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Tomiyoshi et al.

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[54] SPERGUALIN-RELATED COMPOUND AND USE THEREOF

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[30] **Foreign Application Priority Data**

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[51] **Int. Cl.**[5] **A61K 31/195**

[52] **U.S. Cl.** **514/563;** 514/558; 562/439; 562/560; 554/53

[58] **Field of Search** 562/560, 439; 514/563, 514/558; 260/404.5

[56] **References Cited**

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4,430,346	2/1984	Umezawa et al.	424/311
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4,556,735	12/1985	Umezawa et al.	564/157
4,851,446	7/1989	Umezawa et al.	514/620

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0347820	12/1989	European Pat. Off. .

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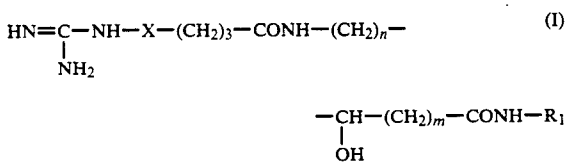
Primary Examiner—Michael L. Shippen

Attorney, Agent, or Firm—Nields & Lemack

[57] **ABSTRACT**

Novel spergualin-related compounds represented by the general formula (I):



(I)

wherein X represents $-(CH_2)_{1-5}-$ or a phenylene group which may be substituted; m represents 0, 1 or 2; n represents 1 or 2; and R_1 represents $-(CH_2)_{1-3}-COOH$, and pharmacologically acceptable salts thereof, possess an immunopotentiating activity, and are expected to be useful as immunopotentiators applicable to warm blooded animals.

9 Claims, No Drawings

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SPERGUALIN-RELATED COMPOUND AND USE THEREOF

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a novel spergualin-related compound which is useful for an immunopotentiator and which has a high specificity, and to medical use of the compound.

2. Related Art Statement

Spergualin is a compound having an anti-tumor activity and immunosuppressive activity which is obtained from the culture broth of *Bacillus laterosporus* (U.S. Pat. No. 4,416,899) and many derivatives of spergualin have been synthesized (cf., U.S. Pat. No. 4,430,346, U.S. Pat. No. 4,518,532, U.S. Pat. No. 4,529,549, U.S. Pat. No. 4,556,735, U.S. Pat. No. 4,851,446, EP-A-213526, EP-A-241,797). These compounds are expected to be drugs as carcinostatic agents or immunosuppresants.

Currently, some immunopotentiators have been developed but new immunopotentiators having a higher specificity have still been desired.

SUMMARY OF THE INVENTION

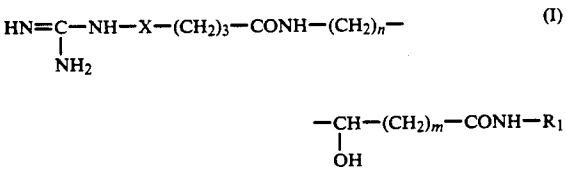
Therefore, an object of the present invention is to provide a novel compound useful as an immunopotentiator.

Another object of the present invention is to provide a pharmaceutical composition comprising the novel compound as an active ingredient, which is particularly useful for an immunopotentiator.

A further object of the present invention is to provide a method for immunopotentiation which comprises administering the novel compound to a warm-blooded animal.

A still further object of the present invention is to provide use of the novel compound as an immunopotentiator.

A first aspect of the present invention relates to a novel spergualin derivative represented by the general formula (I):



(I)

wherein X represents $-(CH_2)_{1\text{-}5}-$ or a phenylene group which may be substituted; m represents 0, 1 or 2; n represents 1 or 2; and R_1 represents $-(CH_2)_{1\text{-}3}-COOH$; and a pharmacologically acceptable salt thereof.

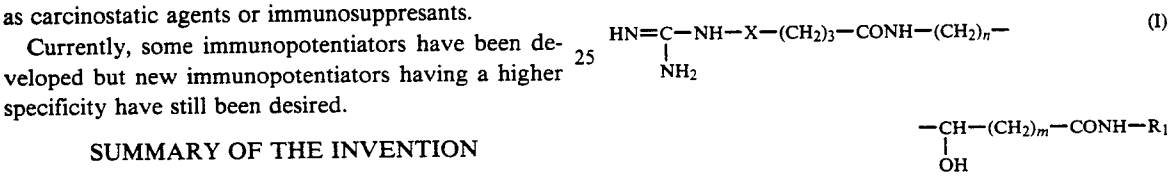
A second aspect of the present invention relates to a pharmaceutical composition for immunopotentiation comprising as an active ingredient the novel spergualin derivative represented by the general formula (I) or the pharmaceutically acceptable salts thereof, together with a pharmaceutically carrier.

A third aspect of the present invention relates to a method for immunopotentiation which comprises administering an effective amount of the novel spergualin derivative represented by the general formula (I) or the pharmaceutically acceptable salt thereof to a warm-blooded animal having a reduced immunity.

A fourth aspect of the present invention relates to use of the novel spergualin derivative represented by the general formula (I) or the pharmaceutically acceptable salt thereof for the production of a pharmaceutical composition for immunopotentiation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The novel spergualin derivatives are represented by the general formula (I):



(I)

wherein X represents $-(CH_2)_{1\text{-}5}-$ or a phenylene group which may be substituted; m represents 0, 1 or 2; n represents 1 or 2; and R_1 represents $-(CH_2)_{1\text{-}3}-COOH$.

The phenylene group of X may be substituted with a halogen atom such as chlorine, fluorine and bromine atom; a lower alkyl group such as methyl, ethyl, propyl, t-butyl and pentyl group; or a lower alkoxy group such as methoxy, ethoxy, propoxy, t-butoxy and pentoxy group. X is preferably $-(CH_2)_3$ or $-(CH_2)_5-$, more preferably $-(CH_2)_3-$. m is preferably 0 or 1. n is preferably 1. R_1 is preferably $-(CH_2)_2-COOH$ or $-(CH_2)_3-COOH$.

The compounds represented by the general formula (I) may form salts with acids. As the acids for forming the salts, any of inorganic acids and organic acids may be used as long as they are non-toxic. As the inorganic acids, there are no particular limitation but hydrochloric acid, sulfuric acid, nitric acid and phosphoric acid are preferred. As the organic acids, there are no particular limitation but preferred are acetic acid, propionic acid, succinic acid, fumaric acid, maleic acid, malic acid, tartaric acid, glutaric acid, citric acid, benzenesulfonic acid, toluenesulfonic acid, methanesulfonic acid, ethanesulfonic acid, propanesulfonic acid, aspartic acid, and glutamic acid.

In the spergualin derivative of the general formula (I) of the present invention, steric configuration of the carbon atom to which the hydroxy group is bound indicates S, R or RS form. In particular, S or RS form are preferable. The representative compounds in the present invention are listed in the following Table 1.

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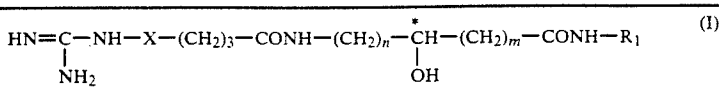
TABLE 1



(I)

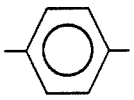
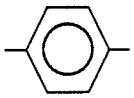
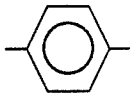
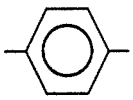
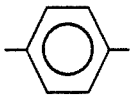
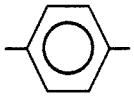
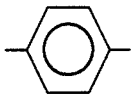
Compound No.	X	n	m	Steric Configuration of C*	R_1
1	$-(CH_2)_3-$	1	1	S, R or RS	$-CH_2-COOH$
2	$-(CH_2)_3-$	1	1	S, R or RS	$-(CH_2)_2-COOH$
3	$-(CH_2)_3-$	1	1	S, R or RS	$-(CH_2)_3-COOH$
4	$-CH_2-$	1	1	S, R or RS	$-CH_2-COOH$
5	$-CH_2-$	1	1	S, R or RS	$-(CH_2)_2-COOH$
6	$-CH_2-$	1	1	S, R or RS	$-(CH_2)_3-COOH$
7	$-(CH_2)_2-$	1	1	S, R or RS	$-CH_2-COOH$
8	$-(CH_2)_2-$	1	1	S, R or RS	$-(CH_2)_2-COOH$
9	$-(CH_2)_2-$	1	1	S, R or RS	$-(CH_2)_3-COOH$
10	$-(CH_2)_4-$	1	1	S, R or RS	$-CH_2-COOH$
11	$-(CH_2)_4-$	1	1	S, R or RS	$-(CH_2)_2-COOH$
12	$-(CH_2)_4-$	1	1	S, R or RS	$-(CH_2)_3-COOH$
13	$-(CH_2)_5-$	1	1	S, R or RS	$-CH_2-COOH$
14	$-(CH_2)_5-$	1	1	S, R or RS	$-(CH_2)_2-COOH$
15	$-(CH_2)_5-$	1	1	S, R or RS	$-(CH_2)_3-COOH$
16		1	1	S, R or RS	$-CH_2-COOH$
17		1	1	S, R or RS	$-(CH_2)_2-COOH$
18		1	1	S, R or RS	$-(CH_2)_3-COOH$
19	$-(CH_2)_2-$	1	0	S, R or RS	$-CH_2-COOH$
20	$-(CH_2)_2-$	1	0	S, R or RS	$-(CH_2)_2-COOH$
21	$-(CH_2)_2-$	1	0	S, R or RS	$-(CH_2)_3-COOH$
22	$-(CH_2)_3-$	1	0	S, R or RS	$-CH_2-COOH$
23	$-(CH_2)_3-$	1	0	S, R or RS	$-(CH_2)_2-COOH$
24	$-(CH_2)_3-$	1	0	S, R or RS	$-(CH_2)_3-COOH$
25	$-(CH_2)_4-$	1	0	S, R or RS	$-CH_2-COOH$
26	$-(CH_2)_4-$	1	0	S, R or RS	$-(CH_2)_2-COOH$
27	$-(CH_2)_4-$	1	0	S, R or RS	$-(CH_2)_3-COOH$
28	$-(CH_2)_5-$	1	0	S, R or RS	$-CH_2-COOH$
29	$-(CH_2)_5-$	1	0	S, R or RS	$-(CH_2)_2-COOH$
30	$-(CH_2)_5-$	1	0	S, R or RS	$-(CH_2)_3-COOH$
31		1	0	S, R or RS	$-CH_2-COOH$
32		1	0	S, R or RS	$-(CH_2)_2-COOH$
33		1	0	S, R or RS	$-(CH_2)_3-COOH$
34	$-(CH_2)_3-$	2	0	S, R or RS	$-CH_2-COOH$
35	$-(CH_2)_3-$	2	0	S, R or RS	$-(CH_2)_2-COOH$
36	$-(CH_2)_3-$	2	0	S, R or RS	$-(CH_2)_3-COOH$
37		2	0	S, R or RS	$-CH_2-COOH$

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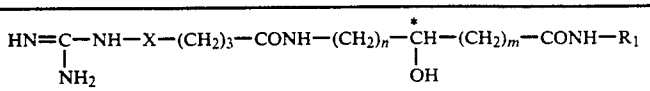
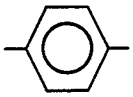
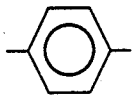
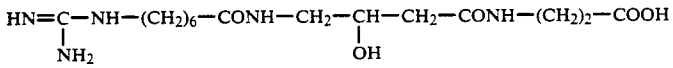
TABLE 1-continued



(I)

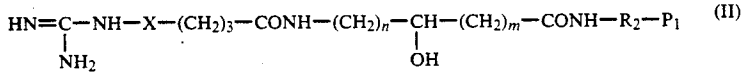
Compound No.	X	n	m	Steric Configuration of C*	R_1
38		2	0	S, R or RS	$-(CH_2)_2-COOH$
39		2	0	S, R or RS	$-(CH_2)_3-COOH$

Among the compounds listed in Table 1, the following compound is the most preferable compound. The compound No. 2



The steric configuration of the asymmetric carbon atom in Compound No. 2 is preferably S form.

The compounds listed above are all novel and may be prepared by the following process. That is, the compounds are obtained by removing a protective group from compounds represented by the formula (II):

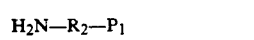


(II)

wherein X, m and n are as defined above; R2 represents $-(CH_2)_{1-3}-COO-$; and P_1 represents a protective group of the carboxy group.

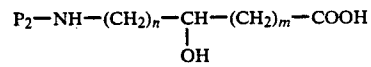
The protected compounds of the general formula (II) which are the starting compounds of the present invention may be synthesized by the following method.

Protected amino acids represented by the formula (III):



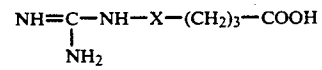
(III)

wherein R_2 and P_1 are as defined above; are reacted with reactive derivatives of protected amino acids represented by the formula (IV):



(IV)

wherein P_2 represents a protective group of the amino group which is different from P_1; and n and m are as defined above. Then the amino protective group P_2 is removed and reacted with reactive derivatives of ω-guanidino-fatty acids represented by the formula (V):



(V)

wherein X is as defined above. Thus, the compounds represented by the formula (II) are obtained.

The condensation reaction as described above may be carried out by methods generally used in peptide chemistry. Examples of these methods include a carbodiimide method using dicyclohexylcarbodiimide, 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide or the like; a mixed acid anhydride method using ethyl chlorocarbonate, isobutyl chlorocarbonate or the like; an activated ester method using a cyanomethyl ester, a vinyl ester, a substituted or unsubstituted phenyl ester, a thiophenyl ester or a hydroxysuccinimide ester or the like; an O-acylhydroxylamine derivative method using acetoxime, cyclohexanone oxime or the like; an N-acyl compound method using carbonyldiimidazole; and a carboxylic acid activation method using 1,3- thiazolidine-2-thione or the like.

As a solvent for the condensation reaction, any solvent applied to conventional peptide bond-forming reactions may be used. Examples of such a solvent include ethers such as diethyl ether, tetrahydrofuran and dioxan; esters such as ethyl acetate; ketones such as acetone and methyl ethyl ketone; halogenated hydrocarbons such as methylene chloride and chloroform; amides such as dimethylformamide and dimethylacetamide; nitriles such as acetonitrile. These solvents may be used singly or in combination thereof. Where the solvent is miscible with water, the solvent may be used as an admixture with water.

As the protective group which may be used in the present invention, there are a lower alkyl group, t-butyl group, benzyl group, a substituted benzyl group, and the like.

The protective group in the compounds shown by the general formula (II) may be split off by reactions such as reduction, hydrolysis and acid decomposition. Such reactions are carried out generally in a solvent at −60° C. to the boiling point of a solvent, preferably at −50°

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to 100° C. Examples of the solvent used include water and hydrophilic organic solvents, for example, a lower alcohol such as methanol and ethanol; a ketone such as acetone and methyl ethyl ketone; an amide such as dimethylformamide and dimethylacetamide; a cyclic ether such as tetrahydrofuran and dioxane; a lower fatty acid such as acetic acid and trifluoroacetic acid; liquid ammonia; and liquid hydrogen fluoride. These solvents may be appropriately used.

The compounds of general formula (I) may be isolated from the reaction solution of the compounds from which the protective group has been split off, by conventional methods for purification, for example, where the protective group is removed by catalytic reduction using palladium black, the filtrate obtained by filtering the catalyst off is concentrated under reduced pressure and the residue is purified by known method using chromatography using CM-Sephadex® (Na^+) or Sephadex® LH-20. Where the protective group is removed with trifluoroacetic acid, the reaction solution is also concentrated under reduced pressure and the residue is purified by the method described above. Thus, the desired compounds may be purified.

By the purification method described above, the compounds of the general formula (I) may be obtained in the form of hydrochloride. The salt may also be converted into other salts. For example, the hydrochloride is dissolved in water and the resulting aqueous solution is passed through a strongly basic ion exchange resin. The non-adsorbed fraction containing the compounds of general formula (I) is collected and a desired acid is added thereto for neutralization. The mixture is then evaporated to dryness under reduced pressure. In this case, water, or, if necessary, a hydrophilic organic solvent such as methanol, ethanol, acetone, tetrahydrofuran, dioxan, or the like is added. When the organic solvent is contained, the solvent is distilled off under reduced pressure and freeze-dried to give the desired salts. The desired salts may also be obtained by adding an aqueous solution of silver hydroxide to the hydrochloride of the compounds of the general formula (I) to neutralize hydrochloric acid, filtering insoluble silver chloride, adding a desired acid to the filtrate to form the salts, and freeze-drying.

The physiological activity of the compounds of the present invention is demonstrated by the following experiments wherein the effects of potentiating antibody production were determined.

EXPERIMENT 1

1. Method

Sheep red blood cells (SRBC) were intravenously injected to CDF_1-SLC mice (5 mice in each group) in a dose of 1×10^8/0.2 ml for booster. The compound of the present invention was diluted with physiological saline in various concentrations. Each diluted solution was administered once in a daily dose of 0.1 ml per 10 g of body weight (0.1 ml/10 g/day) for consecutive 3 days from the next day after sensitization. Mice were sacrificed 4 days after the sensitization. The count of anti-SRBC antibody-producing cells (plaque forming cell, PFC) in the spleen cells was determined and the PFC count was calculated per 10^6 of the spleen cells. As is shown in the following equation, the effect of the compound of the present invention is expressed by a potentiation rate (%) of the PFC count in the group administered with the compound of the present invention, as compared to the PFC count in the control group.

$$\text{Potentiation rate (\%)} = \left(\frac{\text{PFC count in the administered group}}{\text{PFC count in the control group}} \right) \times 100$$

2. Results

The effects of representative examples of the compounds of the present invention on potentiating antibody production is shown in Table 2.

TABLE 2

Effects of Compounds of This Invention on Potentiating Antibody Production

Compound No.	Effect of Potentiating Antibody Production (control: 100%) Dose 3 mg/kg	3 mg/kg
2*	127	151
3*	128	128
5*	93	127
14*	84	113
21*	152	173
24*	169	166
Control: Physiological saline	100	100

*RS form

EXPERIMENT 2

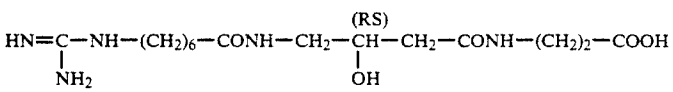
1. Method

Methylprednisolone was intraperitoneally administered to BALB/C mice (female, age of 8 weeks, Japan Kurea) in a dose of 400 mg/kg for 9 days to prepare immunosuppressed mice. On Day 6, the mice was boosted by intravenous injection of sheep red blood cells (SRBC, Japan Biological Material Center) in a dose of 1×10^6 cells. On Day 6 after the sensitization the count of antibody-producing cells (plaque forming cell, PFC) in the spleen cells was determined. A test compound was intravenously administered in each dose shown in Table 3 only on the next day after the sensitization.

In the same way as in Experiment 1, a potentiation rate of PFC count was determined.

The tested compounds were as follows:

Compound No. 2 (RS form)



Compound No. 2 (S form)

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Sync your system to PACER to automate legal marketing.