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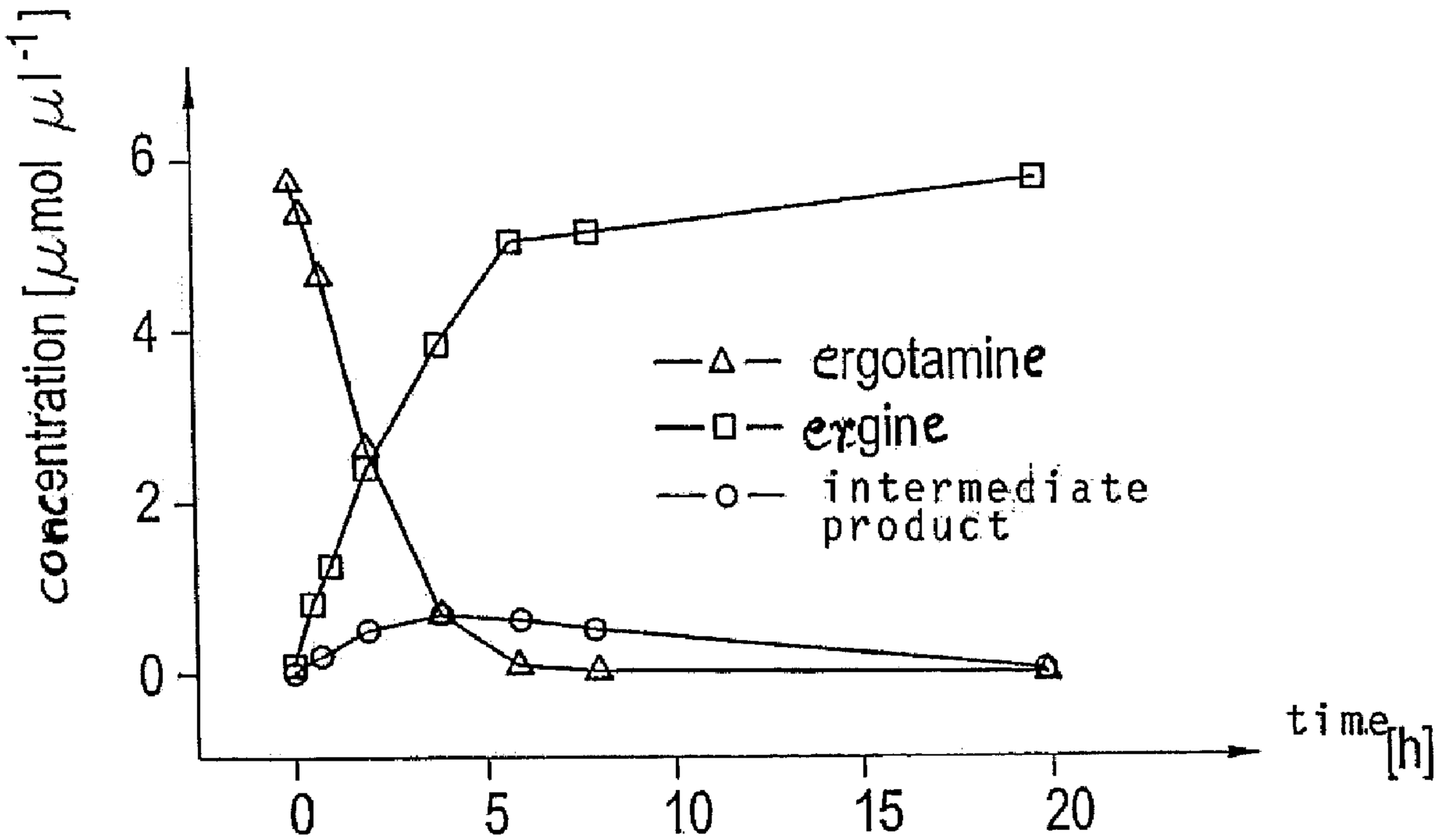
(54) **Titre : ENZYMES DE TRANSFORMATION D'ERGOPEPTINES ET PROCEDE CORRESPONDANT**
(54) **Title: ENZYMES FOR TRANSFORMING ERGOPEPTINES AND METHOD THEREFOR**



(57) **Abrégé/Abstract:**
Enzymes for transforming, in particular hydrolytically cleaving, ergopeptines, which ergopeptines are α/β-hydrolases hydrolytically cleaving ergopeptines in the cyclol ring, for the transformation of ergopeptines, and method for producing ergopeptine-metabolizing enzymes.

Abstract:

Enzymes for transforming, in particular hydrolytically cleaving, ergopeptines, which ergopeptines are α/β-hydrolases hydrolytically cleaving ergopeptines in the cyclol ring, for the transformation of ergopeptines, and method for producing ergopeptine-metabolizing enzymes.

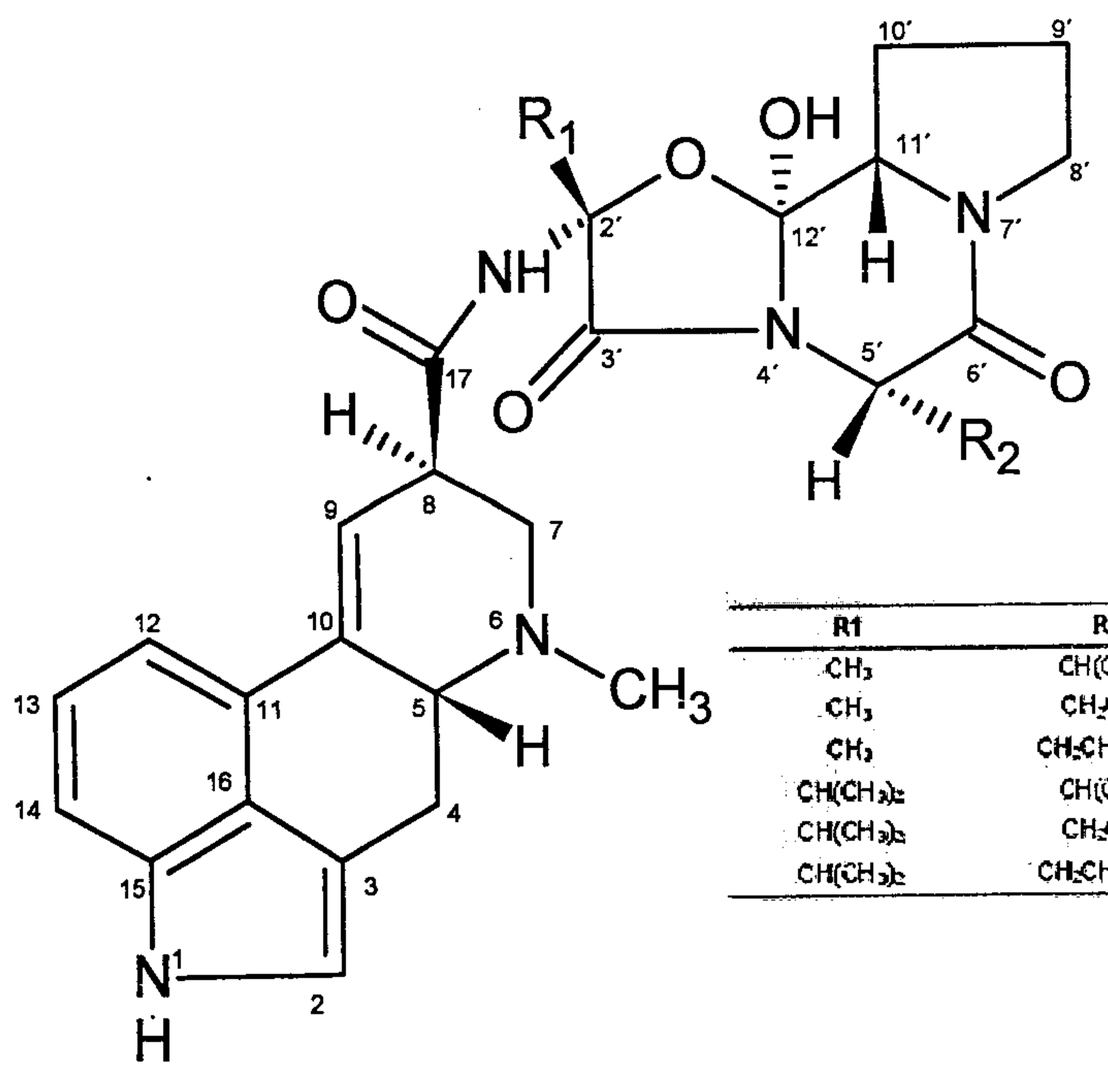
(Fig. 1)

ENZYMES FOR TRANSFORMING ERGOPEPTINES AND METHOD THEREFOR

The present invention relates to enzymes for transforming, in particular hydrolytically cleaving, ergopeptines, a method for transforming ergopeptines, and a method for producing ergopeptine-metabolizing enzymes.

Ergopeptines are a group of ergot alkaloids and are, moreover, secondary metabolic products formed by plant-associated fungi of the genus *Claviceps* belonging to the Clavicipitaceae family. The most prominent member of this genus is *Claviceps purpurea*, which above all affects cereals like rye, wheat, tricitale, barley and maize. Another member, namely *Claviceps africana*, is widely found in millet. Further ergot alkaloid-producing fungi of this family include grass endophytes of the genus *Epichloë*, *Neotyphodium* and *Balansia*, yet also *Aspergillus fumigatus* and various *Penicillium* spp. are able to produce ergot alkaloids.

In general, ergot alkaloids have a characteristic skeletal structure with a tetracyclic ergoline ring that comprises a methylated nitrogen at the 6-position and may have different substituents at the C-8 position. Based on these substituents, ergot alkaloids are categorized into clavines, simple lysergic acid amides, ergopeptines and ergopeptams.



R1	R2	Ergopeptin
CH_3	$CH(CH_3)_2$	Ergovalin
CH_3	$CH_2C_6H_5$	Ergotamin
CH_3	$CH_2CH(CH_3)_2$	α-Ergosin
$CH(CH_3)_2$	$CH(CH_3)_2$	Ergocornin
$CH(CH_3)_2$	$CH_2C_6H_5$	Ergocristin
$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	α-Ergocryptin

Due to their structural similarity with neurotransmitters, ergot alkaloids interact with the receptors of the latter, causing a plurality of effects such as intoxications, yet also positive actions in the pharmaceutical field. Today, ergot alkaloids no longer constitute problems in the

human field because of improved cleaning techniques in mills. However, they still contribute to problems in animal husbandry, causing a plurality of adverse symptoms. The symptoms caused by ergot alkaloids in animals, in particular, comprise gangrene, lameness, a reduced weight gain, an increased respiratory frequency, a reduced serum prolactin level, a reduced milk production, and a low reproduction rate. In this respect, the endophytes encountered in pasture grasses in America, New Zealand and Australia first of all raise problems in animal husbandry. Thus, the endophyte infection of tall fescue by *Neotyphodium coenophialum* has caused high losses to livestock producers.

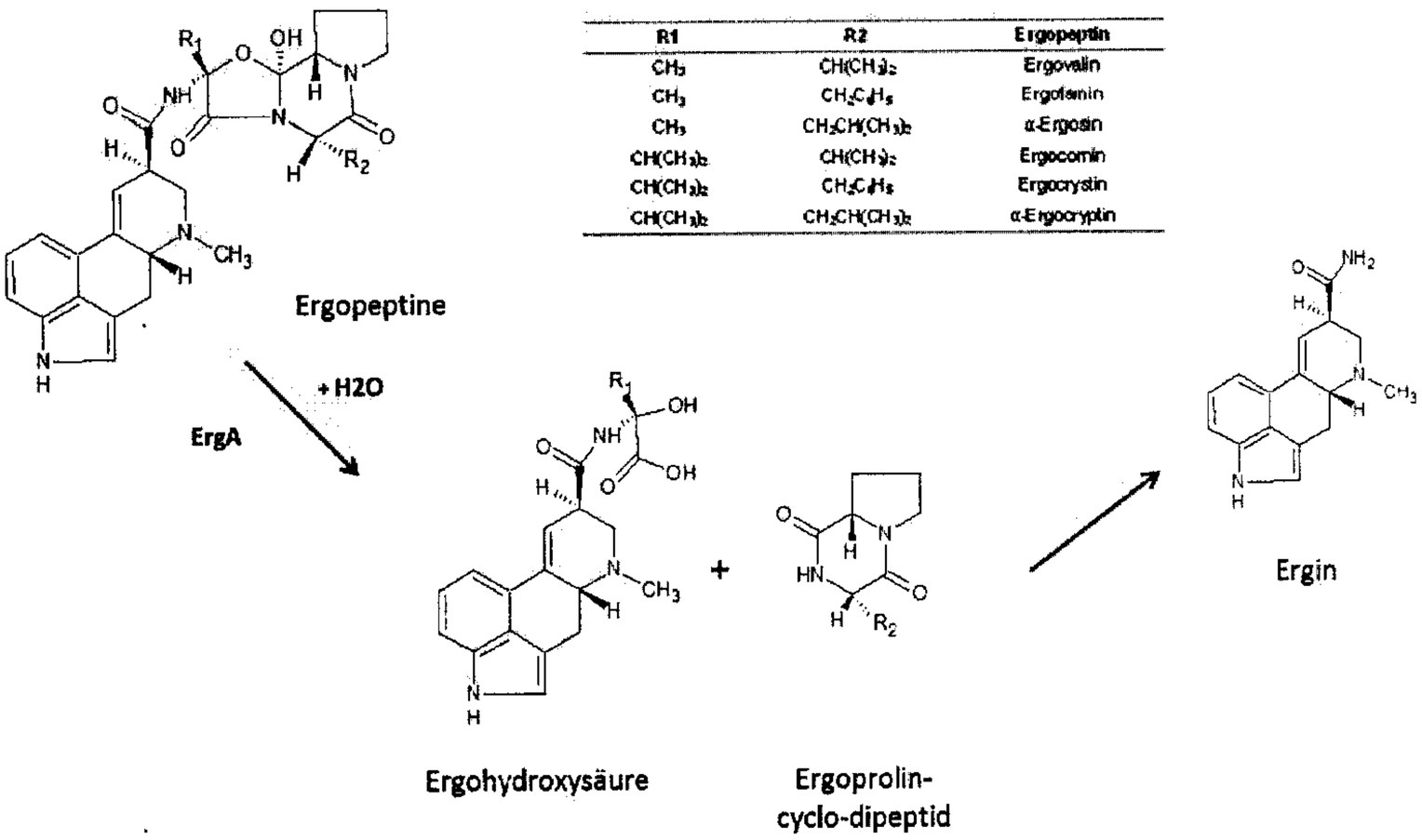
For the majority of the above-described effects or symptoms, ergopeptines, which constitute the group with the highest multiformity of the ergot alkaloids, are responsible, which, in turn, are themselves categorized according to the amino acid directly bound to D-lysergic acid. In this respect, the characteristic oxazolidin-4-ring of the ergopeptines is referred to as cyclol ring according to the nomenclature used by Schardl et al. Members are represented by the ergotamine group comprising, *inter alia*, ergotamine, ergovaline and ergosine, in which the first amino acid is L-alanine. A further group is the ergotoxine group, in which the first amino acid bound to D-lysergic acid is L-valine. Representatives of the latter include ergocristine, ergocryptine or ergocornine. Still a further member is the ergoxine group, in which the first amino acid bound to lysergic acid is an α-aminobutyric acid. Representatives are ergostine and ergonine.

Among these, ergovaline is one of the main alkaloids of the *Neotyphodium* and *Epichloë* species endophytically growing in pasture grasses and is of veterinary-toxicological relevance, e.g. in fescue toxicosis. 9,10-dihydroergopeptines only rarely occur in nature and have so far only been detected in *Sphacelia sorghi*. Partially synthetically obtained dihydroergopeptines such as dihydroergotamine and dihydroergotoxine have therapeutic relevance in the treatment of migraine and cardiovascular diseases. Apart from the described positive effects, in particular the therapeutic relevance of ergopeptines, their toxic action is, however, of non-negligible significance, since, in particular, their toxicity due, for instance, to the consumption of contaminated grains or toxic endophytes will result in the destruction or impairment or damage of numerous physiological systems such as the reproductive organs, the growth-oriented systems and the cardiovascular structures within the body of an animal or human being. Furthermore, there is presently no doubt that the consumption of grains infested with ergotamines or ergopeptines will also directly affect the gastrointestinal system and hence strongly impair not only the health of animals, but also their performances.

The present invention aims to provide enzymes and enzyme preparations as well as genes from which such enzymes and enzyme preparations are derived, which enable the degradation of ergopeptines to less toxic metabolites, in particular ergine.

To solve this object, the invention is essentially characterized in that said enzymes are α/β-hydrolases hydrolytically cleaving ergopeptines in the cyclol ring. The oxazolidin-4-on ring of the ergopeptines is defined as cyclol ring. By enzymatically cleaving ergopeptines in the cyclol ring using an ergopeptine-specific α/β-hydrolase, it has become possible to degrade the ergopeptines to ergine in a multistep reaction partially occurring spontaneously. α/β-hydrolases are members of an enzyme class with different catalytic functions, which, inter alia, are able to attack the cyclol ring of native ergopeptines and degrade the latter to ergine via a secondary lysergic acid amide (ergo hydroxy acid).

According to a further development of the invention, said enzymes are essentially characterized in that they comprise a catalytic triad consisting of a nucleophilic amino acid and histidine and an acidic amino acid, and that the triad is contained in a peptide chain with an α/β-hydrolase fold. A particularly complete enzymatic cleavage will be achieved in that the catalytic triad consists of the nucleophilic amino acid serine, of histidine and one of the acidic amino acids, aspartate or glutamate, and that the triad is contained in a peptide chain with a fold of an ergopeptine-specific α/β-hydrolase. The use of an enzyme comprising the above-defined catalytic triad has enabled the complete enzymatic cleavage of ergopeptines to ergine in a surprising manner. The enzymatic cleavage occurs at the 3'-site of the cyclol ring of ergopeptines, during which hydrolytic cleavage the group of three rings, namely the cyclol ring, the lactam ring and the pyrolidine ring, is cleaved in several steps to finally form ergine, as can be taken from the following reaction scheme.



R1	R2	Ergopeptin
CH_3	$CH(CH_3)_2$	Ergovalin
CH_3	$CH_2C_6H_5$	Ergotamin
CH_3	$CH_2CH(CH_3)_2$	α-Ergosin
$CH(CH_3)_2$	$CH(CH_3)_2$	Ergocomin
$CH(CH_3)_2$	$CH_2C_6H_5$	Ergocrystin
$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	α-Ergocryptin

According to a further development of the invention, said enzyme is essentially characterized in that the α/β-hydrolase comprises a nucleophilic elbow having the sequence Gly-Gln-Ser-Arg-Asn-Gly. If the α/β-hydrolase comprises a nucleophilic elbow having the sequence Gly-Gln-Ser-Arg-Asn-Gly, a particularly rapid enzymatic degradation of ergopeptine to ergo hydroxy acid will be possible, the latter being spontaneously transformed into ergine. In this case, the nucleophilic elbow is a central element of the α/β-hydrolases. It comprises the catalytically active amino acid with the nucleophilic side chain in a structure having unusual bond angles located in unfavorable regions of the Ramachandran plot (Ollis et al., 1992). The amino acid sequence of the nucleophilic elbow, in the present case Gly-Gln-Ser-Arg-Asn-Gly, is conserved and can, for instance, also be used for classifying α/β-hydrolases (Kourist et al., 2010).

According to a preferred further development of the invention, a complete degradation will be enabled in that said enzyme comprises the sequence ID No. 1. The enzyme comprising the sequence ID No. 1 has turned out to be particularly effective in the catalytic cleavage of ergopeptines to ergine. The nucleophilic amino acid serine assumes a central role in a conserved structure, i.e. the nucleophilic elbow. The nucleophilic elbow is localized between the β5-strand and the consecutive α-helix, and comprises the consensus sequence Sm-X-Nu-X-Sm, wherein Sm is a small amino acid, X is any amino acid, and Nu represents a nucleophilic amino acid. The sequence ID No. 1 is G-Q-S-R-N. The α/β-hydrolase having sequence ID No. 1 belongs to the enzymes that do not require any cofactors for their mode of action.

According to a preferred further development of the invention, said enzyme is characterized in that it comprises at least 96% sequence identity with sequence ID No. 1, wherein the catalytic

properties of said enzyme are substantially maintained. In a surprising manner, it could be demonstrated that in addition to the enzyme with sequence ID No. 1, modifications thereof can also be used, and that good results are still possible with the modified enzymes, as was shown by way of the enzyme with sequence ID No. 5.

According to a preferred further development of the invention, said enzyme is characterized in that it comprises an, in particular extended, N- or C-terminal sequence different from the sequence ID No. 1, in particular an enzyme having sequence ID No. 5, and that it exhibits at least 96% sequence identity with sequence ID No. 1. It could be demonstrated in a surprising manner that, in addition to the sequence ID No. 1, a modification width thereof may also be provided, wherein, in particular, the N-terminus can be modified. Especially good results will be achieved if the enzyme with a modified starting sequence exhibits a sequence identity with sequence ID No. 1 of at least 96%.

Enzymes with an N-terminus deviating from the sequence ID No. 1 are equally apt to completely degrade ergotamine.

In order to completely degrade and detoxify ergopeptines, the present invention, furthermore, aims to provide a method for enzymatically transforming ergopeptines.

To solve this object, the method according to the invention is essentially characterized in that the ergopeptines are hydrolytically cleaved in the cyclol ring to primary metabolites.

It turned out in a surprising manner that, following the hydrolytic cleavage of ergopeptines in the cyclol ring to ergot hydroxy acid and ergoproline cyclodipeptide, a spontaneous reaction of these intermediate products to, in particular, ergine and pyruvate takes place. The thus formed reaction products exhibit a toxicity that is significantly reduced, if not negligible, relative to that of the starting product.

In a preferred manner, the method according to the invention is substantially performed such that said cleaving is effected by a nucleophilic attack on the C3'-atom of the cyclol ring. Particularly advantageous and complete results will be achieved in that the nucleophilic attack on the C3'-atom of the cyclol ring is effected by a catalytic triad contained in a peptide chain with an α/β-hydrolase fold and consisting of the nucleophilic amino acid serine, of histidine and one of the acidic amino acids, aspartate or glutamate. Such a process control allows for the achievement of a rapid and complete degradation of the ergopeptines to primary metabolites, which, as in correspondence with a preferred further development of the invention, are further

converted into ergine. Such a reaction, according to a preferred further development of the invention, is effected by a spontaneous reaction, to which end the ambient conditions are selected such that the intermediate products of the degradation are directly and completely further transformed into ergine.

As in correspondence with a further development of the invention, the method is performed such that the further reaction of the primary metabolites formed by the hydrolytic cleavage with the α/β-hydrolase is effected by enzymes occurring in the reaction medium. Such a process control uses the enzymes always present in natural surroundings, which are surprisingly able to completely degrade the primary metabolites to ergine.

The present invention, moreover, aims to provide a method for producing ergopeptine-metabolizing enzymes. To solve this object, the method according to the invention is performed such that a gene for an enzyme coding according to the invention is cloned in an expression vector, transformed into prokaryotic and/or eukaryotic host cells, and expressed in a host cell. Such a procedure enables the provision of high enzyme concentrations which are able to completely convert to ergine six ergopeptines, namely ergotamine, ergovaline, ergocornine, ergocristine, ergocryptine and ergosine as well their respective isomeric forms, namely ergotaminine, ergovalinine, ergocominine, ergocristinine, ergocryptinine and ergosinine. In this case, a gene having sequence ID No. 2, 4 or 6 is preferably used to enable a further increase in the enzyme combinations formed.

Particularly high enzyme activities will be achieved according to the present invention, if the method is performed such that the gene is transformed into, and expressed in, one of the microorganisms selected from *Pichia pastoris*, *E. coli* or *Bacillus subtilis* as host cell. The name *Pichia pastoris* used in the present application is a synonym for the name *Komagataella pastoris*, *Pichia pastoris* being the older and *Komagataella pastoris* being the systematically newer name (Yamada et al., 1995).

An even further increase in the enzyme activity will be achieved in that the method is performed such that the enzyme having sequence ID No. 1, in particular the his-tagged enzyme having sequence ID No. 5, is purified by affinity chromatography. A purified enzyme having sequence ID No. 5 not only enables the complete conversion of ergopeptines into ergine, but such a purified enzyme will, in particular, display an especially high catalytic activity, in particular in a pH range between about 6 and about 9.

According to a preferred further development of the method, the first step of the reaction is carried out such that the cyclol ring is cleaved by the enzyme of sequence ID No. 1. Such a method control enables the ergopeptines to be almost completely converted into metabolites having a low vasoconstrictive activity.

The enzyme preparation according to the present invention is preferably applied in a feed or silage additive. Such use enables the detoxification of the ergopeptines present on feed or silage additives, partially prior to feeding and partially in the gastrointestinal tracts of the animals, merely by admixing said enzyme preparation.

In the following, the invention will be explained in more detail by way of exemplary embodiments and Figures. Therein,

Fig. 1 illustrates the kinetics of the reaction of ergotamine to ergine with the enzyme having sequence ID No. 1;

Fig. 2 illustrates the reaction of the ergopeptines ergocornine, ergocryptine, ergosine, ergovaline and ergotamine by the enzyme of sequence ID No. 1 with the exemplary negative controls for ergocryptine and ergosine;

Fig. 3 is an illustration of the *P.pastoris* expression vector pGAPZ alphaC with the gene sequence ID No. 2; and

Fig. 4 is an illustration of the *B. subtilis* expression vector pET43 with the gene sequence ID No. 2.

Example 1:

Determination of the catalytic activity of the enzyme with the sequence ID No.1.

The gene with the sequence ID No. 2, which codes for an α/β-hydrolase comprising a catalytic triad of S94-D234-H270, was cloned into the expression vector pET28a(+) by applying standard methods, transformed and expressed in *E. coli*. Following the expression in *E. coli* BL21(DE3), the his-tagged enzyme was purified by affinity chromatography. The enzyme concentration was determined using a Pierce BSA Protein Assay Kit, and the enzyme was used in activity assays. The assays were carried out in 50 mM sodium phosphate buffer (pH 7.0) at 25°C.

In the context of the detoxification assays, enzyme concentrations of 0.079 µg/ml and ergotamine concentrations of 5 mg/kg were used.

A further assay for reacting the six ergopeptines, namely ergotamine, ergovaline, ergocornine, ergocristine, ergocryptine or ergosine, and their respective isomeric forms, namely

ergotaminine, ergovalinine, ergocorninine, ergocristinine, ergocryptinine and ergosinine, used 1.58 µg/ml of the enzyme with sequence ID No. 1 and 10 mg/kg ergotamine, or the equimolar (summation) concentrations of the remaining ergopeptines or their epimers. The results are indicated in Fig. 2.

The samples were analyzed using HPLC-FLD or HPLC-MS/MS, each by analytically determining the respective concentration of the sum of the respective epimers. Simultaneously with the determination of the ergopeptine concentration during the enzymatic reaction, the formation of the ergo hydroxy acid (metabolite 1) and of the ergoproline cyclodipeptide (metabolite 2) was observed. During the continued reaction course, the conversion of metabolite 1 to ergine was detected.

Fig. 1 exemplarily shows the kinetics of the reaction of ergotamine with sequence ID No. 1. During said reaction, slight amounts of an instable intermediate product were detected, and the end production of the reaction was ergine. From Fig. 1, it is apparent that an almost complete degradation of ergotamine to ergine by sequence ID No. 1 occurred within 4 hours. The reaction courses of all other ergopeptines, namely ergovaline, ergocomine, ergocristine, ergocryptine or ergosine, as well as their respective isomeric forms, namely ergovalinine, ergocominine, ergocristinine, ergocryptinine and ergosinine, are comparable.

Example 2:

Identification of the N-terminus of the enzyme with the sequence ID No. 1

To identify the N-terminus of the enzyme with sequence ID No. 1, the genes having sequences ID No. 2 and ID No. 6 were cloned into PET28a(+) and transformed into *E. coli* using standard methods.

Following the expression, the bacteria cells were taken up in 50 mM sodium phosphate buffer and lyzed using a French press (20,000 psi). The lysates were used in dilutions of 1:10, 1:100 and 1:1000 in degradation batches of 5 mg/kg ergotamine. The batches were incubated at 25°C, and the samples were analyzed using HPLC-FLD.

The results of the degradation test indicated that both of the enzymes were able to transform ergotamine. However, the enzyme with the shorter nucleotide sequence displayed a significantly higher activity, this variant thus having been able to completely transform ergotamine even in the 1:1000 dilution, the longer variant displaying only little activity already in the 1:100 dilution.

Example 3:
Determination of the temperature range of the activity, and the temperature stability, of the enzyme with the sequence ID No. 1

In order to determine the optimum temperature for the activity of the enzyme with the sequence ID No. 1, 0.1 µg/ml enzyme was incubated with 5 mg/kg ergotamine in Teorell-Stenhagen universal buffer (pH 9.0) at varying temperatures ranging from 10°C to 50°C. The enzyme displayed activity in a range of 10°C to 35°C with an optimum at 35°C, based on the starting speed.

In order to determine the temperature stability, the enzyme was incubated for 1 h at varying temperatures ranging from 10°C to 60°C. After this, the enzyme solutions were incubated at concentrations of 0.1 µg/ml in Teorell-Stenhagen universal buffer (pH 7.0) with 0.1 mg/ml BSA and 5 mg/kg ergotamine at 25°C. The results indicate that the enzyme is stable up to a temperature of 30°C, still displaying some activity after incubation at 40°C, yet showing a decrease of activity between 35° and 40°C. To sum up, it has turned out that the enzyme with the sequence ID No. 1 substantially shows the temperature optimum at the temperature conditions found in the gastrointestinal tract.

Example 4:
Determination of the pH optimum of the activity, and the pH stability, of the enzyme with the sequence ID No. 1

In order to determine the optimum pH range for the activity of ErgA, 0.1 µg/ml enzyme was incubated with 5 mg/kg ergotamine at varying pH values using Teorell-Stenhagen universal buffer at 25°C. Said buffer was chosen, since the combination of citrate, phosphate and borate allows for the adjustment of the same buffer capacity in a range of pH 2 to pH 12 by hydrochloric acid. The enzyme displayed activity in a range of pH 6 to pH 11 with a small activity plateau at pH 8 to pH 9.

In order to determine the pH stability, the enzyme was incubated for 1 h at 25°C at varying pH values ranging from pH 2 to pH 12. After this, the enzyme solutions in concentrations of 0.1 µg/ml were incubated with 0.1 mg/ml BSA and 5 mg/kg ergotamine in Teorell-Stenhagen universal buffer (pH 7.0) at 25°C. Also in this case an activity plateau appeared, this time in the range of pH 6 to pH 9, with a strongly decreasing activity outside this range. The activity in this

range ensures the technological application of the enzyme with the sequence ID No. 1 as a feed additive.

Example 5:
Expression of the enzyme with the sequence ID No. 1 in *Picha pastoris*

The gene with the sequence ID No. 2 was cloned into pGAPZ alpha C, transformed into *P. pastoris*, and expressed using standard methods. The expression vector pGAPZ alphaC with the gene having the sequence ID No. 2 is illustrated in Fig. 3. A degradation assay was carried out in 50 mM sodium phosphate buffer (pH 7.0) with 5 mg/kg ergotamine at 25°C. From the culture supernatant, a 1:100 dilution was used. The samples were analyzed by HPLC-FLD. Based on the results from SDS-PAGE and degradation assays, an expression of the enzyme with the sequence ID No. 1 in the culture supernatant could be confirmed.

Example 6:
Expression of the enzyme with the sequence ID No. 1 in *Bacillus subtilis*

The gene with the sequence ID No. 2 was cloned into pHT43, transformed into *B. subtilis*, and expressed using standard methods. The expression vector pHT43 with the gene having the sequence ID No. 2 is illustrated in Fig. 4. A degradation assay was carried out in 50 mM sodium phosphate buffer (pH 7.0) with 5 mg/kg ergotamine at 25°C. From the culture supernatant, a 1:10 dilution was used. The samples were analyzed by HPLC-FLD. Based on the results from SDS-PAGE and degradation assays, an expression of ErgA in the culture supernatant could be confirmed.

Example 7:
Degradation assay in the rumen model

The activity of the ergot alkaloid-degrading enzyme of the enzyme with the sequence ID No. 1 was tested in an in-vitro rumen model. To this end, fresh rumen juice was diluted 1:1 using a solution consisting of synthetic rumen juice, hay and a cereal mixture of wheat, maize and soy. To demonstrate the reaction of the ergopeptines, a batch was supplemented with the enzyme of sequence ID No. 1 (1 µg/ml) and 5 mg/kg ergotamine. Fermentation tubes were used over septums, and the batches were incubated in water bath at 39°C. Analytics by means of HPLC/ESI-MS/MS showed that ergotamine had been completely converted into ergine and lysergic acid in the rumen model.

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SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 31816-30 Seq 06-MAR-15 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

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CLAIMS:

1. An enzyme for transforming ergopeptines by hydrolytic cleavage, said enzyme being an α/β-hydrolase that hydrolytically cleaves ergopeptines in the cyclol ring, said enzyme having at least 96% sequence identity with SEQ ID NO: 1.

2. The enzyme according to claim 1, comprising a catalytic triad consisting of a nucleophilic amino acid, histidine, and an acidic amino acid, wherein the triad is contained in a peptide chain with an α/β-hydrolase fold.

3. The enzyme according to claim 2, wherein the catalytic triad consists of the nucleophilic amino acid serine, histidine, and one of the acidic amino acids aspartate or glutamate, and that the triad is contained in a peptide chain with an α/β-hydrolase fold.

4. The enzyme according to claim 1, 2 or 3, wherein the α/β-hydrolase comprises a nucleophilic elbow having the sequence Gly-Gln-Ser-Arg-Asn-Gly.

5. The enzyme according to any one of claims 1 to 4, said enzyme having SEQ ID NO: 3.

6. The enzyme according to any one of claims 1 to 4, said enzyme having SEQ ID NO: 1.

7. The enzyme according to any one of claims 1 to 4, said enzyme comprising SEQ ID NO: 5.

8. A method for enzymatically transforming ergopeptines, comprising bringing the enzyme according to any one of claim 1 to 7 into contact with said ergopeptines in an aqueous environment at a temperature of 10°C to 35°C and a pH of 6 to 11, whereby the ergopeptines are hydrolytically cleaved in the cyclol ring to primary metabolites.

9. The method according to claim 8, wherein said cleaving is effected by a nucleophilic attack on the C3'-atom of the cyclol ring.

10. The method according to claim 9, wherein the nucleophilic attack on the C3'-atom of the cyclol ring is effected by a catalytic triad contained in a peptide chain with an α/β-hydrolase fold and consisting of the nucleophilic amino acid serine, histidine, and one of the acidic amino acids aspartate or glutamate.

11. The method according to any one of claims 8 to 10, wherein the primary metabolites formed by the hydrolytic cleavage of the cyclol ring of the ergopeptines are further reacted.

12. The method according to claim 11, wherein the further reaction of the primary metabolites is effected by a spontaneous reaction.

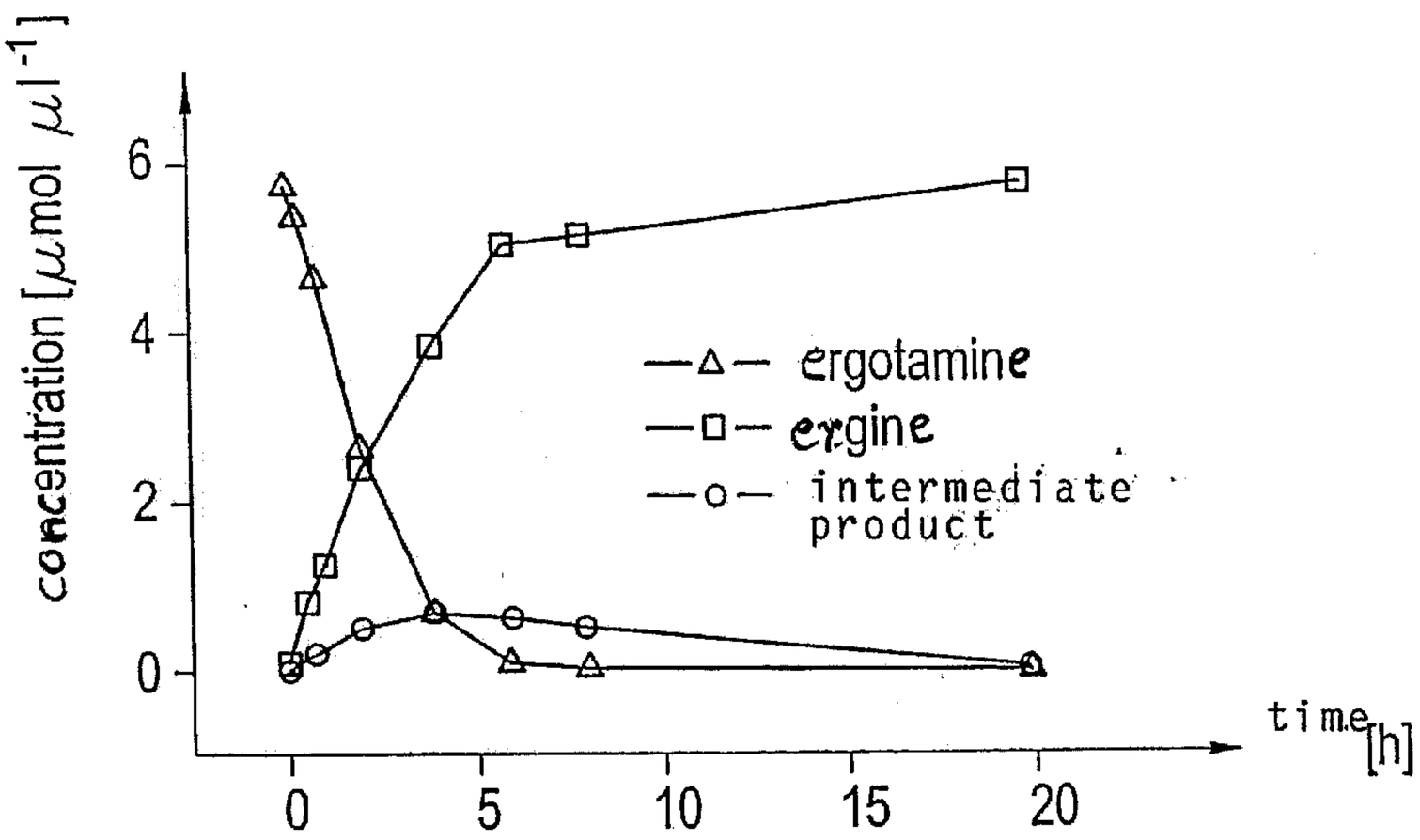
13. The method according to claim 11 or 12, wherein ergine is formed in the further reaction of the primary metabolites.

14. The method according to claim 11, 12, or 13, wherein the further reaction of the primary metabolites is effected by enzymes occurring in the reaction medium.

15. A method for producing the enzyme according to any one of claims 1 to 7, comprising cloning a nucleic acid molecule encoding said enzyme into an expression vector, transforming said expression vector into a prokaryotic or eukaryotic host cell, and expressing said nucleic acid molecule in said host cell.

16. The method according to claim 15, wherein said nucleic acid molecule comprises SEQ ID NO: 2, 4, or 6.

17. The method according to claim 15 or 16, wherein said host cell is *Pichia pastoris*, *E. coli* or *Bacillus subtilis*.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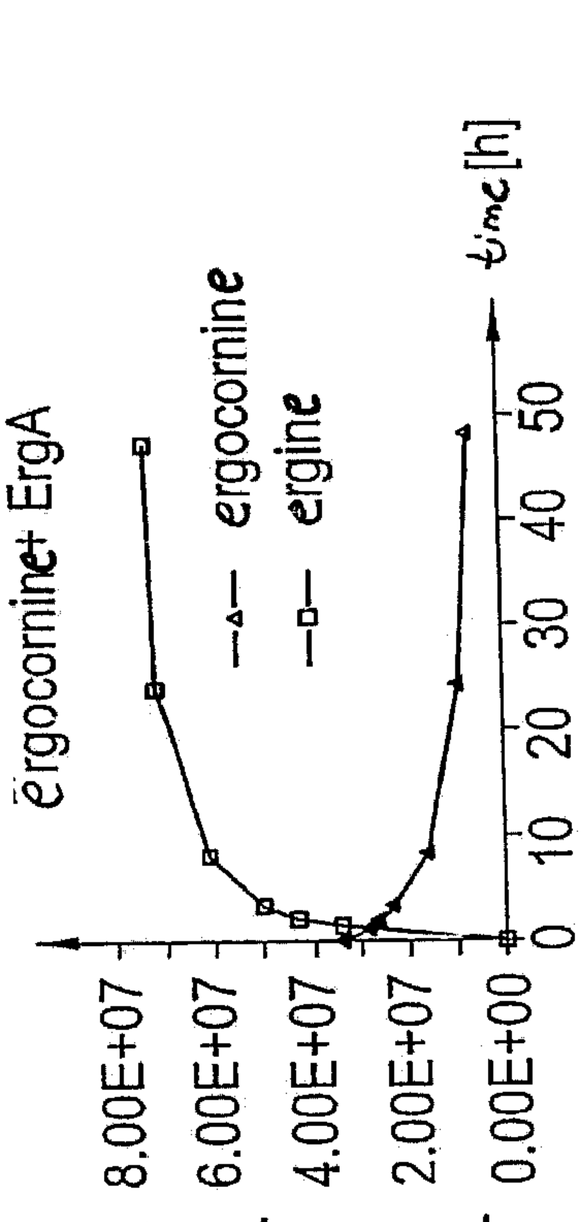
ergine
intermediate product

Fig. 1

Fig. 2

Fig. 2a



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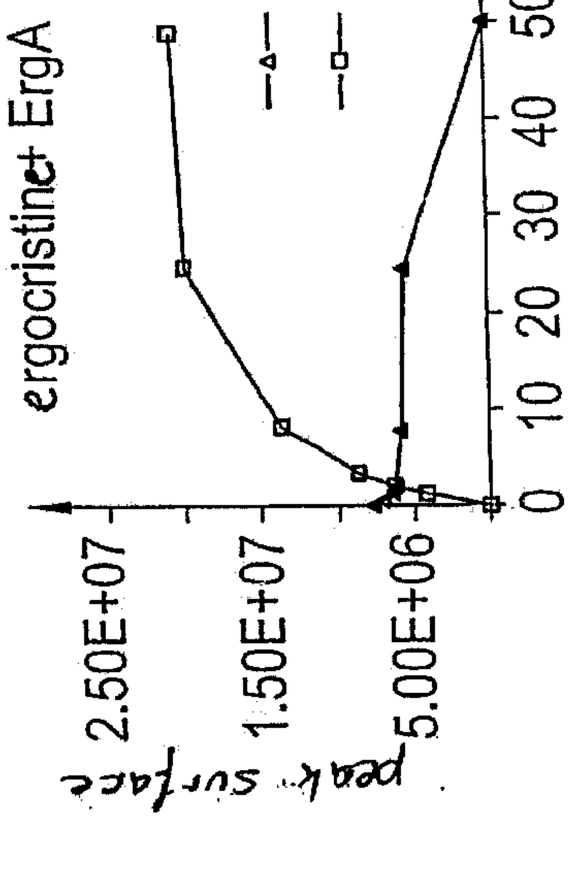

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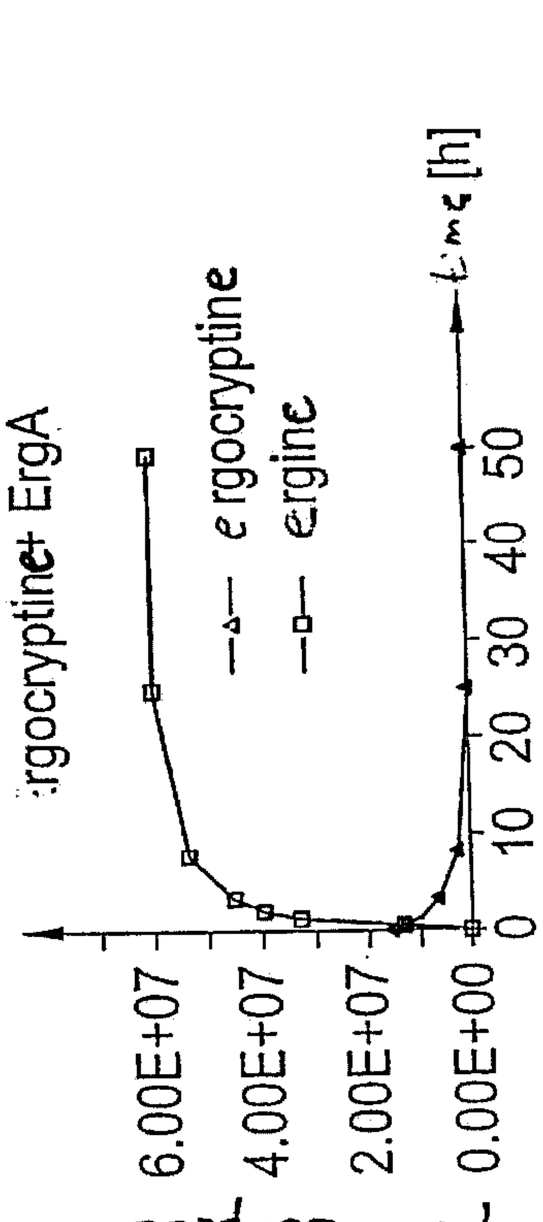
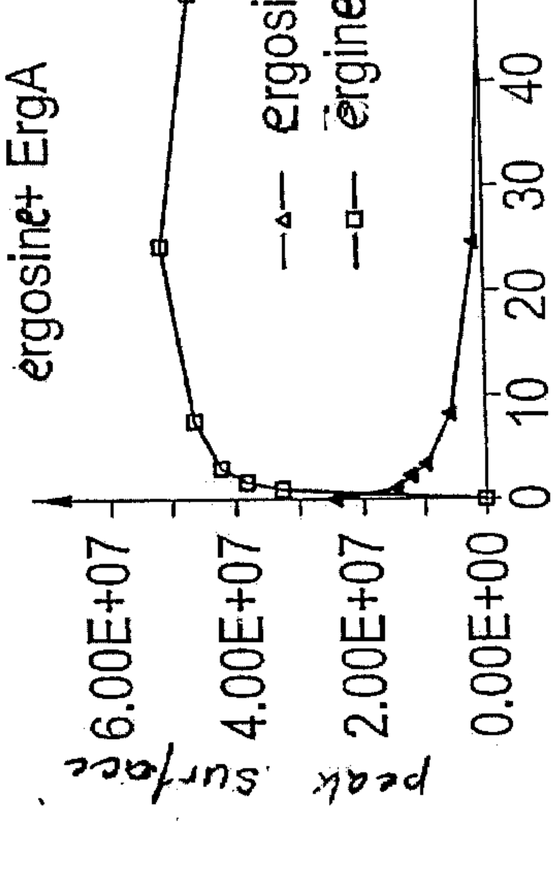


Fig. 2d



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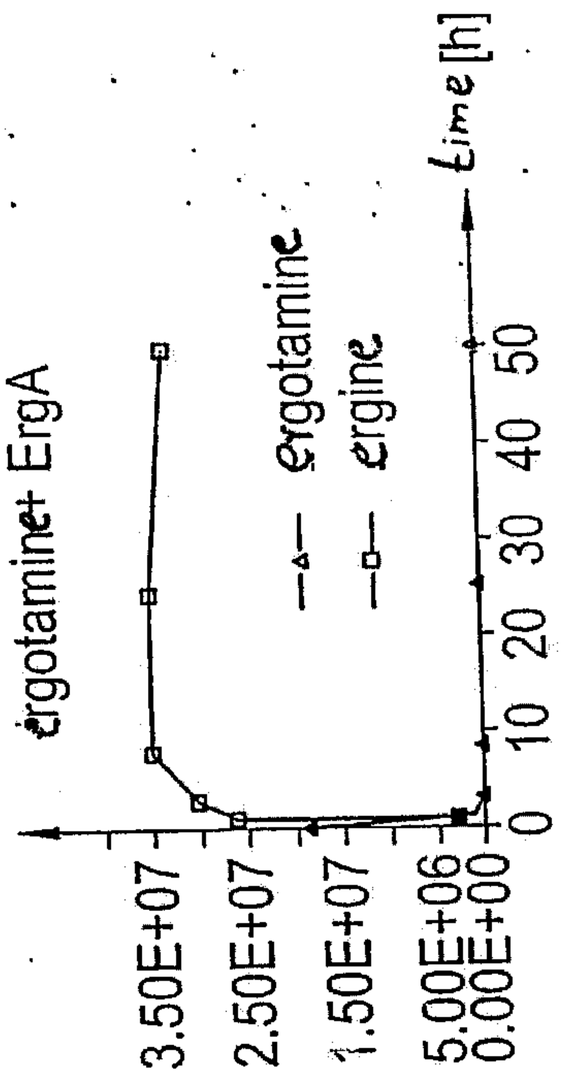

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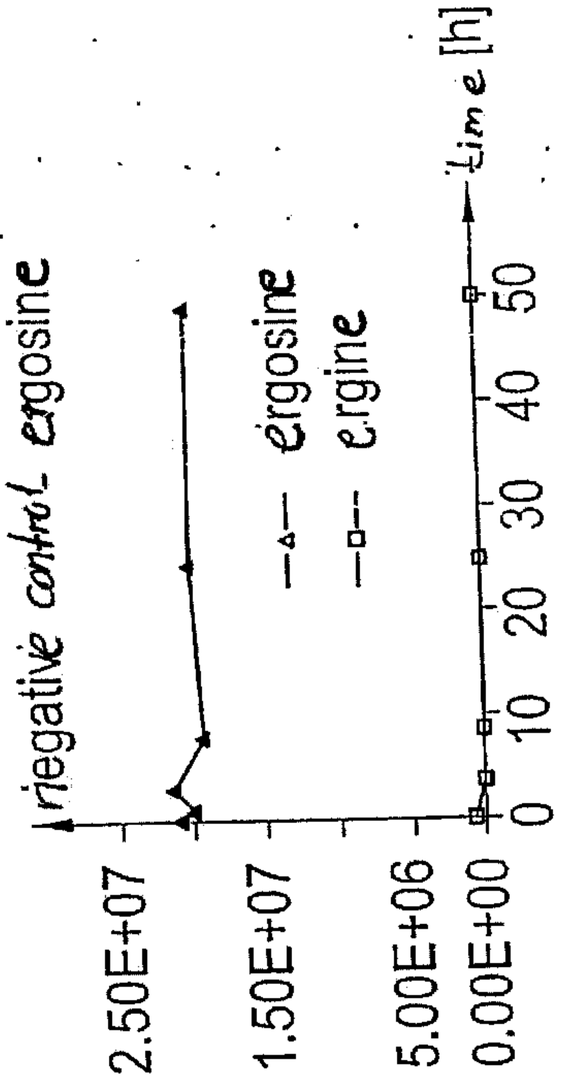

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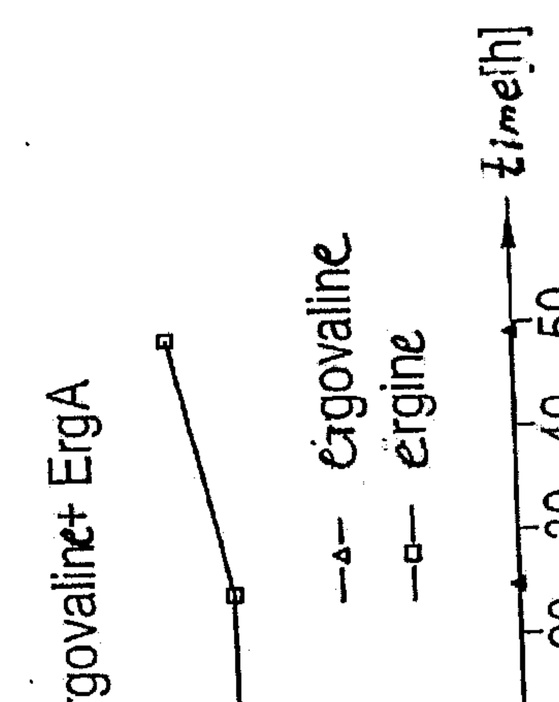

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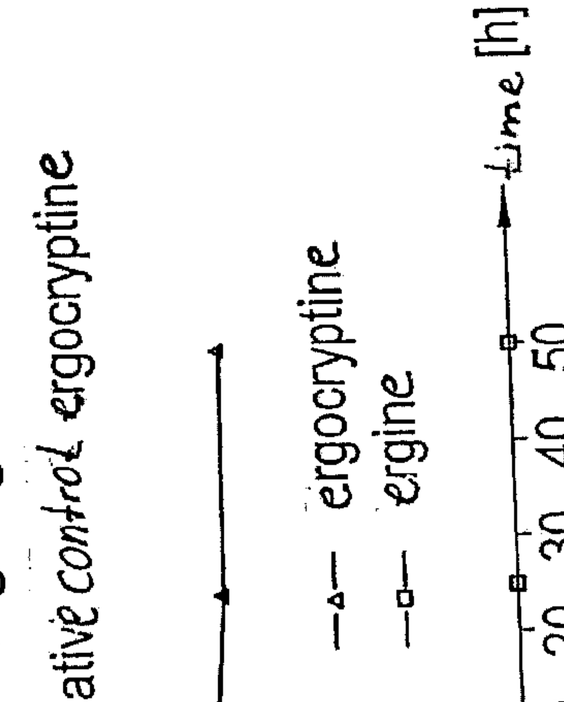

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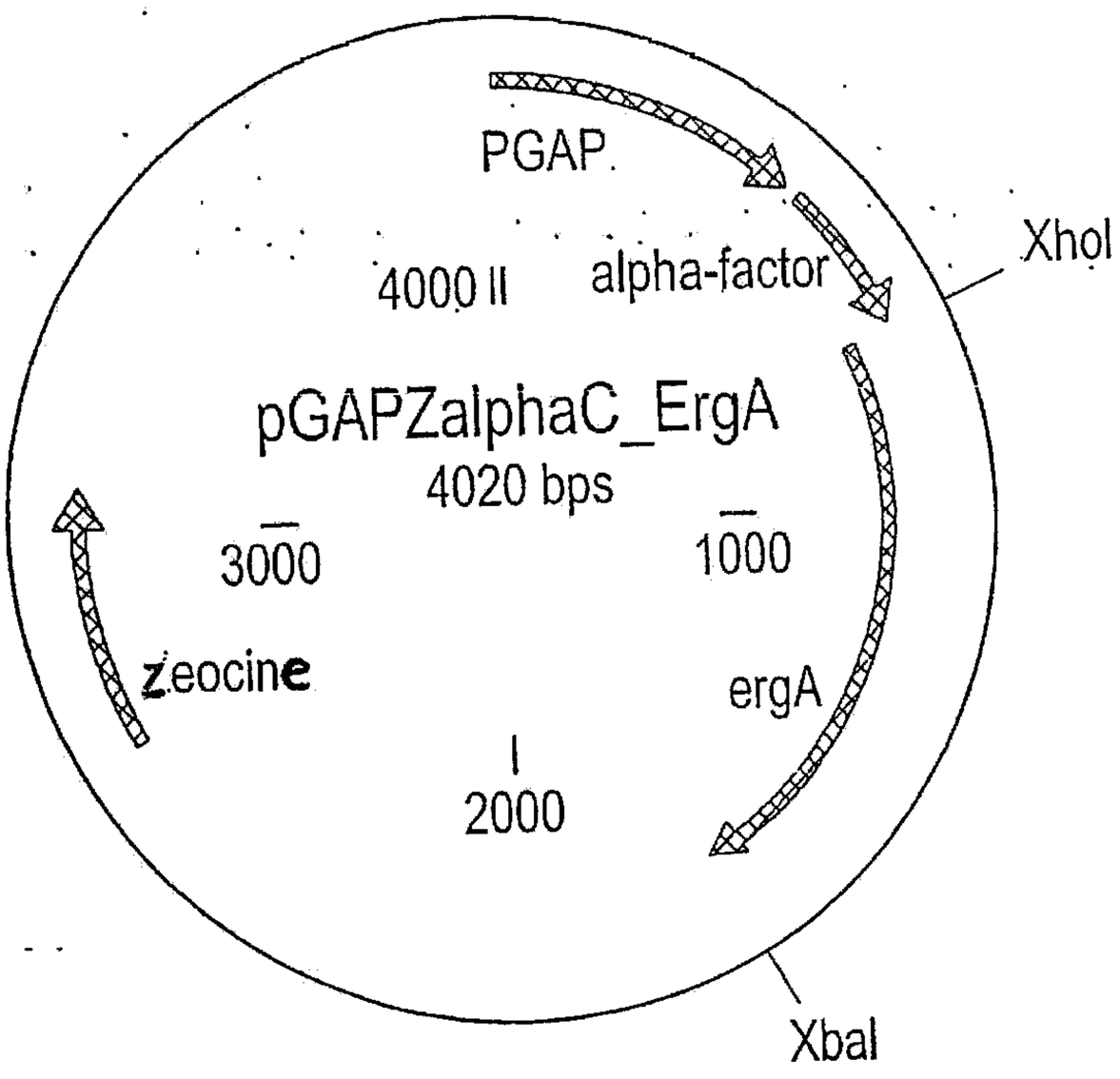
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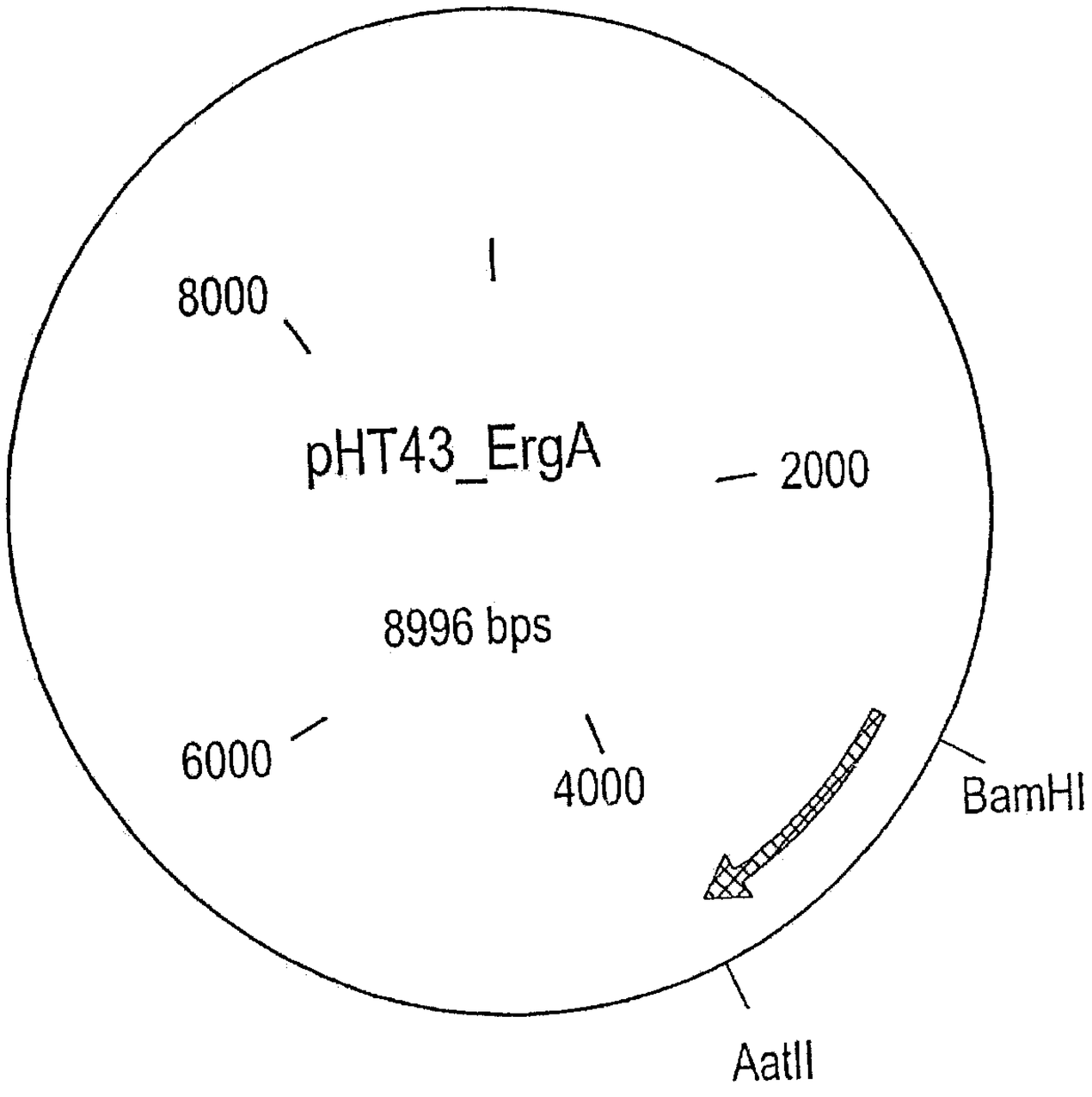
Fig. 4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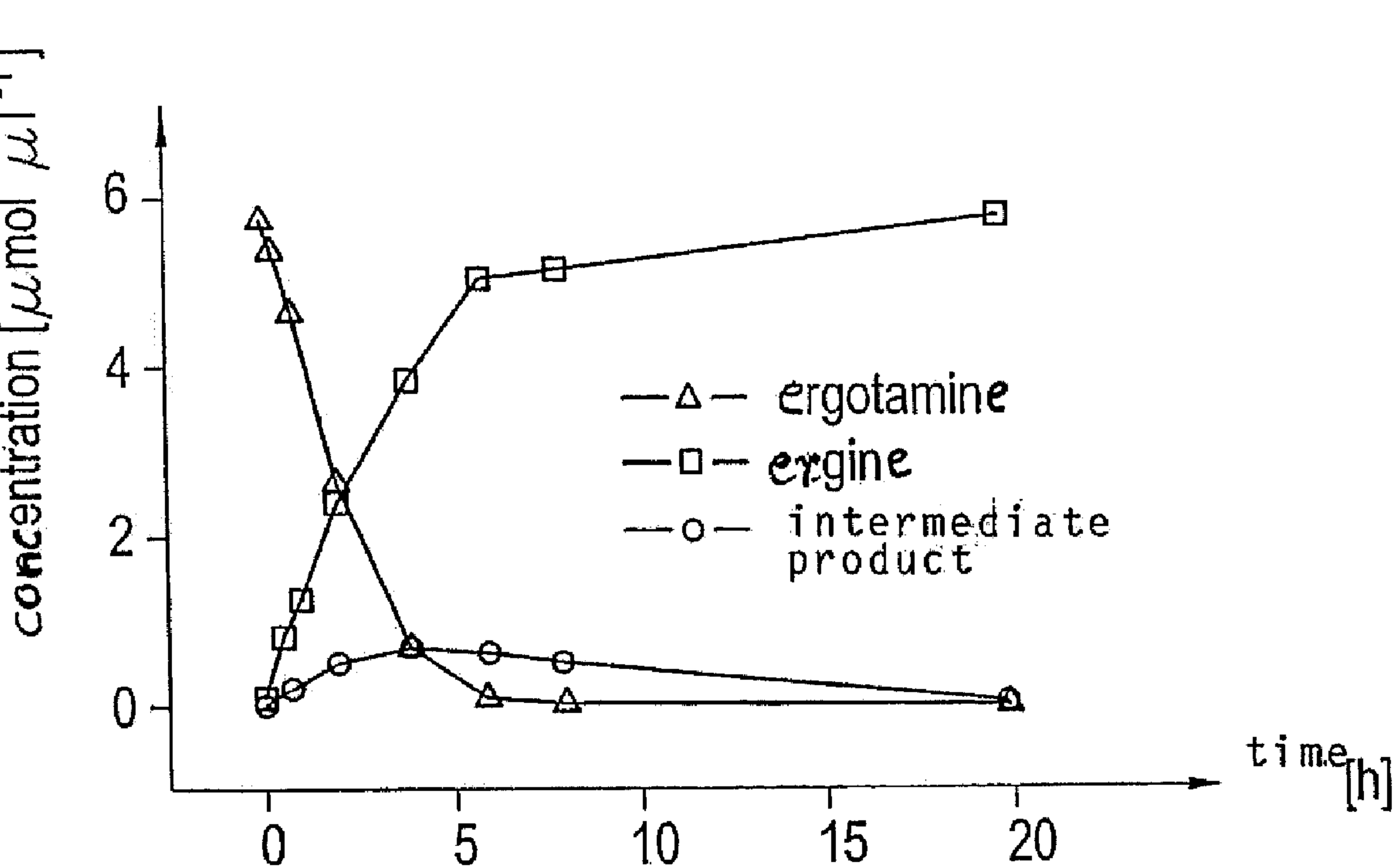
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