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[54] Title: METHOD OF INDUCING IMMUNOSTIMULATING ACTIVITY

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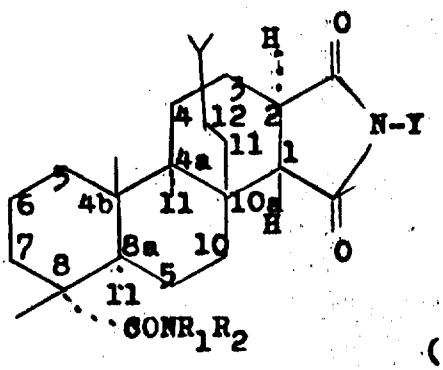
[57]

A B S T R A C T

A method of inducing immunostimulating activity in warm-blooded animals comprising administering to warm-blooded animals an immunostimulating amount of at least one maleo-pimaric acid compound of the formula



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5 Claims. Specification: 12 page (s): Drawings: None sheet (s)

Examiner: L. M. Cruz

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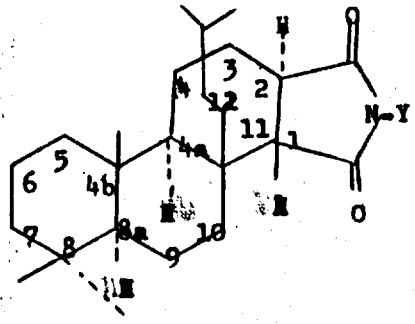
wherein R_1 and R_2 together with the nitrogen to which they are attached form an optionally substituted saturated heterocycle optionally containing at least one other heteroatom, Y is selected from the group consisting of hydrogen, amino, alkyl of 1 to 5 carbon atoms optionally substituted with at least one hydroxy, dialkylamino with alkyl of 1 to 5 carbon atoms, alkoxy carbonyl of 2 to 5 carbon atoms, acyl of an organic carboxylic acid of 1 to 5 carbon atoms and $-XO-\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}-N\begin{matrix}R_1\\R_2\end{matrix}$, X is an alkylene of 1 to 5 carbon atoms and R_1 and R_2 have the above definition.

METHOD OF INDUCING IMMUNOSTIMULATING ACTIVITY

ABSTRACT OF THE DISCLOSURE

A method of inducing immunostimulating activity in warm-blooded animals comprising administering to warm-blooded animals an immunostimulating amount of at least one maleo-pimaric acid compound of the formula



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$CONR_1R_2$

wherein R_1 and R_2 together with the nitrogen to which they are attached form an optionally substituted saturated heterocycle optionally containing at least one other heteroatom, Y is selected from the group consisting of hydrogen, amino, alkyl of 1 to 5 carbon atoms optionally substituted with at least one hydroxy, dialkylamino with alkyl of 1 to 5 carbon atoms, alkoxy carbonyl of 2 to 5 carbon atoms, acyl of an organic carboxylic acid of 1 to 5 carbon atoms and $-XO-\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}-N\begin{matrix}R_1\\R_2\end{matrix}$, X is an alkylene of 1 to 5 carbon atoms and R_1 and R_2 have the above definition.

STATE OF THE ART

US patent No. 3,998,823 describes novel maleo-pimaric acid compounds having a hepato-protective activity.

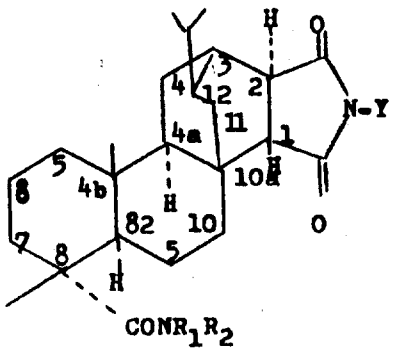
OBJECTS OF THE INVENTION

It is an object of the invention to provide a novel method of inducing immunostimulating activity in warm-blooded animals including humans.

This and other objects and advantages of the invention will become obvious from the following detailed description.

THE INVENTION

The novel method of the invention of inducing immunostimulating activity in warm-blooded animals comprises administering to warm-blooded animals an immunostimulating amount of at least one maleo-pimaric acid compound of the formula



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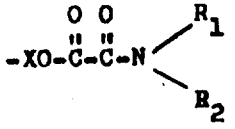
wherein R_1 and R_2 together with the nitrogen to which they are attached form an optionally substituted saturated heterocycle optionally containing at least one other heteroatom, Y is selected from the group consisting of hydrogen, amino, alkyl of 1 to 5 carbon atoms optionally substituted with at least one hydroxy, dialkylamino with alkyl of 1 to 5 carbon atoms, alkoxy carbonyl of 2 to 5 carbon atoms, acyl of an organic carboxylic acid of 1 to 5 carbon atoms and  , X is an alkylene of 1 to 5 carbon atoms and R_1 and R_2 have the above definition. The usual daily dose is 0,30 to 40 mg/kg depending on the specific compound, the method of administration and the condition treated. The compounds may be administered orally, parenterally or topically.

While US Patent No. 3,998,823 describes the malee-pimaric acid compounds as having hepato-protecting properties, this would in no way suggest to one skilled in the art that the compounds of formula I would have remarkable immuno-modulating properties. Immuno-modulator is understood to "regulate the immunizing reactions by inhibiting them or stimulating them" (Messieurs Garnier - Delamare - Dictionnaire des termes techniques de Medicine p. 421 - 21st edition 1985).

R_1 and R_2 together with the nitrogen atom may for example, be a saturated hetdrocycle containing from 4 to 6 carbon atoms such as, for example, morpholino, piperidino,

piperazin-1-yl, pyrrolidino or hexamethylene imino, this latter group being able to carry a substituent at either on the carbon atoms, i.e., the substituent being alkyl, hydroxyalkyl of phenyl, or on the nitrogen with the substituent then being phenyl, alkyloxycarbonyl, sulfonyl or acyl, such alkyl, alkyloxy or acyl containing at the most 5 carbon atoms Y may be, for example, hydroxyalkyl, dialkylaminoalkyl or alkyloxycarbonyl with the alkyls containing 1 to 5 carbon atoms.

Among the preferred compounds of the invention are those of formula I wherein R_1 and R_2 together with the nitrogen atom represent pyrrolidino, morpholino, piperazin-1-yl, 4-alkyl-piperazin-1-yl or 4-hydroxyalkyl-piperazin-1-yl group and those wherein Y is hydrogen, amino or β-hydroxyethyl and preferably those of formula I wherein R_1 and R_2 together with the nitrogen atom are morpholino and those wherein Y is hydrogen or β-hydroxyethyl.

The preferred compounds of formula I are 8β-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl-1β,3β, 4,4aβ-4bα, 5,6,7,8,8aβ, 9,10,10a-tetradecahydro-3,10a-ethenophenantro-[1,2-c]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione and 8β-(morpholino carbonyl)-4bα, 8α-dimethyl-12-isopropyl-1β,2β,3β, 4,4aβ, 4bα, 5,6,7,8,8aβ,9,10,10,10a-tetradecahydro-3,10a-ethenophenantro-[1,2-c]2',5'-pyrrolidinedione.

The derivatives with the formula I possess remarkable immunomodulating properties and are further distinguished by a very weak toxicity and a remarkable tolerance. Because of these properties, the immunomodulating compositions may be administered as prophylactic or curative means. They are useful in the treatment of auto-immune diseases, whether concerned with non-specific attacks on organs (rheumatoid polyarthritis, erythematous lupus, hemolytic anemia, autoimmune leukopenia, etc.) or with specific diseases of organs (throiditis, Graves' disease, Addison's disease, disseminated sclerosis, pemphigus, hemorrhagic rectocolitis, certain nephropathies etc.). The compositions are also useful in the treatment of hemopathies, of cancer, of aida, of viral and microbial infections, particularly chronic and recurrent (bronchitis, influenza, etc.), diseases of the oral cavities, etc. They can be adjuvants of viral therapy, of antibiotic therapy or of anti-cancerous chemiotherapy.

They are also useful in the treatment of numerous secondary or acquired immune deficiencies observed during very different affections: deficiencies associated with metabolic disturbances, deficiencies of iatrogenic origin (corticoids, ionising radiations), deficiencies observed in those suffering from severe burns, etc.

The compositions may be in the form of tablets, dragees, gelules, capsules, granules, gels, ointments,

creams and injectable solutions or suspensions. Examples of suitable excipients are talc, gum arabic, lactose, starch, magnesium stearate, cocoa butter, acqueous or non aqueous vehicles, fatty substances of animal or vegetable origin, paraffin derivatives, glycols, the various moistening, dispersing or emulsifying agents, and preservatives.

In the case of use of the compounds as an adjuvant treatment of antibiotic therapy or anti-viral therapy, the duration of treatment will for example be equal to or greater than that of the antibiotic therapy or of the anti-viral therapy. In other cases, the administration will be extended for a long time, for example 3 months to 2 years or more, and could be done in a discontinuous manner.

The compounds of formula I may be prepared by known methods such as described in US patent No. 3,998,823.

In the following examples there are described several preferred embodiments to illustrate the invention. However, it should be understood that the invention is not intended to be limited to the specific embodiments.

EXAMPLE 1

Tablets were containing 200 mg of 8B-morpholinocarbonyl-4bα, 8α-dimethyl-12-isopropyl(1B,2B,3B,4,4aB,4ba, 5,6,7,8,8aB,9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-g]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione and

sufficient excipient of lactose, starch, talc and magnesium stearate for a final tablet weight of 300 mg.

EXAMPLE 2

Tablets were prepared containing 200 mg of 8β-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl(1β,2β,3β,4,4aβ,4bα,5,6,7,8,8aβ, 9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-2',5'-pyrrolidinedione (maleopimarimidyl) and sufficient excipient of lactose, starch, talc and magnesium stearate for a final tablet weight of 300 mg.

EXAMPLE 3

An injectable solution was prepared containing 150 mg of 8B-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl-1β,2β,3β,4, 4aβ,4bα, 5,6,7,8,8aβ,9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione and sufficient aqueous solvent, q.s. for 2 ml.

EXAMPLE 4

An injectable solution was prepared containing 150 mg of 8B-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl-1β,2β,3β,4,4aβ,4bα,5,6,7,8,8aβ,9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-2',5'-pyrrolidinedione and sufficient aqueous solvent, q.s. for 2 ml.

EXAMPLE 5

Gelules were prepared containing 200 mg of 8β-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl-1β,2β,3β,4,4aβ,4bα,5,6,7,8aβ,9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione and an excipient of mannite, citric acid, sodium chloride, thiurea, sodium diaminotetracetate ethylene, lactose, methylcellulose and magnesium stearate, q.s. for one gelule.

EXAMPLE 6

An ointment was prepared containing 1.5 g of 8β-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl-1β,2β,3β,4,4aβ,4bα,5,6,7,8,8aβ,9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione and excipients of cetyl alcohol, saturated vegetable triglycerides, esters of polyethylene glycol 2000 with fatty acids of 12 to 14 carbon atoms, Tween 80, paraoxybenzoates, sorbitol, carboxyvinyl polymer, sodium sulfite, triethanolamine, lecithin, purified water and lactic acid, q.s. for 100 g.

EXAMPLE 7

A cream was prepared containing 1.5 g of 8β-morpholinocarbonyl-4bα-8α-dimethyl-12-isopropyl-1β,2β,3β,4,4aβ,4bα-5,6,7,8,8aβ, 9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione and sufficient excipients of 2-octyl-2-dodecanol alcohol, cetostearyl alcohol, sodium cetostearyl sulfate, methyl and propyl parahydroxybenzoates and purified water, q.s. for 100 g.

PHARMACOLOGICAL STUDY

The study was done with the following products:

PRODUCT A

8B-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl-1β,2β,3β,4,4aβ,4bα,5,6,7,8,8aβ-9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione.

PRODUCT B

8β-morpholinocarbonyl-4bα,8α-dimethyl-12-isopropyl-1β,2β,3β,4,4aβ,4bα,5,6,7,8,8aβ,9,10,10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-2',5'-pyrrolidinedione.

1- Rosette test with red corpuscles of sheep

The administration to animals of a product revealed by an increase in their capacity to react to the injection of an immunizing product the ability to stimulate the activity of the immune systems. Male rats aged 3 months were sensitized intraperitoneally with sheep erythrocytes (day 0). 7 days later (day 7), their spleens were removed and the spleenocytes were put into contact with the erythrocytes of sheep. A count was then made of the percentage of leucocytes around which the erythrocytes had formed rosettes. The products under study were administered orally daily from day -1 to day 1. A dose of a product was considered to be immunostimulant if it multiplied by about 2 the percentage of rosettes observed in the control animals. The active doses for products A and B were 1 and 2 mg/kg, respectively, orally.

2- Test of antibody formation determination of the level of hemagglutinine in mice.

Mice received an intravenous administration of red corpuscles of sheep on the first day and the products were administered orally at a dose of 100 mg/kg from the 1st to the 3rd day. On the fourth day, the animals were killed and the levels of seral antibodies were determined by hemagglutination. With the product A, an increase of 23% of the level was observed as compared with the level of control animals.

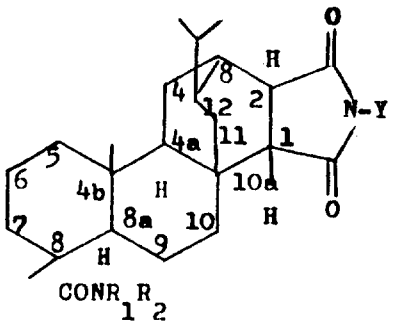
3- Acute toxicity

The products tested were very well tolerated by rodents after acute oral administration. In fact, for product A, the LD 50 was 10 g/kg for the mouse and 6.3 g/kg for the rat, respectively.

Various modifications of the method of the invention may be made without departing from the spirit or scope thereof and it should be understood that the invention is intended to be limited only as defined in the appended claims.

CLAIMS:

1. A method of inducing immunostimulating activity in warm-blooded animals in need of said activity comprising administering to warm-blooded animals in need of immunostimulating activity an effective immunostimulating amount of maleo-primaric acid compound of the formula



"wherein R_1 and R_2 together with the nitrogen atom form a group selected from the group consisting of morpholino, piperazin-1-yl, pyfrolidino, 4-alkylpiperazin-1-yl of 1 to 5 alkyl carbon atoms and 4-hydroxyalkyl-piperazin-1-yl of 1 to 5 alkyl carbon atoms and Y is selected from the group consisting of hydrogen, amino and B-hydroxyethyl".

2. The method of claim 1 wherein R_1 and R_2 together with the nitrogen atom form morpholino.

3. The method of claim 1 wherein Y is hydrogen or β-hydroxyethyl.

4. The method of claim 1 wherein the compound is 8β-morpholinocarbonyl-4bα, 8a-dimethyl-12-isopropyl-1β, 2β, 3β, 4, 4aβ, 4bβ, 5, 6, 7, 8, 8aβ, 9, 10, 10a-tetradecahydro-3,10a-ethenophenanthro-[1,2-c]-1'-(2-hydroxyethyl)-2',5'-pyrrolidinedione.

5. The method of claim 1 wherein the compound is 8β-morpholinocarbonyl-4bα, 8a-dimethyl-12-isopropyl-1β, 2β, 3β, 4, 4aβ, 4bα, 5, 6, 7, 8, 8aβ 9, 10, 10a-tetradecahydro-3,10a-ethenophenophenanthro-[1,2-c]-2',5'-pyrrolidinedione.

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