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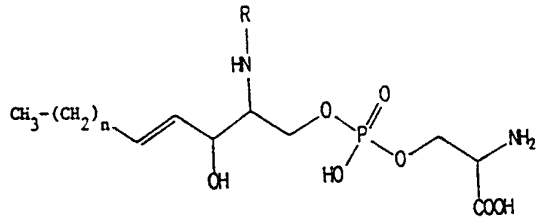

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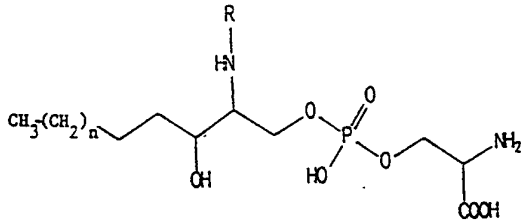

(II)

(57) Abstract

The present invention relates to the therapeutical use of phosphoryl-L-serine-N-acyl-sphingosine composed of a mixture of compounds of formula (I) or (II), in which n = 6-16 and R means an acyl residue of a higher aliphatic acid having between 16 and 24 carbon atoms, the distribution of the values for n and R being those of a natural sphingomyelin. The invention also includes a novel process for the preparation of these compounds, as well as pharmaceutical preparations containing them as active ingredients.

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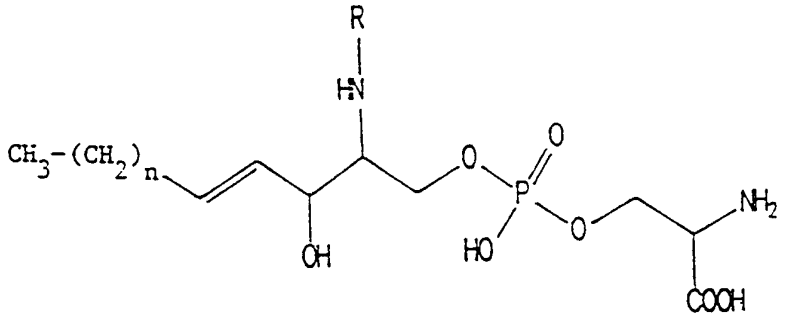
THERAPEUTIC USE OF PHOSPHORYL-L-SERINE-N-ACYL-SPHINGOSINE

BACKGROUND AND SUMMARY OF THE INVENTION

The present invention concerns the therapeutical use of phosphoryl-L-serine-N-acyl-sphingosine composed of a mixture of compounds of formula I or II,



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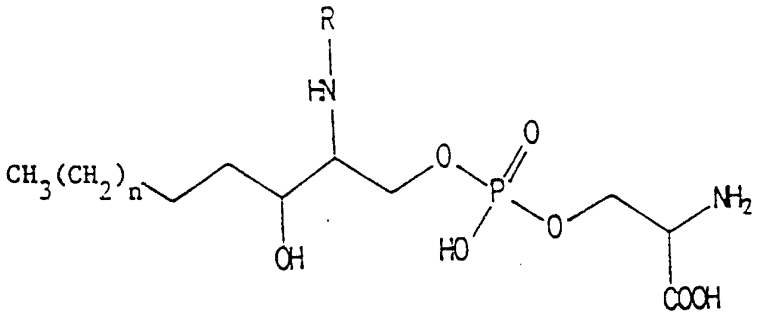


II

in which n is an integer of 6-16 and R means an acyl residue of a higher aliphatic acid having between 16 and 24 carbon atoms, the distribution of the values for n and R being those of a natural sphingomyelin. The invention also includes a new process for the preparation of these compounds, as well as pharmaceutical preparations containing said compounds as active ingredients. The ratio of the mixture of compounds of formulas (I) and (II) is not critical in order to use the compound of the present invention. The compound, as previously defined, was prepared by treatment with phospholipase D, as described in Biochemistry vol. 28, No. 8, 1989, 34-57, starting from a derivative of sphingomyelin (i.e. phosphorylcholine-N-acyl-sphingosine, in which the acyl group was derived from higher aliphatic acids of different molecular size and was variable between C12 and C24), and particularly to an analog of a sphingomyelin having an acyl group derived from 4-doxyl-pentanoic acid on the sphingosine amino group instead of the acyl residue R. In the cited paper there are no indications about pharmacological properties of the derivative.

DETAILED DESCRIPTION OF THE INVENTION

It has now been found that the compound has various interesting pharmacological properties and can be used therapeutically, especially in human medicine. More precisely the phosphoryl-L-serine-N-acyl-sphingosine of

the present invention has an inhibiting action on the activation of protein kinase C, which activation can be undesired or have negative effect, in particular conditions of unbalance of the normal mechanisms of neurotransmittal functions. Activation is caused by an increased concentration of excititatory amino acids, like glutamic and/or aspartic acid; these acids have a direct toxic action on neuronal cells in the above mentioned abnormal conditions.

Phosphoryl-L-serine-N-acyl-sphingosine can therefore be used in the therapy of many diseases of the central nervous system, as those arising after cerebral degenerations or lesions, like ischaemia, hypoxia, epilepsy, traumas and compressions, metabolic dysfunctions, aging, toxic-infective and chronic degenerative diseases, like Alzheimer's, Parkinson's and Huntington's diseases.

Phosphoryl-L-serine-N-acyl-sphingosine is moreover able to increase glycemia and histamine levels, resulting in cerebral glucose accumulation, which has important reflexes in the functionality of CNS. Also an activating influence on cerebral neurotransmitter systems was shown; particularly at cholinergic level, the compounds of the present invention can increase the release of acetylcholine (ACh) in the cerebral cortex area. The action on the cholinergic system is also reinforced by the efficacy of the aforesaid compounds in influencing cognitive functions, like e.g. learning and

memorizing, in various behavioral tests (e.g. the capacity to invert scopolamine caused amnesia). The compounds I and II can also be used in therapies associated with slowdown of cerebral metabolism, in involutive cerebral syndromes of different origins (due e.g. to senile decay and/or vascular pathologies), in Alzheimer's disease, memory loss and senile dementia, anoxic and oedematous conditions, cerebral tiredness, chronic vascular cerebropathies of aging, and pre-senile dementia following trauma, postanoxic cerebropathies and extrapyramidal syndromes. Moreover the compounds of the present invention perform a modulatory action on the immune system and, particularly, an inhibitory action on the secretion of TNF (tumor necrosis factor). They can therefore be favorably used in the therapy of pathologies characterized by a modified immunological reactivity and/or by autoimmunological symptoms like multiple sclerosis, cachectic situations associated with infections and neoplastic diseases, pulmonary fibrosis, rheumatoid arthritis, bacterial and protozoic infections, HIV (human immunodeficiency virus), cerebral complication of malaria, insulin dependent diabetes and several pathologies linked to organ transplantation or allergy caused pathologies.

Another object of the invention is directed to the use of phosphoryl-L-serine-N-acyl-sphingosine of the formula I or II as defined above, in the preparation of pharmaceutical compositions to be employed in the

treatment of diseases or conditions as explained above.

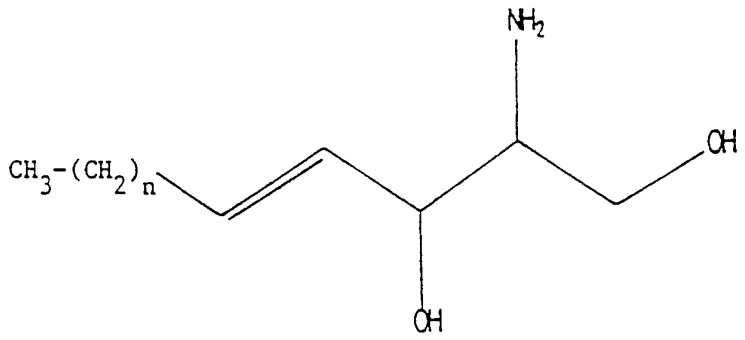
Another object of the present invention relates to pharmaceutical preparations containing phosphoryl-L-serine-N-acyl-sphingosine of formula I as active ingredient, optionally together with usual pharmaceutical excipients to be administered to a patient in need thereof. These pharmaceutical preparations can be for oral, rectal, parenteral, local or transdermic use. The pharmaceutical preparations can be presented in solid or semisolid form, e.g. as pills, tablets, gelatine covered capsules, or soft gelatine capsules. For the parenteral use it is possible to use formulations for intramuscular, subcutaneous or transdermic use, or formulations suitable for infusions or intravenous injections, and they can therefore be prepared as solutions of active components or as a lyophilized powder of the active components, eventually to be added to one or more excipients or pharmaceutically acceptable solvents, which are usable for the aforesaid purpose and osmolar with physiological fluids. For the local application spray preparations, e.g. nasal sprays, can be considered, or ointments for topical use, or plasters for transdermal administration. The preparations of the invention can be used both in humans and animals. They contain preferably between 0.01 % and 10 % of the active components for solutions, sprays, ointments and creams, and between 1 % and 100 %, preferably between 5 % and 50 % of the active component,

for the solid form preparation, the remaining being made up of excipients. The dosage will be determined according to the indication, the desired effect and the route of administration. The daily dosage for the injectable (subcutaneous or intramuscular) or transdermic or oral administration in humans of phosphoryl-L-serine-N-acyl-sphingosine of formula I varies between 0.5 and 12 mg/kg body weight of active substance.

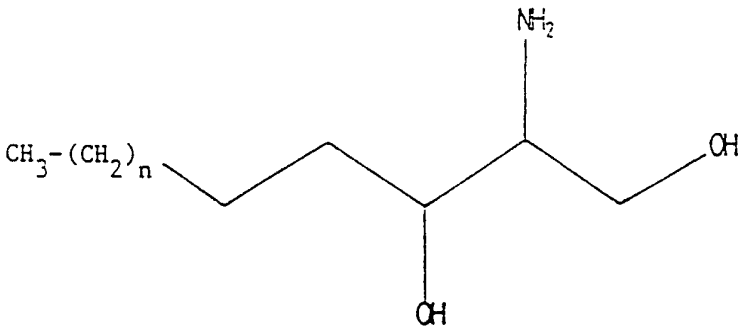
Phosphoryl-L-serine-N-acyl-sphingosine, as described by formulae I or II, is a derivative of the natural compound sphingomyelin and, concerning the N-acyl-sphingosine component, it is identical with the composition of the natural product, i.e. it is a mixture of different acylamino-alkenyl residues corresponding to the above mentioned formula, in which both n and R vary within the defined ranges, which are those of sphingomyelin. According to the starting material from which sphingomyelin is extracted, R can be a mixture of acyl residues belonging to palmitic, stearic, nervonic, lignoceric and, in minor amounts, to other acids of different saturated and unsaturated molecular species of the same order.

The present invention also concerns the corresponding compounds of formulae I and II in unitary composition, i.e. in which both n and R have a defined value within in the defined range. These compounds are novel. The compounds I and II can be prepared according

to already known methods starting from an alcohol of formula III or of formula IV



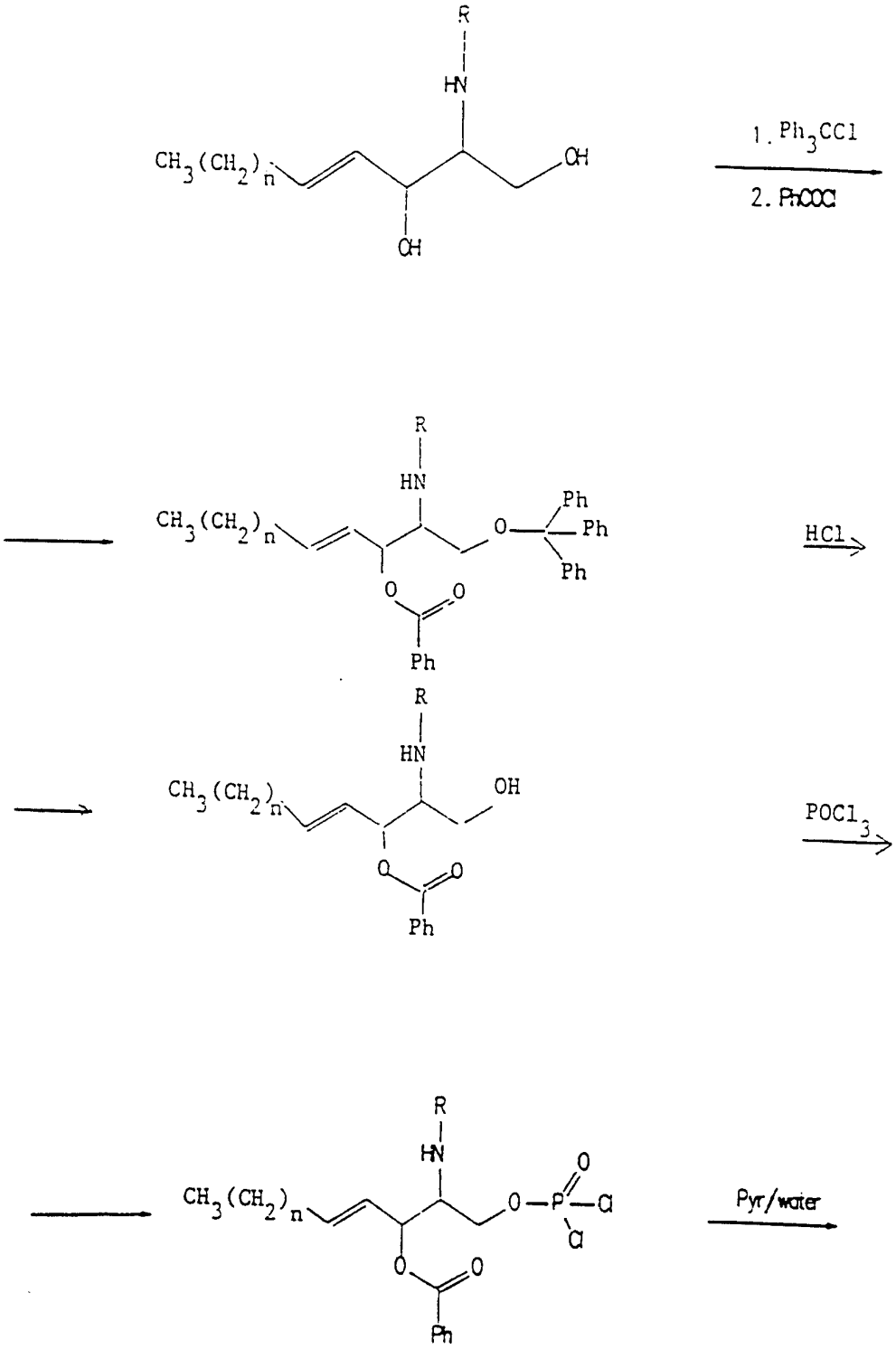
III

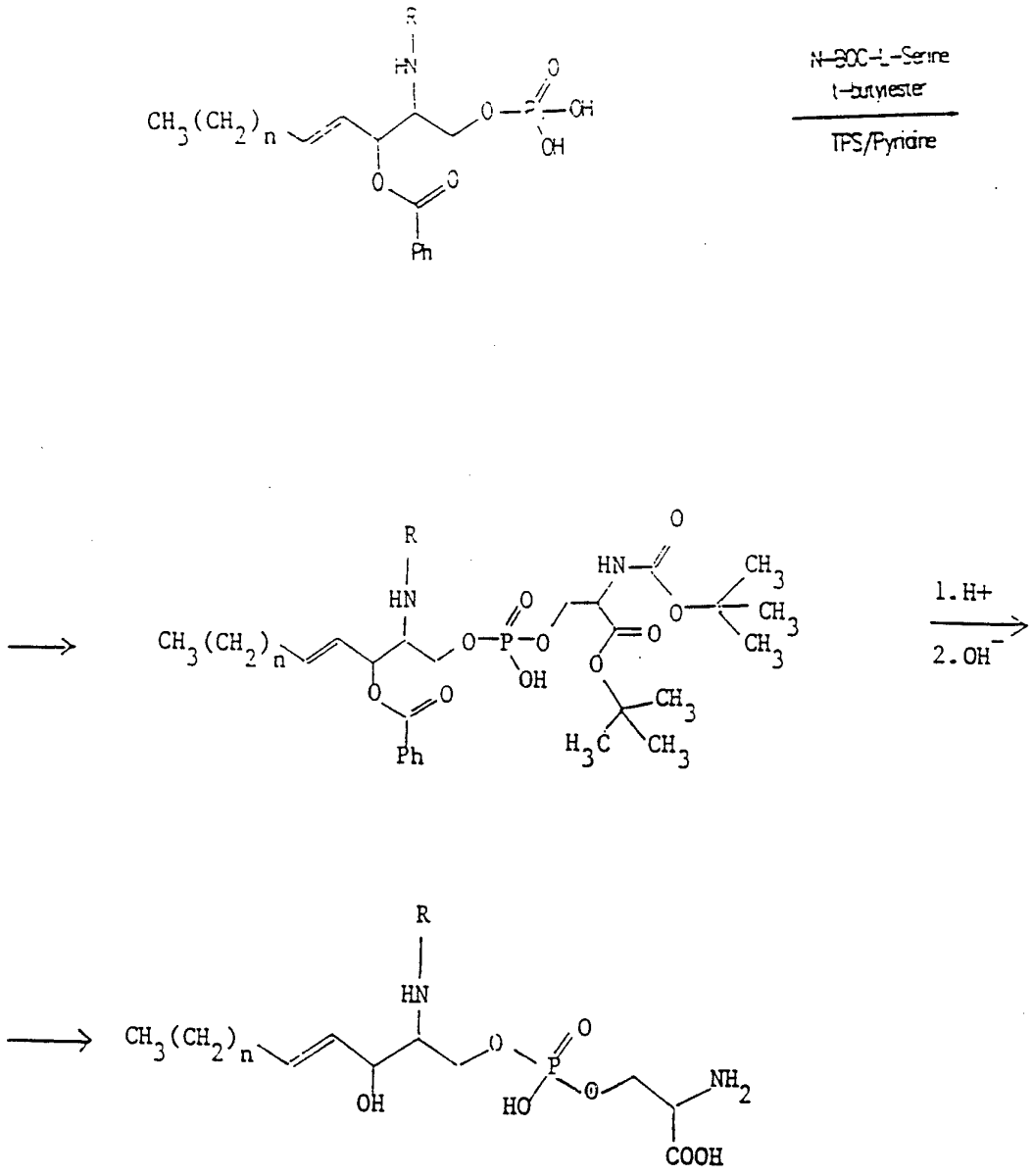


IV

where n = 6 - 16, which is reacted with phosphoric acid or a reactive derivative thereof, L-serine and a fatty acid R-COOH, where R has between 16 and 24 carbon atoms, in random sequence. The process involves acylating the amino-alcohol on the nitrogen atom with the mentioned acid and then reacting the obtained derivative with phosphoric acid and L-serine, or the steps can be carried out in different sequences. These new derivatives have the same pharmacological activities as reported for the product of formula I or II and can be

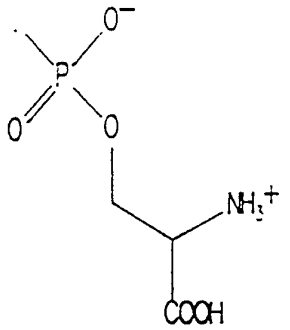
used in therapy for the same indications. The following scheme shows a preferred sequence of the aforesaid preparation, which can also be used for the preparation of compounds of formula I or II

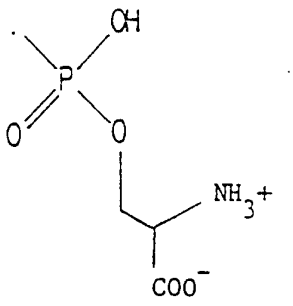




where Ph designates a phenyl group and TPS designates triisopropyl-benzenesulfonyl chloride.

Phosphoryl-L-serine-N-acyl-sphingosine of formula I or II is generally present in the betaine form, i.e. according to formula





of the serine component, and that is also true for the aforesaid chemically unitary compound. If desired, also these betaines can be transformed into acid addition salts or basic salts, i.e. into alkaline, alkaline-earth or magnesium or heavy metal salts, or into salts of hydrohalogenic acids, as e.g. chlorides, bromides or iodides, in sulfates, sulfonates, or in organic acid salts. Also these salts, if therapeutically compatible, can be used for therapeutic applications. Therapeutically non-acceptable salts are also part of the invention, and can optionally be used for the purification of the compounds. Compounds of formula I or II can be prepared by an already known procedure, e.g. according the aforesaid method and described in Biochemistry, vol. 28, No. 8, 1989, 3457.

It has now been found that the exchange between choline and serine can be done also directly utilizing phospholipase D, reacting sphingomyelin and serine, without the use of the above mentioned intermediate,

i.e. the N-acyl derivative of 4-doxyl-pentanoic acid of lyso-sphingomyelin. This process is, therefore, still another object of the present invention, and the resulting great technical advantage is clear. This procedure is illustrated in Ex.1.

The starting materials for the preparation of phosphoryl-L-serine-N-acyl-sphingosine and the other analogous compounds are known or can be obtained according methods described in literature. The invention is illustrated in the following non-limiting Examples:

Example 1:

5 gr of chromatographically pure sphingomyelin were dissolved in a mixture of 80 ml of ethyl acetate and 80 ml of acetate buffer pH 5.6, 0.2 M, $CaCl_2$ 0.04 M saturated with serine. 320 U of phospholipase D from Streptomyces sp. were added and the reaction was conducted for 24 hours at 45 C. At the end 80 ml of chloroform/methanol 2:1 were added in order to extract the compound. After partitioning with 50 ml of HCl 0.5N, the organic layer was washed with 50 ml of water and then precipitated in 500 ml of ethanol containing 10 ml of sodium acetate 4.5M. The final purification was performed through chromatography on silica gel using a mixture of chloroform/methanol/water 60:30:3 as eluent. The pure fractions were gathered, concentrated and finally precipitated in 250 ml of acetone. Yield: 2.7

gr. After thin layer chromatography on silica gel, the compound is ninhydrine and phosphorus positive having Rf = 0.27 (sphingomyelin 0.20) and 0.11 (sphingomyelin 0.13) respectively, utilizing as eluent chloroform/methanol/water 60:30:4 and chloroform/ethyl acetate/n-propanol/methanol/ NH3 0.1N 25:25:25:22:13.

Example 2:

Pharmaceutical preparations in solution for injection

Preparation No. 1

A vial of 2 ml contains:

phosphoryl-L-serine-N-acyl-sphingosine	mg 5
sodium chloride	mg 16
citrate buffer pH 6 in distilled water,	ad 2 ml

Preparation No. 2

A vial of 2 ml contains:

phosphoryl-L-serine-N-acyl-sphingosine	mg 50
sodium chloride	mg 16
citrate buffer pH 6 in distilled water,	ad 2 ml

Preparation No. 3

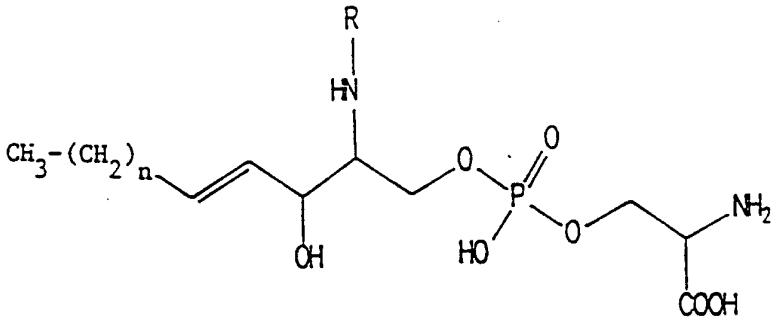
A flacon of 4 ml contains:

phosphoryl-L-serine-N-acyl-sphingosine	mg 100
sodium chloride	mg 32
citrate buffer pH 6 in distilled water,	ad 4 ml

The invention being described in this way, it is clear that these methods can be modified in several ways. These modifications cannot be considered different from the concept of the invention and all modifications, which can be considered as evident for an expert in the field, are included in the following claims.

CLAIMS

1. Use of phosphoryl-L-serine-N-acyl-sphingosine formed by a mixture of compounds of formula I or II



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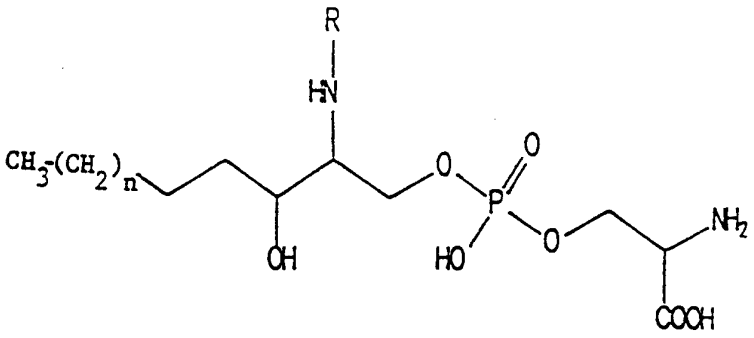


II

in which n is 6-16 and R means an acyl residue of a higher aliphatic acid having between 16 and 24 carbon

atoms, the distribution of the values for n and R being those of a natural sphingomyelin, or salts thereof, in the preparation of pharmaceutical compositions.

2. Use of phosphoryl-L-serine-N-acyl-sphingosine according to claim 1 wherein the pharmaceutical compositions are for the therapy of diseases or disorders of the nervous system.

3. Use of phosphoryl-L-serine-N-acyl-sphingosine according to claim 1 wherein the pharmaceutical compositions are for the therapy of pathologies characterized by a modified immunological reactivity and/or symptoms of autoimmunity.

4. Use according to claim 2 wherein the pharmaceutical compositions are for the treatment of the nervous system as outcomes of neuronal lesions or degenerations.

5. Use according to claim 2 wherein the pharmaceutical compositions are for the treatment of one of the following affections or diseases: ischemia, hypoxia, epilepsy, traumas or compressions, metabolic dysfunctions, aging, toxic-infective and chronic neurodegenerative diseases.

6. Use according to claim 5 wherein the

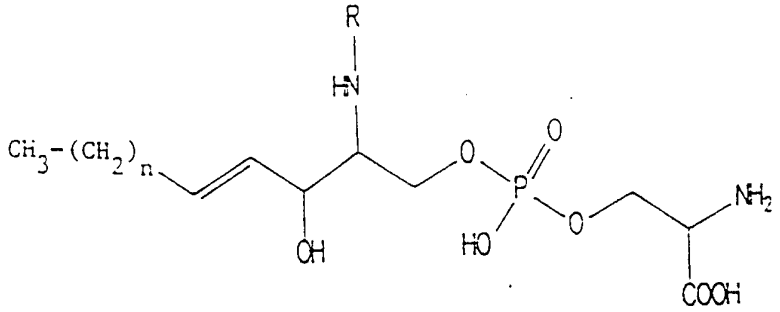
pharmaceutical compositions are for the treatment of Alzheimer's, Parkinson's and Huntington's diseases.

7. Use according to claim 2 wherein the pharmaceutical compositions are for the treatment of psychomotric involutive senile syndromes, chronic vascular cerebropathies of aging, senile dementia, and pre-senile dementia following trauma, postanoxic cerebropathies and extrapyramidal syndromes.

8. Use according to claim 3 wherein the pharmaceutical compositions are for the treatment of one of the following disorders or diseases: multiple sclerosis, cachectic situations associated with infections and neoplastic diseases, pulmonary fibrosis, rheumatoid arthritis, bacterial, protozoic and HIV (human immunodeficiency virus) infections, cerebral complications of malaria, insulin dependent diabetes.

9. Use according to any of claims 1-8 in which acid addition salts or salts of metal bases or organic bases of phosphoryl-L-serine-N-acyl-sphingosine are used.

10. A process for the preparation of phosphoryl-L-serine-N-acyl-sphingosine formed by a mixture of compounds of formula I or II



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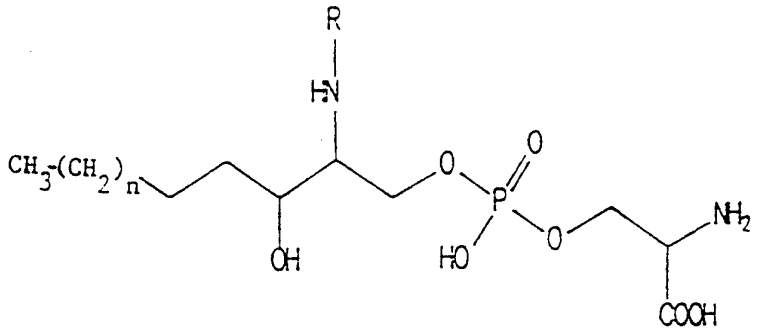
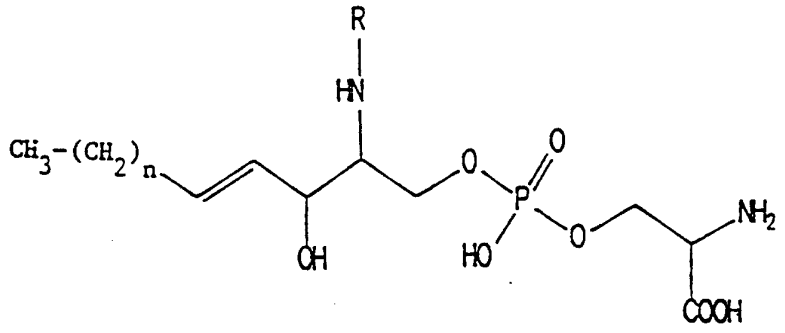


II

in which n is 6-16 and R means an acyl residue of a higher aliphatic acid having between 16 and 24 carbon atoms, the distribution of the values for n and R being those of a natural sphingomyelin, or salts thereof, in which sphingomyelin is reacted with phospholipase D.

11. A process for the preparation of phosphoryl-L-serine-N-acyl-sphingosine formed by a mixture of compounds of formula I or II



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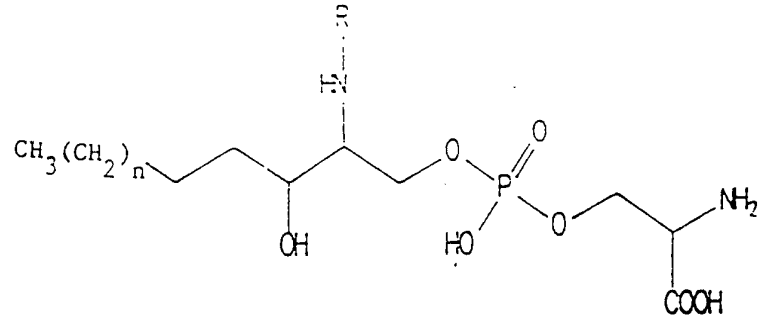
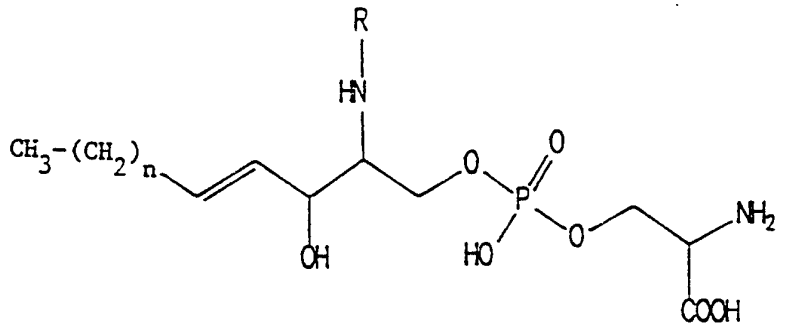


II

in which n is 6-16 and R means an acyl residue of a higher aliphatic acid having between 16 and 24 carbon atoms, the distribution of the values for n and R being those of a natural sphingomyelin, or salts thereof, in which N-4-doxyl-pentanoyl-lyso sphingomyelin is reacted with phospholipase D.

12. Compounds of formula I or II



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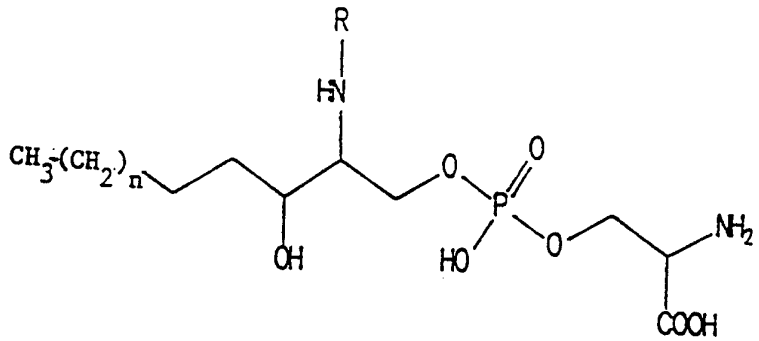
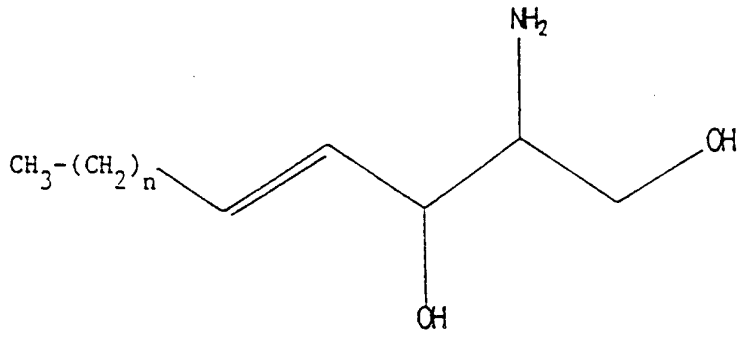


II

wherein n = 6 - 16 and R means an acyl residue of a higher aliphatic acid having between 16 and 24 carbon atoms, in chemically unitary form.

13. A process for the preparation of compounds defined in claim 12 or a mixture of these compounds, in which an aminoalcohol of formula III or IV



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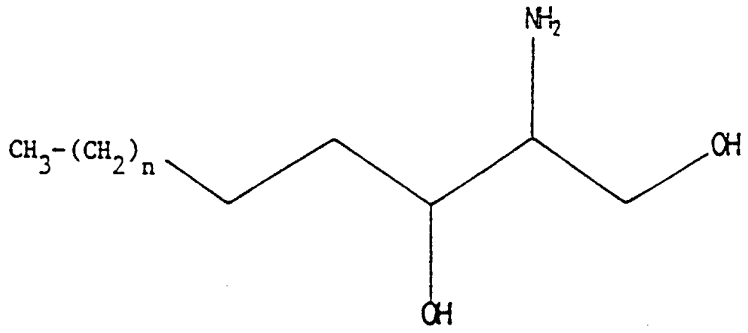


IV

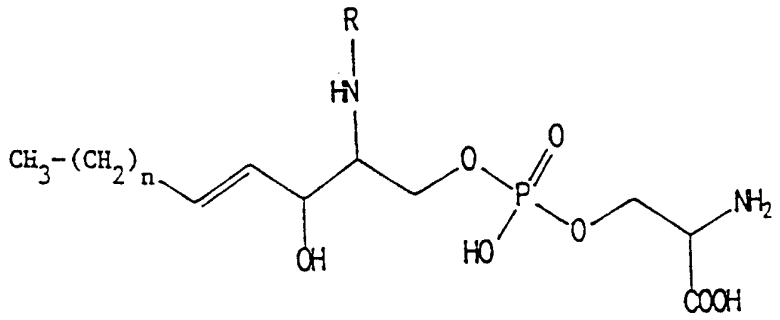
in which n = 6 - 16, is reacted with phosphoric acid or a reactive derivative thereof, L-serine and a fatty acid R-COOH, in random sequence.

14. A pharmaceutical preparation containing a compound of formula I or II in which n is 6-16 and R means an acyl residue of a higher aliphatic acid having

between 16 and 24 carbon atoms, the distribution of the values for n and R being those of a natural sphingomyelin, or a salt thereof as active ingredient together with a pharmacologically acceptable excipient.

15. A pharmaceutical composition containing a compound according to claim 12 as active ingredient together with a pharmacologically acceptable excipient.

16. A method for treating disorders of the nervous system or pathologies characterized by a modified immunological reactivity and/or symptoms of autoimmunity wherein phosphoryl-L-serine-N-acyl-sphingosine formed by a mixture of compounds of formula I or II



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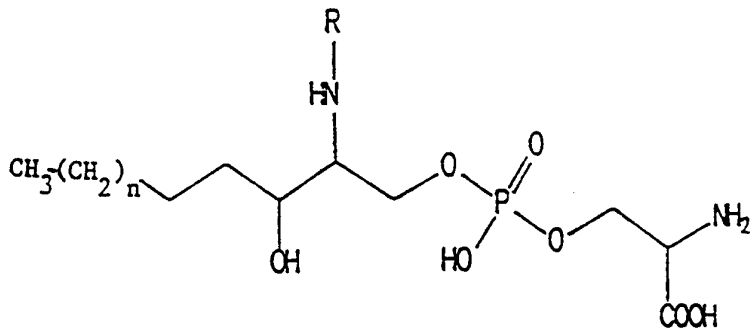
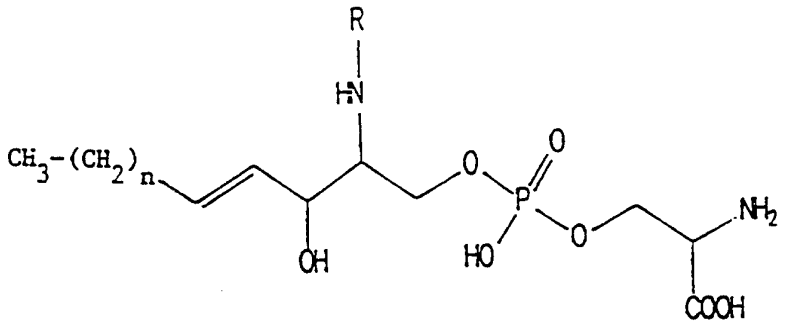


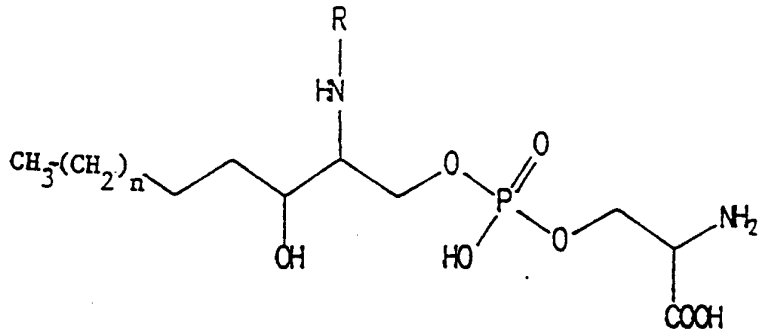
II

in which n is 6-16 and R means an acyl residue of a higher aliphatic acid having between 16 and 24 carbon atoms, the distribution of the values for n and R being those of a natural sphingomyelin, or salts thereof is administered to a patient in need thereof.

17. A method for treating disorders of the nervous system or pathologies characterized by a modified immunological reactivity and/or symptoms of autoimmunity wherein compounds of formula I or II



I



II

in which n is 6-16 and R is an acyl residue of a higher aliphatic acid having 16-24 carbon atoms, in chemically unitary form, is administered to a patient in need thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 93/02061

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/66 C07F9/113 C07F9/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOORG. KHIM. vol. 7, no. 9 , 1981 pages 1388 - 1394 N.N. KARPYSHEV ET AL. 'Synthesis of serine phosphatides' see abstract	12
Y		1,2,4-7, 9,16,17

X	BIOORG. KHIM. vol. 5, no. 9 , 1979 pages 1422 - 1423 N.N. KARPYSHEV 'Synthesis of ceramidephosphoserine' see abstract	12
Y	see page 1422	1,2,4-7, 9,16,17

	-/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 December 1993	11.01.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Krautbauer, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 93/02061

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHEMISTRY vol. 28, no. 8 , 1989 pages 3456 - 3462 G. MORROT ET AL. 'Aminophospholipid translocase of human erythrocytes: Phospholipid substrate specificity and effect of cholesterol' cited in the application see page 3457	10,11
Y	DATABASE WPI Week 8927, Derwent Publications Ltd., London, GB; AN 89-197154 & JP,A,1 135 720 (EISAI KK) 29 May 1989 see abstract	1,2,4-7, 9,16,17

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 93/ 02061

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 16 and 17 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alle ged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.