MEDICINAL PLANTS

THE CHEMISTRY OF PEPTIDE ERGOT ALKALOIDS. PART 1. CLASSIFICATION AND CHEMISTRY OF ERGOT PEPTIDES

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INTRODUCTION

Ergot alkaloids is a group of natural biologically active compounds belonging, according to the general alkaloid classification, to the class of indole derivatives. Ergot alkaloids are found in largest amounts in the fungal species of *Claviceps* genus, the most widely occurring source of these substances being purple ergot (*Claviceps purpurea Fr. Tul.*) [1 – 6]. Another well-known fungal species producing alkaloids of the clavine series is *Claviceps paspali* [1, 3, 4, 6 – 8]. Ergot alkaloids are rarely encountered in higher plants; examples are offered by the *Ipomoea* genus of the *Convolvulaceae* family occurring in Central America [4, 9, 10].

The life cycle of ergot consists of three sequential stages, including the (i) conidial stage of sphacelia, (ii) sclerotium (resting stage), and (iii) sephalated stroma with perithecium. The alkaloids are obtained from ergot sclerotium called "spurred rye" (*Secale cornutum*) [4 – 6, 11].

The ergot sclerotium contains up to 30 – 40% of fatty oils and up to 2% of alkaloids. The other components of sclerotium are free amino acids, ergosterin, choline, acetylcholine, betaine, ergothionine, uracil, guanidine, free aromatic and heterocyclic amines (tyramine, histamine, agmatine), and alkylamines (the natural representatives of which were originally found in ergot). The outer shell of sclerotium contains acid pigments belonging to anthraquinolinic acid derivatives, including orange-red (endocrocin, clavorubin) and light-yellow (ergochromes, ergochrysins). These pigments impart to the sclerotium shell the characteristic grayish-brown-violet color [6, 12 – 15]. Also encountered are the albino ergot strains incapable of producing pigments [16].

Investigation of the wild-growing *Cl. purpurea* species revealed the existence of numerous geographic types differing in both qualitative and quantitative composition of alkaloids. To the present, ergot strains were specially selected that are capable of producing predominantly a single alkaloid or a certain group of alkaloids: ergotamine, ergotoxin, ergocristine, etc. [3, 4, 6, 16 – 27].

Owing to a high biological activity and broad spectrum of pharmacological effects, ergot alkaloids are of considerable importance for medicine. These compounds are now obtained both by methods of artificial parasitic cultivation on rye [11, 14, 19 – 22, 25 – 30] and by saprophytic growth techniques [31 – 35].

The most widely known pharmacological effect of ergot alkaloids is the ability to induce uterine contraction. Ergot extracts were officially used for the first time in gynecology. The best known drugs of this type are ergotamine and ergometrine.

As the chemical structure and pharmacological properties of ergot alkaloids were studied, the scope of their application considerably expanded. Ergot alkaloids exhibit a complicated action upon the organism, which accounts for a variety of possible therapeutic applications. Dihydroxy derivatives of ergotoxin alkaloids produce α-adrenoblocking and "venotonic" action. These compounds are administered in cases of hypertension, cerebral vasospasms, peripheral circulation disorders, etc. [36 – 39].

Many ergot alkaloids produce a more or less pronounced dopaminergic effect. Specific dopamine agonists are 2-Br-α- and 2-Br-β-ergocryptines, which influence the secretion of hypophyseal anterior hormones and produce a hypotensive action upon the CNS, sympathetic nerve endings, and smooth vascular muscles. The drug abergin (Russia) [36, 37] or parlodel (bromergon, bromocriptine mesylate) are used for the treatment of a wide spectrum of disorders, including galactorrhea, acromegalia, mastites, prolactinomas, amenorrhea, and infertility and for the suppression of lactation, Cushing's disease, some hormonal tumors, etc. [1, 36 – 44].

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The ergot alkaloids are administered both separately and in numerous complex compositions [39]. At present, extensive investigations aimed at the selection of new ergot strains, creation of new original domestic drugs based on ergot alkaloids, and development of the corresponding technologies are in progress in the All-Russia Research Institute of Medicinal and Aromatic Plants.

CLASSIFICATION OF ERGOT ALKALOIDS

The structure of ergot alkaloids is built around a tetracyclic ring system of ergoline (Fig. 1). Depending on the structure of ring D in the ergoline nucleus and the types of substituents at C^8, all ergot alkaloids can be divided into three biogenetically related classes: clavine ergot alkaloids, simple lysergic acid derivatives, and peptide ergot alkaloids (ergot peptides). The biosynthesis of ergot alkaloids is genetically controlled [1, 4 – 6, 32, 41, 45 – 52].

The classes of simple lysergic acid derivatives and peptide ergot alkaloids are based on the lysergic acid (LA) fragment (Fig. 1), the structure of which was elucidated in 1938. The two classes are distinguished by the type of substituents in the acid radical [1, 4, 6 – 8, 53]. The LA molecule possesses two centers of symmetry (C^5 and C^8, Fig. 1). The active LA form was assigned a stereoconfiguration of 8R : 5R. Thus, all the natural pharmacologically active argot alkaloids are derivatives of *D*-6-methyl-9,10-ergoline-8β-carboxylic acid, representing a series of left-hand rotation isomers [4, 6 – 8, 53 – 58].

In 1964, H. Kobel et al. [59] isolated paspalic acid from the *Cl. paspali* ergot species (Fig. 1), which differs from LA by the presence of a double bond C^8=C^9. Paspalic acid is the nucleus in some ergot alkaloids of the clavine series and is frequently employed as an initial compound in the synthesis of various LA derivatives.

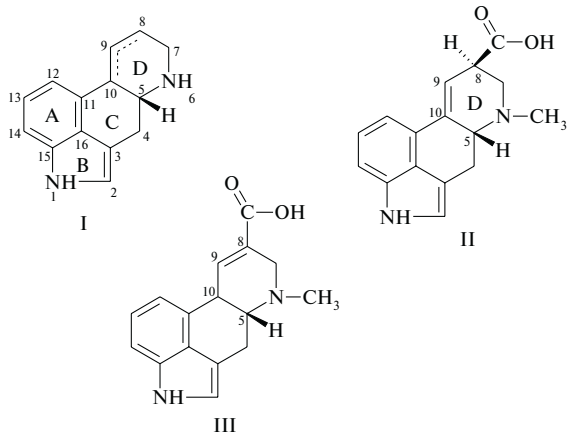

Fig. 1. The structural formulas of ergoline (I), lysergic acid (II), and paspalic acid (III).

THE CHEMISTRY OF PEPTIDE ERGOT ALKALOIDS

This review addresses the chemistry of ergot peptides. This class includes both natural ergot alkaloids and their modified derivatives, ergot peptides isolated from saprophytic ergot cultures, and synthetic ergot alkaloids.

TABLE 1. Classification of Peptide Ergot Alkaloids

R^1	R^2								Configuration at C^8
	$-CH_3$		$-C_2H_5$		$-CH(CH_3)_2$		$-CH(CH_3)C_2H_5$		
	Ergotamine group	Ergotaman group	Ergoxin group	Ergoxam group	Ergotoxin group	Ergotoxam group	β-Ergoannam group		
$-CH(CH_3)_2$	Ergovaline	[Ergovalam][1]	Ergonine	[Ergonam]	Ergocornine	Ergocornam	–		R
	Ergovalinine	–	Ergoninine	–	Ergocorninine	–	–		S
$-CH_2CH(CH_3)_2$	α-Ergosine	[α-Ergosam]	α-Ergoptine	[α-Ergoptam]	α-Ergocryptine	α-Ergocryptam	α,β-Ergoannam[3]		R
	α-Ergosinine	–	α-Ergoptinine	–	α-Ergocryptinine	–	–		S
$-CH(CH_3)C_2H_5$	β-Ergosine[2]	[β-Ergosam]	β-Ergoptine[2]	[β-Ergoptam]	β-Ergocryptine	β-Ergocryptam	α,β-Ergoannam[3]		R
	β-Ergosinine	–	β-Ergoptinine[2]	–	β-Ergocryptinine	–	–		S
$-CH_2C_6H_5$	Ergotamine	[Ergotaman]	Ergostine	[Ergostam]	Ergocristine	Ergocristam	–		R
	Ergotaminine	–	Ergostinine	–	Ergocristinine	–	–		S
$-CH_2CH_3$	Ergobine[3]	–	Ergobutine[3]	–	Ergobutinine[3]	–	–		R
$-CH_3$	Ergoalanine[2]	–	–	–	–	–	–		R

Notes. [1] Names in square brackets refer to ergot alkaloids suggested to exist; [2] synthetic ergot alkaloids; [3] ergot alkaloids isolated from saprophytic ergot cultures.

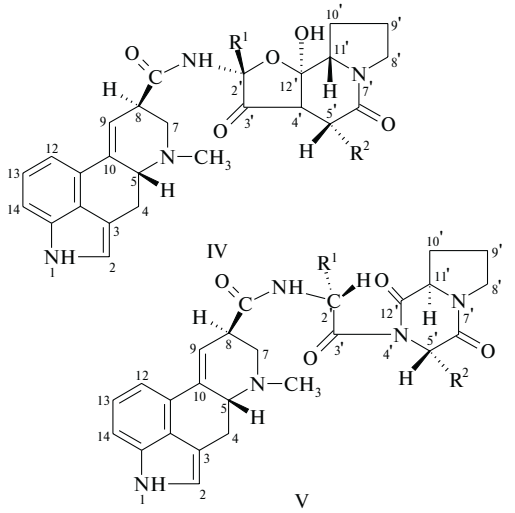

Fig. 2. The structural formulas of classical peptide ergot alkaloids: ergopeptines (IV) and lactam ergot alkaloids – ergopeptams (V).

Peptide ergot alkaloids contain the LA fragment and a tripeptide group (Fig. 2), the structure of the latter group determining classification of the ergot peptides (Table 1). The LA fragment is responsible for the basic biological activity of the compounds (serotonin antagonism, oxytocin activity, etc.), while the substituent imparts certain specificity to this general activity.

The class of ergot peptides can be subdivided into two main types (Table 1). Ergopeptines represent the classical cyclic ergot alkaloids (cyclopeptides) with the oxazolopyrrolopyrazine system (cyclole) as substituent in the LA radical (Fig. 2) [4, 56, 61 – 63]. Ergopeptines are the most widely spread natural type of peptide ergot alkaloids.

The second type of peptide ergot alkaloids is represented by the group of lactam ergot alkaloids – ergopeptams (E-*seco*-ergot alkaloids) with diketopiperazine peptide substituents (Fig. 2) [4, 63 – 67]. It was suggested that ergopeptams are formed as a result of competitive epimerization at the last stage of cyclopeptide biosynthesis [46, 62 – 66]. Individual representatives of ergopeptines and ergopeptams possess different substituents R^1 and R^2 in the peptide fragment, which serves as a basis for their classification (Fig. 2, Table 1) [1, 4, 6 – 8, 53].

Individual Representatives of Ergot Peptides

Attempts at isolating individual ergot alkaloids were undertaken since the beginning of the 19th century. The first alkaloid (ergotinine) was isolated by Tanret in 1875. A mixture of peptide ergot alkaloids called "ergotoxin" was isolated in 1906 [1, 6]. In 1918, Stoll isolated ergotamine – the first clinically useful ergot alkaloid – from *Cl. purpurea* (Table 2). By the 1990s, most of the natural ergopeptines were isolated and structurally characterized (Table 2) [1, 4, 53, 68 – 79].

Ergopeptams (Fig. 2) were originally found in small amounts in certain ergot strains as substances accompanying the classical ergot alkaloids. The first lactam ergot alkaloid – ergocristam – was isolated in 1973 from an ergocristine strain of *Cl. purpurea*. Note that, to the present, only ergopeptams of the ergotoxam group were isolated from natural ergot [4, 64 – 66] (Table 2). The probability of existence of this alkaloid type decreases with decreasing volume of radical R^1, which is explained by the high lability of ergopeptams. The presence of a branched radical R^1 (Fig. 2, Table 1) favors the formation of a more stable configuration of the tripeptide group in lactam alkaloids. In 1995, we obtained a mutant sclerotium of the ergocryptine *Cl. purpurea* strain [67], which gave a progeny with genetically fixed biosynthesis of the lactam ergot alkaloid ergocornam (Table 1).

In 1984, unusual $C^{5'}$-epimers of lactam alkaloids were isolated from a saprophytic culture of the ergocryptine ergot species, which opened a new group of ergot alkaloids called β-ergoannams. It should be noted that ergot alkaloid derivatives untypical of natural ergot are frequently found in saprophytic cultures. Examples are offered by 12′-O-methyl derivatives of ergocornine and α-ergocryptine (Table 2), 8-hydroxyergotamine, and some other alkaloids [34, 77].

In attempts to obtain more active analogs of the natural ergot alkaloids, a large number of derivatives were synthesized by modifying both the LA fragment and the peptide part of the molecule [1, 35, 68]. For example, a series of synthetic ergot alkaloids were obtained by modifying the proline moiety of the peptide fragment, where *L*-proline was replaced by *D*-proline, α-methyl-*L*-proline, and *L*-pipecoline acid; analogs free of the proline cycle were synthesized as well [1].

Another series of ergot alkaloid derivatives were obtained by modifying peptide fragments in $C^{5'}$ position with methyl, *p*-methoxy-benzyl, *L*-norvalyl, and other R^2 substituents (in Table 1, these derivatives are represented by ergoalanine). Some of the analogs contained two substituents in $C^{5'}$ position or represented epimers with respect to $C^{5'}$ and $C^{13'}$ positions ($R^2 = -C^{13'}H(CH_3)C_2H_5$) of the peptide fragment. Most of the synthetic alkaloids were reported in the 1960 – 1980s [1].

C^8-Epimerization of Ergot Peptides

Lysergic acid derivatives readily exhibit epimerization, especially in the presence of alkalis, with respect to the center of symmetry C^8 with the formation of a series of right-hand rotation (S)-isomers representing isolysergic acid (*iso*-LA) derivatives (Table 1). This group of ergot alkaloids exhibits a relatively weak pharmacological activity [3, 6 – 8]. The epimerization is due to the presence of a double bond $C^9=C^{10}$, which favors enolization of the carboxy group (Fig. 3) [60]. The *iso*-LA derivatives represent LA diastereomers with a stereoconfiguration of the 8S : 5R type [49, 53, 55, 60]. The results of investigations showed that ring D in both diastereomers occurs in a "pseudo-chair" conformation [4, 6, 8, 49, 55 – 58, 60, 79, 80].

According to the international classification, the left-hand rotation isomers of ergot alkaloids representing LA

derivatives (C^8(R) configuration) are termed ergopeptines and ergopeptams, while the right-hand rotation diastereomers representing the *iso*-LA derivatives (C^8(S) configuration) are termed ergopeptinines (Table 1).

The conditions for and the rate of epimerization in ergopeptines depend on the structure of substituent R at the carboxamide group of the LA fragment (Fig. 3). No (S)-isomers were reported for ergopeptams, which is related to a high lability of the lactam ergot alkaloids: in the presence of bases, these compounds readily decompose into simpler derivatives [4, 63 – 66, 81, 82].

In nature, ergopeptinines always accompany ergopeptines. Considerable amounts of the former compounds may form in the course of storage of raw materials for prolonged time or under improper conditions [83], in the process of ergot alkaloid extraction from the raw materials, and in other stages of the chemical processing [13, 30, 83 – 88]. Some investigations were devoted to the influence of external factors on the stability of ergot alkaloids in relation to the search for stabilizers, creation of stable medicinal preparations, and development of epimerization reversal [13, 89, 90]. It was found that ergopeptinines can be converted into ergopeptines in media containing an organic solvent (ethanol, methanol, acetone, etc.), water (10 to 95%), and acid (pH 18.5 – 5). The most pronounced epimerization activity was observed for mixtures containing phosphoric acid [91, 92].

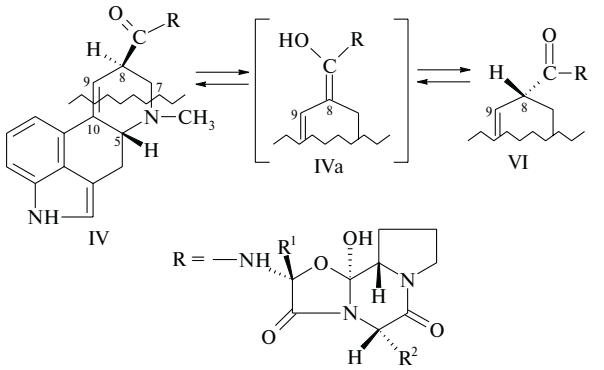

Fig. 3. The scheme of epimerization in LA derivatives (IV) with respect to C^8 position with the formation of *iso*-LA derivatives (VI). Here and below R is a polypeptide moiety with R^1 and R^2 indicated in Table 1.

TABLE 2. Most Known Representatives of Peptide Ergot Alkaloids

No.	Alkaloid name	Source: natural, saprophytic, synthetic	First isolated (year)	Ref.
1	Ergotamine	*Cl. purpurea*	1918	4, 7, 13, 56
2	α-Ergosine	Ergotamine-producing strains of *Cl. purpurea* and *Ipomoea argyrophylla Vatke*	1936	4, 6, 68 – 69, 71
3	Ergovaline	Synthesis, later isolated from *Cl. purpurea* and *Epichloe typhina* fungal culture (*Clavicepitaceae*)	1964	4, 53, 71 – 72
4	β-Ergosine β-Ergosinine	Synthesis	1977	1, 4, 68, 71
5	β-Ergoptine β-Ergoptinine	Synthesis	1977	1, 4, 68, 74
6	Ergostine Ergostinine	Ergotamine-producing strain of *Cl. purpurea*	1964	1, 4, 73
7	Ergonine α-Ergoptine	Ergocornine-ergocryptine strain of *Cl. purpurea*	1970	4, 8, 68, 73, 74
8	Ergobutine Ergobutyrine	Saprophytic culture *Cl. purpurea* 231 F. J.	1982	6, 35
9	Ergocristine	*Cl. purpurea*	1937	4, 70, 75
10	Ergocornine Ergocryptine	*Cl. purpurea*	1943	4, 70, 75
11	α-Ergocryptine β-Ergocryptine	*Cl. purpurea* *Hypomyces amantius* fungi (*Hypocreaceae*)	1967	4, 61, 70, 75, 76, 78
12	Ergocristam	Ergocristine strain of *Cl. purpurea*	1973	4, 64
13	Ergocryptam Ergocornam and isomers	Ergocristine strain of *Cl. purpurea*	1981	4, 46, 53
14	β-Ergoannams ($C^{5'}$-epimers of lactam ergot alkaloids)	Saprophytic ergocryptine culture	1984	4, 34
15	12′-O-Methyl derivatives of ergocornine and α-ergocryptine	Saprophytic culture *Cl. purpurea* 231 F. L.	1987	77

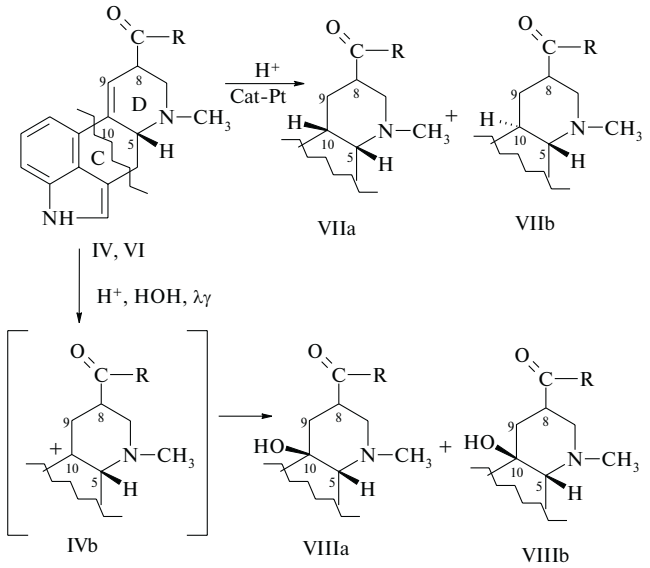

Fig. 4. The schemes of modification of ergopeptines (IV, VI) at C^9=C^{10}: (A) hydrogenation with the formation of *cis* and *trans* isomers of the 9,10-dihydro-LA or *iso*-LA derivatives (VIIa and VIIb, respectively); (B) hydroxylation with the formation of *cis* and *trans* isomers of the lumi-derivatives of LA or *iso*-LA (VIIIa and VIIIb, respectively).

Derivatives of the optical isomer opposite to *D*-LA (i.e., *L*-LA) are not encountered in nature. Such derivatives were obtained by chemical synthesis [57, 60]: *L*-LA exhibits a 5α-configuration of hydrogen at the asymmetry center C^5 (5S), whereas the natural ergot alkaloids exhibit the 5β configuration (Fig. 1) [4, 6, 7, 55]. For the first time, *L*-LA hydrazide was obtained in 1943 as a derivative formed in the course of racemization of peptide ergot alkaloids heated in hydrazine [13, 60]. The opposite optical isomer of ergotamine was obtained by stereoselective synthesis from *L*-LA and an opposite optical isomer of the peptide fragment [1, 7, 13].

A change in the configuration at C^5 may take place in the course of thermal treatment. Isomerization of this type is related to the presence of a double bond C^9=C^{10} in the molecules of ergot alkaloids. Under certain conditions, this bond can migrate to the C^5=C^{10} position. The formation of 5α-isomers was observed upon thermal treatments of solutions of simpler LA derivatives in a temperature interval from 130 to 200°C [93].

Derivatives of Ergot Alkaloids Modified at the C^9–C^{10} *Bond Position*

The presence of a double bond in C^9–C^{10} position explains a number of features characteristic of ergot alkaloids (including both (R) and (S) derivatives) and facilitates some chemical transformations. This bond constitutes a conjugated system of bonds with the indole fragment of a molecule, which is manifested in a typical UV spectrum of ergot peptides with λ_{max} = 313 nm and λ_{min} = 270 nm. The ergot peptides are also characterized by a bright-blue fluorescence. Violation of the conjugated bond system leads to a shift of the absorption maximum toward shorter wavelengths.

The C^9–C^{10} double bond favors the formation of lumi-, methoxy-, dihydro-, and some other derivatives of ergot alkaloids. Dihydro derivatives were obtained for the first time in 1943 by Stoll et al. [94]. In nature, the 9,10-dihydro derivatives of ergot alkaloids are very infrequent: it was not until 1968 that 9,10-dihydro-α-ergosine was isolated from sclerotium of *Sphacelia sorghi (McRae)* and *Sorghum vulgare* (*Claviceps*) [7, 95]. In 1979, up to 12% of 9,10-dihydroergotamine was isolated from the *Cl. purpurea* $PCCT_1$ strain culture [84].

The chemical reduction and catalytic hydrogenation of ergot alkaloids leads to the formation of 9,10-dihydro derivatives (Fig. 4) [96, 97], some which are used in medicine. This is accompanied by the appearance of another center of asymmetry in C^{10} position in the molecule (Fig. 4), which makes possible existence of this molecule in two isomer forms with *cis* and *trans* arrangement of rings C and D [6, 7, 55, 60, 98 – 100]. It was established that the hydrogenation of LA and its derivatives leads to the formation of *trans* isomers (as the more favorable confirmation) [7, 96]. In the case of hydrogenation of *iso*-LA and related compounds, the formation of *cis* isomers is more favorable. The possible conformations of 9,10-dihydro derivatives of ergot alkaloids were studied in [96].

The photochemical attachment of a water molecule at the C^9–C^{10} double bond in ergot alkaloids leads to the formation of 10-hydroxy-dihydro (or lumi-) derivatives of ergot alkaloids characterized by a high intensity of fluorescence. The structure of lumi-derivatives formed in aqueous solutions (where the process is catalyzed by light and the presence of hydrogen protons) was confirmed in 1955 (Fig. 4) [6, 101 – 103]. It was suggested that the formation of lumi- and methoxy-dihydro derivatives of ergot alkaloids proceeds via the formation of carbonium ions (Fig. 4). In these derivatives, as well as in the 9,10-dihydro derivatives, an additional center of asymmetry appears in C^{10} position of the lysergic acid fragment, which also makes possible the existence of molecules in two isomer forms with *cis* and *trans* arrangement of rings C and D (Fig. 4) [49, 102, 103]. For hydroxy-dihydro-LA and methoxy-dihydro-LA, the formation of *trans* isomers is more favorable.

In the presence of acids, the solutions of lumi-derivatives in methyl alcohol exposed to light exhibit methanolysis with the formation of 10-methoxy-dihydro derivatives. Lumi-derivatives are stronger bases than the natural ergot alkaloids of their 9,10-dihydro derivatives, which is explained by the ability of the OH group in position C^{10} to increase the basicity of nitrogen atom N^6. For this reason, the hydroxy group in position C^{10} is readily methylated in acid methanol solutions with the formation of less polar 10-methoxy derivatives of ergot alkaloids. Some ergot alkaloids are capable of directly converting into 10-methoxy-dihydro derivatives in acid methanol solutions [1, 6, 102, 104].

Derivatives of Ergot Alkaloids Substituted in C^2, C^3, C^{12}, *and* C^{13} *Positions*

Ergot alkaloids exhibit some characteristic chemical reactions due to the presence of the indole fragment. The nitrogen atom in this indole fragment, as well as in other indole derivatives, possesses amphoteric properties. Weak acidity of the NH proton is manifested in the formation of salts during the interaction with metallic potassium or concentrated alkali solutions. Weak basicity is manifested in the reaction of formation of 1-acyl derivatives of ergot alkaloids [1, 105]. Similar to indole and pyrrole, ergot alkaloids are readily oxidized in air to form dark-colored compounds. Under the action of acids, these alkaloids form condensed products also colored brown or dark grayish brown.

From the standpoint of various modifications, the most readily accessible site is C^2 position of the indole fragment (Fig. 2) [1, 106, 107]. In 1957, Troxler and Hofmann [105, 106] produced selective halogenation of ergot alkaloids in C^2 position with the formation of 2-Cl, 2-Br, and 2-I derivatives under mild conditions (with N-Br-succinimide in dioxane, N-I-succinimide, or N-Cl-2,6-dichloro-4-nitroacetanilide). Investigation of the physicochemical properties and pharmacological activity of 2-halogen derivatives of ergot alkaloids led to the discovery of a therapeutically important compound 2-Br-α-ergocryptine mesylate [42, 108].

The bromination of ergot alkaloids with an HBr – HBrO mixture in excess pyridine leads to the formation of doubly substituted 2,13-dibromo derivatives. Some other C^2-substituted derivatives were obtained as well (e.g., 2-nitro derivatives, etc.), but these compounds still did not find any application [1].

The reduction of ergot alkaloids with zinc dust in the presence of hydrochloric acid leads to the formation of 2,3-dihydro derivatives possessing a new center of asymmetry in position C^3 with a favorable 3β-configuration. Stadler et al. [61] suggested a scheme of this transformation and showed that the selective hydrogenation is possible only under mild conditions. More severe reduction leads to saturation of the double bond in position C^8–C^9 and of the benzene ring in the indole fragment of ergot alkaloid molecules [109].

The interaction of 9,10-dihydro-lysergol with CF_3COOH and BF_3-Et_2O or $POCl_3$ leads to the formation of a dimeric derivative substituted in position C^2 [110]. An analogous derivative was isolated during investigations of the ergot alkaloid metabolites. This derivative exhibited decomposition in an acid medium with the formation of a compound analogous to the metabolite formed as a result of enzymatic oxidation of ergot alkaloids [1, 87, 88, 110].

Investigation of the oxidized metabolites (LA derivatives) showed that the enzymatic oxidation leads to the formation of physiologically inactive 2-oxo derivatives of ergot alkaloids [1, 111]. Analogous compounds were found upon the reduction of 2-oxo-3-hydro derivatives of ergot alkaloids with zinc dust (the products of oxidation of ergot alkaloids with hypochloric acid). The physicochemical properties of

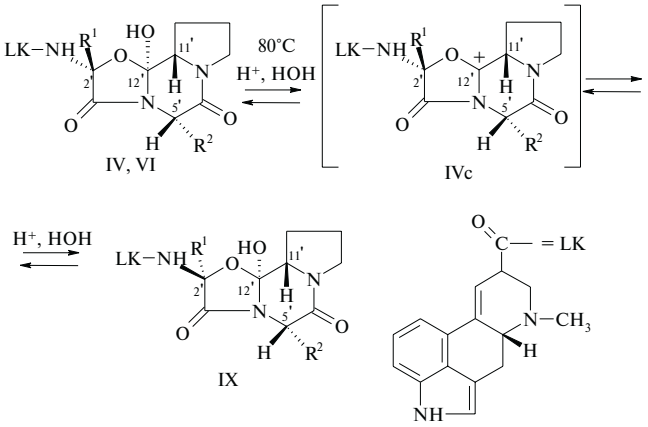

Fig. 5. The scheme of $C^{2'}$ epimerization of ergopeptines (IV, VI) into *aci*-ergopeptines (IX): the transformation begins with the formation of an intermediate cation (IVc). Here and below LK denotes the LA or *iso*-LA residue.

2-oxo-2,3-dihydro and 2-oxo-2,3,9,10-tetrahydro derivatives of ergot alkaloids are different from those of the natural analogs, which is explained by modification of the indole fragments of the molecules. The alkaline hydrolysis of oxindoles takes place at the amide bond with the formation of amphoteric compounds containing amino groups and featuring a color reaction of diazocoupling [110]. Oxidation of the 2,3-dihydro derivatives of ergot alkaloids with $NO(SO_3K)_2$ led to the formation of a series of products representing 12-hydroxy-2,3-dihydro derivatives of these alkaloids [1, 109].

The 1- and 2-substituted derivatives of ergot alkaloids lead to a change in color or a negative response in qualitative color reactions with specific reagents [105].

The Structure and Stereoconfiguration of the Peptide Fragment of Ergot Alkaloids

The peptide fragment structure in cyclopeptides was determined by Stoll and Hofmann [7, 112, 113] using the results of investigation of the products of thermal decomposition, hydrolysis, and oxidation of ergot alkaloids. These data were confirmed by the stereoselective synthesis of ergotamine, which showed evidence of the 2′R : 5′S : 11′S : 12′S configuration (Fig. 2). Thus, the cyclole fragment of the ergot alkaloid molecule contains four centers of asymmetry at which the formation of isomers may take place.

All natural ergot alkaloids possess a 5′-(S)-configuration. There are only several $C^{5'}$-epimers (5′R) isolated from saprophytic ergot cultures, including 5′-*epi*-β-ergocryptine and a group of β-ergoannams (Tables 1 and 2) [1, 34]; a series of $C^{5'}$-epimers (5′R) were obtained by synthetic methods [1]. The configuration of the peptide fragment of lactam ergot alkaloids differs from that of cyclopeptides in position $C^{11'}$ (11′R) (Fig. 2). Attempts at the synthesis of cyclole $C^{11'}$-(R)-epimers failed because of poor stability of the products [1].

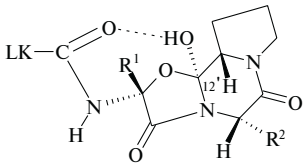

Fig. 6. The scheme of intramolecular interaction between cyclole hydroxy group at $C^{12'}$ and carboxamide group of LA fragment (LA).

The isomerization most readily takes place at the center of asymmetry in $C^{2'}$ position of the cyclole fragment of the molecules. The $C^{2'}$-(S)-isomers of ergopeptines are referred to as *aci*-ergopeptines [113 – 116]. The $C^{2'}$-isomerization takes place on heating aqueous solutions of ergot alkaloids. The reaction starts with the formation of carbocations that appear as intermediate products (Fig. 5) [116]. In the natural ergot alkaloids, this type of isomerization accompanied the isomerization in C^8 position of the ergoline nucleus.

In 1966, the structure of *aci*-isomers was confirmed by x-ray diffraction. The rate of isomerization and the isomer ratio depend on the conditions (temperature, pH) and the ergot alkaloid structure. These isomers can form as a result of degradation of ergot alkaloid based drugs stored for a prolonged time or under improper conditions in the form of aqueous solutions [113 – 116].

The *aci*-isomers are characterized by smaller values of pK_a and the optical rotation angle as compared to those of the natural ergot alkaloids. The cyclole hydroxy groups in *aci*-isomers are subject to methylation under milder conditions than those required for the natural ergot alkaloids [113]. The formation of 12′-OCH_3 derivatives is considered as an artifact accompanying the isolation of ergot alkaloids from methanol solutions: these isomers are never encountered in natural ergot.

In the course of investigation of the stereoconfiguration of the cyclole fragment of ergot alkaloids, it was suggested that $C^{12'}$-hydroxy derivatives may exist in the form of two epimers [7, 115]. It was established that all natural ergot peptides represent 12′-α-epimers (12′S) [6, 8]. These epimers are characterized by intramolecular interaction between the cyclole hydroxy group and the carboxamide group of LA (Fig. 6) [116]. The ^1H NMR spectra of ergot alkaloids show evidence of ω-interaction between the hydrogen atom in the cyclole hydroxy group and hydrogen atom in position $C^{11'}$ (J = 1.7 Hz), which is characteristic of this type of bonding.

Hydrolysis, Pyrolysis, and Oxidation of the Peptide Fragment of Ergot Alkaloids

The cyclole fragment of ergopeptines can be readily decomposed. In the course of metabolism of ergot alkaloids, the decomposition (catabolism) begins with the proline fragment. Depending on the conditions of hydrolysis, the decomposition of the cyclole fragment may proceed in different ways (Fig. 7) [112, 117, 118]. In the case of weak hydrolysis, the fragment exhibits an incomplete decomposition with the

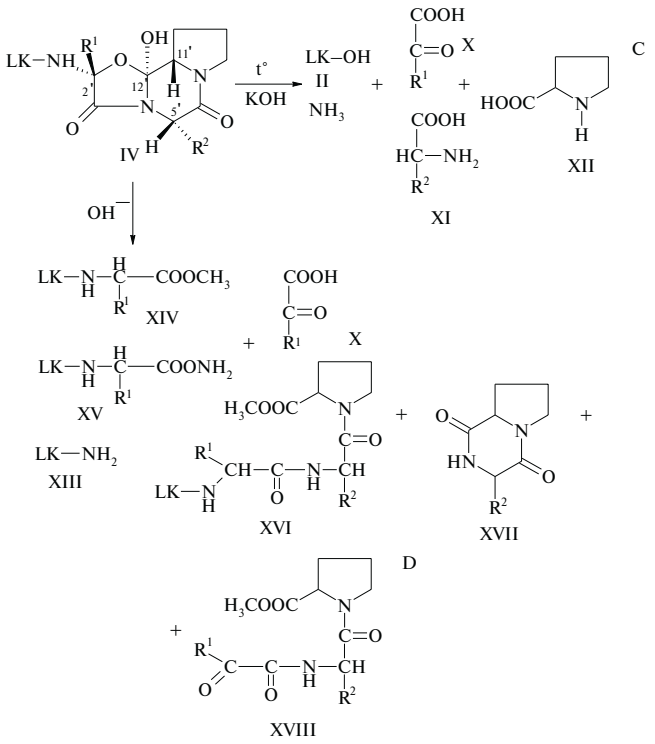

Fig. 7. The schemes of hydrolysis of the peptide fragment of an ergopeptine molecule (IV): (C) complete hydrolysis (in concentrated alkali solution or by heating) with the formation of LA (II), α-ketoacid (X) (with the structure depending on the radical R^1, Table 1), and two amino acids (one being proline XII and the other depending on the radical R^2, Table 1); (D) incomplete hydrolysis with the formation of α-ketoacid (X), LA amide (XIII), peptide and tripeptide N-(*D*-lisergyl) derivatives (XIV, XV, XVI), 2,5-dioxopiperazine derivatives (XVII), and di- or tripeptides (XVIII, for R^1 and R^2 see Table 1).

formation of LA amide and incomplete hydrolysis products (Fig. 7). Rigid hydrolysis leads to the complete decay of the cyclole fragment with the formation of LA, ammonia, two amino acids, and α-ketoacid (Fig. 7). One of the two amino acids is proline, which is a common molecular fragment for all peptide ergot alkaloids and appears upon the hydrolysis of any such compound. The alkaline hydrolysis leads to the formation of *L*-proline, while the acid hydrolysis is accompanied by the appearance of *D*-proline [1, 7, 8, 53].

Depending on the type of substituent R^2 in position $C^{5'}$, the second amino acid can be phenylalanine, leucine, valine, or 2′-aminobutyric acid [1, 6, 53]. The type of α-ketoacid formed as a result of the hydrolysis depends on the structure of substituent R^1 in position $C^{2'}$ and plays a key role in the classification of ergot alkaloids (Table 1).

The lactam ergot alkaloids are more reactive than cyclopeptides. In methanol solutions, especially in the presence of alkalis, the former alkaloids immediately decompose with

the formation of simpler derivatives, some of which correspond to the products of hydrolysis of ergopeptines (Fig. 8) [4, 64 – 66, 81].

The pyrolysis and oxidation of ergot alkaloids are also accompanied by the formation of simpler derivatives, some of which are identical to the products of hydrolysis of these ergot alkaloids [1, 6, 53, 56, 112 – 116]. The pyrolysis leads to the formation of a pyro derivative, the structure and stereoconfiguration of which were studied in [118 – 120]. Under the action of alkalis, this derivative decomposes with the formation of 2,5-dioxopiperazine, a compound considered as an artifact accompanying the isolation of ergot alkaloids [81, 119, 120].

In conclusion, it should be emphasized that peptide ergot alkaloids are labile compounds the decomposition of which is catalyzed by temperature, light, oxidizers, reducers, alkalis, acids, etc. These compounds readily decompose under improper conditions of storage, isolation, and purification and in the course of chemical analysis and modification. This circumstance must be taken into account during technological treatments and the chemical and analytical handling of ergot peptides, in developing stable medicinal forms, and in the storage of raw materials, semiproducts, parent substances, and ready-to-use medicinal preparations. Optimum storage implies low temperatures (not above 5°C), inert gas atmosphere, and hermetic and light-tight package.

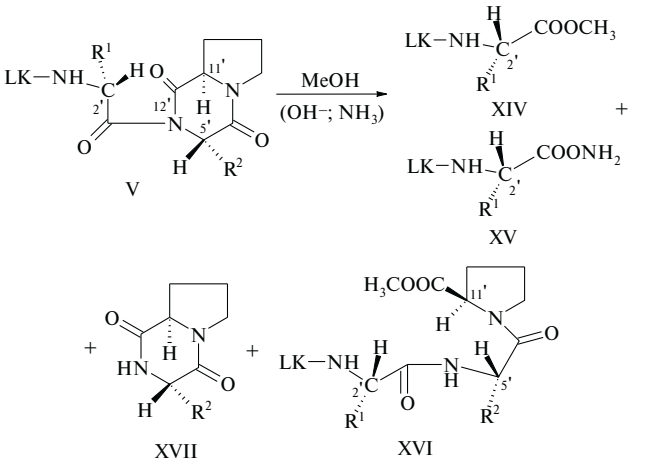

Fig. 8. The scheme of methanolysis of ergopeptams (V) with the formation of compounds corresponding to the products of incomplete hydrolysis of ergopeptines (XIV – XVII), where LK denotes the LA residue (*D*-lisergyl).

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