at pH 7 (a, a') and at pH 11 (b, b').

In connection with this study, it was interesting to investigate whether the lack of toxicity of methyl-α-aldo-amanitin[4] and of naturally occuring amanullin[5] is due to conformational changes of these 2 compounds.

As can be seen from the CD-spectrum of methyl-α-amanitin (I) (Figure 3a), methylation of the phenolic 6-hydroxy group causes only a slight spectral change at about 240 nm, similar to that induced by organic solvents (Figure 2b). This result was expected, because the *O*-methylether (I) exhibits the same toxicity as does α-amanitin. Nontoxic methyl-α-aldoamanitin (II), however, lacks the 220 nm negative Cotton effect and shows higher ellipticity at 205 nm (Figure 3b). We conclude that the aldehyde group in the γ-position causes a conformational change resulting in the nontoxicity of the peptide. The structural change may be explained by the proton-acceptor properties of the carbonyl group, which perhaps bring about an intramolecular hydrogen bond in competition with another one, which normally stabilizes the toxic conformation. The resulting spectral effect observed is similar to that of α-amanitin in dimethyl-sulfoxide (Figure 2c). As this solvent is also a very good acceptor for protons, the mechanism of the conformational changes may be similar in both cases.

By treatment with sodium borohydride, WIELAND and FAHRMEIR[4] reduced the carbonyl group of the non-toxic aldehyde (II) to a primary hydroxyl group:




Side chain in I Side chain in II Side chain in III

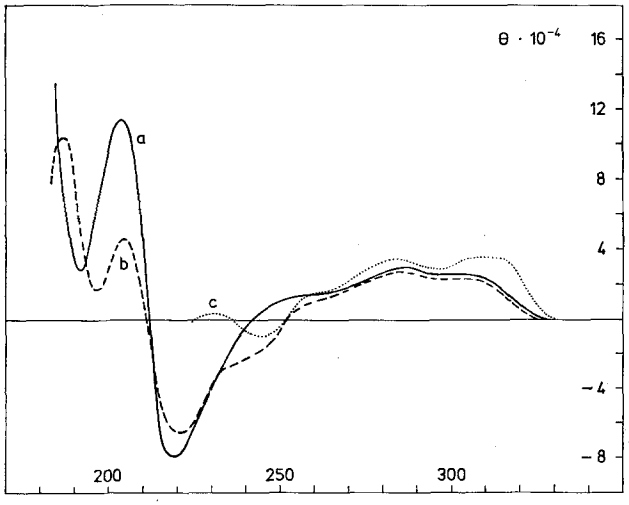



Fig. 2. CD-spectra of α-amanitin in water (a), acetonitrile (b) and dimethylsulfoxide (c).

The resulting compound, γ-desmethyl-methyl-γ-amanitin (III) was found to be toxic, though less so than I. Also, the CD-spectrum of III is almost identical to the CD-spectrum of toxin I. This means that on reduction of the carbonyl group the toxic conformation is restored, but that the decrease of toxicity of III is not reflected by the CD curve.

Moreover, the CD-spectrum of the non-toxic compound amanullin, in which the hydroxylated isoleucine of the toxin (I) is substituted by isoleucine, is identical with that of compound I (Figure 3c). Therefore we must conclude that neither the lack of the γ-hydroxylgroup in amanullin nor the lack of the methyl- or hydroxymethyl group in III have any influence on the conformation of these peptides. So, other factors than conformation must be responsible for the decrease or the lack of toxicity in compound III and amanullin respectively.

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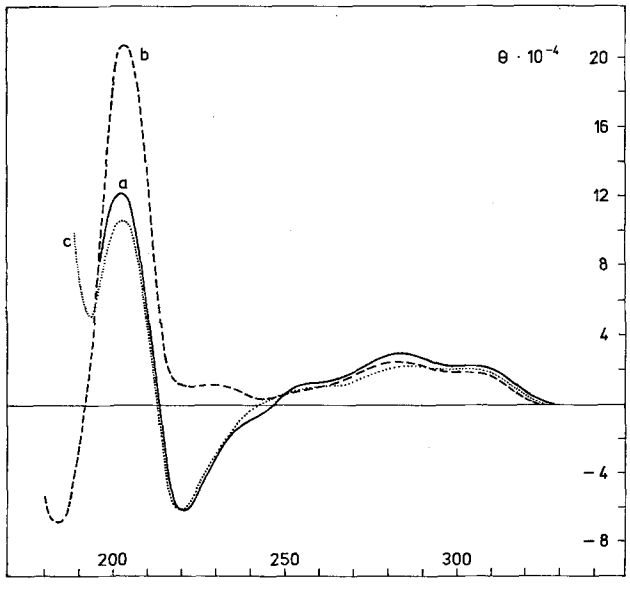


Fig. 3. CD-spectra of methyl-α-amanitin (a), methyl-aldo-α-amanitin (b) and amanuliln (c) in water.

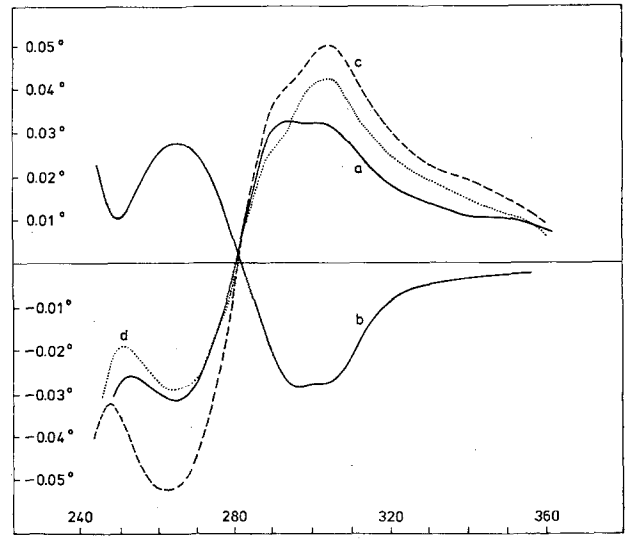


Fig. 4. ORD curves of the 2 diastereomeric sulfoxides of phalloidin (a, b) of amanin (c) and of acetylated γ-amanitin (d); $1.35 \times 10^{-5} M$ solutions in water (a, b, c) or methanol (d), (1 cm cuvette).

We also succeeded in coordinating by chir-optical methods the chiral sulfoxide centre of amatoxins to the sulfoxides prepared by oxydation of phalloidin. The ORD curves of the 2 diastereomeric phalloidin sulfoxides are mirror images[6] (Figure 4a and b). This confirms the observations of HENSON and MISLOW[7], who found that the sign of the ORD curves of sulfoxides is determined predominantly by the chirality at the sulfur atom. Only the phalloidin sulfoxide, having positive Cotton effects in the region of 290–360 nm (Figure 4a) possess the toxicity of phalloidin[8].

Amanin is an amatoxin without a phenolic hydroxyl group and therefore possesses the same chromophoric system as the sulfoxides of phalloidin[8]. It has an ORD curve (Figure 4c) similar to the toxic phalloidin-sulfoxide. Comparison of amanitin sulfoxide was achieved by acetylation of the phenolic group[9] in position 6, which shifts the absorption of amanitins 10 nm to shorter wavelengths, so that it corresponds to those of amanin and phalloidin sulfoxides. The ORD-curve of *O*-acetyl-$\gamma$-amanitin (Figure 4d) resembles also that of the toxic phalloidin sulfoxide. From this it must be concluded that the configuration of the sulfoxides in amanin and in the amanitins is identical with that in the toxic phalloidin sulfoxide. The absolute configuration, however, of this centre of chirality still remains to be elucidated.

*Zusammenfassung.* Die Cottoneffekte der Amanitine konnten dem Indolteil bzw. den Amidgruppen des Molekühls zugeordnet werden. Das untoxische Amanullin besitzt die gleiche Konformation wie die toxischen Peptide, das untoxische Aldoamanitin dagegen eine andere. Die absolute Konfiguration der Sulfoxidgruppe der Amatoxine ist identisch mit der des toxischen Phalloidinsulfoxids.

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[10] Acknowledgment. The authors wish to express their gratitude to Dr. A. BUKU for preparation of methyl-α-aldoamanitin.

## Acquisition of an Embryonal Biochemical Feature in the Rat Liver after Portacaval Shunt

The end-to-side portacaval shunt (PCS) leads to a marked alteration in quantity and composition of the blood reaching the liver. Consequently, shunt-induced alterations in metabolic stimulation might, in a general way, explain the various morphological and functional changes of the liver[1–4]. To our knowledge the specific hypothesis has not been tested whether some of the hepatic consequences of a PCS might be regarded as a regression of the hepatocytes to a more immature or embryonal state. A study of biochemical features which differ significantly in fetal and adult animals might shed light on this question.

In the mature rat liver, the enzyme $\gamma$-glutamyl transpeptidase (GGTP, $\gamma$-glutamyl transferase, EC 2.3.2.1) is barely measurable[5], whereas in the newborn rat it exhibits 10 to 20 times more activity[6]. Derepression of this enzyme has also been reported in transplantable chemically induced rat hepatomas[7]. Hepatic GGTP was, therefore, measured in rats with a PCS and in appropriate controls.

The experimental details of our animal model have been described previously[1,3]. Adult male Sprague-Dawley rats were examined for liver GGTP activity 10, 20 and 30 days after an end-to-side portacaval shunt. Non-operated control animals from the same batch were sacrified simultaneously. Enzyme assays were carried out on 10% (w/v) liver homogenates in 0.9% saline solution, using $\gamma$-glutamyl-*p*-nitroanilid as substrate[5].

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$\gamma$-glutamyl transpeptidase activity after end to side portacaval anastomasis ($\bar{x} \pm$ SD)

| Experimental procedure | $n$ | Liver weight (g/100 g) | Enzyme activity ($\mu$moles/min/g wet wt. |
|---|---|---|---|
| Unoperated controls | 6 | $3.66 \pm 0.33$ | $0.006 \pm 0.012$ |
| Portacaval anastomosis | | | |
| after 10 days | 7 | $2.38 \pm 0.41$ | $0.060 \pm 0.018$[a] |
| after 20 days | 5 | $2.16 \pm 0.27$ | $0.067 \pm 0.027$[a] |
| after 30 days | 5 | $2.35 \pm 0.5$ | $0.104 \pm 0.043$[a] |

[a] Significantly different from unoperated controls ($p < 0.001$).