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Poisonous Principles of Mushrooms of the Genus Amanita

Four-carbon amines acting on the central nervous system and cell-destroying cyclic peptides are produced.

Theodor Wieland

The true poisonous mushrooms are of the genus *Amanita*. The best known of them, widely spread throughout the world, except for the tropic zones, is undoubtedly the fly agaric, *Amanita muscaria* (L. ex Fr.) Pers. ex Gray; its toxicity, however, is generally overestimated. Much more capable of inciting fatal poisoning is the white mushroom *A. verna* (Bull. ex Fr.) Pers. ex Vitt. sensu Fr. 1821 non al. (*A. virosa* Lam. ex Secr.). *Amanita tenuifolia* (*1*), and *A. bisporigera* Atk. (*2*) are probably closely related varieties. In Central Europe, the green death-cap *A. phalloides* (Vaill. ex Fr.) Secr., the *grüne Knollenblätterpilz*, is noted for its toxicity. The more frequently occurring yellow mushroom *A. citrina* (Schaeff.) Gray [*A. mappa* (Batsch ex Lasch) Quél.] definitely contains no peptidic toxins—unlike *A. phalloides* in this respect—but it does contain bufotenine (5-hydroxy-*N*-dimethyltryptamine) in relatively high concentration (*3*) and some other indole amines (*4*). It is of some interest that bufotenine occurs also in several other plants, and that other components of poisonous amanitas have been found also in mushrooms of different *Galerina* species (*5*). I shall outline the present state of knowledge of the components of *A. muscaria* and give a summary of the chemistry and toxicology of the poisonous peptides of *A. phalloides*.

Amanita muscaria (*6*), the fly agaric, grows from July until the end of autumn, preferably under fir or birch trees, as an egg-shaped white cap. Within 1 to 2 days the cap spreads and bursts open the outer shell, the surface then becoming brilliant red with the residue which remains as white spots regularly distributed over it. The white veil protecting the lamellae in the young mushroom now hangs at the stem as a cuticular ring. The stem, which grows up to 25 centimeters, has a cup at its bottom. The cap may reach 25 centimeters in diameter; the weight of an average mushroom is 60 to 70 grams. Different subspecies of varying colors, like the native North American one that is orange yellow, have been described.

The name "fly" agaric was derived from an insect-killing property of its extracts; however, this characteristic is inconspicuous compared to modern insecticides and can only be observed under special conditions. Wasson (*7*) interprets the "flies" as a symbol for the demonic power of the mushroom, which has been used in Siberia as a hallucinogen because of its psychotropic principles. The symptoms of intoxication from the use of *A. muscaria* are very complex and resemble those of drugs that act on the central nervous system, but with the addition of associated peripheral phenomena attributable to muscarine.

Problem of Muscarine

The search for the toxic principle of *Amanita muscaria* started over 100 years ago and led Schmiedeberg and Koppe in 1869 to a substance that excites the parasympathetic nervous system. This substance they named "muscarin" (*8*, *9*). The sensation caused among pharmacologists by the first

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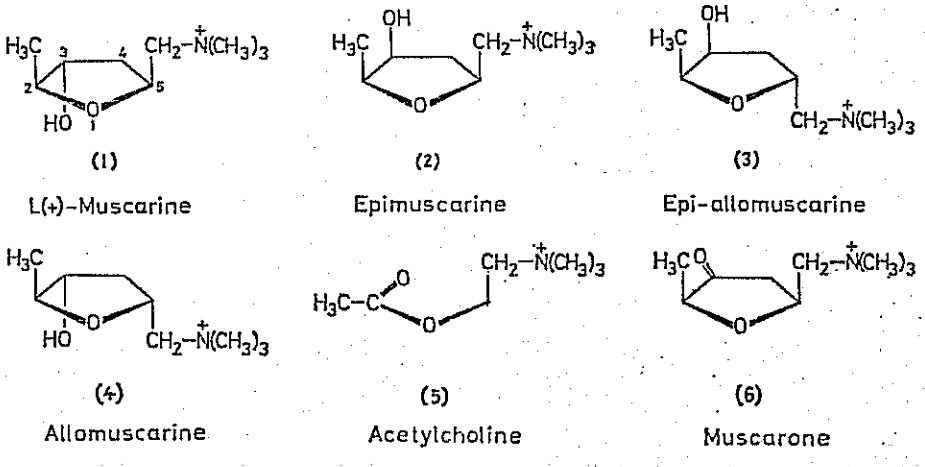
totally impure drug substance was surpassed only by the later discovery of the Vagusstoff, acetylcholine (No. 5), by Loewi (*10*). Incidentally, acetylcholine was also isolated from *A. muscaria* by Kögl *et al.* in 1957 (*9*). Attempts to prepare muscarine (No. 1) in the pure state and to establish its structural formula were successfully completed in 1954, with the crystallization of muscarine chloride and the determination of its composition as $C_9H_{20}O_2N^+Cl^-$ by Eugster and Waser (*7*); in 1957 the same team and the Dutch group of Kögl and Jellinek (*11*) settled the structure of the molecule mainly by the use of x-ray crystallography. Accordingly, muscarine (compound 1) is an oxoheterocyclic quaternary salt, 2-methyl-3-hydroxy-5-trimethyl-ammoniummethyltetrahydrofuran chloride with S-configuration at position-2, R- at position-3, and S- at position-5 of the ring.

The total synthesis of compound 1, which succeeded immediately, disclosed the whole area. In view of the three centers of asymmetry, we anticipate four diastereoisomeric racemates, compounds 1 to 4, that is, eight antipodes; nearly all have been obtained. I do not intend to enumerate here all the synthetic approaches; the first syntheses of (±)-muscarine and of its diastereoisomers (*12*) may stand as examples. From the furan derivative (No. 7), obtained by condensation of ethylacetoacetate with glucose, through a reaction sequence (shown at lower right), the important (±)-normuscarone (No. 8) was prepared. From compound 8, not only normuscarine and, by quaternization, (±)-muscarine were available, but also with the use of various reducing agents the tertiary compounds of the salt compounds 2, 3, and 4 became accessible. The racemic muscarine was resolved into the antipodes with the (+)- and (−)-ditoluoyltartrates.

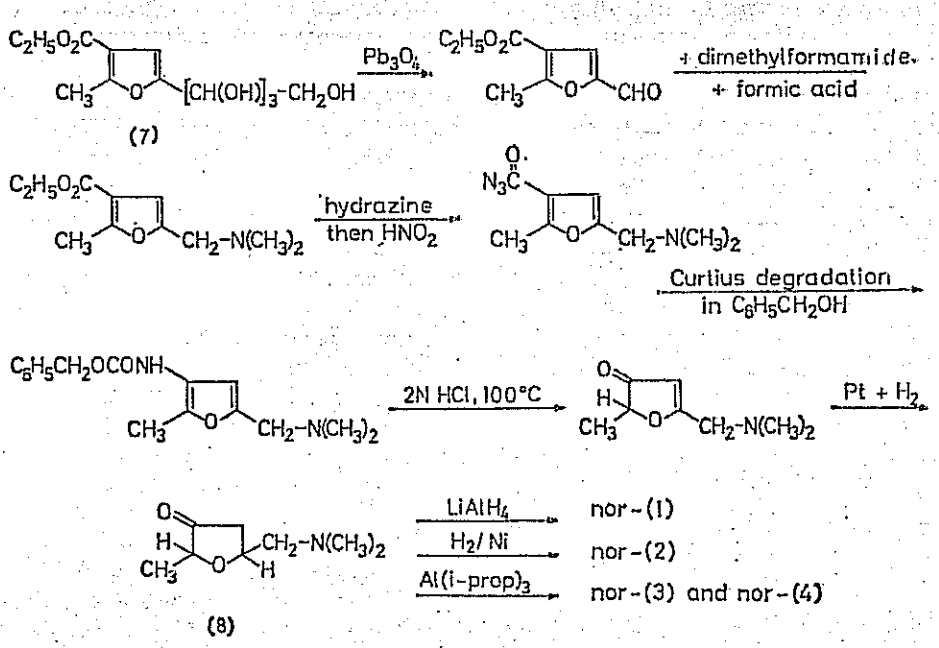
The enantiomorphs of (±)-muscarine differ greatly in potency, which is almost exclusively associated with the (+)-isomer. This alkaloid exhibits a highly specific activity at postganglionic parasympathetic effector sites. Its effect as measured by the decrease of blood pressure of anesthetized cats (minimum dose of the magnitude of 10^{-8} gram per kilogram of body weight) or by the reduction of amplitude and rate of the beat of the perfused frog heart, is nearly equal to that of acetylcholine, the (−)-isomer being about 1000 times less active. The same relation holds for (+)-muscarine, as



compared with the diastereomeric racemates. No muscarine isomers have any significant nicotine-like action on skeletal muscles. The closely related (+)- and (−)-muscarone iodides (*6*) have even greater activity in the blood-pressure test than acetylcholine, and they exhibit strong nicotine-like activity on the isolated rectus abdominalis of the frog. Certain similarities of the structures of acetylcholine (No. 5) and particularly of ketone (No. 6) point to analogous mechanism of physiological action.

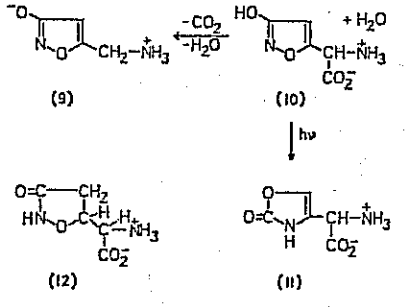
Further Search for Active Principles

To judge from results of numerous pharmacological experiments and in view of the extremely low concentration of muscarine in *A. muscaria* (0.0002 percent of the fresh mushroom), it must be concluded that the famous alkaloid cannot possibly be the poisonous principle. Other mushrooms, particularly of the genus *Inocybe*, contain much larger amounts of muscarine; for example, *Inocybe patouillardi* (Bres.) *s. lateraria* Ricken contains 0.037 percent. These mushrooms cause muscarine toxicoses which are distinctly different from the toxic state produced by *A. muscaria*. In the latter case there are symptoms of hallucinogenic and centrally acting substances. The 5-hydroxyindole alkaloid, bufotenine, seemed to appear in paper chromatograms in my laboratory (*3*), an observation not confirmed by others; but other unidentified indole bases are said to occur in *A. muscaria*. This is of great interest in connection with the psychotropic 4-hydroxyindole derivatives psilocybin and psylocin from the Mexican teonanacatl (*13*) (Psilocybe, sp. *Stropharia cubensis*). In view of the fact that atropine and scopolamine in *Amanita muscaria* of South African and Polish origin was not also found in Dutch

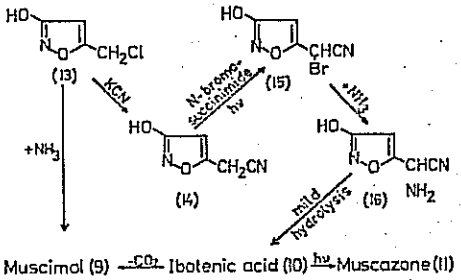


and German specimens (*6*), it is apparent that the investigation of the toxic drugs has not yet come to a satisfactory end.

Chemically rather simple, centrally active crystalline substances, muscimol (No. 9), the fly killing ibotenic acid (No. 10), and the related muscazone (No. 11) have been discovered in the fly agaric and in other mushrooms. Compounds 9 and 10 strengthen the narcotic power without being narcotics. Nevertheless, muscimol (No. 9), which easily results from ibotenic acid (No. 10) by decarboxylation, may be considered as an essential principle of *Amanita muscaria*, merely on account of the high concentration of 0.03 to 0.1 percent of compound 10 in fresh tissue.



The isoxazole derivatives No. 9 and No. 10 were isolated in 1960 and shortly afterward by Eugster and his colleagues and by other research groups independently. These derivatives were isolated from several mushrooms, and they were provided with several names. Consensus in nomenclature has now been achieved (*14*). Muscimol (No. 9) stands for the former *Pyroibotensäure* and for agarine similarly obtained from *Amanita muscaria* by English chemists (*15*). Ibotenic acid (No. 10) has also been isolated from *A. strobiliformis* (Vitt.) Quél.—which is the equivalent of *A. solitaria* (Fr.) Quél.—and from *A. pantherina*. Tricholomic acid (No. 12), a compound with insecticidal properties obtained from the mushroom *Tricholoma muscarium* Kawamura (*16*), is the erythro-2,3-dihydro derivative of ibotenic acid (No. 10).



One sequence out of several syntheses is represented (*17*) by the reaction of compound No. 13, 3-hydroxyl-5-chloromethylisoxazol, with ammonia to give muscimol (No. 9) in good yield. The same compound with cyanide forms the cyanomethylisoxazole (No. 14), whose α-position of the side chain is brominated with *N*-bromosuccinimide in the light. The bromonitrile (No. 15) is converted to the aminonitrile (No. 16) with ammonia, and mild hydrolysis of the amino nitrile leads to ibotenic acid (No. 10); decarboxylation of compound 10 gives compound 9. An interesting photoreaction—comparable to a Lossen rearrangement of hydroxamic acid derivatives—converts the isoxazol system of compound 10 into the 2-(3*H*)-oxazolone ring of muscazone (No. 17). Tricholomic acid (No. 12) and its threo-isomer have been synthesized from erythro- and threo-3-hydroxyglutamic acid (*18*). Both tricholomic acid (No. 12) and ibotenic acid (No. 10) were discovered in Japan as a result of research conducted to elucidate the fly-killing property of the corresponding mushrooms. However, an accidental finding, that these substances have great tastiness, their flavors being about 20 times more intense than that of sodium glutamate, was reported by the Japanese investigators. This effect and an additional synergism in taste with nucleotide seasonings make the new compounds interesting with regard to their possible use in food formulation.

Amanita phalloides and Related Species

The deadly poisonous, green mushroom *Amanita phalloides* (Vaill. ex Fr.) Secr., is sometimes called the "death cap"; it grows in Central Europe from July until the end of October and is associated with deciduous trees; it grows preferably under beeches in loose forests that are not too dry. It develops from an egg-shaped white state within 1 to 2 days to a height of about 10 to 15 centimeters. The slightly vaulted cap reaches diameters up to 12 centimeters; it is smooth, more or less deep olive-green, and often patterned with darker, radially extending, filamentous streaks. The lamellae are white, the stem, which sometimes shows pale greenish cross stripes, bears at its upper part a big white cuff and ends in a large tuber which is surrounded by a leafed sheath. The toxic mushroom has no specific smell or taste, in contrast to the related nontoxic *A. citrina* (Schaeff.) Gray. This yellow mushroom is easily discernible by its odor of raw potato. The white toxic variety, *A. verna* (Bull. ex Fr.) Pers. ex Vitt., appears during the summer, in deciduous forests; in Europe its occurrence is not common. It is the "destroying angel" or "deadly agaric" of the North American continent. The white *A. virosa* (Lam. ex Secr.) is now considered identical to *A. verna*. It contains the same toxic peptides that *A. phalloides* does. The other varieties in North America (*1*, *2*), *A. tenuifolia* and *A. bisporigera*, which are extremely rich in toxins, have not yet been observed in Europe according to my knowledge.

The white or green *Amanita* causes more than 95 percent of fatal mushroom poisoning. These mushrooms are mistakenly confused with the delicious champignon *Tricholoma equestre* (the white forms with *Agaricus campestris*), which appears in similar places, but which bears from the beginning faintly pink, and later darker red, lamellae

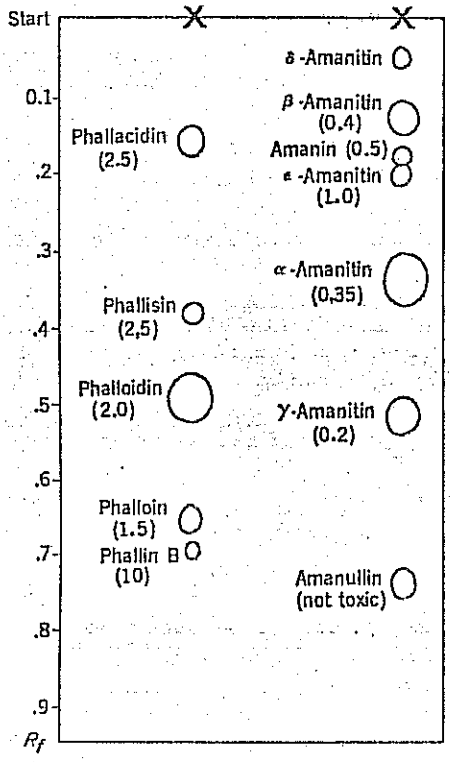


Fig. 1. Descending paper chromatogram in a butanone, acetone, water system (30 : 3 : 5) of the identified ingredients of *Amanita phalloides*. For clarity, the phallotoxins (left) are arranged separately from amatoxins (right). Size of spots is a rough indication of the relative amounts; figure in parentheses is the minimum lethal dose per kilogram of body weight of the white mouse.

and has a typical anise-like smell. The high percentage of fatalities occurs from *Amanita phalloides* and related mushrooms because the first symptoms of intoxication—vomiting and diarrhea, which are not caused by the lethal toxins—do not become apparent until several hours after ingestion, and during this time the deadly toxins have already reached the liver. We know with certainty that the slow-acting amatoxins cause death by destroying the liver cells. Their fatal action, however, starts shortly after the toxins reach the organ and is irreversible.

The exploration of the poisonous substances of *A. phalloides* started at the beginning of the last century. A survey was made in 1959 (*19*) and more recently (*20*). In 1937, F. Lynen and U. Wieland (*21*) in the Munich Chemical Laboratories of the Bavarian State succeeded in isolating a toxic material in crystalline form which they called "phalloidin." Four years later, H. Wieland and R. Hallermayer (*22*) obtained an even more toxic component and named it "amanitin." Both these toxins are representatives of two families of poisonous peptides. My collaborators and I have resolved amanitin into a neutral (α-amanitin) and an acidic (β-amanitin) toxin; and added to the poisonous substances already discovered γ-amanitin, δ-amanitin, ϵ-amanitin, and amanin. This group is called the family of amatoxins. The phallotoxins include phalloidin, phalloin, phallacidin, phallisin, and phallin B. The chromatogram of a variety of substances, including the nontoxic amanullin, are schematically represented in Fig. 1.

Paper chromatography has proved very useful for analysis of crude mushroom extracts and of the various fractions obtained during isolation work. After numerous experiments, a mixture of butanone, acetone, and water (30 : 3 : 5 by volume) was found to allow separation of nearly all of the phytotoxins (Fig. 1). The solvent mixture has been modified by addition of some *n*-butanol by Block *et al.* (*23*). In thin-layer analysis on silica gel G, mixtures of butanone and methanol (1 : 1) or of *n*-butanol, acetic acid, and water (4 : 1 : 1) were successful (*2*). The amatoxins (except amanin) give an immediate violet color with cinnamic aldehyde in an atmosphere of hydrochloric acid gas; the phallotoxins and amanin slowly give a weaker blue color. With diazotized sulfanilic acid (Pauly reagent), the first group forms an intense red compound, whereas the phallotoxins give only a weak yellow color. The much greater sensitivity of the amanitins to color reagents may be the reason why the spot of phalloidin has not been observed in mushroom analyses of other laboratories. Here a column chromatographic separation combined with ultraviolet spectroscopy of the eluent seems indispensable (*24*). In the poisonous species there are about 10 milligrams of phalloidin, 8 milligrams of α-amanitin, 5 milligrams of β-amanitin, and 0.5 milligrams of γ-amanitin per 100 grams of fresh tissue (corresponding to about 5 grams of dry weight). Similar values for α- and β-amanitin have been reported by Tyler *et al.* for *A. phalloides* and *A. verna*, but a higher content (up to 5 milligrams per gram of dry material) in *A. bisporigera* (*2*). Since the lethal dose of the amanitins is lower than 0.1 milligram per kilogram of body weight for human beings, it is conceivable that the toxin content of one mushroom weighing 50 grams (about 7 milligrams of amanitins) may be sufficient to kill a man.

In cases of poisoning, a prominent feature is always the long period of latency between ingestion and the appearance of the first symptoms. Usually not until 10 to 24 hours have elapsed do abrupt violent emesis and diarrhea begin, and the illness sometimes culminates in rapid death, with cholera-like manifestations. These gastrointestinal signs have nothing to do with the toxic peptides. If this phase is overcome, a transient and false remission takes place and gradually more pronounced signs of injury to parenchymatous organs appear, chiefly the liver. A hemolytic agent which is precipitated by organic solvents and is very labile on heating, was obtained from *Amanita phalloides* as early as 1891 by Kobert and was called "phallin." This substance is presumably also destroyed in the gastrointestinal tract and therefore is scarcely responsible for lethal poisoning.

Chemistry of the Toxic Peptides

Chemically, the toxins have some characteristics in common. All of them are cyclopeptides (with a molecular weight of about 1000) composed of only a few amino acids, some of which do not occur in proteins. Each cyclopeptide contains a gamma-hydroxylated amino acid and a sulfur atom derived from a cysteine residue which is connected to the indol nucleus of a tryptophan side chain, thus dividing the cycle into two rings. In the ultraviolet range, they exhibit intensive absorption maxima near 300 nanometers (Figs. 2 and 3). One of the differences between the two families lies in the rapidity and mode of their toxic action. The phallotoxins act quickly and, at higher doses, death in animals occurs within 1 to 2 hours. In contrast, the action of the amatoxins is delayed

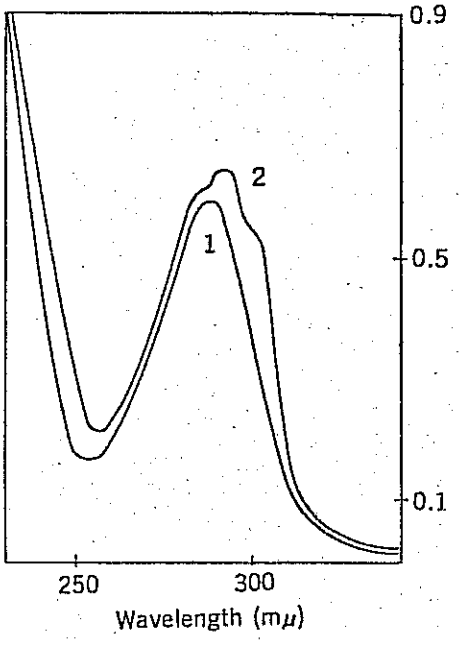


Fig. 2. Qualitative ultraviolet absorption spectra in water of amanin (curve 1) and phalloidin (curve 2).

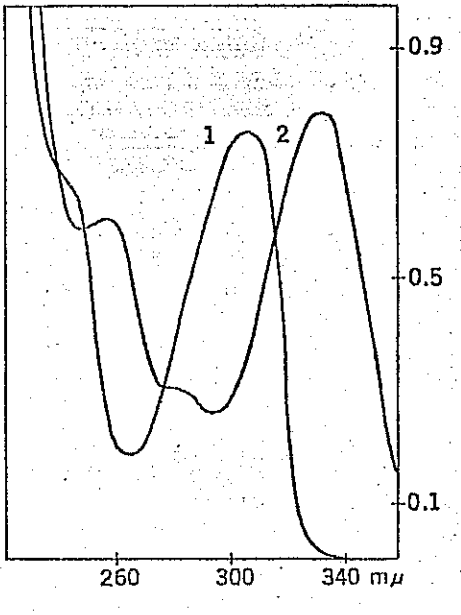
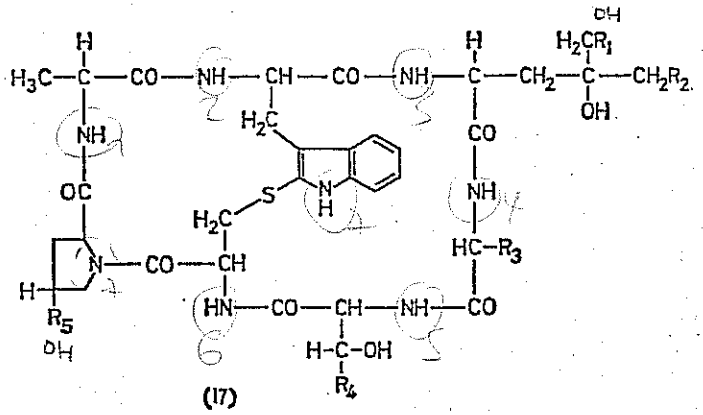


Fig. 3. Qualitative ultraviolet spectra in water of α-amanitin in neutral solution (curve 1), and after addition of a few drops of very diluted NaOH (curve 2).



(17)

		R_1	R_2	R_3	R_4	R_5
a	Phalloidin	OH	H	CH_3	CH_3	OH
b	Phalloin	H	H	CH_3	CH_3	OH
c	Phallisin	OH	OH	CH_3	CH_3	OH
d	Phallicidin	OH	H	$CH(CH_3)_2$	CO_2H	OH
e	Phallin B (tentatively)	H	H	$CH_2C_6H_5$	CH_3	H

so that with very high doses it is not possible to reduce the time required for lethal action to less than 15 hours. The relative toxicity is, however, just the opposite, α-amanitin being 10 to 20 times more toxic than phalloidin and therefore the major poisonous constituent of deadly amanita.

Phallotoxins

The group of phallotoxins consists of at least five members (Fig. 1), whose common cyclic heptapeptide skeleton is shown in formula 17.

After these toxins are heated with 6*N* hydrochloric acid, six different amino acids are liberated, and in the process the thioether bridge is split into L-β-oxindolyl-3-alanine (No. 18) and L-cysteine (No. 19) ("nucleophilic" hydrolysis at the α-carbon).

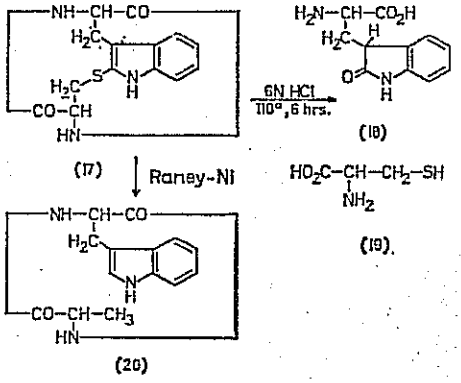
The characteristic ultraviolet spectrum of phallotoxins (Fig. 2) is due to the thioether of tryptophan. Compounds of this type can be synthesized by coupling β-substituted indoles with sulfenyl chlorides. The 2-thioether obtained from β-indolylacetic acid and methylsulfenyl chloride, CH_3SCl, is identical to phalloidin in the spectrum (curve 2) of Fig. 2. Curve 1 represents amanin, which is also a derivative of tryptophan, but—as a sulfoxide—it belongs to the family of amatoxins.

When treated with Raney-nickel in boiling methanol, the thioether bridge of the phallotoxins is opened hydrogenolytically, thus giving nontoxic dethio compounds (No. 20). The only amino acids common to all of the five phallotoxins are L-alanine and the coupling product, called tryptathionine, of L-cysteine and L-tryptophan. Other amino acids present in the different toxins are: L-allohydroxyproline (No. 21) in nearly all of them (No. 17, a to d) except in phallin B (17e) which contains instead proline; D-threonine (No. 22) in (No. 17, a–c and e); L-valine, and D-erythrohydroxyaspartic acid (No. 23) in phallacidin (No. 17d); and phenylalanine in phallin B (No. 17e).

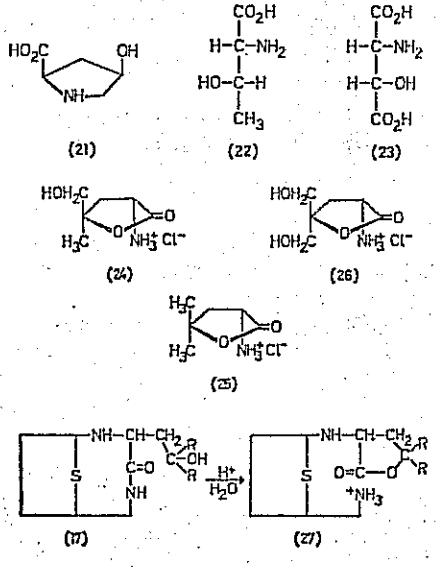
In all toxic amanita peptides, including the amatoxins, a γ-hydroxylated amino acid occurs. The γ-hydroxy group in the side chain causes a selective and mild splitting of the peptide bond (50 to 80 percent trifluoroacetic acid at 20°C, 2 hours) by its neighboring group effect through γ-lactone formation. The seco- compounds (No. 27) obtained by such a partial hydrolysis are totally nontoxic like the dethio compounds (No. 20).

From a hydrolyzate of phalloidin (17a) the γ-lactone-hydrochloride of one diastereoisomer of γ,δ-dihydroxyleucine has been isolated, to which we ascribed the erythro structure (No. 24). This does not permit a conclusion to the native configuration; during hydrolysis of the peptide, an inversion at the γ-carbon might have occurred. Under the same conditions, phalloin (17b) yields the lactone of γ-hydroxyleucine (*25*). This compound, among other aminolactones, was also discovered recently in hydrolyzates of gelatin (*25*). In phallisin (No. 17c) a trihydroxylated amino acid, γ,δ,δ'-trihydroxyleucine-lactone (No. 26) could be disclosed as a result of periodate oxidation: aspartic acid was formed from the open acid of compound 27 by an excessive glycol-splitting reaction (*26*).

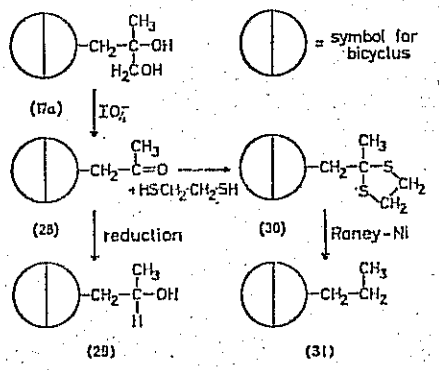
So it appears that all phallotoxins contain γ-hydroxyamino acids derived from L-leucine. As to the function of the γ-hydroxy group as a prerequisite of toxicity, several experiments have been conducted to remove or to substitute this group in the intact molecule. The following sequence of reactions gave the decision: phalloidin (No. 17a) was oxidized by periodate to give the still toxic "ketophalloidin" (No. 28). The toxic properties of compound 28 can still be referred to a hydroxyl group in compound 29, which may be formed



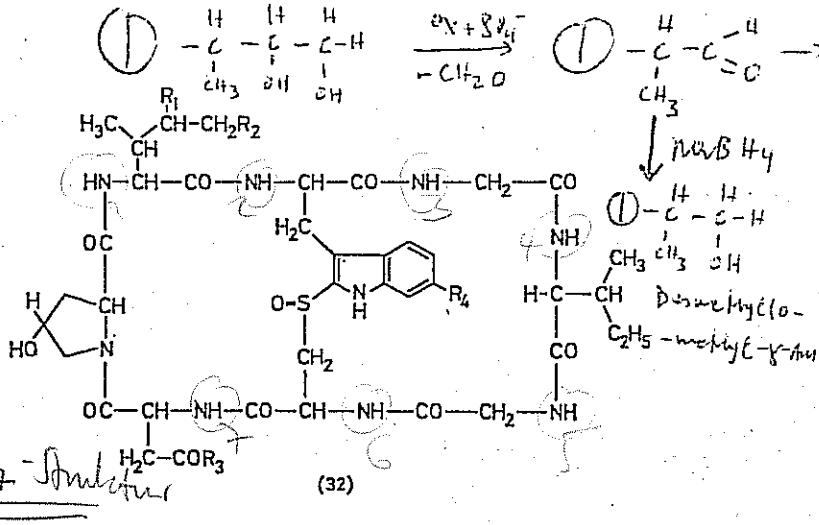
(17) (18) (19) (20)



(21) (22) (23) (24) (25) (26) (17) (27)



(17a) (28) (29) (30) (31)



(32)

		R_1	R_2	R_3	R_4
a	α-Amanitin	OH	OH	NH_2	OH
b	β-Amanitin	OH	OH	OH	OH
c	γ-Amanitin	H	OH	NH_2	OH
d	Amanin	OH	OH	OH	H
e	Amanullin	H	H	NH_2	OH

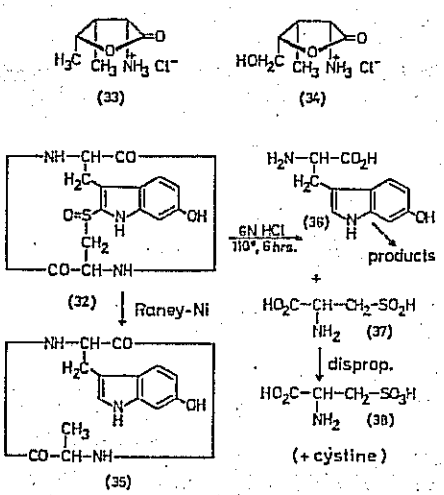
by enzymatic hydrogenation of the carbonyl of structure 28 in the liver. Therefore, a dithiolane (No. 30) was prepared from compound 28 (*27*) which was again toxic. Finally, the sulfur atoms of compound 30 could be substituted by hydrogen without attacking the thioether bridge by means of Raney-nickel catalyst (*28*). The resulting norphalloin (No. 32) proved as toxic as phalloidin, thus eliminating the presence of a γ-hydroxyl group as a requirement for toxicity of the phallotoxins.

Amatoxins

The group of amatoxins has at least six members, whose common cyclic octapeptide skeleton is shown in formula No. 32. The minor compounds δ- and ε-amanitins have been isolated in a pure state, but their exact structure has not yet been elaborated. To this group also belong the "outsiders" amanin (No. 32d) and amanullin (No. 32e). The former differs from β-amanitin (No. 32b) only in its lacking of a phenolic hydroxyl group in position-6 of the indole nucleus. Therefore, the color reactions of amanin resemble these of the phallotoxins, but the toxicological (slow) action is that of the amatoxins.

α-Amanitin (No. 32a) is the amide of the carboxylic acid β-amanitin (No. 32b). During acid hydrolysis the amino acids not concerned in the thioether bridge are liberated in the original state: glycine (2 moles), L-aspartic acid, L-hydroxyproline, L-isoleucine and, varying among the members, L-γ-hydroxyisoleucine as lactone (No. 33) from γ-amanitin (No. 32c) and γ,δ-dihydroxyisoleucine (No. 34) from α- or β-amanitin (No. 32, a and b) and from amanin (No. 32d). Thus the hydroxylated amino acids of amatoxins are derived from isoleucine.

Amanullin (No. 32e) is totally nontoxic. It contains no γ-hydroxylated side chain, but rather a second molecule of isoleucine (*28a*). In contrast to the phallotoxins, the presence of a γ-hydroxy group is apparently a prerequisite for toxic activity. The identification of the natural γ-hydroxyisoleucine with one of the eight possible diastereoisomeric antipodes as compound 33 has been made very recently chiefly by nuclear magnetic resonance spectroscopy (*29*). It is supposed that the corresponding γ-, δ-dihydroxyamino acid of α-amanitin has an analogous structure (No. 34). Likewise the phallotoxins (No. 27), also



nontoxic secoamatoxins, are formed by treatment with trifluoroacetic acid at room temperature. Similarly, the dethio compounds (No. 35) obtained with Raney-nickel in the amanitin series are also nontoxic.

The chromophoric part of the amatoxins until very recently was supposed to consist of a similar 2-thioether of an indole, the sole difference from phallotoxins being a hydroxyl in the 6-position. This phenolic group causes a shift of the ultraviolet maximum to longer wavelengths (302 mμ) as compared with phallotoxins, and an additional bathochromic shift after the addition of a little alkali as a consequence of phenate ion formation (Fig. 3). From oxidation and reduction experiments with model thioethers, H. Faulstich arrived at a sulfoxide structure of the amatoxins (*30*).

Accordingly, on acid hydrolysis the thioether bridge is broken in a way different from that in phallotoxins. Here a predominantly "electrophilic" hydrolysis is observed, one which results in 6-hydroxytryptophan (No. 36) and cysteinesulfinic acid (No. 37). The latter compound manifests itself as cysteic acid (No. 38), a product of disproportionation. The tryptophan derivative (No. 36) is very labile to acids and can be detected only in traces.

Amanin (No. 32d), which has an unsubstituted tryptophan moiety like phalloidin, is also a sulfoxide and is hydrolyzed in an analogous way, and tryptophan and cysteic acid are formed.

On the Mechanism of Toxic Action

The first point of attack of both the toxins is the liver (*31*). There are, however, considerable differences in toxicities and in modes of action. Phalloidin has a marked affinity to the microsomal fraction of the liver cell. This has been shown by perfusing an isolated rat liver with radioactive toxin, and homogenizing, and fractionating the homogenate by centrifugation. Only the microsome fraction retained a constant activity after several washings (*32*). This result corresponds well to that of von der Decken *et al.* (*33*), who found that incorporation of amino acids into the protein of the liver microsome fraction was inhibited when the animals were poisoned with phalloidin 1 to 2 hours before.

Here, and in its other effects on liver metabolism, phalloidin acts only if it has been administered to the intact

animal. This fact led the Swedish group (*33*), and recently Fiume (*34*), to the idea that phalloidin is not toxic in itself, but is converted into a toxic compound by drug-metabolizing enzymes of the liver. Newborn rats endure highly toxic doses of the substance without being killed. Apparently a toxic effect is absent as long as the system of drug-metabolizing enzymes is very rudimentary. If these enzymes are damaged by carbon tetrachloride or other liver poisons (*35*), toxicity of phalloidin is reduced, too. In spite of all of these observations, there has been no consistent evidence for a toxification in liver up to now.

Further insight into the mechanism of the toxic action was obtained by electron microscopy (*36*). This method confirmed that only the cells of liver are changed by phalloidin. The earliest ultrastructural changes—observable 15 minutes after injection of phalloidin—are dilatation of the endoplasmic reticulum, which occasionally forms very large vacuoles, swelling of mitochondria, and deposition of fat droplets. The alterations of the reticulum offer a plausible explanation of the reduced protein synthesis in the liver of intoxicated animals (*33*) and are also consistent with the observation of a preferred adsorption of radioactive toxin by the microsomal elements of liver (*32*).

The mechanism of the action of the amanitins is distinctly different from that of the phallotoxins. As already mentioned, the evidence of intoxication appears considerably later. Here the nuclei are primarily affected, as recent electron micrographs of Fiume and Laschi (*36*) demonstrate. Without any damage in the endoplasmic reticulum, the nucleoli of liver cells begin to fragment 15 hours after α-amanitin is given to rats. Cytoplasmatic lesions follow the nuclear ones and presumably are a consequence of the nuclear damage. The cytotoxic effects of the amanitins are not restricted to the liver cells; cytological changes are also observed in kidney. Fiume and Stirpe (*37*) have observed that, in mouse liver nuclei, the RNA content decreases progressively during the first 24 hours of an intoxication of the animals with α-amanitin. Since the toxin prevents neither the incorporation of ^{3}H-uridine and ^{3}H-thymidine into the nucleic acids of human tumor cells of the KB-Eagle line, nor the replication of viruses, nor the reproduction of bacteria, it is suggested that RNA synthesis is not impaired, at least in these systems (*38*). Mouse liver cells, however, showed a clear response to α-amanitin in their reduced ability to incorporate ^{14}C-orotic acid into RNA, and an effect in vitro has been found after all by the Italian pathologists. α-Amanitin added to normal mouse liver nuclei inhibited the RNA polymerase reaction activated by manganous ion and ammonium sulfate (*39*) by about 80 percent at 10^{-7} molar concentration (*40*).

Accordingly, as in the case of phalloidin and also with the amanitins, an insight into the mechanism of action on a molecular basis has not yet been achieved. One might expect that this would also be of general interest in cell physiology.

Mushrooms of the genus *Amanita* contain numerous biologically active substances. Muscarine, exciting the parasympathetic nervous system, has been isolated from *Amanita muscaria* (fly agaric), its chemical structure having been established and synthesized mainly by Swiss workers. It is presumably not the poisonous principle. Research in Swiss, Japanese, and British laboratories has led to the discovery of fly-killing and centrally active substances of the isoxazole type like "muscimol," "ibotenic acid," and "tricholomic acid." The green, deadly, agaric *Amanita phalloides* and its white relatives contain two groups of lethal sulfur-containing cyclopeptides, the "phallotoxins" and the "amatoxins," whose structures have been determined. Among other unusual amino acids, they contain leucine and isoleucine residues wich are hydroxylated in γ- and δ-positions. The phallotoxins act on the endoplasmatic reticulum of liver cells, whereas the nucleus is the target of the cytopathogenic action of the amatoxins.

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