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(72) Inventeurs/Inventors:
JESCHKE, PETER, DE;
HARDER, ACHIM, DE;
MENCKE, NORBERT, DE;
KLEINKAUF, HORST, DE;
ZOCHER, RAINER, DE

(73) Propriétaire/Owner:
BAYER AKTIENGESELLSCHAFT, DE

(74) Agent: FETHERSTONHAUGH & CO.

(54) Titre : DEPSIPEPTIDES AVEC CYCLE DE 18 ATOMES, RENFERMANT DE L'ACIDE LACTIQUE, AGENTS ENDOPARASITICIDES; METHODE DE PREPARATION

(54) Title: LACTIC-ACID-CONTAINING CYCLIC DEPSIPEPTIDES HAVING 18 RING ATOMS AS ENDOPARASITICIDAL AGENTS, AND PROCESS FOR THEIR PREPARATION

(57) Abrégé/Abstract:
The present invention relates to a process for the preparation of lactic-acid-containing, optically active, cyclic depsipeptides having 18 ring atoms with the aid of fungal strains of the species Fusarium or enzymatic preparations isolated therefrom.

Lactic-acid-containing cyclic depsipeptides having 18 ring atoms as endoparasiticidal agents, and process for their preparation

A b s t r a c t

The present invention relates to a process for the preparation of lactic-acid-containing, optically active, cyclic depsipeptides having 18 ring atoms with the aid of fungal strains of the species Fusarium or enzymatic preparations isolated therefrom.

The present invention relates to a new process for the preparation of lactic-acid-containing cyclic depsipeptides having 18 ring atoms, some of these depsipeptides being known.

Certain lactic-acid-containing cyclic depsipeptides having 18 ring atoms (enniatins) and their use as endoparasiticides are already the subject of an earlier patent application (DE-OS (German Published Specification) 4 317 458).

A series of chemical and microbial processes exist for the preparation of cyclic depsipeptides which have 18 ring atoms and contain D-2-hydroxy-isovaleric acid.
(for example by synthesis, cf.: P. Quitt et al., Helv. Chimica Acta 46 (1963) pp. 1715-1720; P. Quitt et al., Helv. Chimica Acta 47 (1964) pp. 166-173 [enniatin A]; Pl. A. Plattner et al., Helv. Chimica Acta 46 (1963) pp. 927-935 [enniatin B]; Yu. A. Ovchinnikov et al., Tetrahedron Lett. 2 (1971) pp. 159-162; R. W. Roeske et al., Biochem. Biophys. Res. Commun. 57 (1974) pp. 554-561 [beauvericin]; for example by fermentation, cf.: R. Zocher et al., J. Antibiotics 45 (1992) pp. 1273-1277 [enniatins A, B and C]; A. Visconti et al., J. Agric. Food Chem. 40 (1992) pp. 1076-1082 [enniatin B_4]; Hiroshi Tomoda et al., J. Antibiotics 45 (1992) pp. 1207-1215 [enniatins A, A_1, B, B_1, D, E and F]).

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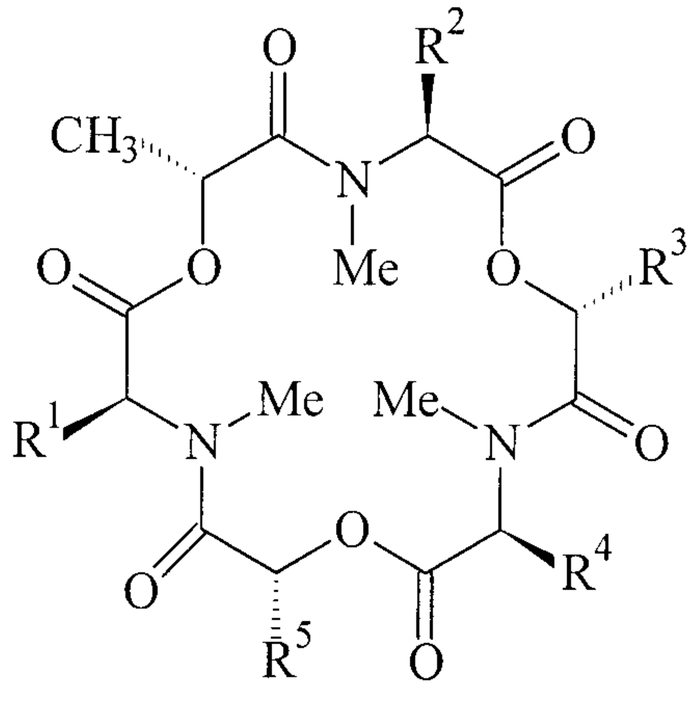
The fermentation of a cyclohexadepsipeptide which contains D-2-hydroxy-sec-caproic acid is described in a Japanese patent (cf. synthesis of MK 1688: JP Patent 02 229 177 A2; Ref. C.A. 114 (23): 227 487k).

However, nothing has been disclosed about synthesizing lactic-acid-containing cyclohexadepsipeptides (enniatins) by means of fermentation.

The present invention, according to one aspect, relates to a use of a fungal strain of the species Fusarium or an enzymatic preparation isolated therefrom for the preparation of a lactic-acid-containing, optically active, cyclic depsipeptide having 18 ring atoms, wherein the optically active, cyclic depsipeptide having 18 ring atoms is of the formula (I):



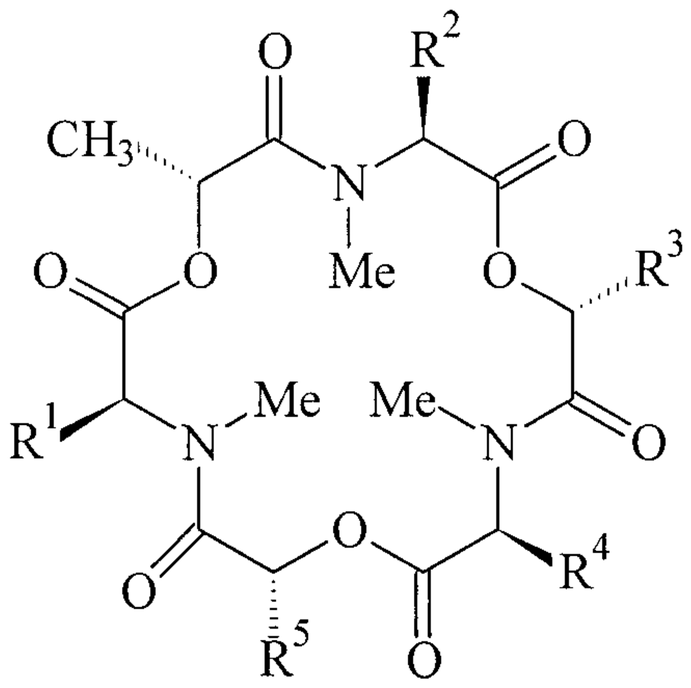
(I)

in which R^1, R^2 and R^4 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atcms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl, alkylsulphinylalkyl, alkylsulphonylalkyl, carboxyalkyl, alkoxycarbonylalkyl, carbamoylalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, guanidinoalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, or

optionally substituted arylalkyl or hetarylalkyl, substituents being halogen, hydroxyl, alkoxy, alkyl, nitro or amino, R^3 and R^5 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl, alkylsulphinylalkyl, alkylsulphonylalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, or optionally substituted arylalkyl or hetarylalkyl, substituents being halogen, hydroxyl, alkoxy, alkyl, nitro or amino.

The present invention relates, in another aspect, to a process for the preparation of lactic-acid-containing, optically active, cyclic depsipeptides having 18 ring atoms with the aid of fungal strains of the species Fusarium, or enzymatic preparations isolated therefrom.

In the process according to the invention, the lactic-acid-containing, optically active, cyclic depsipeptides having 18 ring atoms (enniatins) of the general formula (I)



(I)

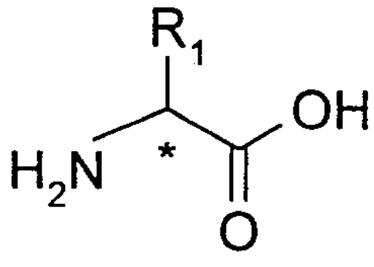
in which

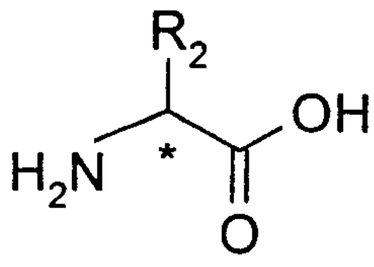
R^1, R^2 and R^4 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl, alkylsulphinylalkyl, alkylsulphonylalkyl, carboxyalkyl, alkoxycarbonylalkyl, carbamoylalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, guanidinoalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, and optionally substituted arylalkyl or hetarylalkyl, substituents which may be mentioned being halogen, hydroxyl, alkoxy, alkyl, nitro or amino,

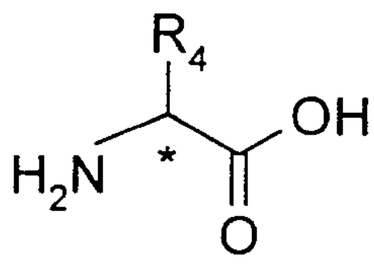
R^3 and R^5 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl, alkylsulphinylalkyl, alkylsulphonylalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, and optionally substituted arylalkyl or hetarylalkyl, substituents which may be mentioned being halogen, hydroxyl, alkoxy, alkyl, nitro or amino,

are prepared by

reacting optically active racemic amino acids of the formulae (II), (III) and (IV)


(II)

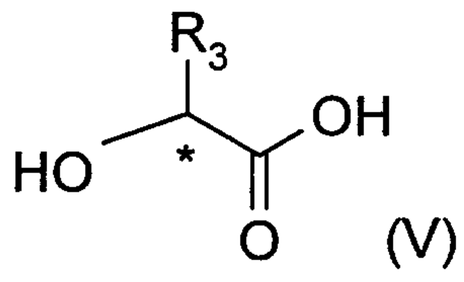

(III)

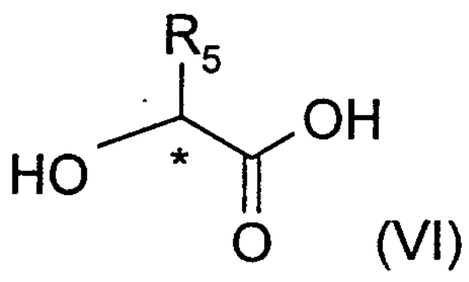

(IV)

in which

R^1, R^2 and R^4 have the abovementioned meaning,

with optically active or racemic 2-hydroxy-carboxylic acids of the formulae (V) and (VI)


(V)


(VI)

in which

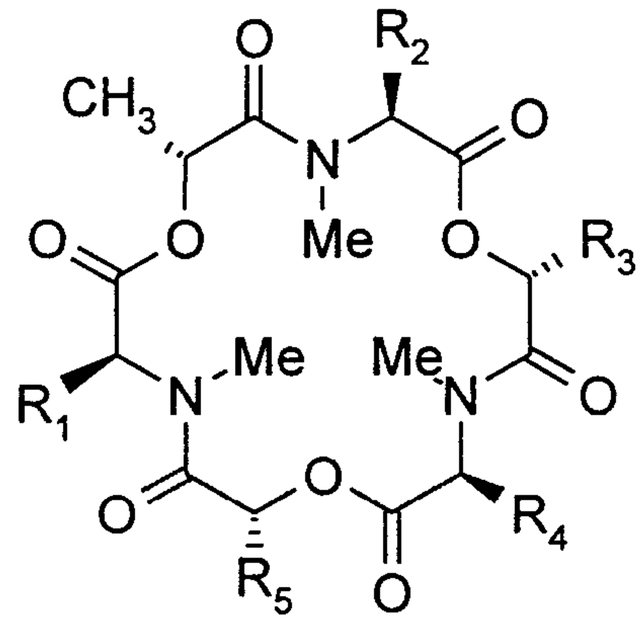
R^3 and R^5 have the abovementioned meaning,

and optically active or racemic lactic acid in the presence of fungal strains of the species Fusarium in suitable nutrient solutions or in a buffer system in the presence of synthetases isolated from microorganisms, and subsequently isolating the desired lactic-acid-containing cyclic depsipeptides having 18 ring atoms (enniatins).

The lactic-acid-containing cyclic depsipeptides having 18 ring atoms (enniatins) of the general formula (I) are

outstandingly suitable for combating endoparasites, in particular in the field of medicine and veterinary medicine

The general formula (I) provides a general definition of the lactic-acid-containing cyclic depsipeptides having 18 ring atoms (enniatins) according to the invention.



(I)

Preferred compounds of the general formula (I) are those in which

R^1, R^2 and R^4 independently of one another represent hydrogen, straight-chain or branched C_1-C_8-alkyl, in particular methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, sec-pentyl, 1,2-dimethylpropyl, neo-pentyl, 1-ethyl-propyl, 1,1,-dimethyl-propyl, hexyl, isohexyl, sec-hexyl, heptyl, isoheptyl, sec-heptyl, tert-heptyl, octyl, isooctyl, sec-octyl, hydroxy-C_1-C_4-alkyl, in particular hydroxymethyl, 1- and 2-hydroxoxyethyl,

mercapto-C_1-C_4-alkyl, in particular mercaptomethyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, in particular methylthiomethyl, methylthioethyl, C_1-C_4-alkylsulphinyl-C_1-C_4-alkyl, in particular methylsulphinylmethyl, methylsulphinylethyl, C_1-C_4-alkylsulphonyl-C_1-C_4-alkyl, in particular methylsulphonylmethyl, methylsulphonylethyl, carboxy-C_1-C_6-alkyl, in particular methoxycarbonylmethyl, ethoxycarbonylethyl, carbamoyl-C_1-C_6-alkyl, in particular carbamoylmethyl, carbamoylethyl, amino-C_1-C_6-alkyl, in particular aminopropyl, aminobutyl, C_1-C_4-alkylamino-C_1-C_6-alkyl, in particular methylaminopropyl, methylaminobutyl, C_1-C_4-dialkylamino-C_1-C_6-alkyl, in particular dimethylaminopropyl, dimethylaminobutyl, guanido-C_1-C_6-alkyl, in particular guanidopropyl, C_2-C_6-alkenyl, in particular vinyl, allyl, butenyl, hexenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, in particular cyclopropylmethyl, cyclopentylmethyl, cyclohexylmethyl, cycloheptylmethyl, hetaryl-C_1-C_4-alkyl, in particular thien-2-yl-methyl, thien-3-yl-methyl, indol-3-yl-methyl, phenyl-C_1-C_4-alkyl, in particular phenylmethyl which can optionally be substituted by radicals from the series comprising halogen, in particular fluorine, chlorine, bromine or iodine, hydroxyl, nitro, amino, C_1-C_4-alkoxy, in particular methoxy or ethoxy and C_1-C_4-alkyl, in particular methyl,

R^3 and R^5 independently of one another represent hydrogen, straight-chain or branched C_1-C_8-alkyl, in particular

methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, sec-pentyl, 1,2-dimethylpropyl, neo-pentyl, 1-ethyl-propyl, 1,1-dimethyl-propyl, hexyl, isohexyl, sec-hexyl, heptyl, isoheptyl, sec-heptyl, tert-heptyl, octyl, isooctyl, sec-octyl, hydroxy-C_1-C_4-alkyl, in particular hydroxymethyl, 1- and 2-hydroxyoxyethyl, mercapto-C_1-C_4-alkyl, in particular mercaptomethyl, C_1-C_4-alkyl-thio-C_1-C_4-alkyl, in particular methylthiomethyl, methylthioethyl, C_1-C_4-alkylsulphinyl-C_1-C_4-alkyl, in particular methylsulphinylmethyl, methylsulphinyl-ethyl, C_1-C_4-alkylsulphonyl-C_1-C_4-alkyl, in particular methylsulphonylmethyl, methylsulphonylethyl, C_2-C_6-alkenyl, in particular vinyl, allyl, butenyl, hexenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, in particular cyclo-propylmethyl, cyclopentylmethyl, cyclohexylmethyl, cycloheptylmethyl, hetaryl-C_1-C_4-alkyl, in particular thien-2-yl-methyl, thien-3-yl-methyl, phenyl-C_1-C_4-alkyl, in particular phenylmethyl which can optionally be substituted by radicals from the series comprising halogen, in particular fluorine, chlorine, bromine or iodine, hydroxyl, nitro, amino, C_1-C_4-alkoxy, in particular methoxy or ethoxy, and C_1-C_4-alkyl, in particular methyl.

Particularly preferred compounds of the general formula (I) are those in which

R^1, R^2 and R^4 independently of one another represent straight-chain or branched C_1-C_8-alkyl, in particular

methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl, sec-pentyl, 1,2-dimethyl-propyl, neo-pentyl, 1-ethyl-propyl, 1,1-dimethyl-propyl, hexyl, isohexyl, sec-hexyl, heptyl, isoheptyl, sec-heptyl, octyl, isooctyl, sec-octyl, hydroxy-C_1-C_4-alkyl, in particular hydroxymethyl, 1- and 2-hydroxyoxyethyl, mercapto-C_1-C_4-alkyl, in particular mercaptomethyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, in particular methylthiomethyl, methylthioethyl, C_2-C_6-alkenyl, in particular vinyl, allyl, butenyl, hexenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, in particular cyclopropylmethyl, cyclopentylmethyl, cyclohexylmethyl or cycloheptylmethyl,

R^3 and R^5 independently of one another represent straight-chain or branched C_1-C_8-alkyl, in particular methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, sec-pentyl, 1,2-dimethyl-propyl, neo-pentyl, 1-ethyl-propyl, 1,1-dimethyl-propyl, hexyl, isohexyl, sec-hexyl, heptyl, isoheptyl, sec-heptyl, tert-heptyl, octyl, isooctyl, sec-octyl, hydroxy-C_1-C_4-alkyl, in particular hydroxymethyl, 1- and 2-hydroxyoxyethyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, in particular methylthiomethyl, C_2-C_6-alkenyl, in particular vinyl, allyl, butenyl, hexenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, in particular cyclopropylmethyl, cyclopentylmethyl, cyclohexylmethyl or cycloheptylmethyl.

Very particularly preferred compounds of the formula (I)

are those in which

R^1, R^2 and R^4 independently of one another represent straight-chain or branched C_1-C_8-alkyl, in particular methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl, sec-pentyl, 1,2-dimethyl-propyl, neo-pentyl, 1-ethylpropyl, 1,1-dimethyl-propyl, hexyl, isohexyl, sec-hexyl, heptyl, isoheptyl, sec-heptyl, octyl, isooctyl, sec-octyl, C_2-C_6-alkenyl, in particular vinyl, allyl, butenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, in particular cyclohexylmethyl,

R^3 and R^5 independently of one another represent straight-chain or branched C_1-C_8-alkyl, in particular methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, sec-pentyl, 1,2-dimethyl-propyl, neo-pentyl, 1-ethyl-propyl, 1,1-dimethyl-propyl, hexyl, isohexyl, sec-hexyl, heptyl, isoheptyl, sec-heptyl, tert-heptyl, octyl, isooctyl, sec-octyl, C_2-C_6-alkenyl, in particular vinyl, allyl, butenyl, hexenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, in particular cyclohexylmethyl.

The following optically active compounds of the general formula (I) may be mentioned individually:

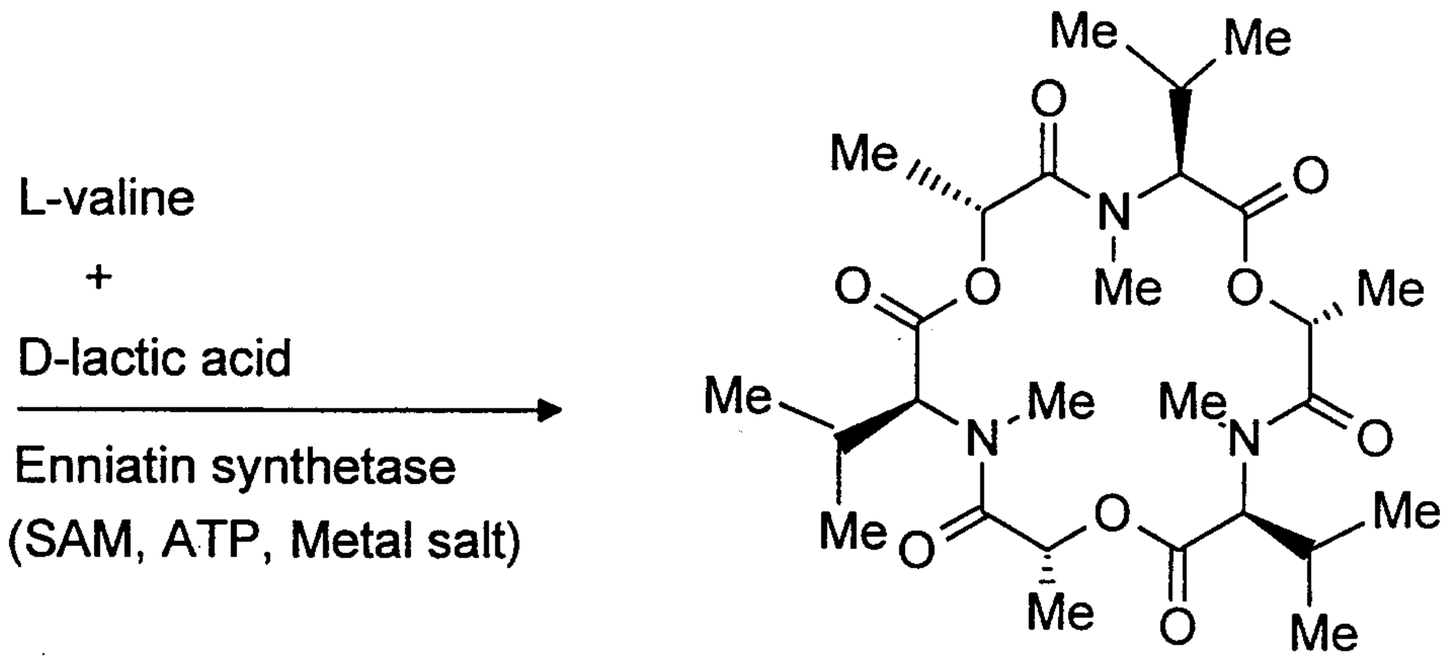
cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-valyl-D-lactyl-),
cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-norvalyl-D-lactyl-),

cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-leucyl-D-lactyl-),
cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-norleucyl-D-lactyl-),
cyclo(-N-methyl-L-valyl-D-lactyl-N-methyl-L-valyl-D-2-hydroxy-isovaleryl-N-methyl-L-valyl-D-lactyl-),
cyclo(-N-methyl-L-valyl-D-2-hydroxy-isovaleryl-N-methyl-L-valyl-D-2-hydroxy-isovaleryl-N-methyl-L-valyl-D-lactyl-),
cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-alloisoleucyl-D-lactyl-N-methyl-L-alloisoleucyl-D-lactyl-),
cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-alloisoleucyl-D-lactyl-N-methyl-L-alanyl-D-lactyl-),
cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-2-amino-butyryl-D-lactyl-),
cyclo(-N-methyl-L-alanyl-D-lactyl-N-methyl-L-alloisoleucyl-D-lactyl-N-methyl-L-2-amino-butyryl-D-lactyl-).

Some of the compounds of the general formula (I) are known (cf., for example V. Z. Pletnev et al. Bioorg. Khim. 1 (2) (1975) pp. 160-165; ref. C.A. 83 (13): 114 872e; DE-OS (German Published Specification) 4 317 458 and can also be obtained by the chemico-synthetic processes described therein.

If, in the process according to the invention for the preparation of the lactic-acid-containing cyclic depsipeptides (enniatins) (I), L-valine (R^1, R^2 and R^4:

-isopropyl) is used as compounds of the formulae (II) to (IV) and D-lactic acid (R^3 and R^5: -methyl) as compounds of the formulae (V) and (VI), the process can be described, for example, by the following equation:



SAM: S-adenosyl-L-methionine

ATP: Adenosine triphosphate

Metal salt: For example alkaline earth metal salt (Mg^{2+} salt) or Mn^{2+} salt.

Formulae (II) to (IV) provide general definitions of the amino acids required as starting compounds for carrying out the process according to the invention. In these formulae, R^1, R^2 and R^4 preferably represent those radicals which have already been mentioned in connection with the description of the substances of the formula (I) according to the invention as being preferred for these substituents.

If the natural or synthetic amino acids which are used as starting substances are chiral, they can exist in the D

or L form. However, alpha-amino acids in the L configuration are preferred.

Examples which may be mentioned are:
Aad, Abu, jAbu, ABz, 2ABz, ϵAca, Ach, Acp, Adpd, Ahb, Aib, ßAib, Ala, ßAla, ΔAla,
Alg, All, Ama, Amt, Ape, Apm, Apr, Arg, Asn, Asp, Asu, Aze, Azi, Bai, Bph, Can, Cit, Cys, $(Cys)_2$, Cyta, Daad, Dab, Dadd, Dap, Dapm, Dasu, Djen, Dpa, Dtc, Fel, Gln, Glu, Gly, Guv, hAla, hArg, hCys, hGln, hGlu, His, hIle, hLeu, hLys, hMet, hPhe, hPro, hSer, hThr, hTrp, hTyr, HyI, Hyp, 3Hyp, Ile, Ise, Iva, Kyn, Lant, Lcn, Leu, Lsg, Lys, ßLys, ΔLys, Met, Mim, Min, nArg, Nle, Nva, Oly, Orn, Pan, Pec, Pen, Phe, Phg, Pic, Pro, ΔPro, Pse, Pya, Pyr, Pza, Qin, Ros, Sar, Sec, Sem, Ser, Thi, ßThi, Thr, Thy, Thx, Tia, Tle, Tly, Trp, Trta, Tyr, Val, Nal, Tbg, Npg, Chg, Thia, (cf., for example, Houben-Weyl, Methoden der Organischen Chemie [Methods in Organic Chemistry], Volumes XV/1 and 2, Stuttgart, 1974).

Formulae (V) to (VI) provide general definitions of the 2-hydroxy-carboxylic acids required as starting substances for carrying out the process according to the invention.

In these formulae: R^3 and R^5 preferably represent those radicals which have already been mentioned in connection with the description of the substances of the formula (I) according to the invention as being preferred for these substituents.

If the 2-hydroxy-carboxylic acids which are used as starting substances are chiral, they can exist in the D or L form. However, the 2-hydroxycarboxylic acids which have the D configuration are preferred.

Examples which may be mentioned are the following: Hyac, Hyba, Hydd, Hyde, Hyic, Hyiv, Hymb, Hypp, Hypr (Lac), Hytd, Hyud, Hyva, (cf., for example, Houben-Weyl, Methoden der Organischen Chemie [Methods in Organic Chemistry], Volumes XV/1 and 2, Stuttgart, 1974).

Fusarium strains which are suitable for carrying out the process according to the invention are the Fusarium strains which follow.

Fusarium strain		isolated from
Fusarium acuminatum		
BBA 61 148		blue lupin
Fusarium arthrosporoides		
BBA 64 134		bent grass (seeds)
Fusarium avenaceum		
BBA 64 338		winter barley (seeds)
BBA 62 163		cabbage
Fusarium compactum		
BBA 65 671		cotton
Fusarium crookwellense		
BBA 64 297		wheat (stem base)
Fusarium ensiforme		
BBA 64 683		sweet potato
Fusarium equiseti		
BBA 64 814		rye
Fusarium inflexum		
BBA 63 203		field bean
Fusarium gibbosum		
Fusarium lateritium		
BAA 65 090		wheat (stem base)
Fusarium meresmoides		
BBA 64 329		rye (stem base)
Fusarium moniliforme		
Fusarium oxysporum		
BBA 62 057	f. pisi	pea
BBA 62 060	f. lycopersici	tomato
BBA 62 334	f. lupini	white lupin
BBA 64 952	f. batatas	sweet potato

Fusarium proliferatum			
	BBA 63 625		dragon tree
Fusarium redolens			
	BBA 62 390		gillyflower
Fusarium sambucinum			
	BBA 63 933		wheat
	BBA 62 397		potato
	NRRL-13 500		potato
	NRRL-13 503		potato
	R-583		poligonum
	R-5390		potato
	R-7570		soil
	R-5455		cereals
	R-6380		potato
	R-7843		pink
	R-5690		soil
	R-2633		potato
	R-6354		cereals
Fusarium scirpi			
	ETH 1536		grassland soil
Fusarium semitectum			
Fusarium solani			
	BBA 64 953		sweet potato
	BBA 62 420	f. pisi	pea
Fusarium subglutinans			
Fusarium tricinctum			
	BBA 62 446		red clover
Fusarium udum			Cajanus inidians
	BBA 62 451		

Particular mention must be made of the Mintolyte Fusarium strains DSM 8938 and DSM 8939, which were deposited on 31.01.1994 at the Deutsche Sammlung für Mikroorganismen (DSM); (German Collection of Microorganisms) in Brunswick in accordance with the Budapest Treaty.

The process can also be carried out using synthetases isolated from microorganisms. The enniatin synthetases required for this purpose can be isolated from the Fusarium strains mentioned further above using processes known from the literature (cf. for example: R. Pieper, H. Kleinkauf, R. Zocher, J. Antibiot. 45(1993) pp. 1273-1277).

The fungal strains of the species Fusarium are fermented by methods known per se in the presence of suitable nutrient solutions. These nutrient solutions contain the salts which are required for the fungal growth, as well as carbon and nitrogen sources.

Suitable inorganic salts for carrying out the process according to the invention are all alkali metal salts, alkaline earth metal salts and metal salts with elements of sub-groups II to VIII of the Periodic Table.

Examples which may be mentioned are the acetates, chlorides, bromides, iodides, fluorides, nitrates, nitrites, phosphates, hydrogenphosphates, dihydrogen-phosphates, phosphites, hydrogenphosphites, sulphates, hydrogensulphates, sulphites, hydrogensulphites,

carbonates or hydrogencarbonates of lithium, sodium, potassium, caesium, magnesium, calcium, barium, zinc, cadmium, scandium, titanium, zirconium, vanadium, niobium, chromium, molybdenum, manganese, iron, cobalt or nickel.

Substances which are preferably used are acetates, halides, phoshates, hydrogenphosphates, dihydrogen-phosphates, nitrates of the alkali metals, in particular sodium and potassium, the sulphates of the alkaline earth metals, in particular magnesium, and metals of sub-groups II, VII and VIII of the Periodic Table, for example zinc, manganese and iron.

Carbon sources for carrying out the process according to the invention are carbohydrates and carbohydrate-containing products.

Examples which may be mentioned are the monosaccharides, such as pentoses, in particular ribose, the hexoses, in particular glucose and fructose, the oligosaccharides, such as disaccharides, in particular sucrose, maltose and lactose, the trisaccharides, in particular raffinose, as well as tetra-, penta- and hexasaccharides.

Monosaccharides, such as, for example, hexoses, in particular glucose, oligosaccharides, such as, for example, disaccharides, in particular sucrose are preferably used.

Suitable nitrogen sources for carrying out the process

according to the invention are amino acids and nitrogen-containing salts.

Examples which may be mentioned are the natural and synthetic amino acids which have been mentioned further above, or nitrogen-containing salts, such as ammonium nitrate, ammonium nitrite, or the nitrates and nitrites of the metals which have been mentioned further above.

The natural amino acids which have been mentioned further above, and nitrogen-containing salts, such as ammonium nitrate are preferably used.

The Fusarium strains used for the fermentative process are first grown by methods known per se in a medium which is composed of, for example, molasses/cornsteep liquor. After they have been grown, the spores formed are isolated by means of spore filters. To produce the preculture, a Fusarium defined medium (FDM), composed of a carbon source and inorganic salts, is inoculated with approximately 10^9 spores and refermented. After a few days, the FDM main culture can be prepared by inoculation with 1 ml of preculture, and fermentation can be carried out analogously.

The actual fermentation is then carried out in the presence of compounds of the formulae (II) to (IV) or (V) and (VI) and in the presence of optically active or racemic lactic acid.

The fermentation time is 1 to 30 days. The fermentation is carried out at temperatures between +5°C and +40°C, preferably between +15°C and +35°C, particularly preferably between +25°C and +30°C. The process is carried out under sterile conditions and under atmospheric pressure.

To carry out the process, the compounds of the formulae (V) and (VI) are generally employed at a concentration of 5 mM to 50 mM, preferably 5 mM to 15 mM.

After the fermentation has ended, the mycelium of the Fusarium culture is filtered off with suction, homogenized extracted repeatedly with an organic solvent, and then filtered. The culture filtrate obtained is extracted in the customary manner, dried and concentrated in vacuo.

The crude enniatins obtained can be purified in the customary manner by column chromatography or counter-current distribution. The optimum procedure must be determined in each individual case (cf. also the Preparation Examples).

If the process according to the invention is carried out in the presence of isolated synthetases, it is carried out using an aqueous buffer system in the presence of metal salts, S-adenosyl-L-methionine (SAM) and adenosine triphosphate (ATP).

Metal salts which may be mentioned are: acetates, chlorides, bromides, iodides, fluorides, nitrates, phosphates, hydrogenphosphates, phosphites, hydrogenphosphites, sulphates, hydrogensulphates, sulphites, hydrogensulphites, carbonates and hydrogencarbonates of lithium, sodium, potassium, caesium, magnesium, calcium or barium.

Salts which are preferably used are alkaline earth metal salts, such as, for example, magnesium chloride, magnesium sulphate or magnesium acetate.

The process according to the invention is carried out in an aqueous buffer solution.

Examples which may be mentioned are commercially available buffer solutions, for example for a pH of 1.0, in particular glycine/hydrochloric acid, for a pH of 2.0 to 4.0, in particular citrate/hydrochloric acid, for a pH of 5.0 to 6.0, in particular citrate/sodium hydroxide solution, for a pH of 7.0, in particular phosphate, for a pH of 8.0, in particular borate/hydrochloric acid, and for a pH of 9.0 to 10.0, in particular boric acid/ potassium chloride/sodium hydroxide solution.

It is preferred to operate within the "physiological range", i.e. at a pH of 6.0 to 9.0, for which it is preferred to use a phosphate buffer solution, in particular potassium hydrogenphosphate/disodium hydrogenphosphate or potassium hydrogenphosphate/

dipotassium hydrogenphosphate.

To carry out the process, 2 mM to 8 mM, preferably 3 mM to 5 mM of compounds of the formulae (II) to (VI), optically active or racemic lactic acid and S-adenosyl-L-methionine (SAM), 3 mM to 9 mM, preferably 4 mM to 6 mM, of adenosine triphosphate (ATP), 2 mM to 25 mM, preferably 5 mM to 15 mM, of alkaline earth metal salt, 10 mM to 100 mM, preferably 40 mM to 60 mM of buffer, are generally employed together with 100 µg to 1000 µg, preferably 200 µg to 600 µg, of isolated enniatin synthetase in vitro.

The reaction time of the enzymatic in-vitro synthesis is 2 minutes to 24 hours. The enzymatic in-vitro synthesis is carried out in a temperature range of 0°C to +50°C, preferably at +10°C to +35°C, particularly preferably between +20°C and +30°C.

It proceeds in a pH range of 6.5 to 8.5, preferably at 7.0 to 8.0, the pH being kept at a constant 7.3 during the entire reaction by adding a buffer.

The process is preferably carried out under sterile reaction conditions and under atmospheric pressure.

The enzymatic in-vitro synthesis can be stopped by diluting with water.

For working up, the aqueous phase is extracted repeatedly

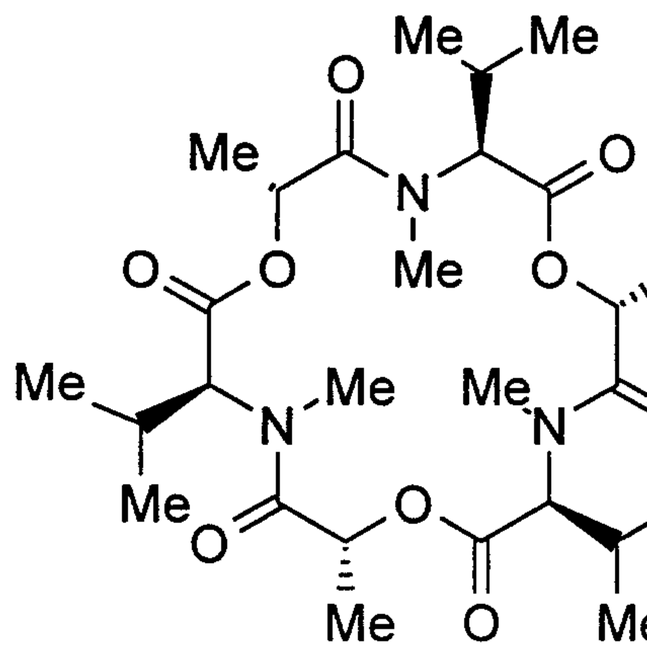
with an organic solvent, and the extract is dried and concentrated in vacuo.

The crude enniatins obtained can be purified in the customary manner by column chromatography or by counter-current distribution. Again, the ideal procedure will have to be determined in each individual case (cf. also the Preparation Examples).

Preparation Examples

Example 1:

Preparation of cyclo(-N-methyl-L-valyl-D-lactyl-N-methyl-L-valyl-D-lactyl-N-methyl-L-valyl-D-lactyl-)



In-vivo incorporation of D-lactic acid

D-lactic acid is added under sterile conditions to a 2-day-old main culture of *Fusarium scirpi*, at a concentration of 10 mM, and the fermentation is continued

for another 3 days. The mycelium of the Fusarium culture is then filtered off with suction, and the filtrate is extracted three times using ethyl acetate. The mycelium is homogenized twice in a mortar using acetone, and the homogenate is subsequently subjected to filtration with suction. The culture filtrate is extracted by shaking three times with in each case 100 ml of ethyl acetate, and the combined organic phases, together with the acetone extract are evaporated to dryness.

Alternatively, the entire Fusarium culture can be extracted overnight in approximately twice its volume of ethyl acetate.

To concentrate the enniatin by column chromatography, the crude enniatin, which is dissolved in a small amount of chloroform, is applied to an Al_2O_3 column (30 × 2 cm) and eluted stepwise.

Enzymatic <u>in-vitro</u> synthesis

300-500 µg of purified enniatin synthetase in 50 mM of phosphate buffer (pH 7.3) are incubated for 10 minutes at 28°C in a total volume of 1.5 ml in the presence of 4 mM L-valine, 4 mM D-lactic acid, 4 mM S-adenosyl-L-methionine (SAM), 5 mM adenosine triphosphate (ATP) and 10 mM $MgCl_2$.

After adding of 2 ml of water.
the mixture is extracted repeatedly using 2 ml portions

portions of ethyl acetate. The organic phase is dried using sodium sulphate and then concentrated in vacuo.

The product which has been obtained by a) or b) is purified by means of preparative HPLC (RP 18/75-80% methanol).

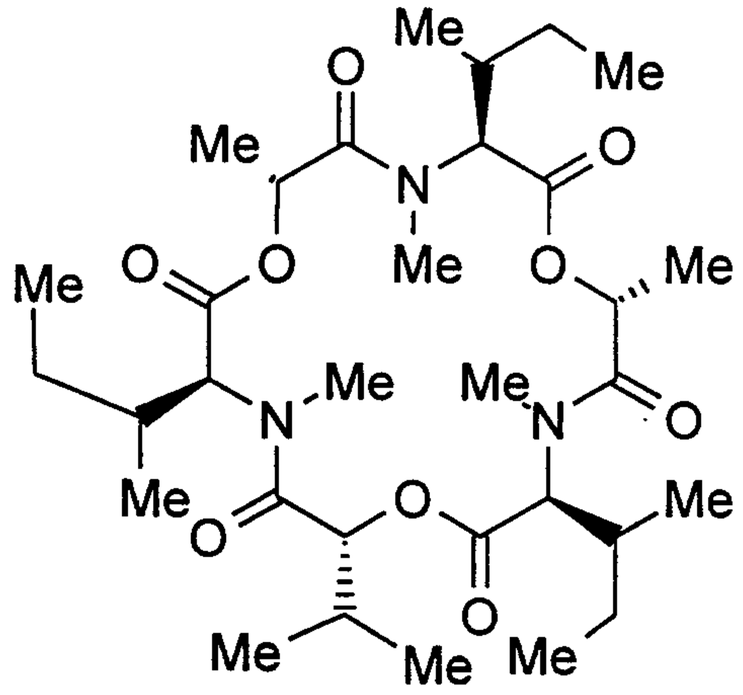
^{1}H NMR (400 MHz, $CDCl_3$, δ): 0.83; 1.03 (d, 18H, 3 × -$CH(C\underline{H}_3)_2$); 1.45 (d, 9H, 3 × -O-CH-$C\underline{H}_3$); 2.27 (m, 3H, 3 × -$C\underline{H}(CH_3)_2$); 3.06 (s, 9H, 3 × -N-$C\underline{H}_3$); 4.43 (d, 3H, 3 × -N-C<u>H</u>-CO-); 5.62 (q, 3H, 3 × -O-C<u>H</u>-CO-) ppm

^{13}C NMR (100 MHz, $CDCl_3$, δ): 16.5 (3 × -$\underline{C}H_3$, D-Lac); 18.5; 20.1 (6 × -$\underline{C}H_3$, MeVal); 27.8 (3 × -$\underline{C}H(CH_3)_2$, L-MeVal); 32.9 (3 × -N-$\underline{C}H_3$, L-MeVal); 63.1 (3 × -N-<u>C</u>H-CO-, L-MeVal); 66.3 (3 × -O-<u>C</u>H-CO-, D-Lac); 169.2 (3 × -<u>C</u>=O, amide); 169.8 (3 × -<u>C</u>=O, ester) ppm

EI MS m/z (%): 555 (M^+, 32); 482 (20); 353 (1); 268 (34); 168 (100); 86 (53)

Example 2

Preparation of cyclo(-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-isoleucyl-D-lactyl-N-methyl-L-isoleucyl-D-2-hydroxy-isovaleryl-)



In-vivo incorporation of D-lactic acid

D-lactic acid is added under sterile conditions to a 2-day-old main culture of Fusarium sambucinum, at a concentration of 10 mM, and the fermentation is continued for another 3 days. The mycelium of the Fusarium culture is then filtered off with suction, and the filtrate is extracted three times using ethyl acetate. The mycelium is homogenized twice in a mortar using acetone, and the homogenate is subsequently subjected to filtration with suction. The culture filtrate is extracted by shaking three times with in each case 100 ml of ethyl acetate, and the combined organic phases, together with the acetone extract are evaporated to dryness.

Concentration and purification of the enniatin are carried out as described in Example 1.

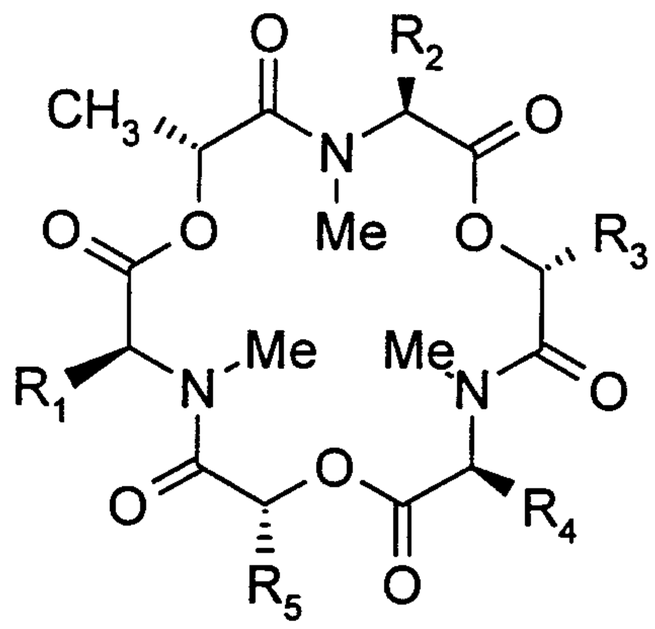
^{13}C NMR (100 MHz, $CDCl_3$), δ): 18.4; 18.0 (2 × -$\underline{C}H_3$, D-HyIv); 16.8; 15.8 (2 × -$\underline{C}H_3$, D-Lac); 16.7; 15.3; 11.4; 10.6; 10.3; 10.0 (6 × -$\underline{C}H_3$, L-Melle); 25.0; 24.9; 24.4 (3 × -$\underline{C}H_2$-, L-Melle); 34.7; 34.1; 32.3 (3 × -$\underline{C}H(CH_3)$-, L-Melle); 35.6; 31.6; 31.2 (3 × -N-CH_3, L-Melle); 29.9 (-$\underline{C}H(CH_3)_2$, D-HyIv); 74.0; 67.5; 66.1 (3 × -O-$\underline{C}H$-CO-, D-HyIv); 65.1; 60.5; 59.5; (3 × -N-$\underline{C}H$-CO-, L-Melle); 169.2; 169.1; 169.0 (3 × -$\underline{C}$=O, amide); 170.6; 170.1; 169.8 (3 × -$\underline{C}$=O, ester) ppm

EI MS m/z (%): 625 (M^+, 23); 552 (14); 409 (5); 296 (20); 182 (43); 100 (100)

Analogously, the compounds of the general formula (I) listed in Table I below can be prepared in the form of LDLDLD stereoisomers.

Table 1

Examples of compounds of the general formula (I)



(I)

Ex.-No.	Radical R^1	Radical R^2	Radical R^3	Radical R^4	Radical R^5	Physical data[a)]
3	-s-C_4H_9	-s-C_4H_9	$-CH_3$	-s-C_4H_9	$-CH_3$	33.9 (-N-$\underline{C}H_3$); 61.9 (-N-$\underline{C}$H-); 66.4 (-O-$\underline{C}$H-); 169.3 (-$\underline{C}$O-N-); 169.9 (-$\underline{C}$O-O-); 598 (M^++H, 12); 597 (37); 541 (42); 524 (14; 182 (100)
4	-i-C_4H_9	-i-C_4H_9	$-CH_3$	-i-C_4H_9	$-CH_3$	32.0 (-N-$\underline{C}H_3$); 55.8 (-N-$\underline{C}$H-); 67.0 (-O-$\underline{C}$H-); 169.4 (-$\underline{C}$O-N-); 170.5 (-$\underline{C}$O-O-); 597 (M^+, 22); 524 (7); 381 (1); 296 (15); 182 (100); 100 (78)
5	$-CH_3$	-s-C_4H_9	$-CH_3$	-s-C_4H_9	$-CH_3$	32.8; 33.9; 34.2 (-N-$\underline{C}H_3$); 56.2; 59.9; 60.5 (-N-$\underline{C}$H-); 66.0; 66.3; 67.7 (-O-$\underline{C}$H-); 168.7; 169.7; 170.1 (-$\underline{C}$O-N-); 169.0; 170.0; 170.4 (-$\underline{C}$O-O-); 555 (M^+, 64); 499 (37); 428 (12); 357 (19); 182 (100); 100 (52)
6	$-CH_3$	-s-C_4H_9	$-CH_3$	$-CH_3$	$-CH_3$	31.8; 33.8; 34.6 (-N-$\underline{C}H_3$); 168.1; 168.7; 169.9 (-$\underline{C}$O-N-); 170.0; 170.4; 170.5 (-$\underline{C}$O-O-); 513 (M^+, 42); 440 (22); 255 (29); 213 (60); 182 (75); 58 (100)
7	-i-C_3H_7	-i-C_3H_7	-i-C_3H_7	-i-C_3H_7	-i-C_3H_7	611 (M^+, 29); 528 (15); 196 (100)

a) ^{13}C NMR (100 MHz, $CDCl_3$, δ); FAB-MS or EI MS m/z (%)

Spore culture, precultures and main cultures

The Fusarium strain in question is grown in a medium composed of molasses/cornsteep liquor (30 g and 10 g/l, respectively).

The culture is grown in 500 ml Erlenmeyer flasks (100 ml of medium) at 100 rpm (26 to 28°C). After 4 to 5 days, the spores which have formed are isolated by means of a spore filter. These spores can be kept for weeks at 4°C.

To prepare a preculture, a flask containing 200 ml of FDM (75.0 g of sucrose, 12.75 g of $NaNO_3$, 15.0 g of NaCl, 7.5 g of $MgSO_4 \cdot 7 H_2O$, 4.0 g of $KH_2PO_4 \cdot 7 H_2O$, 10 g of $ZnSO_4$ per litre) is inoculated with 10^9 spores and fermented as above.

After 2 to 3 days, FDM main cultures are prepared by using in each case 1 ml of preculture as inoculum, and fermented as above.

Preparations for the in-vitro synthesis aimed at producing enniatin

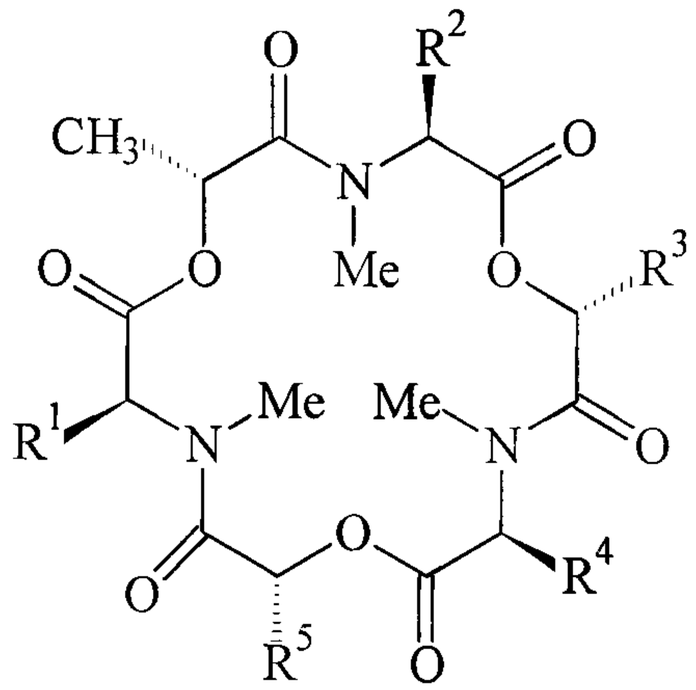
2- to 3-day-old main cultures are first examined for their enniatin titers to ensure that the cells are actively synthetizing. To this end, 3 to 5 ml of culture are sampled under sterile conditions and extracted repeatedly using in each case 2 ml of ethyl acetate. After evaporating the organic phase, the enniatin is examined directly by HPLC (RP 18.80% methanol).

The main cultures in question are treated under sterile conditions with the corresponding precursor hydroxy- or amino acid to an end concentration of 10 mM, and the fermentation is continued as described in Example 1 (total fermentation time approximately 1 week).

30725-72

CLAIMS:

1. Use of a fungal strain of the species Fusarium or an enzymatic preparation isolated therefrom for the preparation of a lactic-acid-containing, optically active, cyclic depsipeptide having 18 ring atoms, wherein the optically active, cyclic depsipeptide having 18 ring atoms is of the formula (I):



(I)

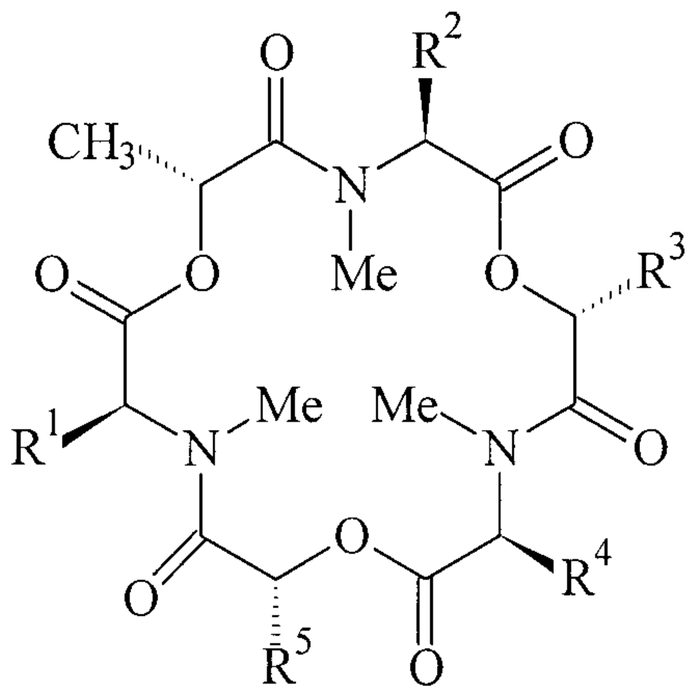
in which

R^1, R^2 and R^4 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl, alkylsulphinylalkyl, alkylsulphonylalkyl, carboxyalkyl, alkoxycarbonylalkyl, carbamoylalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, guanidinoalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, or optionally substituted arylalkyl or hetarylalkyl, substituents being halogen, hydroxyl, alkoxy, alkyl, nitro or amino,

R^3 and R^5 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl,

alkylsulphinylalkyl, alkylsulphonylalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, or optionally substituted arylalkyl or hetarylalkyl, substituents being halogen, hydroxyl, alkoxy, alkyl, nitro or amino.

2. A process for the preparation of a lactic-acid-containing, optically active, cyclic depsipeptide having 18 ring atoms of the formula (I)



(I)

in which

R^1, R^2 and R^4 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl, alkylsulphinylalkyl, alkylsulphonylalkyl, carboxyalkyl, alkoxycarbonylalkyl, carbamoylalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, guanidinoalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, or optionally substituted arylalkyl or hetarylalkyl, substituents being halogen, hydroxyl, alkoxy, alkyl, nitro or amino,

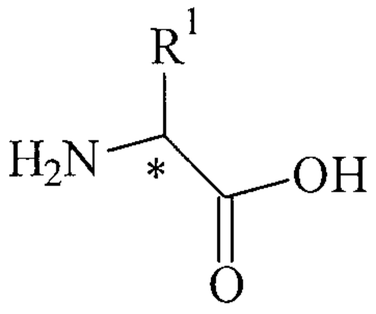
R^3 and R^5 independently of one another represent hydrogen, straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, mercaptoalkyl, alkylthioalkyl,

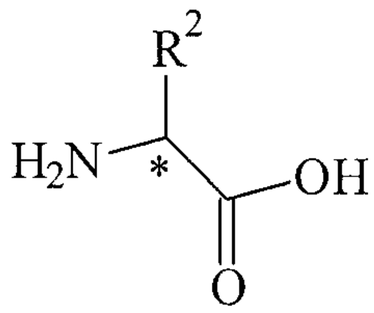
30725-72

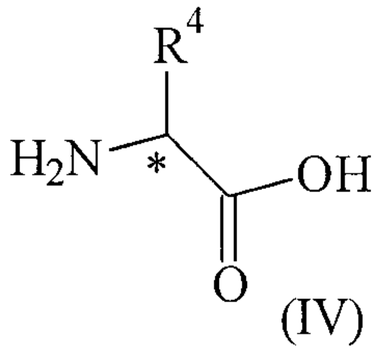
alkylsulphinylalkyl, alkylsulphonylalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, cyclic alkyl having up to 8 carbon atoms, or optionally substituted arylalkyl or hetarylalkyl, substituents being halogen, hydroxyl, alkoxy, alkyl, nitro or amino,

the process comprising reacting:

(i) an optically active or racemic amino acid of the formula (II), (III) or (IV)


(II)

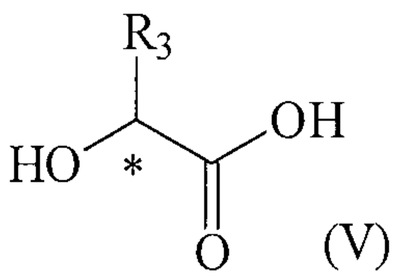

(III)

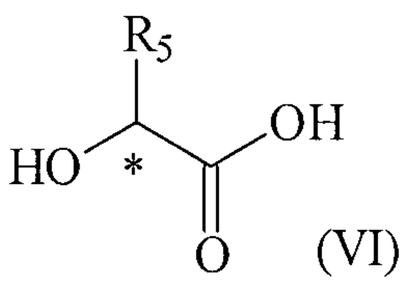

(IV)

in which

R^1, R^2 and R^4 have the abovementioned meaning,

(ii) an optically active or racemic 2-hydroxy-carboxylic acid of the formula (V) or (VI)


(V)


(VI)

in which

R^3 and R^5 have the abovementioned meaning, and

(iii) an optically active or racemic lactic acid,

wherein the reaction is carried out in the presence of a fungal strain of the species Fusarium in a suitable nutrient solution or in a buffer system in the

presence of a synthetase isolated from a fungal strain of the species Fusarium.

3. The process according to claim 2, wherein

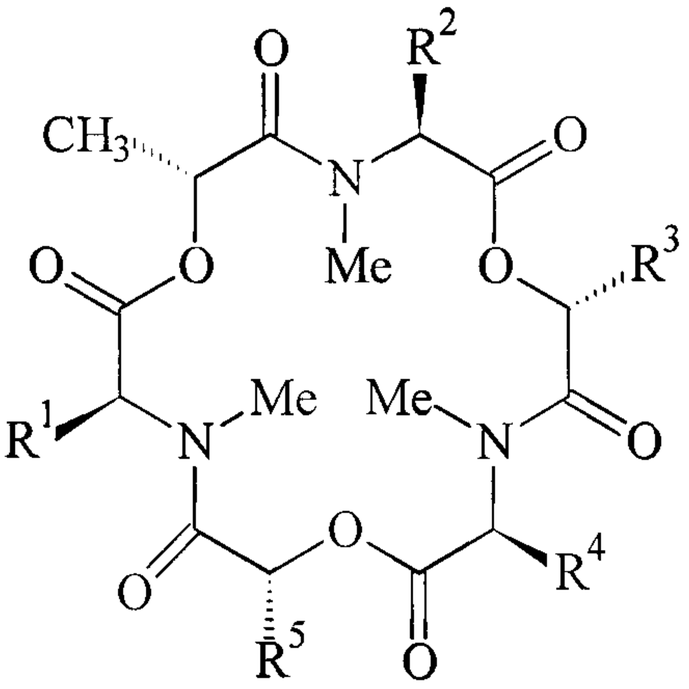
R^1, R^2 and R^4 independently of one another represent hydrogen, straight-chain or branched C_1-C_8-alkyl, hydroxy-C_1-C_4-alkyl, mercapto-C_1-C_4-alkyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, C_1-C_4-alkylsulphinyl-C_1-C_4-alkyl, C_1-C_4-alkylsulphonyl-C_1-C_4-alkyl, carboxy-C_1-C_6-alkyl, carbamoyl-C_1-C_6-alkyl, amino-C_1-C_6-alkyl, C_1-C_4alkylamino-C_1-C_6-alkyl, C_1-C_4-dialkylamino-C_1-C_6-alkyl, guanido-C_1-C_6-alkyl, C_2-C_6-alkenyl, or C_3-C_7-cyclo-C_1-C_4-alkyl,

R^3 and R^5 independently of one another represent hydrogen, straight-chain or branched C_1-C_8-alkyl, hydroxy-C_1-C_4-alkyl, mercapto-C_1-C_4-alkyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, C_1-C_4-alkylsulphinyl-C_1-C_4-alkyl, C_1-C_4-alkylsulphonyl-C_1-C_4-alkyl, C_2-C_6-alkenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, hetaryl-C_1-C_4-alkyl, phenyl-C_1-C_4-alkyl which can optionally be substituted by a halogen radical, hydroxyl, nitro, amino, C_1-C_4-alkoxy, or C_1-C_4-alkyl.

4. The process according to claim 3, wherein the phenyl-C_1-C_4-alkyl is optionally substituted by fluoro, chloro, bromo, iodo, methoxy, ethoxy or methyl.

5. A process for the preparation of a lactic-acid-containing optically active, cyclic depsipeptide having 18 ring atoms of the formula (I):

30725-72



(I)

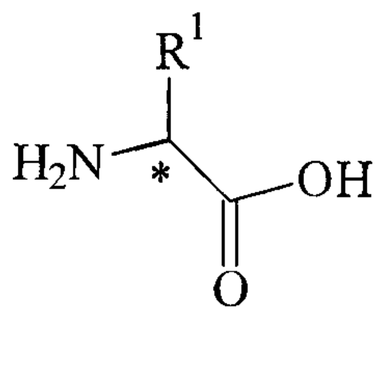
in which

R^1, R^2 and R^4 independently represent straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, alkylthioalkyl, carboxyalkyl, carbamoylalkyl, aminoalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, or cyclic alkyl having up to 8 carbon atoms; and

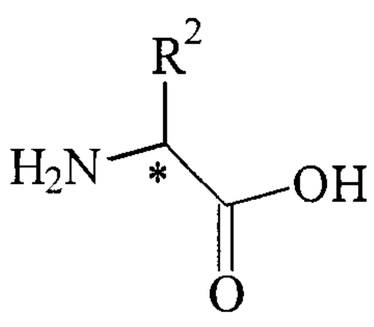
R^3 and R^5 independently represent straight-chain alkyl, hydroxyalkyl, or alkylthioalkyl each having up to 6 carbon atoms;

said process comprising reacting:

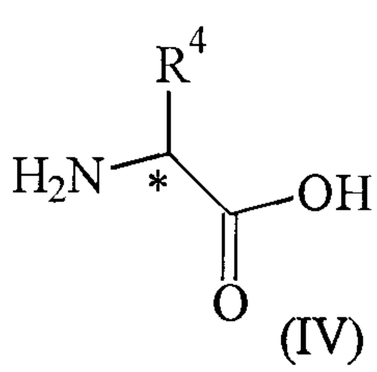
(i) an optically active or racemic amino acid of the formula (II), (III) or (IV):



(II)



(III)



(IV)

in which

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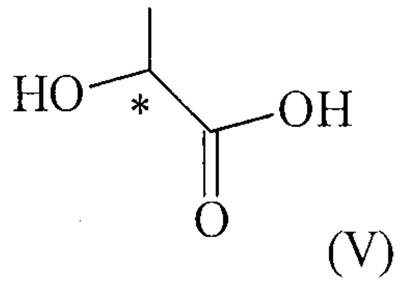
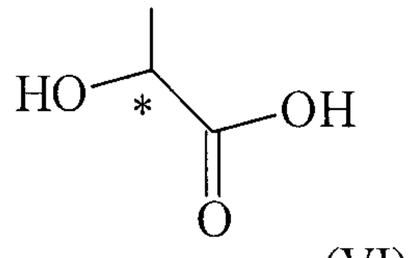
R^1, R^2 and R^4 have the abovementioned meanings;

(ii) an optically active or racemic 2-hydroxy-carboxylic acid of the formula (V) or (VI):



(V)



(VI)

in which

R^3 and R^5 have the abovementioned meanings; and

(iii) an optically active or racemic lactic acid;

wherein the reaction is carried out in the presence of a fungal strain, which is a Fusarium fungal strain, in a suitable nutrient solution.

6. The process according to claim 5,

in which

R^1, R^2 and R^4 independently represent straight-chain or branched alkyl having up to 6 carbon atoms, or straight-chain or branched alkenyl having up to 6 carbon atoms; and

R^3 and R^5 independently represent straight-chain alkyl or hydroxyalkyl having up to 6 carbon atoms.

7. The process according to claim 5,

in which

R^1, R^2 and R^4 independently represent straight-chain or branched alkyl having up to 6 carbon atoms, or

straight-chain or branched alkenyl having up to 6 carbon atoms; and

R^3 and R^5 independently represent straight-chain alkyl having up to 6 carbon atoms.

8. The process according to claim 2, 3, 4, 5, 6 or 7, wherein the fungal strain is Fusarium DSM 8938.

9. The process according to claim 2, 3, 4, 5, 6 or 7, wherein the fungal strain is Fusarium DSM 8939.

10. The use according to claim 1, wherein

R^1, R^2 and R^4 independently of one another represent hydrogen, straight-chain or branched C_1-C_8-alkyl, hydroxy-C_1-C_4-alkyl, mercapto-C_1-C_4-alkyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, C_1-C_4-alkylsulphinyl-C_1-C_4-alkyl, C_1-C_4-alkylsulphonyl-C_1-C_4-alkyl, carboxy-C_1-C_6-alkyl, carbamoyl-C_1-C_6-alkyl, amino-C_1-C_6-alkyl, C_1-C_4alkylamino-C_1-C_6-alkyl, C_1-C_4-dialkylamino-C_1-C_6-alkyl, guanido-C_1-C_6-alkyl, C_2-C_6-alkenyl, or C_3-C_7-cyclo-C_1-C_4-alkyl,

R^3 and R^5 independently of one another represent hydrogen, straight-chain or branched C_1-C_8-alkyl, hydroxy-C_1-C_4-alkyl, mercapto-C_1-C_4-alkyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, C_1-C_4-alkylsulphinyl-C_1-C_4-alkyl, C_1-C_4-alkylsulphonyl-C_1-C_4-alkyl, C_2-C_6-alkenyl, C_3-C_7-cyclo-C_1-C_4-alkyl, hetaryl-C_1-C_4-alkyl, phenyl-C_1-C_4-alkyl which can optionally be substituted by a halogen radical, hydroxyl, nitro, amino, C_1-C_4-alkoxy, or C_1-C_4-alkyl.

11. The use according to claim 10, wherein the phenyl-C_1-C_4-alkyl is optionally substituted by fluoro, chloro, bromo, iodo, methoxy, ethoxy or methyl.

30725-72

12. The use according to claim 1, wherein

R^1, R^2 and R^4 independently represent straight-chain or branched alkyl having up to 8 carbon atoms, hydroxyalkyl, alkylthioalkyl, carboxyalkyl, carbamoylalkyl, aminoalkyl, straight-chain or branched alkenyl having up to 6 carbon atoms, or cyclic alkyl having up to 8 carbon atoms; and

R^3 and R^5 independently represent straight-chain alkyl, hydroxyalkyl, or alkylthioalkyl each having up to 6 carbon atoms.

13. The use according to claim 1, wherein

R^1, R^2 and R^4 independently represent straight-chain or branched alkyl having up to 6 carbon atoms, or straight-chain or branched alkenyl having up to 6 carbon atoms; and

R^3 and R^5 independently represent straight-chain alkyl or hydroxyalkyl having up to 6 carbon atoms.

14. The use according to claim 1, wherein

R^1, R^2 and R^4 independently represent straight-chain or branched alkyl having up to 6 carbon atoms, or straight-chain or branched alkenyl having up to 6 carbon atoms; and

R^3 and R^5 independently represent straight-chain alkyl having up to 6 carbon atoms.

15. The use according to claim 1, 10, 11, 12, 13 or 14, wherein the fungal strain is Fusarium DMS 8938.

16. The use according to claim 1, 10, 11, 12, 13 or 14, wherein the fungal strain is Fusarium DMS 8939.