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(54) Title: MEMNO PEPTIDES, A PROCESS FOR THEIR PREPARATION AND THEIR USE

(57) Abstract: The invention relates to peptide derivatives, called memno peptides, of the formula (I): wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, and (A)n have the meaning herein, obtainable by cultivation of Memnoniella echinata FH2272, DSM 13195 or mutants and variants of this, a process for their preparation and the use of the compounds as pharmaceuticals, for example against cardiac insufficiency.

Memno peptides, a process for their preparation and their use.

The invention relates to novel peptide derivatives, called memno peptides, obtainable by fermentation of Memnoniella echinata FH2272, DSM 13195, in a culture medium, a process for the preparation of the memno peptides, and the use of the memno peptides as pharmaceuticals, for example, for the production of a pharmaceutical for the treatment of cardiac insufficiency.

Cardiovascular disorders still rank first as causes of death in the western industrial countries. A not inconsiderable proportion of these are patients with the diagnosis of cardiac insufficiency. Cardiac insufficiency is understood as meaning the inadequate functioning of the heart. The heart is not able to produce an output corresponding to the mammal's requirements. Cardiac insufficiency is an acute or chronic inability of the heart, under load or even at rest, to muster the blood output necessary for metabolism or to take up the venous return. It is the state of the heart wherein the compensation mechanisms, such as heart rate, stroke volume, or hypertension, no longer suffice for the maintenance of a normal cardiac output. Cardiac insufficiency has a variety of causes, for example inflammatory and degenerative myocardial (heart muscle) changes, coronary circulatory disorders and cardiac infarct. Cardiac insufficiency leads to changes in the peripheral circulation, to impairment of the respiration, the kidney function, and the electrolyte balance, and also to reduced power of the skeletal musculature, and in the end it frequently leads to death.

Cardiac insufficiency generally occurs at an advanced age. The incidence is 3 disorders per 1000 inhabitants per year in 35-64 year-olds and 10/1000/year in the age group from 65 to 94 years. Mortality increases in 75-year-olds almost by a factor of 200 compared with the age group between 35 and 44 years. The mortality rate has remained approximately constant between 1970 and 1983, as investigations in the USA showed. For the Federal Republic of Germany, the same numbers are to be assumed. More than 50% of patients die in the first five years after diagnosis. This statistical examination, in and of itself, shows the great importance of cardiac

insufficiency for the population, but it also confirms the inadequate possibilities of medicinal treatment which are available to the physician today.

In view of the inadequacy of present treatments, new concepts have been developed which should lead to innovative cardiac remedies. The ability of the cardiac and skeletal muscles to contract and thus to perform mechanical work is dependent on (1) contractile structural elements (myofibrils) and (2) chemical energy (ATP) available to the myofibrils, which is converted into mechanical energy in the contraction process. Shortening of the myofibrils occurs in the contraction process. This may be initiated by motor nerve impulses, under the action of which calcium ions (Ca^{2+}) enter into the sarcoplasmatic space from the extracellular space within a few milliseconds and the calcium depots are emptied. In myocardial insufficiency (cardiac insufficiency), the Ca^{2+} concentration in myofibrils may be reduced. Ca^{2+} ions, however, are indispensable for the activation of the contractile apparatus. If there is increased demand, Ca^{2+} is generally pumped into the sarcoplasmatic reticulum (SR) under catalysis of a membranous Ca^{2+}-dependent Mg^{2+}-ATPase: this enzyme is also called Sarco(Endo)plasmatic Reticulum Ca^{2+}ATPase (SERCA2). According to hydropathic analysis, the Ca^{2+}ATPase comprises ten transmembranous helices and a number of extramembranous loops. On the cytosolic side, domains for Ca^{2+} and ATP binding, for phosphorylation and for interaction with the modulator protein phospholamban (PLB) are formed. The latter is a protein pentamer, which is localized in the membrane of the SR and exerts an inhibitory influence on SERCA2 in the unphosphorylated state. Under physiological stress, a phosphorylation of PLB takes place, which increases the Ca^{2+} affinity of SERCA2a and thus increases the transport rate for Ca^{2+} ions in the SR. The phosphorylation of PLB (a 52 amino acid protein) takes place on two amino acid residues: serine-16 may be phosphorylated by the cAMP-dependent protein kinase and threonine may be phosphorylated in position 17 by the Ca^{2+}/calmodulin-dependent kinase. This phosphorylation causes a change in confirmation in PLB followed by an increased affinity of SERCA2 for Ca^{2+}. Anti-PLB antibodies are able to imitate the PLB phosphorylation effect and thus confirm the key role of PLB as a regulator of the contractile activity of the heart (Phospholamban: Protein Structure, Mechanism of Action and Role in Cardiac Function. H. K. Simmerman and L. R. Jones, Physiological

Reviews; Vol. 78, No. 4, 921ff, 1998). Activators of SERCA2 should thus bring about a favorable influence in cardiac insufficiency.

It has surprisingly been found that cultures of the fungal strain Memnoniellla echinata FH 2272, DSM 13195 contain natural substances which are able to display favorable effects on the heart and the circulation. The isolated active compounds, the memno peptides, are natural substances comprising specific constituent groups. These consitiuent groups include terpene units, a so-called polyketide moiety and a nitrogen-containing group.

Terpenes are naturally occurring compounds which can be interpreted formally as polymerization products of the hydrocarbon isoprene. According to the number of isoprene groups, monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}) etc. can be differentiated. A large number of compounds can be formed from the parent structures by substitution, cyclization, rearrangement, oxidation etc.; accordingly, many thousands of terpenes have been described in the literature. Nitrogen-containing compounds originating from the terpenes have also been reported, but these are counted among the alkaloids (e. g. the Gentiana alkaloids) [Römpp Chemie Lexikon [Römpp's Chemical Encyclopedia], 9th Edition, Volume 6, page 4508 ff., Georg Thieme Verlag, Stuttgart/New York, 1992]. These terpenes, however, differ fundamentally from the memno peptides according to the invention, wherein terpenes do not contain a polyketide moiety with which they can bind nitrogen.

Examples of further, known nitrogen-containing terpenes are:

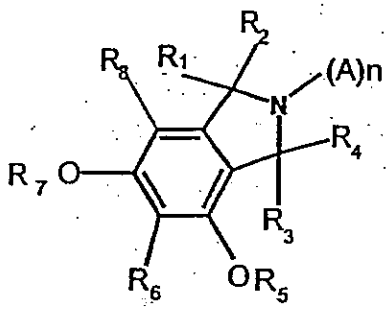
- Stachybocins [J. Antibiotics, 48: 1396 (1995)];
- Stachybotrins [Y. Nozawa et al. J. Antibiotics, 50: 635-645 (1997)];
- Spirodihydrobenzofuran lactams [J. Antibiotics, 49: 13 (1996)];
- Nakijiquinones [Tetrahedron, 51: 10867-10874 (1995)];
- F1839-A to J are nitrogen-containing terpenes having polyketide moieties [Japanese Patent 061864133 and 08283118]. They are cholesterol esterase inhibitors.

These terpene derivatives were synthesized from various strains of the genera Memnoniella echinata and Stachybotrys and others. They were described as antagonists of the endothelin receptor, as inhibitors of HIV-1 protease and of cholesterol esterase and as hair tonics. The inositol monophosphatase inhibitor

L-671,776 was moreover isolated from cultures of the strain Memnoniella echinata, ATCC 20928 [Y.K.T. Lam et al. J. Antibiotics, 45, pp. 1397-1402, (1992)].

The memno peptides according to the invention have a differing spectrum of activity. A conspicuous feature is their activating effect on SERCA2 and thus on the insufficient heart.

A first aspect of the present invention thus relates to compounds of formula (I)



I

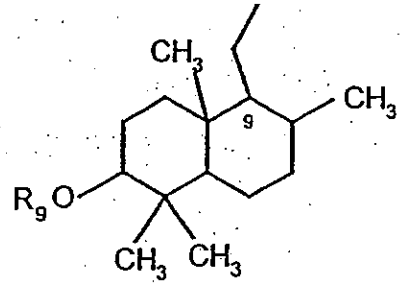
wherein

R_1 and R_2 together are double bonded O, or both hydrogen, or H and OH, or H and O-C_1-C_4-alkyl;

R_3 and R_4 together are double bonded O, or both hydrogen, or H and OH, or H and O-C_1-C_4-alkyl;

R_8 is chosen from H, OH, C_1-C_4-alkyl and O-C_1-C_4-alkyl,

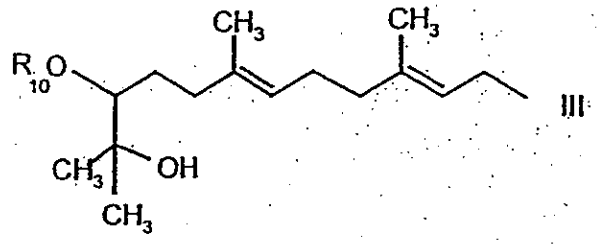
R_6 is a group chosen from a group of formula (II)



II

wherein R_9 is chosen from a hydrogen atom and a glycosidically bonded sugar, and

a group of the formula (III)



III

wherein R_{10} is H or a glycosidically bonded sugar;

and wherein,

if R_6 is a group of the formula (II), then R_5 is a bond to the carbon atom C9 of the formula (II) and R_7 is a hydrogen atom, or R_7 is a bond to the carbon atom C9 of the formula (II) and R_5 is a hydrogen atom, and

if R_6 is a group of the formula III, then R_5 and R_7 are each hydrogen atoms;

A is an amino acid;

n is an integer chosen from 1 to 12, wherein each A is the same or different from every other A;

wherein the nitrogen atom in the isoindole ring of formula (I) is the N-terminal amine nitrogen of the first amino acid of the (A)n group;

or a salt or derivative thereof;

with the proviso that

A is an amino acid other than Glu when n is 1, and R_1 and R_2 together are double bonded O, and R_3 and R_4 together are both hydrogen, and R_6 is a group of formula (II), and R_5 is a bond to the carbon atom C9 of the group of formula (II), and R_7 is H, and R_8 is H and R_9 is H.

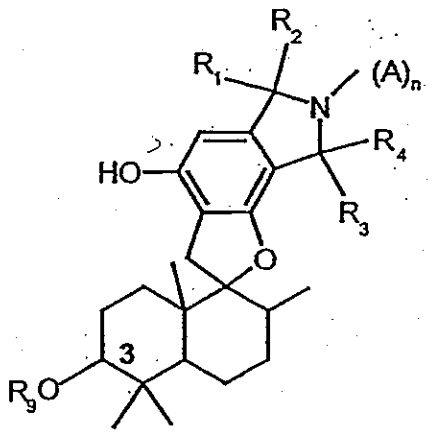
In formula (I), (A)n can be at least one natural amino acid selected from: Gly, Ala, Val, Leu, Ile, Pro, Ser, Thr, Phe, Tyr, Trp, Lys, Arg, Asp, His, Glu, Asn, Gln, Cys and Met. (A)n can be a peptide chain having from 2 to 12 amino acids. In one embodiment (A)n may comprise 10 amino acids.

C_1-C_4-Alkyl is a straight-chain or branched alkyl having 1 to 4 carbon atoms, such as methyl, ethyl, i-propyl and tert-butyl.

The sugar can be a hexose, for example an aldohexose, such as mannose, glucose or galactose, which may be optionally substituted with additional groups, such as C_1 to C_4-Alkyl or NH_2.

An embodiment may relate to all obvious derivatives of the compounds of the formula I. Derivatives may be salts, reduction products, esters, ethers, acetals as well as amides and N-alkylation products, moreover all optical antipodes, diastereomers and all stereomeric forms.

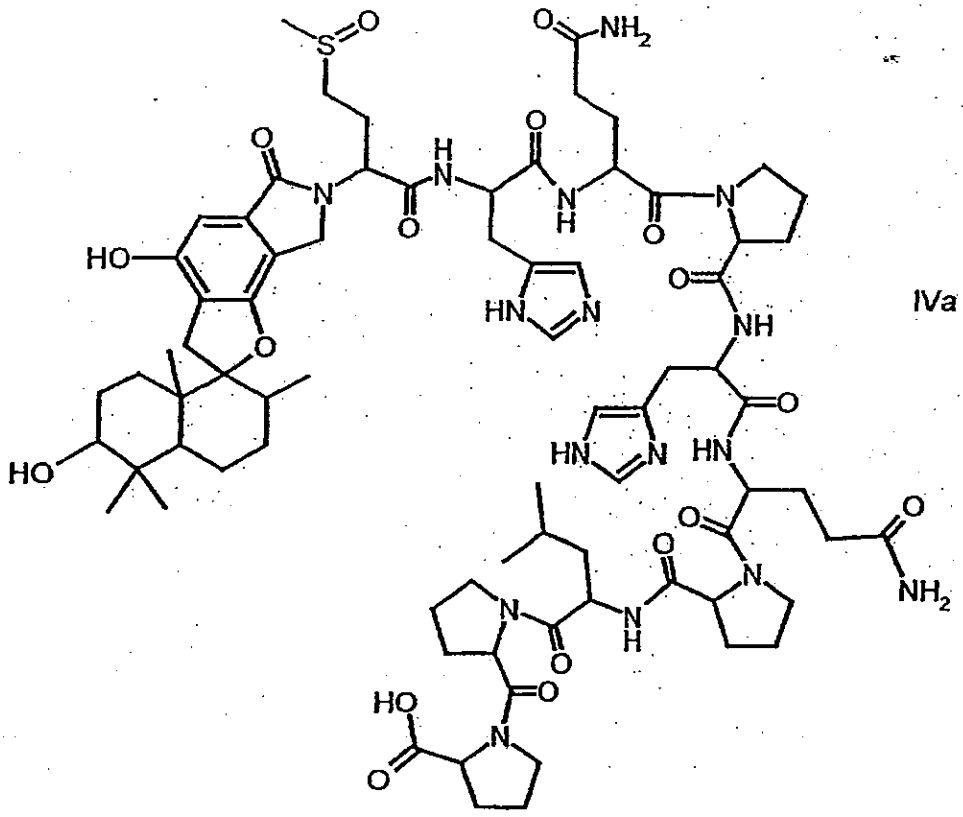
In one embodiment, the compound of formula (I) may have formula (IV)



IV

where the groups R_1, R_2, R_3, R_4, R_9 and (A)n have the meaning as indicated above. In the formula (IV), R_1 and R_2 taken together can be double bonded O, R_3 and R_4 together can be H_2 and R_9 can be H.

In one embodiment, the inventive compound may be memno peptide A, $C_{76}H_{108}N_{16}O_{18}S$, of the formula (IVa)



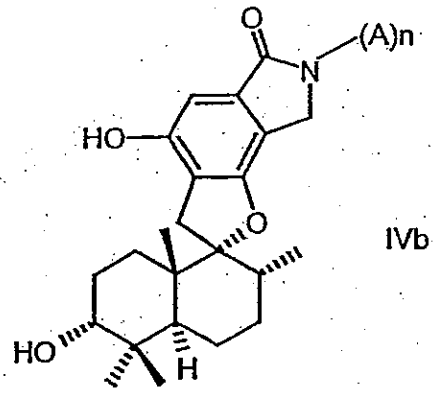
IVa

In this formula, the decalin structure having the 4-methyl and a methylene group may be the terpene moiety; the substituted isoindole ring may be the ketide moiety. The amino acid sequence:

Met His Gln Pro His Gln Pro Leu Pro Pro (SEQ ID NO:1)

is a part of the casein sequence. The methionine (Met) can be oxidized to the sulfoxide.

Formula (IVb) may show a preferred spatial form:



IVb

wherein (A)n may have the meaning as indicated above.

The physicochemical and spectroscopic properties of the preferred compound according to the invention can be summarized as follows:

Memno peptide A

Appearance: colorless substance soluble in polar organic solvents and in water.
It is stable in neutral, mildly acidic medium.
Empirical formula: $C_{76}H_{108}N_{16}O_{18}S$,
Molecular weight: 1565.87 Da,
UV absorption (λ_{max}): 270 nm,
NMR data: see Table 1

The numbering of the carbon atoms and the associated NMR chemical shifts are classified according to the numbering procedure for cyclic sesquiterpenes.

Figure 1: Numbering of a cyclic sesquiterpene ketolide.

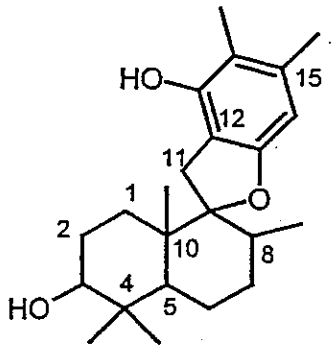


Table 1: NMR data (chemical shifts) of memno peptide A in DMSO - d_6 at 310K.

	δ-^{13}C	m	δ-^{1}H	m	$^{n}J_{HH}$	$^{n}J_{CH}$ (10 Hz)	assignment
1	15.42	q	0.668	d	1.802	1.802	Terpene-8-Me
2	15.72	q	0.978	s	-	2.035, 1.763	Terpene-10-Me
3	20.37	t	1.409 1.460	m	2.035, 1.538 2.035,	2.035	Terpene-6
4	21.43	q	0.873	d	1.678	1.678, 1.428, 0.890	Leu^{8}-δ
5	22.25	q	0.827	s	0.918	0.918, 2.035	Terpene-4-Me
6	22.64 22.80	t t	2.182 2.307 2.19 2.31	ddt ddt ddt ddt	4.892, 2.788 2.632 4.869, 2.757, 2.635	4.892, 2.788, 2.632 4.869, 2.757, 2.635	Met^{1}-β
7	23.08	q	0.890	d	1.678	1.678, 1.428, 0.873	Leu^{8}-δ'
8	23.88 br	d	1.678	m	0.87, 0.88		Leu^{8}-γ
9	23.90	t	1.764 0.962	m m	0.962, 1.416, 1.871	0.978, 1.416, 3.227	Terpene-1
10	24.22	t	1.910	m	2.144, 4.588, 3.467	2.144, 1.771, 4.588, 3.467, 3.689	Pro^{9}-γ
11	24.32	t	1.863	m	2.031, 1.798, 3.625	4.356, 3.625, 2.153, 1.792	Pro^{4}-γ
12	24.37	t	1.927	m	(2.153), (1.814), 3.624	4.388, 3.624, 1.814	Pro^{7}-γ
13	24.43	t	1.926	m	2.144, 1.840, 3.655, 3.538	4.224, 3.655, 3.538	Pro^{10}-γ
14	24.77	t	1.840 1.416	m m	1.416, 1.740, 0.962 1.840, 0.926	-	Terpene-2
15	26.51	t	1.940 1.719	m m	1.719, 4.484, 2.198 1.719, 4.484, 2.198	2.198	Gln^{3}-β
16	26.85	t	1.927 1.722	m m	1.722, 4.468, 2.166 1.927, 4.468, 2.166	2.166	Gln^{6}-β

17	27.04 br	t	2.976 3.067	dd dd	4.598	-	His^{5}-β
18	27.09	t	2.937 3.067	dd dd	4.570	(4.570)	His^{2}-β
19	27.50	t	2.144 1.771	m m	1.771, 4.578,1.905, 1.945 2.144, 4.578,1.905, 1.945	1.945, 3.689, 3.467, 4.578	Pro^{9}-β
20	28.34	t	2.144 1.840	m m	1.840, 4.223, 1.926 2.144, 4.223, 1.926	4.223, 3.669, 3.533	Pro^{10}-β
21	28.50	q	0.918	s	0.827	0.827	Terpene-4-Me
22	28.86	t	1.798 2.031	m m	4.356, 2.031, (1.863)		Pro^{4}-β
23	28.89	t	1.814 2.012	m m	2.012, 4.388, (1.927) 1.814, 4.388, (1.927)	4.388, 3.646	Pro^{7}-β
24	30.63	t	1.538 1.430	m m	1.430, 1.460, (1.409), (1.802) 1.538, 1.460, 1.802	0.667	Terpene-7
25	30.64	t	2.166	t	1.927, 1.722	1.927, 1.722, 4.466, 6.807	Gln^{6}-γ
26	30.65	t	2.192	t	1.940, 1.719	1.940, 1.719, 4.489, 6.789	Gln^{3}-γ
27	31.62	t	3.154 2.792	d d	2.792 3.154	6.598	Terpene-11
28	36.43	d	1.802	ddq	1.538, 1.430, 0.667	0.667, 2.790, 3.156	Terpene-8
29	37.22	s	-	-	-	0.903, 0.824	Terpene-4
30	37.73 37.84	q	2.548 2.546	s	-	2.682, 2.758 2.633, 2.783	Met^{1}-ε
31	39.45	d	2.035	dd	1.409, 1.460	0.903, 0.824, 0.971	Terpene-5
32	40.07 40.03	t	1.428	dt	4.536, 1.678	0.890, 0.873, 4.536	Leu^{8}-β
33	41.71	s	-	-	-	3.156, 2.790, 0.977, (1.764), 2.035	Terpene-10
34	44.25	t	4.332	-	-	4.881	Terpene-8'
35	46.10	t	3.669 3.533		1.934	4.223, 2.147, 1.934, 1.851	Pro^{10}-δ
36	46.53	t	3.689 3.467	m m	1.905, 1.936	4.588, 2.165, 1.781, 1.936	Pro^{9}-δ
37	46.81	t	3.624	m	1.927	1.814, 4.388	Pro^{7}-δ
38	46.87	t	3.644	m	1.863	-	Pro^{4}-δ
39	48.56	d	4.534	dt	7.918, 1.428	7.918, 1.428, 1.678	Leu^{8}-α

40	49.44 49.70	t t	2.682, 2.758 2.633, 2.783	ddt ddt ddt ddt	2.758, 2.162, 2.289 2.682, 2.162, 2.289 2.783, 2.289, 2.162 2.633, 2.289, 2.162	4.892, 2.550 4.869, 2.545	Met^{1}-γ
41	50.18	d	4.466	dt	8.110, 1.927, 1.722	2.166	Gln^{6}-α
42	50.31	d	4.484	dt	8.206, 1.935, 1.707	2.182	Gln^{3}-α
43	51.37	d	4.570	ddd	8.135, 2.975, 3.082	2.975, 3.082	His^{5}-α
44	51.68	d	4.590 4.585	ddd	8.496, 2.934, 3.067 8.506, 2.934, 3.067	2.934, 3.067	His^{2}-α
45	53.30 53.62	d	4.892 4.869		2.162, 2.284 2.162, 2.284	2.162	Met^{1}-α
46	57.33	d	4.588	dd	2.144, 1.771	2.144, 1.771, 1.905, 1.945, 3.684	Pro^{9}-α
47	58.31	d	4.223	dd	2.144, 1.840	3.669, 3.553, 1.926, 2.144, (1.840)	Pro^{10}-α
48	59.20	d	4.388	dd	2.012, 1.814	2.012	Pro^{7}-α
49	59.44	d	4.356	dd	2.031, 1.798	3.635, 1.880	Pro^{4}-α
50	73.50 73.51	d	3.227	dd	1.840, 1.416	0.903, 0.824	Terpene-3
51	97.93 97.91	s	-	-	-	3.154, 2.792, 0.972, 0.668	Terpene-9
52	100.90 100.88	d	6.598 6.596	s	-	-	Terpene-3´
53	112.62 112.55	s	-	-	-	6.593, 4.330	Terpene-5´
54	116.92 116.90	s	-	-	-	3.154, 2.792, 6.597	Terpene-1´
55	116.95	d	7.230	s	8.655	(3.067), 2.937, 8.665	His^{2}-ε
56	117.26	d	7.322	s	8.771	3.092, 3.000, 8.771	His^{5}-ε
57	129.32	s	-	-	-	8.783, 7.322, 3.083 2.976, 4.585	His^{5}-γ
58	129.63	s	-	-	-	7.230, 3.064, 2.937, 4.592	His^{2}-γ
59	133.00 132.98	s	-	-	-	4.330	1-4´
60	133.63	d	8.655	s	7.230	7.230	His^{2}-δ
61	133.68	d	8.771	s	7.320	7.320	His^{5}-δ
62	153.60 153.62	s	-	-	-	6.597, 3.157, 2.792	Terpene-2´
63	155.73	s	-	-	-	(6.597), 3.154, 2.792,	Terpene-6´

						4.330	
64	168.11 168.07	s	-	-	-	6.597, 4.330, 4.881	Terpene-7
65	169.57	s	-	-	-	4.588, (4.223)	Pro^{9}-CO
66	169.59	s	-	-	-	8.110, (4.570)	His^{5}-CO
67	169.69	s	-	-	-	4.534, 1.444	Leu^{8}-CO
68	169.74	s	-	-	-	8.207, 2.937, 4.585	His^{2}-CO
69	169.97 169.89	s	-	-	-	8.513, 4.869, 2.190 8.499, 4.892, 2.182	Met^{1}-CO
70	170.07	s	-	-	-	4.471, 1.733, 1.924, 4.356	Gln^{3}-CO
71	170.21	s	-	-	-	4.466, 1.722, 1.927, 4.388	Gln^{6}-CO
72	170.99	s	-	-	-	4.538, 4.388, 2.012, 1.844, 7.918	Pro^{7}-CO
73	171.32	s	-	-	-	8.138, 4.356, 2.003, 1.805, 4.570	Pro^{4}-CO
74	173.10	s	-	-	-	4.225, 2.144, 1.840	Pro^{10}-CO
75	173.80 173.81	s	-	-	-	2.19, 1.93, 1.73	3-Gln-δ-CO
76	173.88 173.86	s	-	-	-	7.26, 2.19, 1.93, 1.73	4-Gln-δ-CO
	OH		9.75	br		NOE : 6.598	Terpene-2-OH
	NH		8.506 8.496	d d	4.585 4.590	NOE: 4.869 /4.692	His^{2}-NH
	NH		8.206	d	4.484	NOE : 3.072, 2.948, 4.588	Gln^{3}-NH
	NH		8.135	d	4.563		His^{5}-NH
	NH		8.110	d	4.470	NOE : 4.570, (4.470), 1.727, (1.927)	Gln^{6}-NH
	NH		7.918	d	4.534	NOE : 4.388, 1.827, 1.688, 1.427	Leu^{8}-NH
	NH_2		7.260 6.807	s s	(6.807) (7.260)		Gln^{6}-NH_2
	NH_2		7.230 6.789	s s	(6.789) (7.230)		Gln^{3}-NH_2

In a second aspect of the present invention, there is provided a composition comprising at least one compound of formula (I), wherein said compound is as defined above, and an acceptable carrier.

In one embodiment , the compounds of the formula (I) may be obtainable by fermentation of Memnoniella echinata FH 2272, DSM 13195, or of one of its variants or mutants under suitable conditions in a culture medium until at least

one memno peptide of the formula (I) is present in the culture medium, followed by subsequent isolation of the memno peptides.

A third aspect of the present invention therefore furthermore relates to a process for the preparation of a compound of the formula I, or a salt or derivative thereof, comprising cultivation of the microorganism Memnoniella echinata FH 2272, DSM 13195, or one of its variants or mutants, under suitable conditions in a nutrient medium comprising at least one source of carbon atoms and at least one source of nitrogen atoms until at least one compound of formula (I) is present in the nutrient medium and then isolating the said compound from the nutrient medium.

In one embodiment, the strain FH 2272, DSM 13195, its mutants and/or variants may be fermented in a nutrient solution (also called culture medium) which may comprise at least one source of carbon atoms and at least one source of nitrogen atoms and optionally may comprise customary inorganic salts until memno peptides are present in the culture medium. The memno peptides may then be isolated from the culture medium and optionally separated into the individual active components and purified. In one embodiment, memno peptides may accumulate in the culture medium, and may be separated into individual components.

In another embodiment, at least one source of nitrogen atoms used for the culture medium may be chosen from amino acids and peptides. The amino acids and peptides used as sources of nitrogen atoms may be the same as the group (A)n, as defined above.

The process according to the invention can be employed for fermentation on a laboratory scale (milliliter to liter range) and on industrial scale (cubic meter scale).

A strongly producing colony of Memnoniella echinata FH 2272 was grown. An isolate was deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1B, 3300 Brunswick, Germany, according to the rules of the Budapest Convention on December 14, 1999 under the following number: Memnoniella echinata FH 2272, DSM 13195.

Memnoniella echinata FH 2272, DSM 13195, has a brown-green mycelium and is characterized by the conidiophores characteristic of the genus Memnoniella.

Variants and mutants of the strain Memnoniella echinata FH 2272, DSM 13195, may also be employed to synthesize at least one compound of the memno peptides

according to the invention. Such mutants can be produced in a manner known per se by physical means, for example irradiation, such as with ultraviolet rays or X-rays, or chemical mutagens, such as ethyl methanesulfonate (EMS), 2-hydroxy-4-methoxybenzophenone (MOB) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Possible variants may include the closely related fungal species Stachybotrys, such as Stachybotrys atra, Stachybotrys chartarum or Stachybotrys complementi.

Screening for mutants and variants which may synthesize at least one compound of the memno peptides according to the invention may be carried out according to the following scheme:

- Separation of the mycelium after fermentation;
- Extraction of the mycelium with an organic solvent;
- Extraction of the memno peptides from the culture filtrate using solid phases
- Analysis by means of HPLC, DC or by testing the biological activity.

The fermentation conditions described below apply to the fungus Memnoniella echinata FH 2272, the deposited isolate DSM 13195 and mutants and variants thereof.

In one embodiment, a nutrient solution which may comprise at least one source of carbon atoms, casein peptone as a source of nitrogen atoms, and customary inorganic salts, Memnoniella echinata FH 2272, may produce memno peptide A. In one embodiment, Memnoniella echinata FH 2272 may be DSM 13195.

Possible sources of carbon atoms for aerobic fermentation may include assimilable carbohydrates and sugar alcohols, such as glucose, lactose, sucrose, starches, dextrins, fructose, molasses, glycerol, galactose, and D-mannitol, and carbohydrate-containing natural products, such as malt extract. Possible sources of nitrogen atoms may include, for example, amino acids; peptides; proteins; degradation products of amino acids, peptides and of proteins, such as casein, peptones, and tryptones; meat extracts; yeast extracts; peanut extracts; ground seeds, for example, of corn, wheat, beans, soy , and cotton; seed-containing compositions; distillation residues from alcohol production; meat meals; yeast extracts; ammonium salts, and nitrates. In one embodiment, at least one source of nitrogen atoms may be chosen from synthetically obtained peptides and biosynthetically obtained peptides. The nutrient solution

optionally may comprise at least one inorganic salt . In one embodiment, the inorganic salt may be chosen from chlorides, carbonates, sulfates and phosphates. The sulfates and phospates may be chosen from sulfates of the alkali metals, phosphates of the alkali metals, sulfates of the alkaline earth metals, and phosphates of the alkaline earth metals, wherein the alkaline earth metals may be chosen from iron, zinc, cobalt and manganese.

The formation of the memno peptides according to the invention may proceed in a nutrient solution which may comprise casein peptone. In one embodiment, the nutrient solution may comprise a concentration of casein peptone ranging from approximately 0.05 to 5%. Another embodiment may comprise a concentration of casein peptone ranging from 0.1 to 1%. Still another embodiment may comprise a concentration of casein peptone ranging from 0.2 to 5%. The nutrient solution may comprise glucose. One embodiment may comprise a concentration of glucose ranging from 0.5 to 3%. The nutrient solution may also comprise cornsteep. In one embodiment the nutrient solution may comprise a concentration of corn steep ranging from 0.05 to 1%. Another embodiment may comprise a concentration of cornsteep ranging from 0.1 to 0.5%. The nutrient solution may comprise a trace of at least one component chosen from potassium chloride, magnesium sulfate and iron sulfate.

In one embodiment, the nutrient solution may comprise casein peptone, glucose, cornsteep, and traces of potassium chloride, magnesium sulfate and iron sulfate. In another embodiment, the nutrient solution may comprise a concentration of casein peptone ranging from approximately 0.05 to 5%, a concentration of glucose ranging from 0.5 to 3%, a concentration of corn steep ranging from 0.05 to 1%, and traces of potassium chloride, magnesium sulfate and iron sulfate. The data in percent may in each case relate to the weight of the entire nutrient solution.

In this nutrient solution, Memnoniella echinata, which can be Memnoniella echinata FH 2272, DSM 13195, may form a mixture of memno peptides. Depending on the composition of the nutrient solution, the quantitative proportion of one or more of the memno peptides according to the invention may vary. Moreover, the synthesis of individual memno peptides can be controlled by the composition of the media such

that a memno peptide may not be produced at all or may be produced in an amount below the detection limit of the microorganism.

The culturing of the microorganism may be carried out aerobically, i.e., for example, submerging with shaking or stirring in shaker flasks or fermenters, optionally with introduction of air or oxygen. It can be carried out in a temperature range from approximately 18 to 37°C, a narrower range from approximately 20 to 32°C, and a still narrower range of 25 to 30°C. The pH range should be between 6 and 8, such as between 6.5 and 7.5. In general, the microorganism may be cultured under these conditions for a period of 24 to 300 hours, more generally 36 to 140 hours.

Advantageously, culturing may be carried out in a number of steps, i.e. at least one preculture may be prepared in a liquid nutrient medium, and may be then inoculated into the actual production medium, the main culture, for example, in the volume ratio 1:10. The preculture may be obtained, for example, by inoculating a mycelium into a nutrient solution and allowing it to grow for approximately 36 to 120 hours, such as for 48 to 72 hours. The mycelium can be obtained, for example, by allowing the strain to grow for approximately 3 to 40 days, such as 4 to 10 days, on a solid or liquid nutrient medium, for example, malt-yeast agar or potato dextrose agar (standard medium for mold fungi, for example, from Difco).

The course of the fermentation can be monitored by means of the pH of the cultures or of the mycelium volume as well as by chromatographic methods, such as thin-layer chromatography or high-pressure liquid chromatography or testing the biological activity. A compound according to the invention may be in both the mycelium and the culture filtrate, but the largest part is usually found in the culture filtrate.

The isolation process described below may serve for the purification of the memno peptides according to the invention, for example, of memno peptide A. The isolation or purification of a memno peptide according to the invention from the culture medium may be carried out according to known methods taking into account the chemical, physical and biological properties of the natural substances. For the testing of the memno peptide concentrations in the culture medium or in the individual isolation steps, thin-layer chromatography, for example on silica gel, using isopropanol/25%

strength NH_3 as an eluent or HPLC can be used. Detection in thin-layer chromatographic separation can be carried out, for example, by means of color reagents such as chlorosulfonic acid/glacial acetic acid, the amount of the substance formed expediently being compared with a calibration solution.

For the isolation of a memno peptide according to the invention, the mycelium may be generally first removed from the culture broth using the usual procedures and the memno peptides may be then extracted from the cell mass using an optionally water-miscible organic solvent. The organic solvent phase may contain the natural substances according to the invention; it may be optionally concentrated in vacuo and the residue may be further purified as described below. In one embodiment, a memno peptide may be extracted from the culture by adding solvent to the mixture of fungus and broth.

The culture filtrate may be optionally combined with the concentrate of the mycelium extract and extracted with a suitable, water-immiscible organic solvent, for example with n-butanol. The organic phase subsequently removed may be optionally concentrated in vacuo. To defat the valuable products, the concentrate can be diluted with a nonpolar solvent in which the compounds according to the invention may not be very soluble, for example, with hexane, petroleum ether or diethyl ether. In this process, the memno peptides may precipitate, and the lipophilic impurities may remain dissolved and may be removed by customary solid/liquid phase separations.

The precipitate which may comprise the memno peptides, may be dissolved in 1/30 of the original volume of water/methanol. The precipitate may dissolve completely in the course of this and may be lyophilized. The lyophilizate, subsequently called crude product, may comprise 5 to 50% memno peptides and may be employed for further isolation.

The further purification of at least one memno peptide according to the invention may be carried out by chromatography on suitable materials, for example, on molecular sieves, on silica gel, alumina, on ion exchangers or on adsorber resins or on reversed phases (RP). The memno peptides may be separated with the aid of this chromatography. The chromatography of the memno peptides may be carried out using buffered aqueous solutions or mixtures of aqueous and organic solutions.

Mixtures of aqueous and organic solutions may be understood as meaning all water-miscible organic solvents, such as methanol, propanol and acetonitrile, in a

concentration of 5 to 80% of solvent, more generally 20 to 50% of solvent or alternatively all buffered aqueous solutions which may be miscible with organic solvents. The buffers to be used may be the same as indicated above.

Separation of the memno peptides on the basis of their differing polarity may be carried out with the aid of reversed phase chromatography, for example on MCI® (adsorber resin from Mitsubishi, Japan) or Amberlite XAD® (Toso Haas), or further hydrophobic materials, such as on RP-8 or RP-18 phases. Moreover, the separation can be carried out with the aid of normal-phase chromatography, for example on silica gel, alumina and the like.

Chromatography of the memno peptides may be carried out using buffered or acidified aqueous solutions or mixtures of aqueous solutions with alcohols or other, water-miscible organic solvents. In one embodiment, the organic solvent may be chosen from propanol and acetonitrile.

Buffered or acidified aqueous solutions may be understood as meaning at least one solution alone or in combination. For example, said at least one solution may be chosen from water, phosphate buffers, ammonium acetate, citrate buffers, and acids. In one embodiment, citrate buffer may be in a concentration ranging from 0 to 0.5 M. Acids may be chosen from formic acid, acetic acid, trifluoroacetic acid, and all commercially commercially available acid may be in a concentration ranging from 0 to 1%. In yet another embodiment the concentration of acid may be 0.1%.

Chromatography may be carried out using a gradient which may begin with 100% of water and ends with 100% of solvent. At least one solvent may be used. A mixture of two or more solvents may also be used. In one embodiment, a linear gradient may run from 20 to 50% of a solvent chosen from propanol and acetonitrile.

Alternatively, gel chromatography or chromatography on hydrophobic phases can also be carried out.

Gel chromatography may be carried out on polyacrylamide or copolymer gels, such as Biogel-P 2® (Biorad) or Fractogel TSK HW 40® (Merck, Germany or Toso Haas, USA).

A further, very effective process for the purification of the compounds according to the invention may be the use of ion exchangers. For example, the basic memno peptide A can be isolated very advantageously on cation exchangers, such as Fractogel® EMD SO_3^-. Buffer solutions between pH 5 and 8, such as between pH 6 and 7.5, can be used. Elution can be achieved, for example, using a rising salt gradient. In addition to water, it can also be advantageous to use mixtures of aqueous buffer solutions with an organic solvent as solvents. The proportion of the organic solvent may be between 10% and 90%, such as between 30 and 60%.

The sequence of the abovementioned chromatographic processes may be reversible.

A further, very effective purification step for memno peptides may be crystallization. Memno peptides may be crystallized out from solutions in organic solvents and from mixtures of water with organic solvents. Crystallization may be carried out in a manner known per se, for example by concentrating or cooling saturated memno peptide solutions.

The memno peptides according to the invention are stable in the solid state and in solutions in the pH range between 3 and 8, for example 5 and 7, and can thus be incorporated into customary pharmaceutical preparations.

The formation of the nitrogen-containing memno peptides can be favored by adding the desired amino acids or peptides as precursor to the Memnoniella echinata cultures. It may be the peculiarity of the Memnoniella echinata species that they bind the amino groups of the amino acids and peptides supplied with synthesis of a five-membered ring lactam, usually of an isoindole ring system. This binding may take place either starting from the aldehyde precursor in the course of an oxidation or from the lactone oxidation stage in ring-opened form or the cyclic lactone form. This ability of the Memnoniella echinata to bind amines is completely surprising and can be used for obtaining protected, converted amine derivatives, such as of terpene derivatives of amino acids and peptides.

At least one compound of the memno peptides according to the invention may be suitable on account of their valuable pharmacological properties for use in human or veterinary medicine as pharmaceuticals.

A fourth aspect of the present invention thus relates to the use of the compound of the formula (I) or a salt or derivative thereof, for the manufacture of a medicament for the treatment of cardiac insufficiency.

A fifth aspect of the present invention relates to the use of the formula (I) or a salt or derivative thereof, for the manufacture of a medicament for the treatment of diseases wherein cardiac insufficiency is a primary or secondary cause.

The mechanism of action of the memno peptides is uncertain, but a significant effect was detected.

For the detection of the activators of SERCA2 which imitate the phosphorylation effect of PLB, a colorimetric test may be run which determines the activity of the Ca^{2+} ATPase in dog heart microsomes in the presence of test substances. The test may be carried out in 96-well microtiter plates. The enzymatic release of inorganic phosphate from ATP, which may form a blue colored complex with ammonium molybdate which can be measured at 620 nm in a spectrophotometer, may be measured.

Memno peptide A may activate SERCA2 in concentrations from 12.5 µM.

The memno peptides moreover have an antimicrobial action.

A sixth aspect of the present invention thus relates to the use of the compound of the formula (I), or a salt or derivative thereof, for the manufacture of a medicament for the treatment of microbial infections.

treatment of microbial, for example bacterial, infections.

Table 3 shows some minimum inhibitory concentrations (MIC) of the antimicrobial spectrum of memno peptide A against some selected bacteria.

Table 3:

Strain	Resistance against:	MIC [µg/ml]
Staphylococcus aureus SG 511	-	16
Staphylococcus aureus 285	Penicillin	16
Staphylococcus aureus 503	Penicillin	16
Staphylococcus aureus FH 1982	Methicillin	16
Staphylococcus aureus 701E	Methicillin	16

Staphylococcus aureus 707E	Methicillin	16
Staphylococcus aureus 9 Tüb	Ofloxacin	16
Staph. epidermidis ZH 2c	-	16
Staph. epidermidis 763	Methicillin	> 16
Staph. epidermidis 5747IIW	Methicillin	32
Staph. epidermidis 291	Ofloxacin	8
Staph. epidermidis 799	Ofloxacin	8
Enterococcus faecium Md8B	-	8
Enterococcus faecium VR1	Vancomycin	> 64
Enterococcus faecium VR2	Vancomycin	> 64
Streptococcus pyogenes VR3	Vancomycin	> 64
Streptococcus pyogenes 308A	-	8
Streptococcus pyogenes 77A	-	4

In addition to the antibacterial action, the compounds according to the invention may exhibit weak antimycotic, i.e. antifungal, properties, for example against Candida albicans.

Moreover, the substances according to the invention may have a certain favorable inhibitory action on glucose-6-phosphate translocase (G-6-P – TL), an enzyme which may be of importance for glucose metabolism and thus for the treatment of diabetes mellitus. For example, the compound memno peptide A may exhibit selective activities against G-6-P – TL, but it may not exhibit the coenzyme G-6-P phosphatase.

A seventh aspect of the present invention thus relates to the use of the compound of the formula (I), or a salt or derivative thereof, for the manufacture of a medicament for the treatment of diabetes mellitus.

One embodiment may also relate to pharmaceutical preparations of at least one compound of the memno peptides,

At least one compound of the memno peptides according to the invention, can generally be administered as such in undiluted form. Use as a mixture with suitable excipients or vehicles is one embodiment of the invention. Vehicles which can be

used in pharmaceuticals may be the customary and pharmacologically tolerable vehicles and/or excipients.

In general, the pharmaceuticals according to the invention may be administered orally or parenterally, but rectal administration may be also possible. Suitable solid or liquid pharmaceutical preparation forms may be, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, aerosols, drops or injectable solutions in ampoule form and preparations having protected release of active compound, in whose preparation vehicles and additives and/or excipients such as disintegrants, binders, coating agents, swelling agents, glidants or lubricants, flavorings, sweeteners or solubilizers are customarily used. Frequently used vehicles or excipients which may be mentioned are, for example, magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, lactoprotein, gelatin, starch, vitamins, cellulose and its derivatives, animal or vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols.

If appropriate, the dose units can be microencapsulated for oral administration in order to delay release or to extend it over a longer period of time, for example by coating or embedding the active compound in particle form in suitable polymers, waxes or the like.

In one embodiment, the pharmaceutical preparations may be produced and administered in dose units, each unit may comprise, as active constituent, a specific dose of at least one compound of the memno peptides according to the invention. In the case of solid dose units such as tablets, capsules and suppositories, this dose can be up to approximately 500 mg, but more generally can be approximately 0.1 to 200 mg, and in the case of injection solutions in ampoule form up to approximately 200 mg, but usually approximately 0.5 to 100 mg, per day.

The daily dose to be administered may be generally dependent on the body weight, age, sex and condition of the mammal. Under certain circumstances, however, higher or lower daily doses may also be appropriate. Administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else in

a number of smaller dose units and by multiple administration of subdivided doses at specific intervals.

The pharmaceuticals according to the invention may be produced by bringing at least one compound of the memno peptides according to the invention into a suitable administration form using customary vehicles and, if appropriate, additives and/or excipients.

The invention is illustrated further in the following examples. Percentages may relate to weight. Mixing ratios in the case of liquids may refer to the volume, if no other details have been given.

The following examples are intended to illustrate the invention without limiting the scope thereof.

Examples

Example 1. Preparation of a glycerol culture of Memnoniella echinata FH 2272, DSM 13195.

100 ml of nutrient solution (malt extract 2.0%, yeast extract 0.2%, glucose 1.0%, $(NH_4)_2HPO_4$ 0.05%, pH 6.0) in a sterile 300 ml Erlenmeyer flaskwere inoculated with the strain Memnoniella echinata FH 2272, DSM 13195, and incubated on a rotating shaker at 25°C and 140 rpm for 7 days. 1.5 ml of this culture were then diluted with 2.5 ml of 80% strength glycerol and stored at -20°C.

Example 2. Preparation in an Erlenmeyer flask of a culture or a preculture of Memnoniella echinata FH 2272, DSM 13195.

A sterile 300 ml Erlenmeyer flask containing 100 ml of the following nutrient solution, 10 g/l of glucose, 5 g/l of casein peptone, 1.7 g/l of liquid cornsteep, and 7 ml of trace element solution (10 g/l of KCl, 10 g/l of $MgSO_4 \times 7\ H_2O$, 3.6 g/l of $FeSO_4 \times 7\ H_2O$ and 6 g/l of $MgSO_4 \times H_2O$) was inoculated with a culture grown in a slant tube (same nutrient solution, but with 2% agar) or with 1 ml of a glycerol culture (see Example 1) and incubated at 180 rpm and 30°C on a shaker. The maximum production of at least one compounds of the memno peptides according to the invention was achieved after about 120 hours. For the inoculation of 10 and 200 l fermenters, a submerged culture 48 to 96 hours old (inoculation quantity about 10%) from the same nutrient solution sufficed.

Example 3. Preparation of the memno peptides.

A 30 l fermenter was operated under the following conditions:

Nutrient medium:

10 g/l of glucose
0.5 g/l of casein peptone
1.7 g/l of liquid cornsteep
7 ml of trace element solution
pH 6.5 (before sterilization)

Trace element solution : KCl 10 g/l, $MgSO_4 \times 7\ H_2O$ 10 g/l, $FeSO_4 \times 7\ H_2O$ 3.6 g/l and $MnSO_4 \times H_2O$ 6 g/l

Incubation time: 45 hours

Incubation temperature: 28°C

Stirrer speed: 300 rpm

Aeration: 15 l min^{-1}

Foam formation was optionally suppressed by repeated addition of ethanolic polyol solution. The production maximum was achieved after about 35 to 70 hours.

Example 4. Isolation of the memno peptide mixture from the culture solution of Memnoniella echinata FH 2272, DSM 13195.

After completion of the fermentation of Memnoniella echinata FH 2272, DSM 13195, the culture broth of the fermenter, obtained according to Example 3 (200 liters) was filtered with addition of about 2% filter aid (e.g. Celite®) and the cell mass (22 liters) was extracted with 66 liters of methanol. The methanolic solution containing the valuable substance was freed of the mycelium by filtration and concentrated in vacuo. The concentrate was diluted with water and applied to a prepared, 17 liter MCI GEL, CHP20P column together with the culture filtrate (180 liters). It was eluted with a gradient of water after 60% propan-2-ol in water. The column flow (25 liters per hour) was collected in fractions (10 liters each) and the memno peptide-containing fractions (from 25% to 30% propan-2-ol) were combined.

Concentration in vacuo afforded 20 liters of a brown solution. Six liters of cation exchanger, Fractogel® EMD SO_3^-, equilibrated at pH 7 with potassium phosphate buffer were packed into a column (125 mm x 500 mm). After loading the ion exchanger with 20 liters of the concentrate described above, it was eluted with a gradient of 10 mM potassium phosphate buffer, pH 7, after 1 M NaCl in 10 mM potassium phosphate buffer, pH 7 in water/methanol (1:1). The column flow, i.e. the unbound material, contained the neutral memno peptides (49 g). The column flow was 12 liters per hour; 1 liter portions were collected in fractions during the gradient elution. With 0.75 M NaCl (fractions 31 and 32), memno peptide A was obtained.

Fractions 31 and 32 were combined and concentrated to approximately 500 ml in vacuo.

Example 5: Enrichment of memno peptide A by gel chromatography.

8 g of the product obtained according to Example 4 were applied to a column of 3.9 liters capacity packed with Fractogel® TSK HW-40 s (width x height = 10 cm x 50 cm). The eluent:methanol/water (1:1) was pumped through the column at a flow rate of 20 ml per minute and the column outflow was collected in fractions (20 ml). The memno peptides A were found mainly in fractions 75 to 85. They were combined and freed of the methanol in vacuo. They afforded 0.9 g of active compound mixture.

Example 6. HPLC system for the detection of the memno peptides.

The system described below allowed purity testing and also separation and quantification of the memnoterpenes, for example in the crude mixture or in the culture filtrates.

Eluent: 0.1% trifluoroacetic acid in 32% acetonitrile.

Column: Nucleosil 100C_{18}AB 250/4, Macherey-Nagel.

Flow: 1.0 ml/min

Detection: Ultraviolet light absorption at 210 nm.

Under the conditions indicated, memno peptide A can thus have the following retention time: Memno peptide A: 7.0 minutes.

Example 7. Purification of memno peptide A.

500 ml of solution of memno peptide A isolated and enriched according to Example 5 were applied to a 500 ml Nucleosil®100-7 C_{18}AB column and chromatographed using a gradient of 25 to 50% acetonitrile in 0.05% trifluoroacetic acid/water. The flow of the eluent was 50 ml per minute; the fraction size 50 ml. Memno peptide A was found in fractions 71 to 88. Repeated purification of the combined fractions with a constant solvent concentration of 28% of acetonitrile in

0.05% trifluoroacetic acid afforded > 95% of pure memno peptide C after freeze drying (100 mg).

Characterization of memno peptide A:

10 µg of memno peptide A were hydrolyzed in constant-boiling hydrochloric acid and investigated in an amino acid analyzer. The following customary amino acids were found:

Glutamic acid	11 nMol
Proline	22 nMol
Histidine	10 nMol
Leucine	5.6 nMol
Methionine oxide	5.5 nMol

Ultraviolet absorption: λ_{max} at 269 nm, 305 nm (shoulder).

The high-resolution FAB mass spectrum showed an intensive MH^+ at m/z 1565.7819 Da, in good agreement with the calculated mass (for $C_{76}H_{109}N_{16}O_{18}S$, monoisotopic) of 1565.7827 Da. The MS/MS fragmentation corresponded to the formula (IVa).

BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE
ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN
FÜR DIE ZWECKE VON PATENTVERFAHREN

INTERNATIONALES FORMBLATT

Hoechst Marion Roussel
Deutschland GmbH

65926 Frankfurt/Main

LEBENSFÄHIGKEITSBESCHEINIGUNG
ausgestellt gemäß Regel 10.2 von der unten angegebenen
INTERNATIONALEN HINTERLEGUNGSSTELLE

I. HINTERLEGER	II. KENNZEICHNUNG DES MIKROORGANISMUS
Name: Hoechst Marion Roussel Deutschland GmbH Anschrift: 65926 Frankfurt/Main	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER: DSM 13195 Datum der Hinterlegung oder Weiterleitung[1]: 1999-12-09

III. LEBENSFÄHIGKEITSBESCHEINIGUNG

Die Lebensfähigkeit des unter II genannten Mikroorganismus ist am 1999-12-09[2] geprüft worden.
Zu diesem Zeitpunkt war der Mikroorganismus

(X)[3] lebensfähig
( )[3] nicht mehr lebensfähig

IV. BEDINGUNGEN, UNTER DENEN DIE LEBENSFÄHIGKEITSPRÜFUNG DURCHGEFÜHRT WORDEN IST[4]

V. INTERNATIONALE HINTERLEGUNGSSTELLE

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Anschrift: Mascheroder Weg 1b D-38124 Braunschweig	Unterschrift(en) der zur Vertretung der internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten: U. Weis Datum: 1999-12-14

[1] Angabe des Datums der Ersthinterlegung. Wenn eine erneute Hinterlegung oder eine Weiterleitung vorgenommen worden ist, Angabe des Datums der jeweils letzten erneuten Hinterlegung oder Weiterleitung.
[2] In den in Regel 10.2 Buchstabe a Ziffer ii und iii vorgesehenen Fällen Angabe der letzten Lebensfähigkeitsprüfung.
[3] Zutreffendes ankreuzen.
[4] Ausfüllen, wenn die Angaben beantragt worden sind und wenn die Ergebnisse der Prüfung negativ waren.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Hoechst Marion Roussel
Deutschland GmbH

65926 Frankfurt/Main

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
FH 2272	DSM 13195

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
(X) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 1999-12-09 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiss
Address: Mascheroder Weg 1b, D-38124 Braunschweig	Date: 1999-12-14

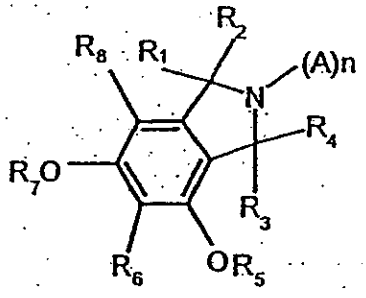
[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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Claims:

1. A compound of formula (I)



I

wherein

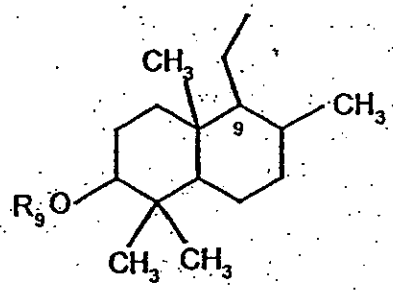
R_1, and R_2 together are double bonded O, or both hydrogen, or H and OH, or H and O-C_1-C_4-alkyl;

R_3 and R_4 together are double bonded O, or both hydrogen, or H and OH, or H and O-C_1-C_4-alkyl;

R_8 is chosen from H, OH, C_1-C_4-alkyl, and O-C_1-C_4-alkyl;

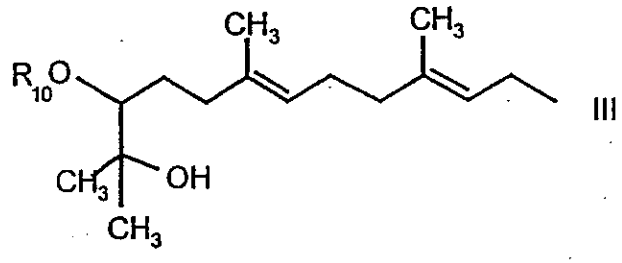
R_6 is a group chosen from

a group of formula (II)



II

wherein R_9 is chosen from a hydrogen atom and a glycosidically bonded sugar, and a group of formula (III)



III

wherein R_{10} is H or a glycosidically bonded sugar,

and wherein,

if R_6 is a group of the formula (II), then R_5 is a bond to the carbon atom C9 of the formula (II) and R_7 is a hydrogen atom, or R_7 is a bond to the carbon atom C9 of the formula (II) and R_5 is a hydrogen atom, and

if R_6 is a group of the formula (III), then R_5 and R_7 are each hydrogen atoms;

A is an amino acid;

n is an integer chosen from 1 to 12, wherein each A is the same or different from every other A;

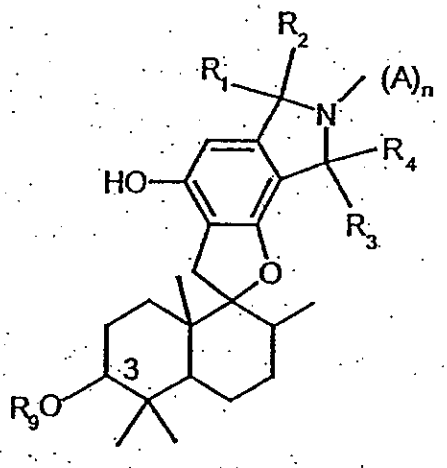
wherein the nitrogen atom in the isoindole ring of formula (I) is the N-terminal amine nitrogen of the first amino acid of the (A)n group;

or a salt or derivative thereof;

with the proviso that

A is an amino acid other than Glu when n is 1, and R_1 and R_2 together are double bonded O, and R_3 and R_4 are both hydrogen, and R_6 is a group of formula (II), and R_5 is a bond to the carbon atom C9 of the group of formula II, and R_7 is H, and R_8 is H and R_9 is H.

2. A compound according to claim 1, of formula (IV)

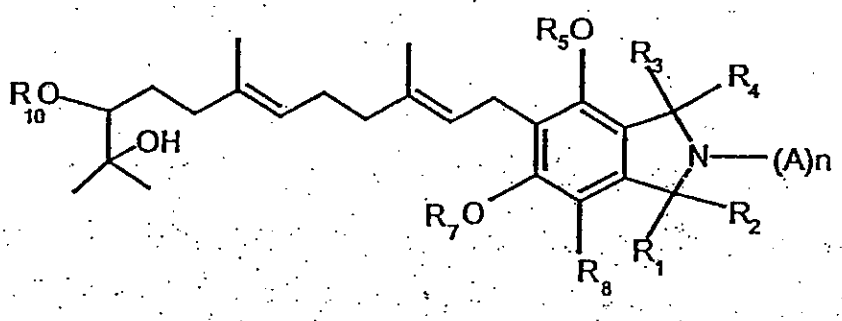


IV

wherein groups R_1, R_2, R_3, R_4, R_9 and (A)n are defined according to claim 1, or a salt or derivative thereof.

3. A compound according to claim 2, or a salt or derivative thereof, wherein R_1 and R_2 together are double bonded O, R_3 and R_4 are both hydrogen, and R_9 is H.

4. A compound according to claim 1, of formula (V)



V

wherein groups R_1, R_2, R_3, R_4, R_5, R_7, R_8, R_{10} and (A)n are defined according to claim 1, or a salt or derivative thereof.

5. A compound according to any one of the preceding claims, or a salt or derivative thereof, wherein (A)n is a peptide chain having 2 to 12 natural amino acid residues.

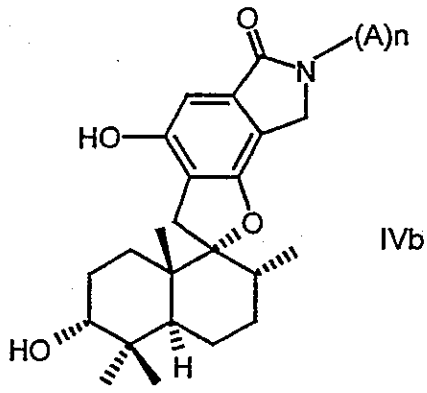
6. A compound according to any one of the preceding claims, or a salt or derivative thereof,wherein (A)n is a peptide chain having the amino acid sequence: Met-His-Gln-Pro-His-Gln-Pro-Leu-Pro-Pro (SEQ ID NO: 1) wherein the Met group is optionally oxidized to form a sulfoxide.

7. A compound according to any one of the preceding claims, or a salt or derivative thereof, wherein R_9 or R_{10} is an aldohexose.

8. A compound according to any one of the preceding claims, or a salt or derivative thereof, obtainable by cultivation of a fungus chosen from Memnoniella echinata FH 2272, DSM 13195, or a mutant or variant thereof, under suitable conditions in a culture medium until the compound is present in the culture medium, and said compound is subsequently isolated.

9. A compound according to claim 8, further comprising conversion of said compound into at least one chemical form chosen from derivatives and physiologically tolerable salts.

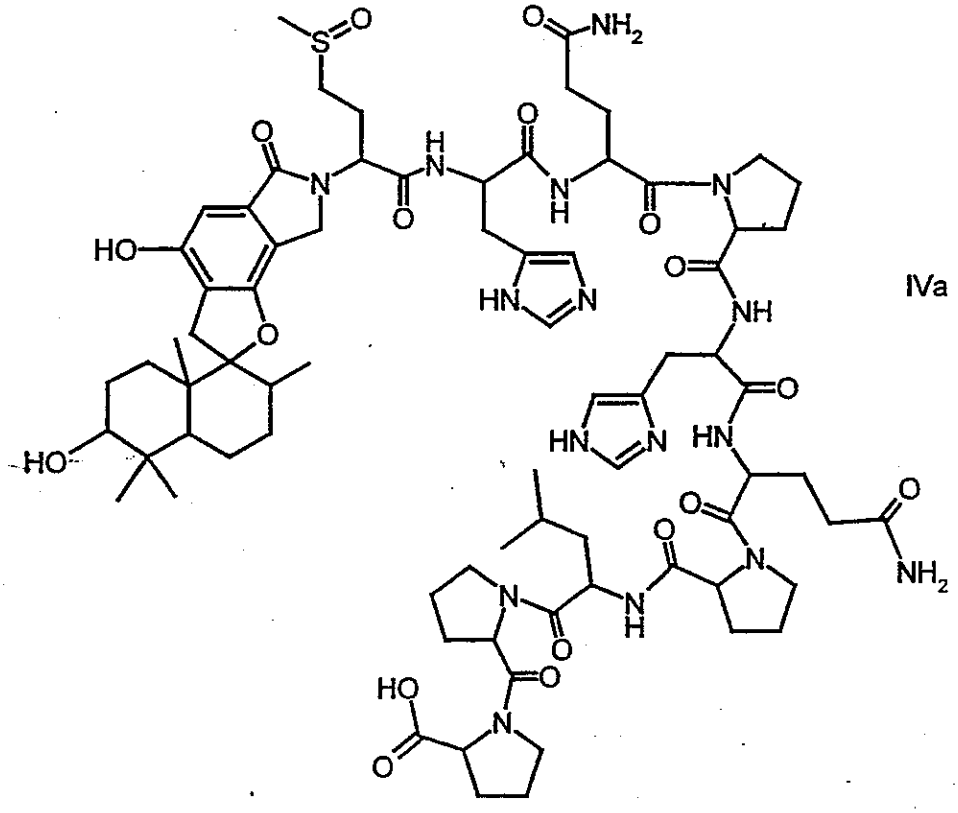
10. A compound according to claim 1, of formula (IVb)



IVb

wherein (A)n is defined according to claim 1.

11. A compound according to claim 1, memno peptide A, $C_{76}H_{108}N_{16}O_{18}S$, of formula (IVa)



IVa

or a salt or derivative thereof.

12. A compound according to any one of the preceding claims, wherein the group -(A)n comprises a part of the amino acid sequence for casein.

13. A compound according to claim 12, wherein (A)n is the amino acid sequence: Met His Gln Pro His Gln Pro Leu Pro Pro (SEQ ID NO:1).

14. A compound according to claim 13, wherein the methionine (Met) is oxidized to form a sulfoxide.

15. A composition comprising at least one compound of formula (I), wherein said compound is defined according to any one of the preceding claims, and an acceptable carrier.

16. A composition according to claim 15, wherein said composition is a pharmaceutical composition and said acceptable carrier is a pharmaceutically acceptable carrier.

17. A process for making a compound of formula (I), or a salt or derivative thereof, as defined in any one of claims1 - 14, comprising cultivation of Memnoniella echinata FH 2272, DSM 13195, or a variant or mutant thereof, under suitable conditions in a nutrient medium comprising at least one source of carbon atoms and at least one source of nitrogen atoms until at least one compound of formula (I) is present in the nutrient medium and said compound is subsequently isolated.

18. A process according to claim 17, further comprising, conversion of the compound into at least one chemical form chosen from derivatives and physiologically tolerable salts.

19. A process according to claim 17 or 18, wherein said cultivation occurs under aerobic conditions.

20. A process according to any one of claims 17 – 19, wherein said at least one source of nitrogen atoms is chosen from amino acids and peptides.

21. A process according to any one of claims 17 - 20, wherein said nutrient medium comprises casein peptone at a concentration ranging from about 0.05% to 5% by weight of the total nutrient solution.

22. A process according to any one of claims 17 - 21, wherein said nutrient medium comprises casein peptone, glucose, cornsteep, and traces of potassium chloride, magnesium sulfate and iron sulfate.

23. Use of a compound of formula (I), or a salt or derivative thereof, as defined in any one of claims 1 – 14, for the manufacture of a medicament for the treatment of cardiac insufficiency.

24. Use of a compound of formula (I), or a salt or derivative thereof, as defined in any one of claims 1 – 14, for the manufacture of a medicament for the treatment of a disease wherein cardiac insufficiency is a primary or secondary cause.

25. Use of a compound of formula (I), or a salt or derivative thereof, as defined in any one of claims 1 – 14, for the manufacture of a medicament for the treatment of diabetes mellitus.

26. Use of a compound of formula (I), or a salt or derivative thereof, as defined in any one of claims 1 – 14, for the manufacture of a medicament for the treatment of a microbial infection.