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INVITED REVIEW

The toxic peptides from Amanita mushrooms

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The results of 50 years of effort in the chemistry of Amanita toxins are reviewed. The phallotoxins, fast acting components, but not responsible for fatal intoxications after ingestion, are bicyclic heptapeptides. They combine with F-actin, stabilizing this protein against several destabilizing influences. The virotoxins likewise fast acting are monocyclic heptapeptides. The amatoxins which are the real toxins lead to death within several days by inhibiting the enzymatic synthesis of m-RNA. They are bicyclic octapeptides. The structures of all of these compounds are described, as well as conformations, chemical reactions and modifications, syntheses and correlations between structures and biological activities.

Key words: amatoxins; cyclic peptides; peptide thioethers; phallotoxins; tryptathionine; virotoxins

Heinrich Wieland (photograph), born in 1877, was one of the most productive and versatile chemists during the first part of this century. In the thirties, at his institute the Chemical Laboratory of the Bavarian Academy of Science at Munich, Germany, students and post doctorals worked on such different themes as steroids, pigments of butterflies, toad and snake venoms, radical reactions, strychnos alkaloids, alkaloids from calabash curare and mechanism of biological oxidation. Natural substances that had any striking properties were of interest.

Theodor and Heinrich Wieland, early 1950s

50 years of Amanita research

In the early thirties, H. Wieland suggested to Hans A. Raab that he attempt the isolation of the poisonous principal of the most dangerous mushroom *Amanita phalloides*, "der grüne Knollenblätterpilz", whose erroneous ingestion yearly accounted for numerous victims. This meant resumption of work which had begun

more than 100 years earlier in French, American and German laboratories, but had not led to a homogeneous substance. It was found that the aqueous extract contained a hemolytic principal (phallin) that was destroyed by acids or by heating, and, therefore, could not be the real toxin which was found to be heat-stable. Raab met with considerable difficulties (1,2) as did his successor J. Renz (3), but they paved the way for a successful continuation of the effort. In 1937, Feodor Lynen and Ulrich Wieland were able to obtain the first crystalline toxic component which they named phalloidin (4). Phalloidin, after intraperitoneal injection, led to death of the experimental animal (white mouse) within 2–5 h. This was much faster than death from *A. phalloides* which typically takes 4–8 days. A slow acting "amanitin" was crystallized by H. Wieland, R. Hallermayer and W. Zilg in 1941 (5). For a detailed history see one of the early reviews (1,6). World War II saw an end to the efforts to determine the chemical structures of the toxins. With phalloidin, B. Witkop skillfully confirmed the suggested peptide nature of phalloidin by isolating from an acid hydrolysate L-alanine, L-cysteine, L-oxindolyalanine ("Oxytryptophan") and L-allohydroxyproline, two amino acids hitherto unknown as building blocks of natural peptides (7).

With new methods of paper chromatography and paper electrophoresis available after the War, work was resumed in the author's laboratory. A sensible test reaction was developed with cinnamaldehyde, which in a strong HCl-atmosphere yields a deep violet color with amanitin and, less sensitive, a blue one with phalloidin (8). A drop of a very dilute solution of amanitin, dried on a free space on a newspaper upon moistening with conc. hydrochloric acid yields a blue colored spot. Neither of these reactions, cinnamaldehyde or newspaper, are yet understood. The latter depends on lignin, set free by the strong acid aldehydes, which, like cinnamaldehyde, can undergo condensation reactions forming conjugated double bond systems. By these analytical tools, a new acidic amanitin was detected and named β-amanitin. This byproduct accompanied the original neutral amanitin, now called α-amanitin (8). In the following years, the family of the amanitin, now summarized as "*amatoxins*", grew with the discovery of γ-amanitin (9), ε-amanitin (10), amanin (11), amanin amide (12), amanullin (10), amanullinic acid (11) and proamanullin (11).

Phalloidin was found to be one member of a whole family of related cyclic peptides called "*phallotoxins*", which included phalloin (13), phallisin (10), phallacidin (14), phallacin and phallisacin (15). The final compound isolated was prophalloin (16). A third group of peptidic toxins was detected when a number of *Amanita virosa* mushrooms were found and collected in the forests of West Virginia. The *virotoxins* are clearly separated from phallotoxins present in *A. virosa* by chromatography on Sephadex LH-20 (17,18). In their mode of action, they are closely related to the phallotoxins (LD_{50} 2 mg/kg white mouse, death within 2–5 h). As it will be shown, a biochemical relationship exists between the phallotoxins and virotoxins.

On looking for minor phallotoxic components in the lipophilic part of extracts of *A. phalloides*, a fraction was obtained which, although containing phallotoxins, proved nontoxic in the animal test. The suspected antitoxic principle was purified, crystallized and characterized as a cyclic decapeptide called *antamanide* (19–21). One mg of antamanide per kg body weight when injected before, and at the latest a few minutes after, a lethal dose of phalloidin, prevented certain death in the mouse. Several additional but biologically inactive lipophilic cyclic peptides have been crystallized from *A. phalloides* and summarized as cycloamanides (CyA) (22). The three-dimensional structure of CyA A has recently been published in this Journal (23).

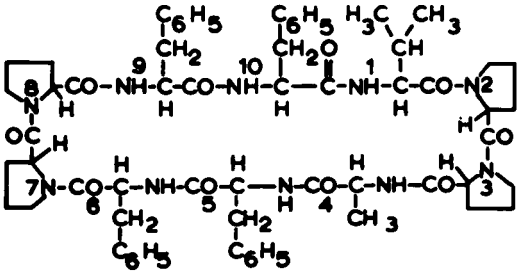


FIGURE 1
Formula of antamanide.

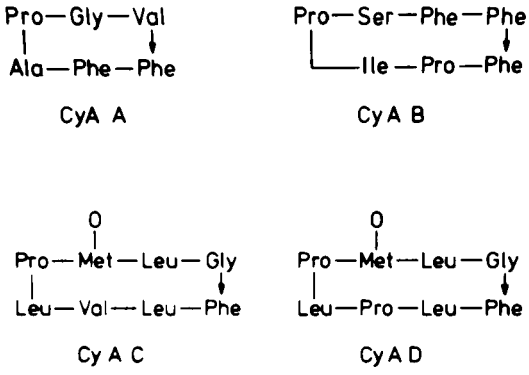


FIGURE 2

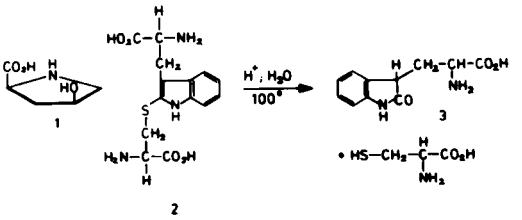
The biologically inert cyclic peptides, cycloamanides (CyA) (23).

THE PHALLOTOXINS

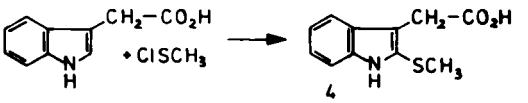
The phallotoxins, with the exception of prophalloin, have the property of specifically binding to F-actin (24); the affinity constant K being 10^{-7}–10^{-8} M (25). By this conjugation F-actin obtains high stability against chemical and physical influences like depolymerization by chaotropic ions (0.6 M KI) (26,27), degradation by proteases (28), cytochalasins (29), or DNAse I (30), ultrasonic vibration (27) or denaturation by heat (31). Since the equilibrium nG-actin ⇌ F-actin by phalloidin is shifted about 30-fold to the right (25), cells may be depleted from G-actin to an intolerable extent. For possible connections between this event and the pathological effect on the liver (swelling by uptake of blood) see reference 32.

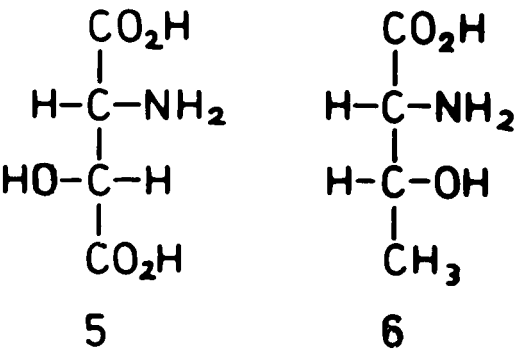
Chemistry of the phallotoxins

The building blocks. The phallotoxins are bicyclic heptapeptides. Common features of all but one are: the constituent amino acids L-alanine, L-allohydroxyproline (4-*cis*-hydroxy-L-proline, 1) (prophalloin has L-proline instead) and the bisfunctional amino acid, L,L-tryptathionine, 2. The latter may be considered a product of an oxidative condensation of L-tryptophan with L-cysteine. Acidic hydrolysis of 2 yields β-oxindolylalanine, 3, and L-cysteine.



Tryptathionine is the chromophoric system of the phallotoxins responsible for the u.v.-maxima around 290 nm (Fig. 3). The existence of a thioether moiety in phalloidin hypothetically suggested by Cornforth *et al.* (33) was proved i) by replacement of the sulfur bridge by hydrogen atoms with the help of Raney nickel (34), forming the u.v.-spectrum of tryptophan (λ_{max} 280 nm), and ii) by synthesis of 2-thioethers, e.g. L-methylthio-indolylacetic acid, 4, from indolylacetic acid and methylsulfenyl chloride (35,36). The u.v.-spectrum of 4 coincided with that of phalloidin (Fig. 3) proving the correctness of its thioether structure.





In addition to phalloidin and three *neutral* phallotoxins [phalloin (13, 37, 38), phallisin (39), and prophalloin (16)], we isolated a group of *acidic* toxins from *A. phalloides* characterized by the term -aci- in their names: phallacidin (14), phallacin, and phallisacin (15). In these peptides β-hydroxy-D-aspartic acid

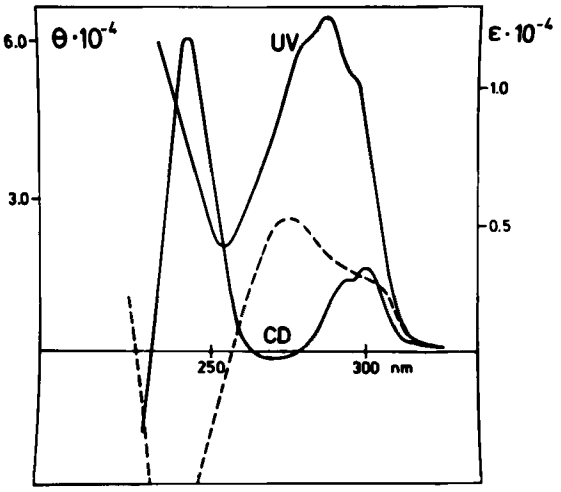
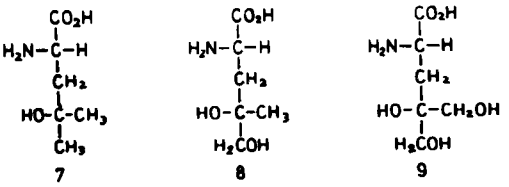
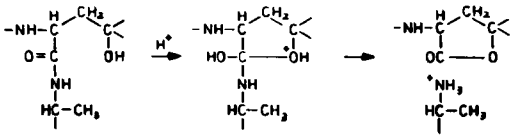


FIGURE 3
U.v. spectrum and CD spectrum of phalloidin in water. Broken line: CD of secophalloidin.

(erythro form, 5) replaces D-threonine (6) at the same position as in the neutral phallotoxins. A second difference is the occurrence of L-valine in the acidic instead of L-alanine in the neutral ones. The individual phallotoxins differ by their number of hydroxyl-groups in the side chain of L-leucine: phalloin and phallacin contain 4-hydroxy-L-leucine ([2 *S*]-2-amino-4-hydroxyisocaproic acid, 7), phalloidin and phallacidin γ, δ-dihydroxy-L-leucine ([2 *S*, 4 *R*]-2-amino-4, 5-dihydroxyisocaproic acid, 8) (40) phallisin and phallisacin γ,δ,δ′-trihydroxy-L-leucine ([2 *S*]-2-amino-4,5,5′-trihydroxyisocaproic acid, 9).



The γ-hydroxylated amino acids allow a mild and very specific cleavage by acids of an adjacent peptide bond, which is split in favor of lactone formation.



SCHEME 1
Peptide bond cleavage by lactone formation.

α-Amino-γ-lactones from the different phallotoxins and amatoxins have been studied *in extenso* in the author's laboratory (40–44). For syntheses, photochlorination of L-leucine and L-isoleucine is a convenient method (45).

Structural and three-dimensional formulae of phallotoxins. By removing the sulfur crosslink of tryptathionine from phalloidin and by acidolysis of the preferentially cleavable peptide bond in dethiophalloidin a linear heptapeptide was obtained. Its stepwise degradation allowed the formulation of a sequence (46), which after a slight modification (38) led to the structural formula of phalloidin and all the phallotoxins (Fig. 4, Table 1).

The LD_{50} values after i.p. adminstration to the white mouse (1.5–3.0 mg/kg body weight) are all in the same order of magnitude. This also applies to their affinity to F-actin, suggesting distinct molecular sites common to all phallotoxins being responsible for strong interaction with the protein. Supporting this view are structure-activity relations of chemically modified or synthesized variants and an inspection of the three-dimensional formula. Such a formula has been constructed according to ^{1}H-

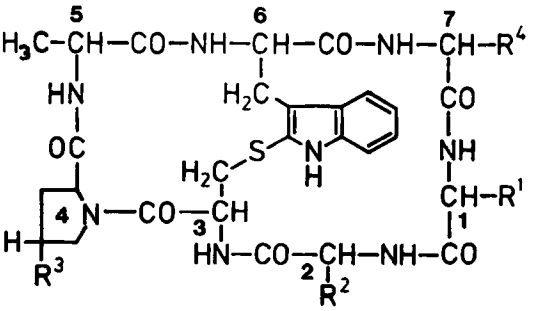


FIGURE 4
Structural formula of phallotoxins.

TABLE 1

The naturally occurring phallotoxins. Positions of residues R^1–R^4 as in Fig. 4

Name	R^1	R^2	R^3	R^4
Prophalloin[a]	CH_3	$CH(OH)CH_3$	H	$CH_2(CH_3)_2OH$
Phalloin	CH_3	$CH(OH)CH_3$	OH	$CH_2(CH_3)_2OH$
Phalloidin	CH_3	$CH(OH)CH_3$	OH	$CH_2(CH_3, CH_2OH)OH$
Phallisin	CH_3	$CH(OH)CH_3$	OH	$CH_2(CH_2OH)_2OH$
Phallacin	$CH(CH_3)_2$	$CH(OH)CO_2H$	OH	$CH_2(CH_3)_2OH$
Phallacidin	$CH(CH_3)_2$	$CH(OH)CO_2H$	OH	$CH_2(CH_3, CH_2OH)OH$
Phallisacin	$CH(CH_3)_2$	$CH(OH)CO_2H$	OH	$CH_2(CH_2OH)_2OH$

[a]Non-toxic with doses up to 30 mg/kg (white mouse). All other phallotoxins show LD_{50} values of 1.5–3.0 mg/kg.

n.m.r. measurements and minimal energy calculation (47). Still undecided was the helical sense of the indolyl-thioether moiety.

Helicity of phallotoxins. The structural element indole-S-CH_2- of the phallotoxins is an inherently dissymmetrical chromophore. It is helical according to the strong positive Cotton effects exhibited in CD around 300 nm and 240 nm (Fig. 3). Helicity of the thioether can be positive or negative (Fig. 5). There is no evidence *a priori* as to which helical orientation is responsible for either positive or negative Cotton effects.

An unambiguous method for solving this problem would be X-ray structure analysis of a phallotoxin, but, unfortunately, these peptides form very thin, long fiber-like crystals which are not suitable for X-ray analysis. Therefore several cyclic peptides having the phallotoxin chromophoric system were synthesized (48). One of them, 2-mercapto-L-tryptophyl-glycyl-L-cysteine cyclic sulfide (1 → 3), **10**, exhibited a CD spectrum mirror imaged to that of the phallotoxins (Fig. 6) and could serve as a probe for elucidating by X-ray the absolute configuration of the thioether moiety, if

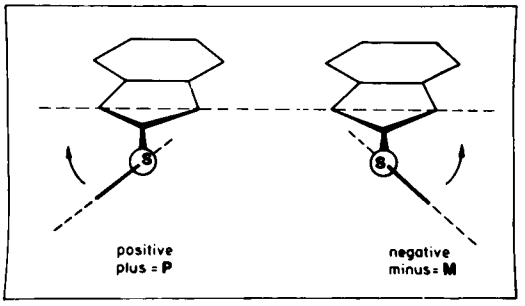


FIGURE 5

M- and P-helicity of an indolylthioether.

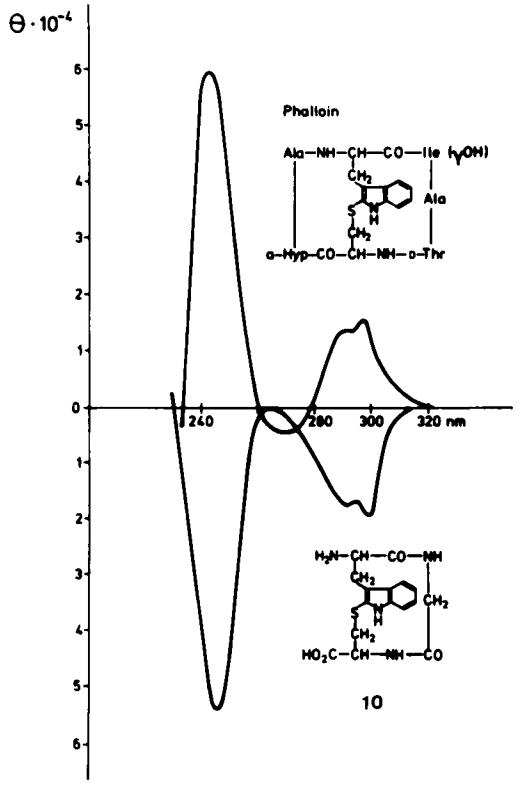


FIGURE 6

CD spectra of phalloin and model thioether **10**.

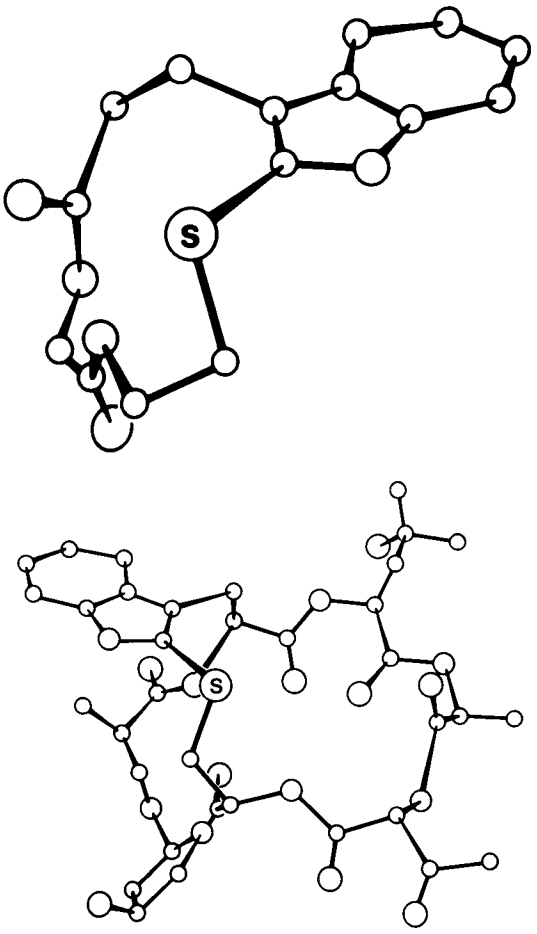


FIGURE 7

Three-dimensional structures (a) of thioether peptide **10** (M-helical) and (b) phalloin (P-helical).

obtained in suitable crystals. An *N-p*-bromobenzene-sulfonyl derivative of 10 was obtained in crystals that revealed M-helicity of the chromophoric system by X-ray diffraction. P-helicity must be ascribed to the phallotoxins with their opposite Cotton effect (49).

The synthetic thioether peptides of ref. 48, including **10**, are biologically inactive. They do not bind to F-actin because they lack the structural elements important for interaction with the protein and visible at the left side of the three-dimensional formula (Fig. 7b): the *cis*-standing OH-group of hydroxyproline (prophalloin, without OH, does not bind at all), the methyl group of alanine, and the aromatic ring in proper arrangement. More insight into the role of the other side chains and of some structural features was gained from chemically modified phallotoxins and synthetic analogs.

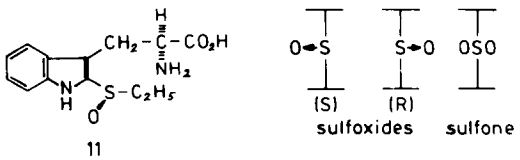
Chemical modifications of phallotoxins

Chemical means of reacting with phallotoxins in a definite way are acids, reductants and oxidants.

Acids preferentially cleave the peptide bond between the carboxyl of the γ-hydroxylated amino acid No. 7 and alanine (resp. valine) No. 1, in favor of formation of a γ-lactone (cf. Scheme 1). The seco-compounds formed are entirely non-toxic, serving as compounds for studying the conditions for syntheses of phallotoxins and analogs as described. The CD spectra of the seco-compounds are quite different from the bicyclic system, with positive Cotton effects around 280 nm and a strong negative one at 235 nm (Fig. 3) pointing to a distortion of the chromophoric and binding moiety (Fig. 3).

Reduction with Raney nickel removes the sulfur crosslink by replacing it by hydrogen atoms. The dethio compounds so formed (34) are non-toxic and exhibit CD spectra without any characteristic features. This points to a loss of stable conformations in solution of the dethio-compounds, the spatial arrangement of the groups responsible for binding to F-actin being disordered.

Oxidants affect the molecules in various ways. Peroxy acids attack the *sulfur* atom yielding the diastereomeric (*R*) and (*S*) sulfoxides and the sulfone (50). The assignment to the (*R*)- and (*S*)-configuration of the sulfoxides has been made by comparison of the ORD curves with that of a synthetic (*R*)-sulfoxide of 2-methylmercapto-L-tryptophan (**11**).



11

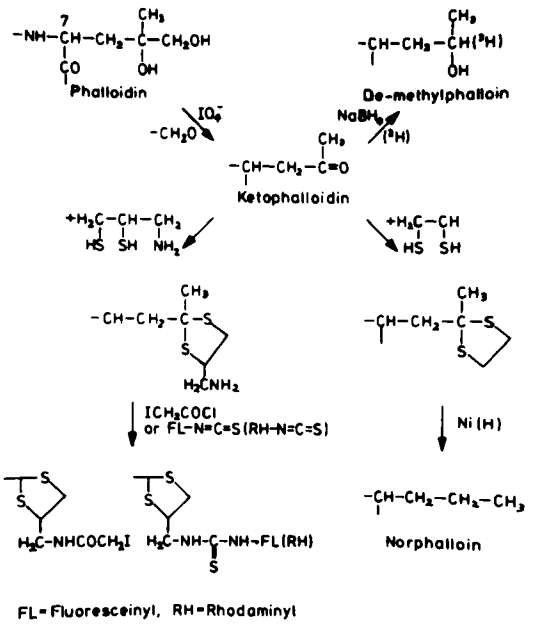
The absolute configuration of **11** was solved by X-ray diffraction analysis (51). Whereas the (*R*)-sulfoxide of phalloidin and the sulfone are

nearly as toxic as the parent substance, and bind to F-actin by comparable affinities, the (*S*)-sulfoxide is not toxic with doses up to 20 mg/kg and does not bind. Since the ORD-curves of (*R*)- and (*S*)-sulfoxides virtually behave like image and mirror image, indicating a pair of enantiomeric sulfoxide moieties, it does not seem probable that a fundamental difference exists in the conformations of the molecules. As will be shown, the amatoxins which are (*R*)-sulfoxides *per se*, reveal an amazing similarity of the three-dimensional structure to that of the much less toxic (*S*)-sulfoxide. The difference in the biological activities may rest upon the opposite directions of the strong S-O dipoles.

Oxidation of a side chain. The glycolic part of the side chain of γ,δ-dihydroxyleucine (No. 7) can be oxidized by periodate (52). The "keto-phalloidin" (α-aminolevulinic acid-7-phalloidin) formed is as toxic as phalloidin, demonstrating that the nature of this side chain does not influence the binding strength to actin. Accordingly, further transformations (53) do not influence the activity: reduction of the keto group by $NaBH_4$ leads to an equally toxic demethyl-phalloin. Radioactivity (^3H) can be introduced (54) by this reaction. The keto group could be reacted with bis-thioglycol to yield a dithiolane which on mild treatment with Raney nickel (without destroying of the tryptathionine moiety) was converted to a methylene of norphalloin (norvaline-7-phalloidin) (53), a similarly toxic compound. With 1-amino-2, 3-dithiopropane an aminomethyl-dithiolane was formed whose amino group served as a function for linking to iodoacetyl (for affinity labeling) or to fluorescein- or rhodamine-isothiocyanate (56, 57).

The fluorescent derivatives still bind strongly to F-actin, thus providing sensitive stains for visualization of F-actin in biological objects (58) (Fig. 8). Fluorescent labels are also introduced in δ-amino-phalloin. This primary amine is obtained from δ-tosylphalloidin (53) by ammonolysis (59).

Other reactions performed with δ-tosylphalloidin were substitution of OTos by methylamine and hydrogensulfide, respectively. With alkali an epoxide is formed which, interestingly,



SCHEME 2

Chemical reactions at side chain No. 7 of phalloidin.

suffers cleavage to secophalloidin under such mild conditions as with aqueous acetic acid at room temperature (53).

The carboxyl group of the acidic phallotoxins. The second side chain modified by chemical means was that of β-hydroxyaspartic acid in phallacidin (PHC). We synthesized the amide,

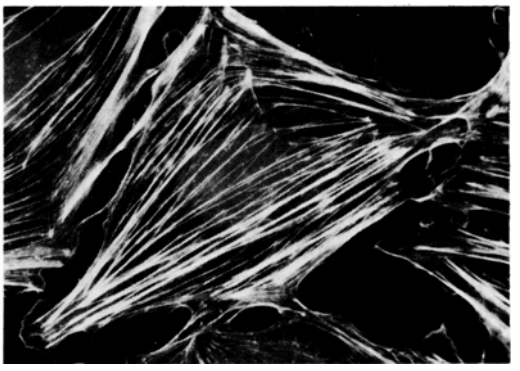
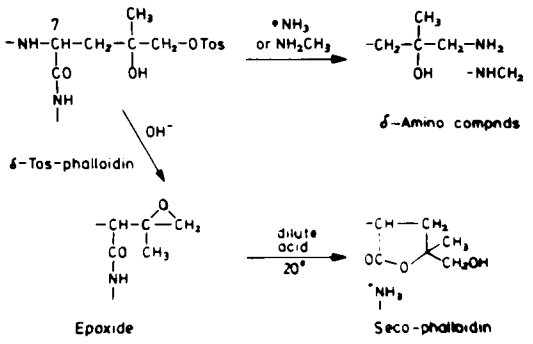


FIGURE 8

F-Actin cables in a fibroblast cell made visible by staining with a fluorescent phallotoxin.



SCHEME 3
Some reactions of δ-toxyl phalloidin.

methylamide and dimethylamide which, in this sequence, were progressively less toxic (15). PHC covalently bound by its carboxylic group to bovine serum albumin using a water soluble carbodiimide proved non-toxic in doses as high as 200 mg/kg white mouse (67). The carboxyl function was also used to introduce the fluorescing 2-nitrobenz-2-oxa-1, 3-diazole residue via the ethylene diamide $-CO-NH-CH_2-CH_2-NH_2$ by Barak *et al.* (61, 62).

At the *indole nitrogen* atom alkylations were carried out in order to study the influence of their bulkiness on the toxicity of the derivatives (63) with the following results: $N-CH_3$ derivative showed an LD_{50} (per kg body weight of the white mouse) like that of phalloidin (2.5 mg); the N-ethyl derivative had 10 mg; the N-propyl compound was non-toxic at doses up to 20 mg/kg. Consequently, N-methylation can be used for introducing radioactive atoms into the molecule without loss of its toxic properties.

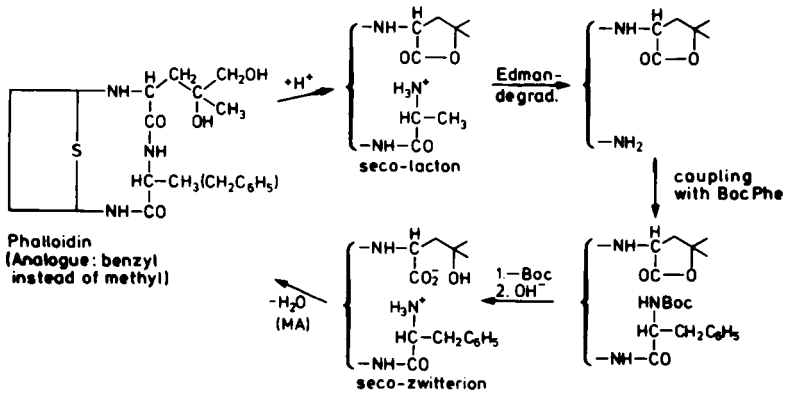
Partial and total syntheses

The synthetic chemistry of natural phallotoxin has a long history beginning in 1958 with the thioether formation by the sulfenylchloride method of appropriate cysteine-peptides with tryptophan-containing counterparts (64). A main obstacle was the γ-hydroxylated amino acid in position 7, which, due to lactone formation, as a component inside of a peptide easily gives rise to a cleaving of the carboxyl-standing peptide bond (65). Therefore as the last step it was intended to close the second ring by a peptide synthesis between the carboxyl function of the γ-hydroxy acid and the amino group of alanine-1. As a relais reaction, the recyclization of secophalloidin (or secophalloin) was attempted without success. Under the conditions of all methods tried, elimination of water took place exclusively by lactone formation. As a result, we turned our attention to seco-ketophalloidin whose *C*-terminal amino acid cannot lactonize. Seco-ketophalloidin was prepared from secophalloidin by oxidation with periodate (52). By the mixed anhydride method using ethoxycarbonylchloride clearly toxic ketophalloidin was obtained and it was demonstrated that, in the monocyclic secopeptide, no odd geometry will prevent a second cyclization (66). When it was recognized that norphalloin with its simple propyl side chain is likewise toxic, its total synthesis was started by F. Fahrenholz (67). He ended up with a toxic preparation identical in every respect to the substance obtained from natural phalloidin via ketophalloidin (Scheme 2).

A breakthrough in the total synthesis of the natural phallotoxin phalloin was achieved by E. Munekata. Although the yields were small, Munekata showed that the recyclization of secophalloidin is feasible. The lactone ring was opened by hydrolysis occurring on passage of the substance through a column of Sephadex LH-20 in 0.004 M ammonia. The zwitterion so formed was subjected to a rapid mixed anhydride procedure at low temperature, and the recyclized toxin was isolated by very careful chromatographic separation of the product mixture (68).

A facile racemization occurs with N-acylated, even urethane-type protected, α-amino-γ-lactones on alkaline ring opening (69). Since, additionally, some racemization occurs in fragment peptide condensations, a diastereomeric phalloidin containing γ,δ-dihydroxy-D-leucine in pos. 7 was also formed and isolated. It was non-toxic with a dose of up to 30 mg/kg (70).

Secophalloidin, easily prepared from phalloidin by mild acid treatment, served as a parent compound for preparing a series of analogs: After removal of the *N*-terminal alanine-1 by Edman degradation, other amino acids were introduced (Scheme 4). Recyclization was also carried out at the de-Ala-secopeptide

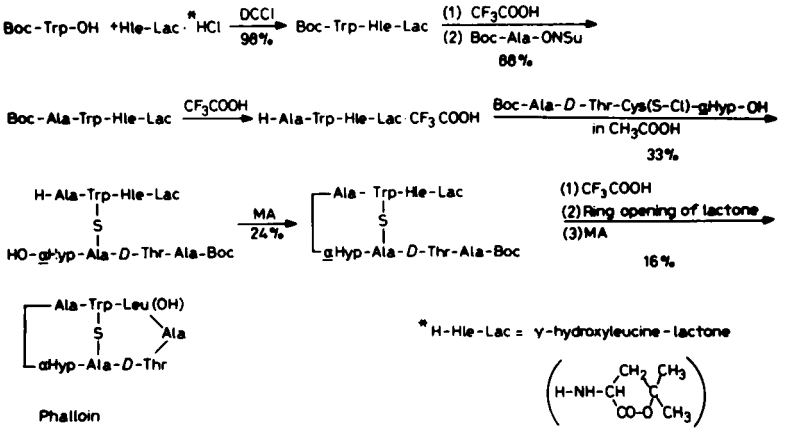


SCHEME 4
Exchange of Ala[1] by Phe in phalloidin.

and after introduction of two alanines yielding a bicyclic hexapeptide, de-Ala[1]-phalloidin and an octapeptide, endo-Ala[1]-phalloidin, respectively. The latter analogs showed no toxicity and, correspondingly, no affinity to F-actin. The difference of their molecular shapes from the normal phallotoxins was also evident from their differing CD curves (71).

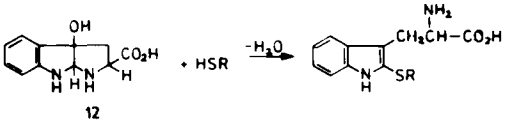
After the conditions for a final ring closure had been elaborated, the first total synthesis of a natural phallotoxin, phalloin, was started (72). All previous syntheses of analogs were with norvaline in pos. 7, avoiding the difficulties caused by lactonization (13,74). Following a similar pathway secophalloin was synthesized. Two appropriate peptides were joined by the classical thioether synthesis via S-chloride (35), and the thioether treated as shown in Scheme 5. Final ring closure yielded phalloin indistinguishable from the natural substance. Analogously, the toxic analog Leu[7]-phalloin and the natural non-toxic prophalloin (16) were obtained by total synthesis. A more detailed review of this synthetic work has been given by Munekata (75).

A second successful approach to the phallotoxins was made possible by Fontana's observation that Savige's oxidation product of L-tryptophan, L-1,2,3,3a,8,8a-hexahydropyrrolo (2,3b) indole-3-carboxylic acid, **12**, abbrev. Hpi



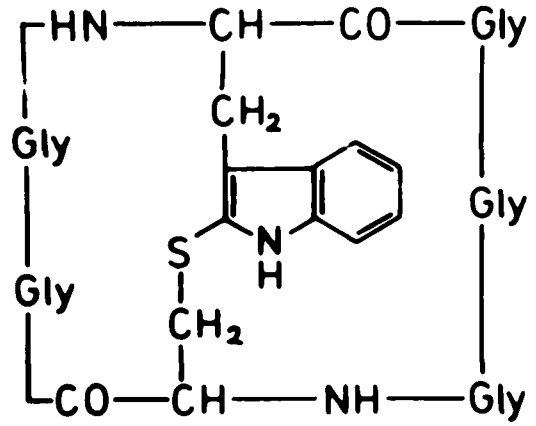
SCHEME 5
Total synthesis of phalloin.

(76), on proton catalysis will react smoothly with thiols yielding 2-thioethers of tryptophan (78,79) (Hpi by photo-oxidation see ref. 77).



12

In our laboratories this reaction was soon applied to Boc-Hpi-(STrt)cysteine methylester affording cyclic thioether **13** which on heating was transformed to the "miniphallotoxin" **14** (Scheme 5). The heptapeptide Boc-Hpi-Gly-Gly-Gly-Cys(STrt)-Gly-Gly-OH on subjection to trifluoroacetic acid loosing the Boc- and Trt-groups was cyclized to form a thioether which by an internal peptide synthesis (mixed anhydride) yielded the simplest *all*-Gly-phallotoxin cyclic (L-cysteinylglycylglycyl-2-mercapto-L-tryptophylglycylglycylglycyl)-cyclic (1–4) sulfide **15** (48).

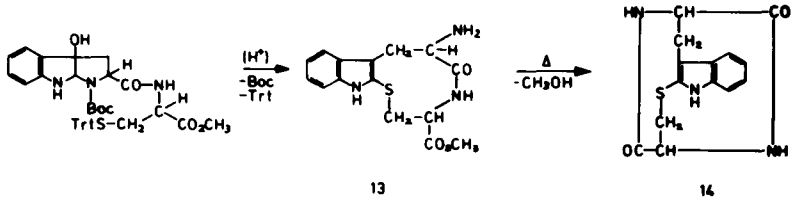


15

The phallotoxin-like bicyclic peptide **15** shows a conformation similar to phalloidin as revealed by CD spectroscopy, but has no biological activity. An F-actin binding analog was also synthesized following the novel route. In order to have available an amino functional phallotoxin independently from natural sources, we synthesized an analog with L-lysine in the "tolerant" 7-position. Since D-α-aminobutyric acid instead of D-threonine in pos. 2 does not influence binding to F-actin (74), D-Abu^2-Lys(Z)^7-phalloin was synthesized via the heptapeptide Boc-Hpi-Lys(Z)-Ala-D-Abu-Cys(STrt)-*a*-Hyp-Ala-*tert*.-butylester. Removal of Boc, Trt and t-butyl by trifluoroacetic acid along with intramolecular thioether formation yielded a monocyclic heterodetic peptide, which after second cyclization with DCC and hydrogenolytical removal of the 7-residue afforded the desired D-Abu^2-Lys^7-phallotoxin. It exhibited an affinity for F-actin of approximately 25% of that of phalloidin and, therefore, was conjugated with rhodamine isothiocyanate to form an excellent fluorescent probe for F-actin in cell preparations (81).

Structure-toxicity relations

Ample information has been collected on the dependence of toxic behavior (affinity to F-actin) on structural details of the phallotoxins. In Table 2 all of the analogs have been listed together with their toxicity in the white mouse. For the contribution of each side chain, the following conclusions can be drawn: the *cis*-standing hydroxyl group in hydroxyproline-4, the methyl group of alanine-5 and the indole moiety are the chief binding sites. Proof of the participation of the aromatic system is an increase of the respective u.v.-absorption on binding to F-actin as shown by difference-spectroscopic measurements (82). The D-side chain of residue 2 belongs to the binding

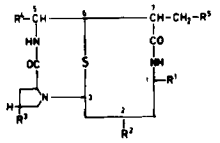


13 14

SCHEME 6
Synthesis of the smallest bicyclic thioether peptide.

TABLE 2

Analogs of phalloidin and their toxicity



	R^1	R^2	R^3	R^4	R^5	Tox.[a]
Norphalloin (NPN)[b]	CH_3	$CH(OH)CH_3$	*cis* OH	CH_3	$CH_2CH_2CH_3$	++
D-Abu^2-NPN	CH_3	CH_2CH_3	*cis* OH	CH_3	$CH_2CH_2CH_3$	++
D-Abu^2-Lys^7-PHN	CH_3	CH_2CH_3	*cis* OH	CH_3	$(CH_2)_4-NH_2$	—[c]
D-Ala^2-Leu^7-PHN	CH_3 (D-)	CH_3	*cis* OH	CH_3	$CH_2CH(CH_3)_2$	—
Pro^4-NPN	CH_3	$CH(OH)CH_3$	H	CH_3	$CH_2CH_2CH_3$	—
Gly^5-NPN	CH_3	$CH(OH)CH_3$	*cis* OH	H	$CH_2CH_2CH_3$	—
Leu^7-PHN	CH_3	$CH(OH)CH_3$	*trans* OH	CH_3	$CH_2CH(CH_3)_2$	++
Gly^1-PHD	H	$CH(OH)CH_3$	*cis* OH	CH_3		+
Val^1-PHD	$CH(CH_3)_2$	$CH(OH)CH_3$	*cis* OH	CH_3		++
Leu^1-PHD	$CH_2CH(CH_3)_2$	$CH(OH)CH_3$	*cis* OH	CH_3		++
Phe^1-PHD	$CH_2C_6H_5$	$CH(OH)CH_3$	*cis* OH	CH_3	H_2COH	±
D-Ala^1-PHD	D-CH_3	$CH(OH)CH_3$	*cis* OH	CH_3	CH_2-C-CH_3	—
D-$(OH)_2$Leu-PHD	CH_3	$CH(OH)CH_3$	*cis* OH	CH_3	OH	—
PHC-amide	$CH(CH_3)_2$	$CH(OH)CONH_2$	*cis* OH	CH		++
PHC-methylamide	$CH(CH_3)_2$	$-CONHCH_3$	*cis* OH	CH_3		+
PHC-dimethylamide	$CH(CH_3)_2$	$-CON(CH_3)_2$	*cis* OH	CH_3		+
des-Ala^1-PHD	cyclic hexapeptide residue no. 1 missing					—
endo-Ala^{1a}-PHD	cyclic octapeptide additional Ala between 1 and 7					—

[a] Toxicity LD_{50} (mg/kg body weight) at white mouse after i.p. injection LD_{50} 1–4 ++, 4–10 +, 10–30 ±, 30—.
[b] NPN, norphalloin; PHD, phalloidin; PHC, phallacidin.
[c] Does not, as a cation, enter the liver cell but has about 20% of PHD-affinity to F-actin.

elements, for it has to have at least two carbon atoms. Minor contribution makes side chain No. 1, which can vary considerably in structure or even be absent. Least important for binding to F-actin is the nature of side chain No. 7, which, consequently, can be modified to a handle for introducing biochemically useful functional groups (56).

THE VIROTOXINS

The virotoxins discovered as components of the white mushroom *Amanita virosa* produce the same toxicological symptoms as the phallotoxins. Surprisingly, the elucidation of the structure of viroidin led to the conclusion that the virotoxins are not bicyclic peptides like the phallotoxins but monocyclic heptapeptides (18). The general formula is shown in Fig. 9. The variations of side chains leading to the six different virotoxins as well as their proportions are given in Table 3.

As shown in Fig. 9, there exist in the amino acid compositions some features common with

TABLE 3

The naturally occurring virotoxins. For meaning of X, R^1 and R^2 see Fig. 9

Name	X	R^1	R^2	% of total
Viroidin	SO_2	$CH(CH_3)_2$	CH_3	18
Desoxo-viroidin	SO	$CH(CH_3)_2$	CH_3	4
Ala^1-viroidin	SO_2	CH_3	CH_3	10
Ala^1-desoxo-viroidin	SO	CH_3	CH_3	
Viroisin	SO_2	$CH(CH_3)_2$	CH_2OH	49
Desoxo-viroisin	SO	$CH(CH_3)_2$	CH_2OH	19

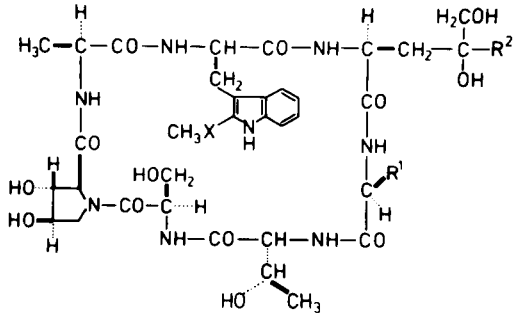
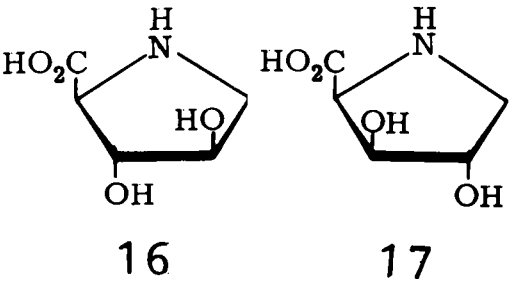


FIGURE 9
Structural formula of virotoxins.

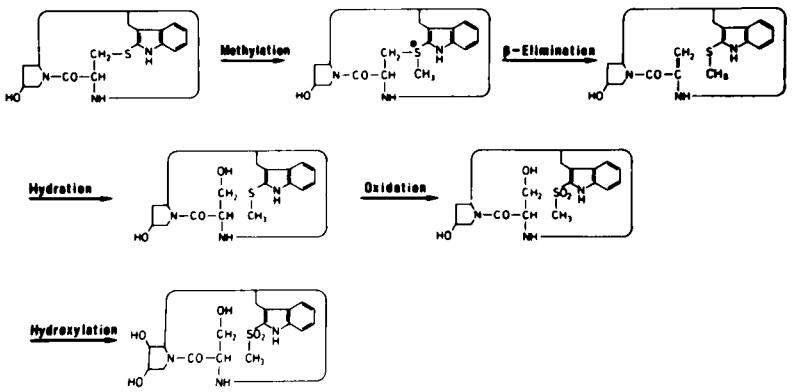
the phallotoxins: alanine (resp. valine) in position 1, D-threonine (pos. 2), L-alanine (pos. 5), and L-leucine, containing a varying number of hydroxy groups, in position 7. Amino acids different from those of phallotoxins are: D-serine instead of the L-cysteine-part of tryptathionine in pos. 3; 2,3-*trans*-3,4-*trans*-3,4-dihydroxy-L-proline, **16**, instead of 2,4-*cis*-4-hydroxy-L-proline in pos. 4 and 2'-(methylsulfinyl)-L-tryptophan or 2'-(methyl-sulfonyl)-L-tryptophan instead of the tryptophan part of tryptathionine in pos. 6. The 3,4-dihydroxyproline (16) was unknown before detection as a component of the virotoxins (83), while the diastereomeric 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline (17) was recognized as a component of a protein in diatoms several years ago (84). Both of these diastereomers have been obtained by synthesis in the author's laboratory (85).



16 17

It is highly probable that the virotoxins are derived from the phallotoxins or from a common precursor molecule. A reasonable biochemical transformation of phalloidin to viroidin is formulated in Scheme 7.

The toxicity of the virotoxins corresponds to that of the phallotoxins (LD_{50} 2,5 mg/kg, white mouse). They also bind strongly to F-actin. This is surprising since the virotoxin molecule is not preformed *a priori* for binding, as its uncharacteristic CD spectrum shows. A similar monocyclic structure in the class of the phallotoxins as a dethiocompound does not exhibit any affinity to the protein. Features of virotoxins not present in dethiophalloidin which could be responsible for such a strong interaction with F-actin by induced fit are the hydroxyl group of D-serine instead of L-alanine, the additional *trans*-hydroxyl group in 3'-position of 4-*cis*-4-hydroxy-L-proline and the methylsufonyl group at the tryptophan moiety. A methyl sulfonyl group has been introduced into dethiophalloidin by A. Buku and J.U. Kahl (personal communication); the product, however, exhibited no affinity for F-actin.



SCHEME 7
Suggested biochemical path from phalloidin to viroidin [from (18)].

The virotoxins, like the phallotoxins, most probably are not involved in the fatal intoxication by *A. virosa*, but it contains the amatoxin amaninamide in a concentration comparable to that of α- and β-amanitin in *A. phalloides* (12).

The white mushroom *A. verna* does not contain virotoxins. Apparently it is a white variety of the green *A. phalloides* as it yields a chromatographic pattern of its components very similar to that of the green mushroom (17). The yellow Amanita mushroom *A. citrina* has no toxic peptides but a rather high content of bufotenine (86).

THE AMATOXINS

The amatoxins are the toxic components of *Amanita species*, which after ingestion can cause death to patients (87, 89). Not every animal species will be intoxicated by peroral administration like man, the dog and guinea pig. These species resorb the toxins from the intestinal tract, whereas rodents don't resorb it at all or only by such a slow rate that the symptoms of intoxication will not occur. The symptoms are: after 8–10 h, nausea, vomiting, diarrhea, and, after a deceptive intercept of 2–3 days, necrosis of the liver and kidneys, and death by coma on the 5th to the 7th day. The lethal dose for an adult person is about 10 mg amatoxins, the contents of one average green death cap mushroom.

The molecular mechanism of action of amatoxins is their strong inhibtion of one of the eukaryotic DNA-dependent RNA polymerases (form II or B) which is responsible for the transcription of the precursor of m-RNA (89). Half inhibition occurs in a concentration as low as 10^{-9} M. The toxins bind to the subunit of 140 KD in a ratio of 1:1 (90). In its presence, one step of elongation, formation of a new phosphodiester bond, still goes on but the next step, translocation of the growing RNA chain to the catalytic site of the enzyme, is blocked. The toxin forms a ternary complex of enzyme, DNA template and nascent RNA which can be isolated by gel chromatography (91). Depletion from m-RNA will lead to a diminished synthesis and eventually arrest of protein formation in the liver and kidneys, leading to necrosis of the parenchymal cells. For a recent survey on toxicological and clinical research see reference 87.

Chemistry of amatoxins

The amatoxins, one family of bicyclic octapeptides, are derived from the same amino acid backbone but differ in the number of hydroxyl groups.

The amatoxins consist exclusively of L-amino acids and glycine. Uncommon building blocks are the crosslinking 6′-hydroxytryptathionine-(R)-sulfoxide (No. 4–8), which is responsible for the u.v.-absorption around 300 nm and for the positive Cotton effects in the same region of circular dichroism (Fig. 11). On addition of alkali the u.v.-maximum is shifted by 30 nm to long wavelengths as a consequence of phenolate-ion formation at the 6′hydroxyl group. This is expressed by the CD spectrum. The OH group is responsible for the very intensive color reaction with cinnamaldehyde plus hydrochloric vapors (deep violet), and for the blue staining on newspaper with 10 N hydrochloric acid (p. 258).

On strong irradiation at 314 nm, i.e. longer than the absorption maxima of proteins, α-amanitin is decomposed, thus leaving the binding site of RNA polymerase B (92). Side chain No. 1 is that of aspartic acid in β-amanitin and of asparagine in α-amanitin. For proof the latter was obtained from β-amanitin by amidation via mixed anhydride plus ammonia (93). In pos. 3 is an L-isoleucine which is monohydroxylated in γ-position (γ-amanitin, ε-amanitin) or bishydroxylated in γ- and δ-position (α-amanitin, β-amanitin). The exact stereochemistry of this

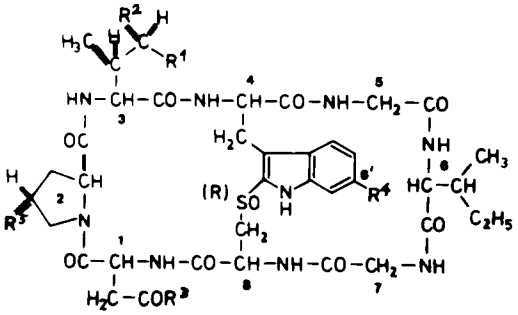


FIGURE 10
Structural formula of amatoxins.

TABLE 4

The naturally occurring amatoxins. Positions of residues R^1–R^5 as in Fig. 10

Name	R^1	R^2	R^3	R^4	R^5	LD_{50} (mg/kg white mouse)
α-Amanitin	CH_2OH	OH	NH_2	OH	OH	0.3
β-Amanitin	CH_2OH	OH	OH	OH	OH	0.5
γ-Amanitin	CH_3	OH	NH_2	OH	OH	0.2
ε-Amanitin	CH_3	OH	OH	OH	OH	0.3
Amanin	CH_2OH	OH	OH	H	OH	0.5
Amaninamide[a]	CH_2OH	OH	NH_2	H	OH	0.3
Amanullin	CH_3	H	NH_2	OH	OH	> 20
Amanullinic acid	CH_3	H	OH	OH	OH	> 20
Proamanullin	CH_3	H	NH_2	OH	H	> 20

[a] In *A. virosa* only.

side chain is: (2S, 3R, 4R)-2-amino-3-methyl-4, 5-dihydroxy-valeric acid (94). Amanullin has a normal L-isoleucine side chain. Amanin and amaninamide differ from β- resp. α-amanitin only by the lack of the phenolic 6′-hydroxyl group at the tryptophan moiety. All of the amatoxins are (*R*)-sulfoxides. A three-dimensional structure formula of α-amanitin in dimethylsulfoxide solution was derived in 1978 from ^{1}H-n.m.r. data (95) and, more recently, for 6′-O-methyl α-amanitin (96). These structures are in accordance with a space formula of β-amanitin obtained by X-ray diffraction analysis (97).

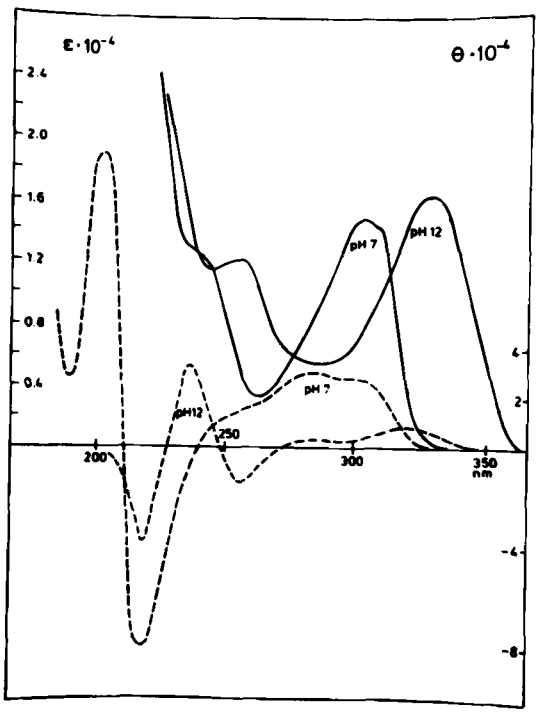


FIGURE 11

U.v.-spectra and CD-spectra (broken lines) of α-amanitin in water at different pH values.

Chemical modifications of amatoxins

As in the phallotoxin series and the amatoxins a preferential cleavage of one peptide bond

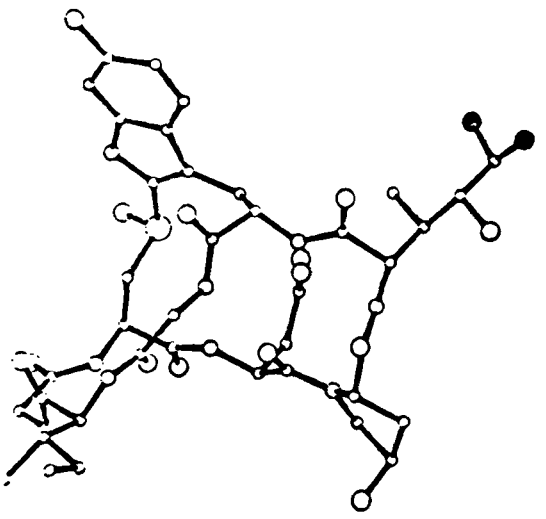


FIGURE 12

Three-dimensional structure of β-amanitin [from (97)].

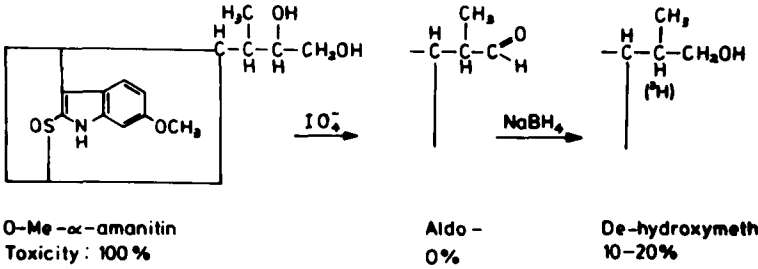
occurs under mild acidolytic conditions between the γ-hydroxylated isoleucine and the tryptophan moiety yielding monocyclic non-toxic seco compounds. Hydrogenolytic removal of the sulfoxide bridge by treatment with Raney-nickel yielded non-toxic monocyclic dethio products. Edman-degradation of a linear dethioseco octapeptide revealed the structure of the amatoxins (98). The oxygen atom of the (*R*)-sulfoxide bridge in 6′-O-methyl-α-amanitin could be removed by treatment with Raney nickel or K_3MoCl_6. Re-oxidation by peroxy acids yielded besides the original (*R*)-sulfoxide the almost non-toxic (*S*)-diastereomer, and, with excessive oxidant, the sulfone which exhibits almost full toxicity (99). The assumption of different conformations of the considerably toxic sulfone and the much less toxic (*S*)-sulfoxide was disproved by X-ray structure analysis which revealed a surprising similarity of both the structures (96). This was confirmed by ^{1}H-n.m.r. spectroscopy in dimethylsulfoxide solution not only for both derivatives but also for the thioether and the (*R*)-sulfoxide. As with an analogous situation in the phallotoxin series, a reason for the differing affinities to RNA polymerase II (or B) of (*R*)- and (*S*)-sulfoxides may be the differing direction of the strong $\overset{\delta+}{S}-\overset{\delta-}{O}$ dipoles in the diasteriomieric compounds.

In the foregoing and following oxidation experiments, the phenolic hydroxyl group susceptible to oxidative attack had to be protected by methylation. 6′-O-methyl-α-amanitin was oxidized by periodate which split the γ, δ-glycol grouping of side chain No. 3 (100). The resulting "aldo-amanitin" proved non-toxic and had only 1% of the affinity to RNA polymerase II. Part of toxicity and binding strength to the enzyme was restored by hydrogenation of the aldehyde group by $NaBH_4$ yielding a primary alcohol function (this is one method for introducing radioactivity, tritium, into the toxin).

The aspartic acid side chain of β-amanitin was derivatized as methyl ester, thiophenylester and as amides of various structures all derivatives being still toxic (93). The carboxylic group was used as a handle for fixation of β-amanitin to serum albumin by the use of a carbodiimide to yield conjugates, which were even more toxic than the drug itself (101).

As a third point for manipulations by chemical means, the phenolic hydroxyl group at the indole nucleus may be mentioned. It is not essential for toxicity, since amanin and amaninamide are toxic inspite of their lacking the 6′-OH group (11, 12]). An inspection of the three-dimensional formula of β-amanitin (Fig. 12) shows that the indole nucleus protrudes distinctly out from the octapeptide cycle which, apparently, forms the binding part, the OH-group being the most separate entity. Hence, it is reasonable to conclude that modifications at the side don't disturb binding to the enzyme. Such modifications are 1) etherification, 2) diazo-coupling, 3) iodination and 4) replacement of OH by tritium.

Ethers. More than six alkyl ethers of growing chain length and a couple of ethers with long amide spacer moieties have been prepared which all exhibit notable binding strength to RNA polymerase (102). An ether prepared with ε-caproylethylenediamine with its free amino group was conjugated with fluoresceine isothiocyanate; the resulting fluorescent amatoxin (FAMA) allowed the visualization of amatoxin-binding substance during mitosis in cultured rat kangaroo (PtK1) cells (103). After succinoylation of the amino group the toxin by its spacer was coupled to diverse proteins, particularly to fetuin. With this antigen antibodies could be raised in rabbits by the use of which a radio-



O-Me-α-amanitin
Toxicity: 100%

Aldo-
0%

De-hydroxymethyl
10–20%

SCHEME 8

immunoassay could be developed for trace amounts of amatoxins (104).

As phenols the amatoxins are prone to *coupling with aromatic diazonium ions* forming highly colored azocompounds. In 1940 this method was used to detect the phenolic nature of amanitin (5), and later for introducing spacer moieties envisioning diverse biological utilizations (105). Most recently it has been extended to several diazonium compounds and studied in detail (106).

Iodination of α-amanitin occurs in 7′-position, and the use of ^{125}I is a further possibility for introducing radioactivity (107). Tritium was introduced into β-amanitin by *hydrogenation* of the 6′-(1-phenyl)tetrazolyl ether. Replacement of the oxygen with hydrogen leads from the amanitin to the amanin-series (108) and use of ^{3}H-containing hydrogen allowed the synthesis of 6′-^{3}H-amanin (109).

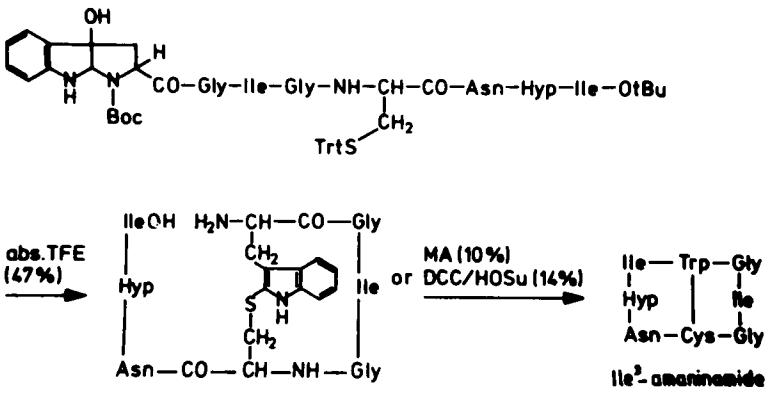
Synthetic approaches

The total synthesis of a natural amatoxin has not been accomplished to date. The main difficulty is the tricky γ-hydroxyisoleucine, which, like γ-hydroxyleucine in the phallotoxin series, as an endo-component of a peptide gives rise to acidolytic cleavage of the adjacent CO–NH–, and, other than with γ-hydroxyleucine doesn't form a trace of peptide bond with its carboxyl group but exclusively the γ-lactone – most probably because of steric hindrance. As a result, it has not been possible to recyclize a seco-amatoxin.

Less grave is the difficult accessibility of 6-hydroxy-L-tryptophan, for the natural amatoxins, amanin and amaninamide lack the 6-OH group. Since the sulfoxide-oxygen may be absent without diminishing the toxicity, several analogs were synthesized containing tryptathionine and amino acids other than γ-hydroxyisoleucine in pos. 3. Using the "classical" thioether formation via S-chlorides we prepared a non-toxic homoserine analog (110) and an isoleucine analog which, like amanullin, was non-toxic but exhibited a considerable affinity to RNA polymerase B (111).

Amanullin, a component of the mushroom, proved non-toxic in mice but had an affinity to RNA polymerase B comparable to that of toxic amatoxins. The reasons for its ineffectivity *in vivo* may be: 1) the rate constant of its dissociation from the enzyme is about 10 times greater than that of α-amanitin (112) and 2) it differs pharmacodynamically from the toxic peptides due to its more lipophilic property.

A fresh impetus was given to amatoxin chemistry by adaptation of the novel indolyl thioether synthesis (78, 79). As with the phallotoxins, the first ring can be closed advantageously by an internal attack of the cysteine sulfur at the Hpi-residue of appropriate octapeptides. Using this method, G. Zanotti synthesized a variety of amatoxin analogs all lacking the γ-hydroxylated isoleucine building block (113, 114). Nevertheless, a comparison of their inhibitory capacity of RNA polymerase B gave much information on the role of the different side chains for binding strength. The synthesis of Ile^{3}-amaninamide is indicated in Scheme 9.



SCHEME 9

Synthesis of Ile^{3}-amaninamide.

Structure-toxicity relations

Summarizing our experiences from natural occurring, chemically modified and synthesized amatoxins and analogs one can draw the following conclusions: For strong inhibition the molecule must be bicyclic. Of the side chains the hydroxyl group of 4-*trans*-hydroxy-L-proline (No. 2) is essential since proamanullin, lacking this OH, binds at least a hundred-fold weaker to the enzyme than amanullin. The chemical nature of the side chain No. 3 is decisive. For strong binding, it must consist of four C-atoms with a methyl branch at the β-C atom (isoleucine). Methyl branching at the γ-C atom (leucine) reduces the affinity to about 5%, whereas omission of the terminal methyl of isoleucine (=valine, β-branched, methyl) has 25% affinity. Optimal binding occurs if the isoleucine side chain No. 3 contains at least one hydroxyl group in γ-position (γ-amanitin) or, as in α- and β-amanitin, an additional one at the δ-carbon. Furthermore isoleucine in pos. 6 takes part in tight binding, since its replacement by alanine in an analog increased the inhibitory constant for RNA polymerase II by about a thousand-fold. From these and additional data (109), it follows that the amatoxins owe their fixation at the protein to hydroxyl and lipophilic groups specifically arranged at the whole peptide ring, and held in the proper position by a very rigid structure.

CONCLUSION

In the present review, the predominant aspects considered have been the chemical rather than the toxicological, clinical or biological; the story of antamanide has not been considered. A more detailed treatment of these questions is offered in the Proceedings of an International Symposium held at the end of 1978 (87) and in a summarizing article with more than 400 references (115). However, a short botanical remark shall finish this review. Phallotoxins occur in *Amanita species* as *A. phalloides, A. verna, A. virosa, A. bisporigera, A. tenuifolia, A. ocreata* and others, compiled in reference 87. In obtaining phallotoxins and amatoxins, which proved valuable tools in cell biology, only the phallotoxins are accessible by synthesis; the others still have to be isolated from mushrooms. Attempts have been made in several laboratories to cultivate toxic Amanitas without any success. Amatoxins, however, occur also in fungi other than *Amanita*. Some *Galerina species* have been found to contain amatoxins, and one of them, *G. sulciceps*, has been reported to grow in greenhouses (116). This observation may set an end to the necessity of *Amanita* mushroom hunting in the forests.

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REFERENCES

1. Raab, H. (1932) *Hoppe-Seylers Z. Physiol. Chem.* **207**, 157–181
2. Raab, H. & Renz, J. (1933) *Hoppe-Seylers Z. Physiol. Chem.* **216**, 224–229
3. Renz, J. (1934) *Hoppe-Seylers Z. Physiol. Chem.* **230**, 245–258
4. Lynen, F. & Wieland, U. (1938) *Liebigs Ann. Chem.* **533**, 93–117
5. Wieland, H., Hallermayer, R. & Zilg, W. (1941) *Liebigs Ann. Chem.* **548**, 1–18
6. Wieland, T. & Wieland, O. (1959) *Pharmacol. Rev.* **11**, 87–107
7. Wieland, H. & Witkop, B. (1940) *Liebigs Ann. Chem.* **543**, 171–183
8. Wieland, T., Wirth, L. & Fischer, E. (1949) *Liebigs Ann. Chem.* **564**, 152–160
9. Wieland, T. & Dudensing, C. (1956) *Liebigs Ann. Chem.* **600**, 156–160
10. Wieland, T., Rempel, D., Gebert, U., Buku, A. & Boehringer, H. (1967) *Liebigs Ann. Chem.* **704**, 226–236
11. Buku, A. & Wieland, T. (1974) *Liebigs Ann. Chem.*, 1587–1595

12. Buku, A., Wieland, T., Bodenmüller, H. & Faulstich, H. (1980) *Experientia* **36**, 33–34
13. Wieland, T. & Mannes, K. (1957) *Angew. Chem.* **69**, 389–390
14. Wieland, T. & Schnabel, H. (1962) *Liebigs Ann. Chem.* **657**, 218–225
15. Faulstich, H., Brodner, O., Walch, St. & Wieland, T. (1975) *Liebigs Ann. Chem.*, 2324–2330
16. Munekata, E., Faulstich, H. & Wieland, T. (1978) *Liebigs Ann. Chem.*, 776–784
17. Faulstich, H., Georgopoulos, D., Bloching, M. & Wieland, T. (1974) *Z. Naturforsch.* **29c**, 86–88
18. Faulstich, H., Buku, A., Bodenmüller, H. & Wieland, T. (1980) *Biochemistry* **19**, 3334–3343
19. Wieland, T., Lüben, G., Ottenheym, H. & Schäfer, H. (1969) *Liebigs Ann. Chem.* **722**, 173–178
20. Wieland, Th., Lüben, G., Ottenheym, H., Faesel, J., de Vries, J.X., Konz, W., Prox, A. & Schmid, J. (1968) *Angew. Chem. Int. Ed. Engl.* **7**, 204–208
21. Wieland, T. (1973) in *Chemistry and Biology of Peptides. Proc. of the 3rd American Peptide Symp.* (Meienhofer, J., ed.), Ann Arbor Science Publ. Inc., pp. 377–396
22. Gauhe, A. & Wieland, T. (1977) *Liebigs Ann. Chem.*, 859–868
23. Chang, Chian Chan, Karle, I.L. & Wieland, T. (1982) *Int. J. Peptide Protein Res.* **20**, 414–420
24. Wieland, T. (1977) *Naturwissenschaften* **64**, 303–309
25. Faulstich, H., Schäfer, A.J. & Weckauf, M. (1977) *Hoppe-Seylers Z. Physiol. Chem.* **358**, 181–184
26. Löw, I. & Wieland, T. (1974) *FEBS Lett.* **44**, 340–343
27. Dancker, P., Löw, I., Hasselbach, W. & Wieland, T. (1975) *Biochim. Biophys. Acta* **400**, 407–414
28. de Vries, J. & Wieland, T. (1978) *Biochemistry* **17**, 1965–1968
29. Löw, I., Dancker, P. & Wieland, T. (1975) *FEBS Lett.* **54**, 263–265
30. Schäfer, A.J., de Vries, J., Faulstich, H. & Wieland, T. (1975) *FEBS Lett.* **57**, 51–54
31. de Vries, J.X., Schäfer, A.J., Faulstich, H. & Wieland, T. (1976) *Hoppe-Seylers Z. Physiol. Chem.* **357**, 1139–1143
32. Wieland, T. (1981) in *Structure and Activity of Natural Peptides* (Voelter, W. & Weitzel, G., eds.), pp. 23–40, Walter de Gruyter, Berlin
33. Cornforth, J.W., Dalgliesh, C.E. & Neuberger, A. (1951) *Biochem. J.* **49**, 598–603
34. Wieland, T. & Schmidt, G. (1952) *Liebigs Ann. Chem.* **577**, 215–233
35. Wieland, T., Weiberg, O., Fischer, E. & Hörlein, G. (1954) *Liebigs Ann. Chem.* **587**, 146–161
36. Wieland, T., Weiberg, O., Dilger, W. & Fischer, E. (1955) *Liebigs Ann. Chem.* **592**, 69–80
37. Wieland, T. & Mannes, K. (1958) *Liebigs Ann. Chem.* **617**, 152–162
38. Wieland, T. & Schnabel, H. (1962) *Liebigs Ann. Chem.* **657**, 225–228
39. Gebert, U., Wieland, T. & Boehringer, H. (1967) *Liebigs Ann. Chem.* **705**, 227–237
40. Wieland, T. & Krantz, H. (1958) *Chem. Ber.* **91**, 2619–2626
41. Wieland, T. & Weiberg, O. (1957) *Liebigs Ann. Chem.* **607**, 168–174
42. Pfaender, P. & Wieland, T. (1966) *Liebigs Ann. Chem.* **700**, 126–132
43. Georgi, V. & Wieland, T. (1966) *Liebigs Ann. Chem.* **700**, 149–156
44. Wieland, T., Hasan, M. & Pfaender, P. (1968) *Liebigs Ann. Chem.* **717**, 205–214
45. Faulstich, H., Dölling, J., Michl, K. & Wieland, T. (1973) *Liebigs Ann. Chem.*, 560–565
46. Wieland, T. & Schön, W. (1955) *Liebigs Ann. Chem.* **593**, 157–178
47. Patel, D.J., Tonelli, A.E., Pfaender, P., Faulstich, H. & Wieland, T. (1973) *J. Mol. Biol.* **79**, 185–196
48. Zanotti, G., Birr, C. & Wieland, T. (1978) *Int. J. Peptide Protein Res.* **12**, 204–216
49. Wieland, T., Beijer, B., Seeliger, A., Dabrowski, J., Zanotti, G., Tonelli, A., Gieren, A., Dederer, B., Lamm, V. & Hädicke, E. (1981) *Liebigs Ann. Chem.*, 2318–2334
50. Faulstich, H., Wieland, T. & Jochum, Ch. (1968) *Liebigs Ann. Chem.* **713**, 186–195
51. Wieland, T., Jordan de Urries, M.R., Indest, H., Faulstich, H., Gieren, A., Sturm, M. & Hoppe, W. (1979) *Liebigs Ann. Chem.*, 1570–1579
52. Wieland, T. & Schöpf, A. (1959) *Liebigs Ann. Chem.* **626**, 174–184
53. Wieland, T. & Rehbinder, D. (1963) *Liebigs Ann. Chem.* **670**, 149–157
54. Puchinger, E. & Wieland, T. (1969) *Liebigs Ann. Chem.* **725**, 238–240
55. Wieland, T. & Jeck, R. (1968) *Liebigs Ann. Chem.* **713**, 196–200
56. Wieland, T., Deboben, A. & Faulstich, H. (1980) *Liebigs Ann. Chem.*, 416–424
57. Faulstich, H., Trischmann, H. & Mayer, D. (1983) *Exp. Cell Res.*, 73–82
58. Wulf, E., Deboben, A., Bautz, F.A., Faulstich, H. & Wieland, T. (1979) *Proc. Natl. Acad. Sci. US* **76**, 4498–4502
59. Wieland, T., Hollosi, M. & Nassal, M. (1983) *Liebigs Ann. Chem.*, in press
60. Wieland, T. & Buku, A. (1969) *FEBS Lett.* **4**, 341–342

61. Barak, L.S., Yocum, R.R., Nothnagel, E. & Webb, W.W. (1980) *Proc. Natl. Acad. Sci. US* **77**, 980–984
62. Barak, L.S. & Yocum, R.R. (1981) *Anal. Biochem.* **110**, 31–38
63. Faulstich, H. & Wieland, T. (1971) *European J. Biochem.* **22**, 79–86
64. Wieland, T., Freter, K. & Gross, E. (1959) *Liebigs Ann. Chem.* **626**, 154–173
65. Faulstich, H. & Trischmann, H. (1970) *Liebigs Ann. Chem.* **741**, 55–63
66. Wieland, T. & Sangl, I. (1964) *Liebigs Ann. Chem.* **671**, 160–164
67. Fahrenholz, F., Faulstich, H. & Wieland, T. (1971) *Liebigs Ann. Chem.* **743**, 83–94
68. Munekata, E., Faulstich, H. & Wieland, T. (1977) *Angew. Chem.* **89**, 274–275
69. Michl, K. (1981) *Liebigs Ann. Chem.*, 33–39
70. Munekata, E., Faulstich, H. & Wieland, T. (1979) *Liebigs Ann. Chem.*, 1020–1027
71. Munekata, E., Faulstich, H. & Wieland, T. (1977) *J. Am. Chem. Soc.* **99**, 6151–6152
72. Munekata, E., Faulstich, H. & Wieland, T. (1977) *Liebigs Ann. Chem.*, 1758–1765
73. Faulstich, H., Nebelin, E. & Wieland, T. (1973) *Liebigs Ann. Chem.*, 50–58
74. Heber, H., Faulstich, H. & Wieland, T. (1974) *Int. J. Peptide Protein Res.* **6**, 381–389
75. Munekata, E. (1981) in *Perspectives in Peptide Chemistry* (Eberle, A., Geiger, R. & Wieland, T., eds.), pp. 129–140, Karger, Basel
76. Savige, W.E. (1975) *Austr. J. Chem.*, 2275–2287
77. Nakagawa, M., Kato, S., Kataoka, S., Kodato, S., Watanabe, H., Okajima, H., Hino, T. & Witkop, B. (1981) *Chem. Pharm. Bull.* **29**, 1013–1026
78. Savige, W.E. & Fontana, A. (1976) *J. Chem. Soc. Chem. Commun.*, 600–601
79. Savige, W.E. & Fontana, A. (1980) *Int. J. Peptide Protein Res.* **15**, 102–112
80. Wieland, T., Birr, C. & Zanotti, G. (1978) *Angew. Chem.* **90**, 67–68
81. Wieland T., Miura, T. & Seeliger, A. (1982) *Int. J. Peptide Protein Res.* **21**, 3–10
82. Wieland, T., de Vries J.X., Schäfer, A.J. & Faulstich, H. (1975) *FEBS Lett.* **54**, 73–75
83. Buku, A., Faulstich, H., Wieland, T. & Dabrowski, J. (1980) *Proc. Natl. Acad. Sci. US* **77**, 2370
84. Nakashima, T. & Volcani, E. (1969) *Science* **164**, 1400
85. Kahl, J.U. & Wieland, T. (1981) *Liebigs Ann. Chem.*, 1445–1450
86. Wieland, T., Motzel, W. & Merz, H. (1953) *Liebigs Ann. Chem.* **581**, 10–16
87. Faulstich, H., Kommerell, B. & Wieland, T. (eds.) (1980) *Amanita Toxins and Poisoning.* Gerhard Witzstrock, Baden-Baden
88. Wieland, T. (1972) *Naturwissenschaften* **59**, 225–231 (Review)
89. Faulstich, H. (1980) in *Progress in Molecular and Subcellular Biology* (Hahn, F.E., ed.), vol. 7, pp. 88–134, Springer, Berlin (Review)
90. Brodner, O.G. & Wieland, T. (1976) *Biochemistry* **15**, 3480–3484
91. Vaisius, A.C. & Wieland, T. (1982) *Biochemistry* **21**, 3097–3101
92. Lutter, L.C. (1982) *J. Biol. Chem.* **257**, 1577–1578
93. Wieland, T. & Boehringer, W. (1960) *Liebigs Ann. Chem.* **635**, 178–181
94. Gieren, A., Narayanan, P., Hoppe, W., Hasan, M., Michl, K., Wieland, T., Smith, H.O., Jung, G. & Breitmaier, E. (1974) *Liebigs Ann. Chem.*, 1561–1569
95. Tonelli, A.E., Patel, D.J., Wieland, T. & Faulstich, H. (1978) *Biopolymers* **17**, 1973–1986
96. Wieland, T., Götzendörfer, Ch., Dabrowski, J., Lipscomb, W.N. & Shoham, G. (1983) *Biochemistry* **22**, 1264–1271
97. Kostansek, E.C., Lipscomb, W.N., Yocum, R.R. & Thiessen, W.E. (1978) *Biochemistry* **17**, 3790–3796
98. Wieland, T. & Gebert, U. (1966) *Liebigs Ann. Chem.* **700**, 157–173
99. Buku, A., Altmann, R. & Wieland, T. (1974) *Liebigs Ann. Chem.*, 1580–1586
100. Wieland, T. & Fahrmair, A. (1970) *Liebigs Ann. Chem.* **736**, 95–99
101. Cessi, C. & Fiume, L. (1969) *Toxicon* **6**, 309–310
102. Faulstich, J., Trischmann, H., Wieland, T. & Wulf, E. (1981) *Biochemistry* **20**, 6498–6504
103. Wulf, E., Bautz, F.A., Faulstich, H. & Wieland, T. (1980) *Exp. Cell. Res.* **130**, 415–420
104. Faulstich, H., Zobeley, S. & Trischmann, H. (1982) *Toxicon* **20**, 913–924
105. Faulstich, H. & Trischmann, H. (1976) Hoppe-Seyler's *Z. Physiol. Chem.* **354**, 1395
106. Falck-Pedersen, E., Morris, P.W. & Venton, D.L. (1983) *Int. J. Peptide Protein Res.*, in press
107. Morris, P.W., Venton, D.L. & Kelly, K.M. (1978) *Biochemistry* **17**, 690–698
108. Buku, A. & Wieland, T. (1975) *Liebigs Ann. Chem.*, 1956–1960
109. Wieland, T. & Brodner, O.G. (1976) *Liebigs Ann. Chem.*, 1412–1414
110. Buku, A., Altmann, R. & Wieland, T. (1974) *Liebigs Ann. Chem.*, 417–431
111. Wieland, T., Buku, A. & Altmann, R. (1976) in *Proc. 14th European Peptide Symp.* (Loffet, A., ed.), pp. 523–526, University of Brussels, Brussels

112. Cochet-Meilhac, M. & Chambon, P. (1974) *Biochim. Biophys. Acta* **353,** 160–184
113. Zanotti, G., Birr, C. & Wieland, T. (1981) *Int. J. Peptide Protein Res.* **18,** 162–168
114. Wieland, T., Götzendörfer, C., Zanotti, G. & Vaisius, A.C. (1981) *European J. Biochem.* **117,** 161–164
115. Wieland, T. & Faulstich, H. (1978) *Crit. Rev. Biochem. CRC* **5,** 185–260
116. Besl, H. (1981) *Z. Mykol.* **47,** 253–255

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