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CA 2330798 C 2008/06/17

(11)(21) 2 330 798

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2000/03/01
(87) Date publication PCT/PCT Publication Date: 2000/09/08
(45) Date de délivrance/Issue Date: 2008/06/17
(85) Entrée phase nationale/National Entry: 2000/11/01
(86) N° demande PCT/PCT Application No.: JP 2000/001192
(87) N° publication PCT/PCT Publication No.: 2000/052005
(30) Priorité/Priority: 1999/03/01 (JP11/52631)

(51) Cl.Int./Int.Cl. *C07D 491/048* (2006.01), *A61K 31/517* (2006.01), *A61P 33/06* (2006.01), *C07D 401/06* (2006.01)

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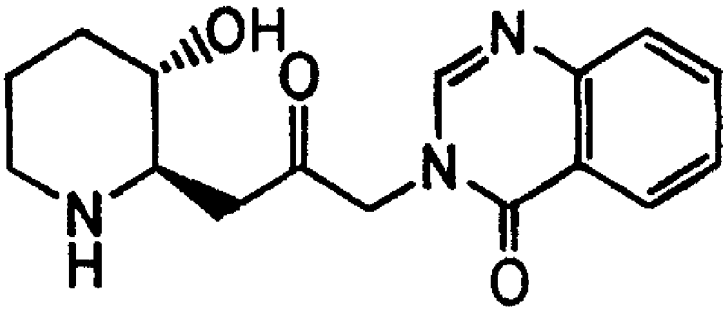
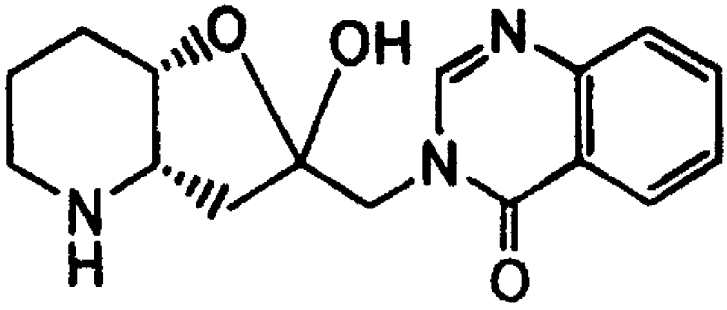
(54) Titre : FEBRIFUDINE ET ISOFEBRIFUGINE, ET METHODE DE PREPARATION
(54) Title: FEBRIFUGINE, ISOFEBRIFUGINE AND METHOD FOR PRODUCING THE SAME



(A)

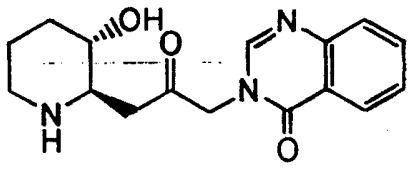


(B)

(57) Abrégé/Abstract:
A febrifugine having formula (A): (see formula A) and an isofebrifugine having formula (B): (see formula B) exhibit extremely strong activities against tropical malarial protozoan.

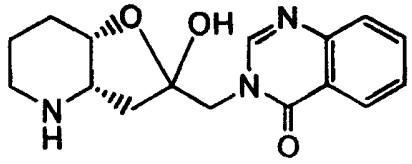
ABSTRACT

A febrifugine having formula (A):



(A)

and an isofebrifugine having formula (B):



(B)

exhibit extremely strong activities against tropical malarial protozoan.

FEBRIFUGINE, ISOFEBRIFUGINE AND METHOD FOR PRODUCING THE SAME

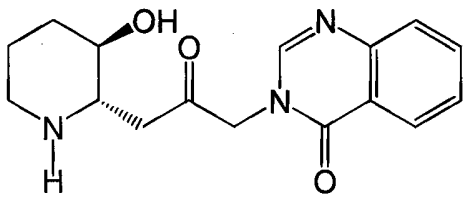
TECHNICAL FIELD

The present invention relates to febrifugine, isofebrifugine, and a method for producing the same.

BACKGROUND OF THE INVENTION

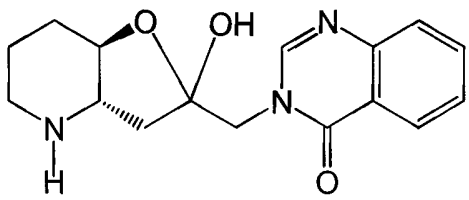
Febrifugine and isofebrifugine derived from Chinese hydrangea are known to have strong activities against tropical malarial protozoan.

The chemical structures of febrifugine and isofebrifugine, known to show such strong activities against malarial protozoan, were reported to be represented by Formulas (A_0) and (B_0):



Febrifugine

(A_0)



Isofebrifugine

(B_0)

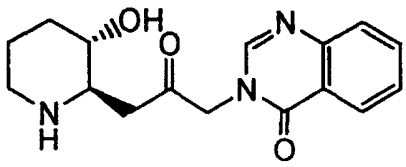
Although the activity of these febrifugine compounds have been known from old times as active ingredients of Chinese medicines such as "JOSAN" practical isolation and utilization of these compounds have been difficult due to their rarity in nature, and efforts to develop an efficient method for synthesizing them under gentle conditions have not been successful.

Therefore, extensive investigations have been desired, from the viewpoint of efficient synthesis of

analogues, and the view point of stereochemistry which enables the exertion of bioactivity.

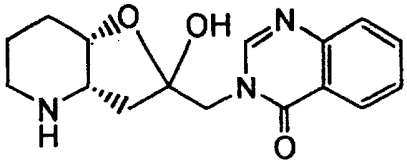
It is therefore an object of the present invention to thoroughly reinvestigate the basis of the strong activity of febrifugines against malarial protozoan in relation to their stereochemistry, to identify actual substances which exhibit extremely strong activity against tropical malarial protozoan, and to establish a total synthetic route which allows efficient large scale synthesis of febrifugines.

According to a first aspect of the present invention, there is provided a febrifugine having formula (A):



(A)

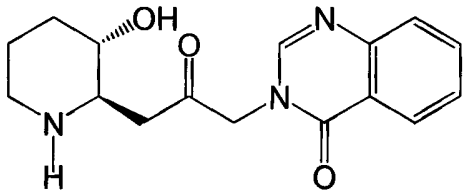
According to a second aspect of the present invention, there is provided an isofebrifugine having formula (B):



(B)

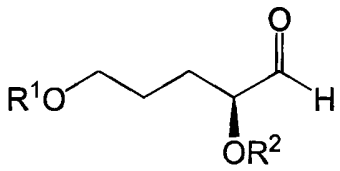
According to a third aspect of the present invention, there is provided a pharmaceutical composition having an anti-malarial activity, which contains as active ingredient, a febrifugine of formula (A) or an isofebrifugine of formula (B) defined above.

According to a fourth aspect of the present invention, there is provided a method for producing a febrifugine of Formula (A) comprising:



(A)

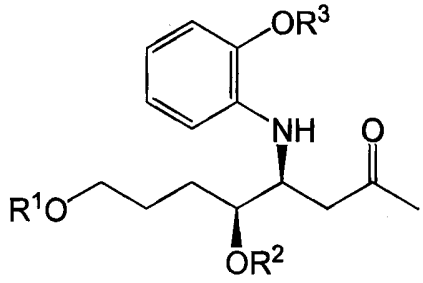
providing an S-aldehyde compound represented by Formula (C):



(C)

wherein R^1 represents a silyl group and R^2 represents a cyclic hydrocarbon group,

subjecting the S-aldehyde compound to a Mannich reaction with a 2-alkoxyaniline compound and a 2-alkoxypropene compound in the presence of a water soluble Lewis acid of a rare earth metal, in an aqueous solvent, to form a diastereomeric mixture of a β-aminoketone compound represented by Formula (D):

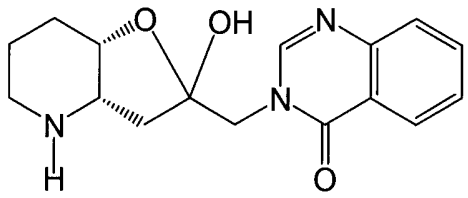


(D)

wherein R^1 and R^2 are as defined above, and R^3 represents an alkyl group which forms an alkoxy group of the 2-alkoxyaniline, the diastereomeric mixture comprising an anti-diastereomer which is isolated and thereafter cyclized to form a piperidine compound, and

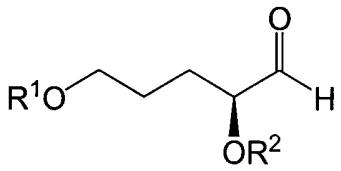
reacting the piperidine compound with a quinazoline compound to obtain a febrifugine of Formula (A).

According to a fifth aspect of the present invention, there is provided a method for producing isofebrifugine of Formula (B):



(B)

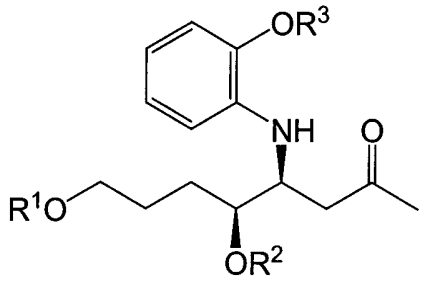
providing an S-aldehyde compound represented by Formula (C):



(C)

wherein R^1 represents a silyl group and R^2 represents a cyclic hydrocarbon group,

subjecting the S-aldehyde to a Mannich reaction with a 2-alkoxyaniline compound and a 2-alkoxypropene compound in the presence of a water soluble Lewis acid of a rare earth metal, in an aqueous solvent, to form a diastereomeric mixture of a β-aminoketone compound represented by Formula (D):

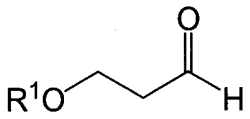


(D)

wherein R^1 and R^2 are as defined above, and R^3 represents an alkyl group which forms an alkoxy group of the 2-alkoxyaniline, the diastereomer comprising a syn-diastereomer which is isolated and thereafter cyclized to form a piperidine compound, and

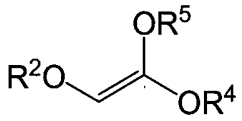
reacting the piperidine compound with a quinazoline compound to obtain a febrifugine represented by Formula (B).

Preferably, the S-aldehyde compound of formula (C) is obtained by subjecting a silyloxypropanal represented by Formula (E):



(E)

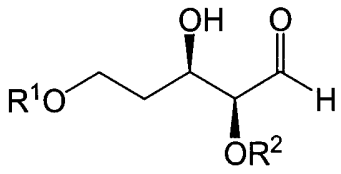
wherein R^1 represents a silyl group, and an ethene compound represented by Formula (F):



(F)

wherein R^2 represents a hydrocarbon group, R^4 represents an aromatic hydrocarbon group and R^5 represents a silyl group,

to an asymmetric aldol condensation in the presence of a chiral tin (II) Lewis acid catalyst, to form an addition reaction product represented by Formula (G),



(G)

wherein R^1 and R^2 are as defined above, which is thereafter dehydroxylated, and reduced to form the S-aldehyde compound of Formula (C), which is then subjected to the Mannich reaction.

The aldehyde compound of formula (C) can be subjected to a Mannich reaction in water, in the presence of a Lewis acid-surfactant-integrated catalyst to form a β-aminoketone compound.

The present invention also provides novel substances having the Formulas (A) and (B), as febrifugine and isofebrifugine expressing strong activity against tropical malarial protozoan.

Moreover, the present invention provides a production method which enables convenient and efficient large scale production of such novel substances.

While the aspects of the invention are as stated above, the embodiments of the invention are as described below.

First, a febrifugine and an isofebrifugine according to the invention may be specified as (2'R, 3'S)-febrifugine represented by Formula (A) and (2'S, 3'S)-isofebrifugine represented by Formula (B), respectively, and are distinct in terms of their absolute configuration, from conventional (2'S, 3,R)- and (2'R, 3'R)-compounds represented by the above Formulas (A_0) and (B_0).

Next, in the production of such compounds according to the present invention, an S-aldehyde compound represented by the above Formula (C), is the first key intermediate in the synthetic route. The second key intermediate is a .beta.-aminoketone compound represented by the above Formula (D).

The symbol R^1 in Formulas (C), (D), (E), (F) and (G) represents a silyl group which may be a hydrocarbon group, same or different, bonded to an Si atom. Examples of a trialkylsilyl group may be t-butyldimethylsilyl, trimethylsilyl groups and so on. $R.^2$ may be any one of various hydrocarbon groups which form protective groups. An example would be a benzyl group. R^3 may also be a

hydrocarbon group, such as an alkyl group including methyl, ethyl, and so on. R^4 is also a hydrocarbon group. An example would be a phenyl group. R^5 is a silyl group, which may vary as indicated above for R^1.

The aldol addition product of formula (G) from which the aldehyde compound of formula (C) is derived, is produced by an asymmetric aldol reaction, in which a chiral metal compound obtained from a metal compound and a chiral compound may be employed as a catalyst. For example, a chiral metal compound catalyst obtained from a triflate or perchlorate such as tin (II) and a chiral amine compound can be used.

The reaction may also be performed in organic solvents such as ethers and nitriles.

A particularly useful is a chiral tin (II) catalyst.

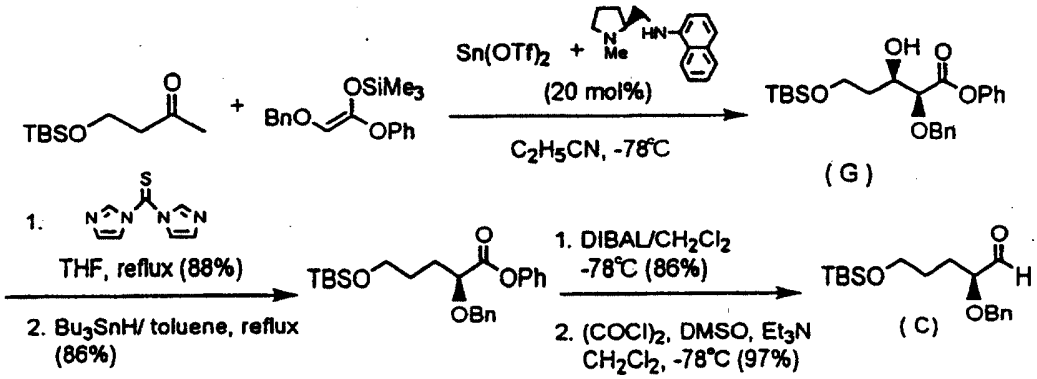
Also, the Mannich reaction by which the β-aminoketone compound of formula (D) is obtained from the aldehyde compound of formula (C), may be performed in the presence of a water soluble Lewis acid of a rare earth metal. For example, a triflate or a perchlorate of a rare earth metal such as ytterbium (Yb) and scandium (Sc) may be used.

Furthermore, a Lewis acid-surfactant-integrated catalyst may be employed in the Mannich reaction described above. Such a catalyst may be any of the various salts of transition metals with surfactant compounds, such as scandium dodecylsulfate (STDS) obtained by mixing scandium chloride and sodium dodecylsulfatein water, as well as sulfonate compounds. The reaction may be performed in water, and the procedures are very simple.

The following non-limiting examples illustrate the invention.

Example 1: Preparation of aldehyde compound of formula (C)

An aldehyde compound of formula (C) was prepared according to the reaction scheme shown below.



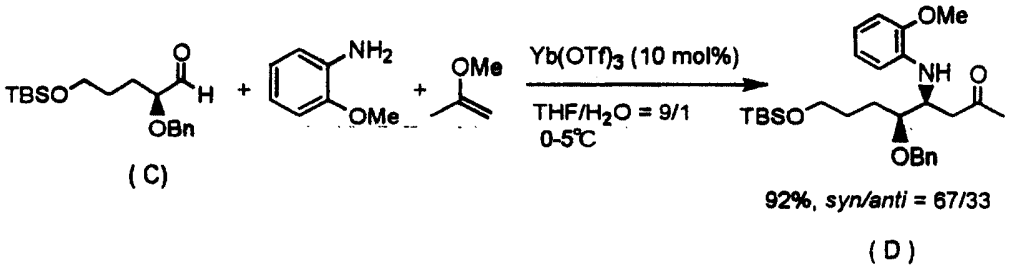
In the presence of a chiral tin (II) Lewis acid (20 mol%) obtained from tin (II) triflate and a chiral diamine compound, 3-t-butyldimethylsilyloxypropanal was reacted with 2-benzyloxy-l-trimethylsilyloxy-l-.phenoxyethene in a solvent of propionitrile, at -78°C, to obtain the corresponding aldol-type addition reaction product, at 70% yield, with an excellent diastereo- and enantio-selectivity.

The product thus obtained was dehydroxylated at the 3-position in two steps, as indicated in the above reaction scheme, after which the ester group was reduced to form an alcohol, which was then subjected to Swerm oxidation (Synthesis, 1978,297) conditions to convert into the intended S-aldehyde compound of formula (C).

The chiral tin (II) Lewis acid can be obtained, for example, from tin (II) triflate and various chiral diamine, and a variety of such substances were proven to be useful in the synthesis of an aldol-type addition reaction product.

Example 2: Preparation of β-aminoketone compound of formula (D)

A β-aminoketone compound of formula (D) was prepared according to the following reaction scheme:

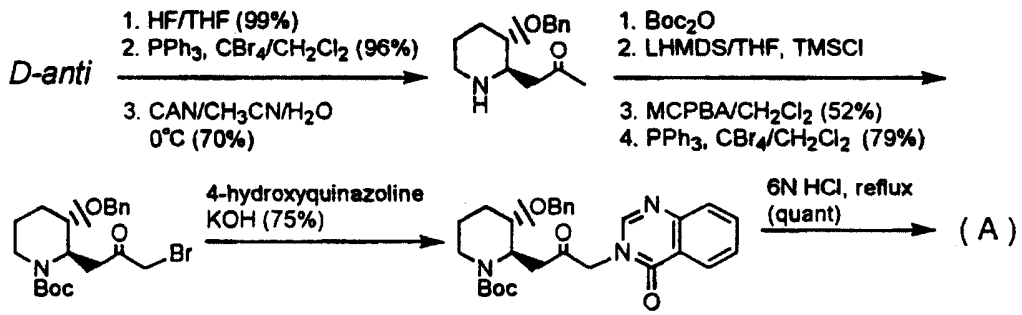


The S-aldehyde compound of formula (C) obtained in Example 1 was reacted with 2-methoxyaniline and 2methoxypropene in the presence of 10 mol % of ytterbium triflate (Yb(OTf)3) in an aqueous solvent consisting of a mixture of tetrahydrofuran (THF) and water (THF/H20=9/1) at a temperature of 0 to 5°C .

A β-aminoketone compound was obtained as the Mannich reaction product at a 92 % yield (Syn/anti=67/33).

Example 3: Preparation of febrifugine of formula (A)

Febrifugine of formula (A) was prepared according to the reaction scheme shown below.



The anti-diastereomer of the β-aminoketone compound obtained in Example 2 as the Mannich reaction product was treated with HF to eliminate the TBS protecting group and cyclized by bromination, after which the 2-methoxyphenyl group as an N-protecting group was eliminated using cerium ammonium nitrate (CAN). As a result, a pyperidine compound was obtained.

Then, the N atom of the pyperidine compound was protected as an N-Boc group and treated sequentially with lithium hexamethyl disilazide (LHMDS) followed by trimethylsilyl chloride (TMSC1).

The silylenol ether thus obtained was oxidized, then brominated, to obtain a pyperidine brominated acetone compound.

This substance was coupled with 4-hydroxyquinazoline using KOH (75 %) and the resulting addition product was treated with 6N HC1 to eliminate the protecting group.

As a result, a febrifugine of formula (A) was obtained quantitatively.

After recrystallization from ethanol, the ^{1}H and ^{13}C NMR spectra and the melting point (MP) were measured.

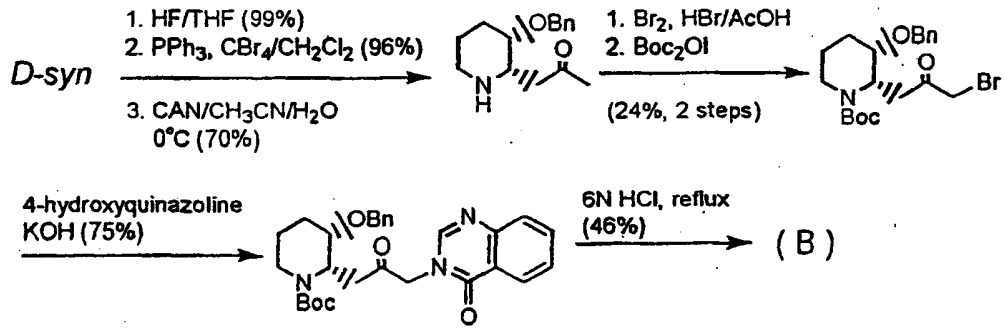
The ^{1}H NMR and ^{13}C NMR spectra were identical to those reported previously, and the melting point was 138 to 140°C which was within the range reported.

The optical rotation, however, was negative as reflected by $[\alpha]d^{24}$ - 28.0° (C=0.24, EtOH) which differed from the previously reported.positive value $[\alpha]d^{25}$ +28° (C=0.5, EtOH) (Koepfly, J.B.: Mead, J.F.; Brockman, Jr., J.A. J. Am. Chem. Soc., 1949, 71, 1048).

Based on the findings described above, the product was identified as a (2'R, 3'S) febrifugine having the above formula (A).

Example 4: Preparation of isofebrifugine of formula (B)

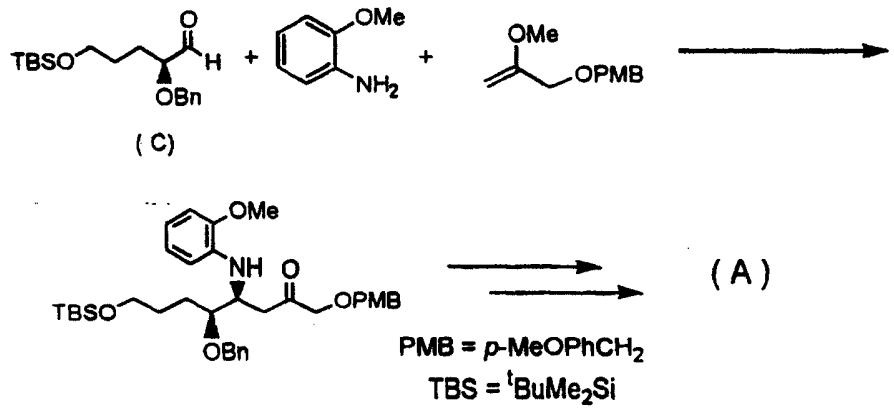
Isofebrifugine of formula (B) was prepared by a procedure similar to that of Example 3 according to the reaction scheme shown below.



The product was identified as the (2'S, 3'S) isofebrifugine having the aforesaid formula (B).

Example 5: Preparation of febrifugine of formula (A)

Using 2-methoxypropene having a p-methoxybenzyloxy group instead of 2-methoxypropene employed in Example 2, and also using a scandium trisdodecylsulfate (STDS) as a Lewis acid-surfactant-integrated catalyst instead of the triflate of ytterbium used as a rare earth metal in Example 2, a β-aminoketone compound was produced in water according to the reaction scheme shown below, and then converted to febrifugine of formula (A) as in Example 3.



Febrifugine of formula (A) was obtained almost quantitatively.

Example 6:

The febrifugine and the isofebrifugine having formulas (A) and (B) obtained by the methods of the present invention were examined for their activity against tropical malarial protozoan, together with previously known compounds having the above formulas (A_0) and (B_0) .

Culture assay of tropical fever malarial protozoan

In this experiment, P.falcipazum FCR-3 strain (ATCC 30932) was employed as the tropical fever malarial protozoan. In order to verify the effect of the commercially available anti-malarial agent chloroquin on resistant strains, a chloroquin resistant malarial protozoan of the P.falciparum K1 strain was employed. The medium used in this experiment was a filter-sterilized RPMI1640 medium which was adjusted to pH 7.4 and supplemented with 10 % human serum. The malarial protozoan was cultured under 5 % 0_2, 4 % CO_2 and 90 % NO_2 at 36.5°C. The hematocrit level (% volume of erythrocyte in erythrocyte suspension) was adjusted to 5 % for use. The initial infection rate with the tropical fever malarial protozoan at the beginning of the cultivation was 0.1 %. The cultivation

was performed using a 24-well cultivation plate, replacing the culture medium everyday, and subcultured at an infection rate of 4 %. The infection rate was obtained by making a thin layer smear preparation, which was subjected to Giemsa staining or Diff-Qick staining, and observed microscopically (immersed in oil, magnified to x 1000) after which the malarial protozoan infection rate was determined using the following equation.

Malarial protozoan infection rate =

[(number of infected erythrocyte) / (total number of erythrocyte)] x 100

Test 1: Screening of malarial protozoan growth inhibition

The cultured malarial protozoan-infected erythrocyte was collected by centrifugation, and washed with a serumsupplemented medium, after which a non-infected erythrocyte was added to adjust the initial infection rate to 0.3 %. At this time, the hematocrit rate was 3 %. The sample used in the test was obtained by dissolving in sterilized water, dimethylformamide (DMF), or dimethylsulfoxide (DMSO) to create samples of desired concentration.

5 to 10µl of the sample were added to a 24-well cultivation plate. The samples were tested in duplicates or triplicates. As a control, 10 µl/well of sterilized water, DMF or DMSO was employed.

Subsequently, to the above medium, 990 to 995 µl of the tropical fever malarial protozoan culture medium previously prepared were added by gentle pipetting to create a uniform suspension. The culture plate was incubated for 72 hours in a $C0_2$-0_2-N_2 (5 %, 5 %, 90 %) incubator, after which thin layer smear preparations of each well was made, stained, and observed microscopically, to determine the infection rate together with the infection rate for the control.

From the malarial protozoan infection rate obtained by the method described above, the reproductive rate was calculated so as to obtain the 50 % growth inhibition concentration (EC_{50}) for malarial protozoan. The results are shown in Table 1.

Reproductive rate = {([b]-[a]) / ([c]-[a])} x 100

A: initial infection rate

B: infection rate with sample added

C: infection rate without sample (Control)

Test 2: Mouse FM3A cell growth inhibition test

A F28-7 strain, a wild cell strain derived from mouse breast cancer FM3A cells, was employed. A culture medium was prepared by supplementing an ES medium with 2 % inactivated fetal calf serum, and incubated at 37°C under 5 % CO_2. Under these conditions, the doubling time of the FM3A cell was about 12 hours.

Following preincubation, the cells in logarithmic growth phase were diluted with the medium to 5 x 10^4 cells/ml. The sample used was one prepared for the anti-malarial activity test of the malaria protozoan. 5 to 10 µl of the samples were added to a 24-well cultivation plate (final concentration after addition of medium was 1 x 10^{-4} to 1 x 10^{-6}) . The compounds were tested in duplicates or triplicates, and wells containing 10 µl of sterilized water, DMF or DMSO were also prepared as a control. Subsequently, 990 to 995 µl of the cultured cell suspension previously prepared were added by gentle pipetting, and uniformly suspended in the medium. After incubating for 48 hours, the number of cells in each well was

counted using SELF CONTROLLER (CC-108, Toa Medical Electrics) and the reproductive rate was calculated by using the following equation:

Reproductive rate (%) = {([C]-[A]) / ([B]-[A])} x 100

A: initial number of cells

B: number of control cell after 48 hours

C: number of cells after 48 hours from sample addition

The cell growth inhibition activity was calculated from the number of cells in the well containing the sample and the number of cells in the control. From the results thus obtained, the cytotoxicity of each sample was evaluated and represented as the cell growth inhibition concentration (EC_{50}) . The EC_{50} value is the concentration (expressed as molar concentration) of a sample capable of inhibiting the reproductive rate of the control by 50 % , wherein the reproductive rate or the rate of malarial protozoan infection for the control in which samples are not added to the medium of malarial protozoan or FM3A cell, is regarded as 100 %. The results are shown in Table 1.

The anti-malarial effect of the sample was evaluated based on the ratio of the ECso of the sample for malarial protozoa to the EC_{50} of the sample for FM3A cell (chemotherapeutic coefficient, see the equation shown below) , from which the drug efficacy was determined.

The results are shown in Table 1.

Chemotherapeutic coefficient = [EC_{50} of the sample for mouse FM3A cell] / [EC_{50} of the sample for tropical fever malarial protozoan]

TABLE 1

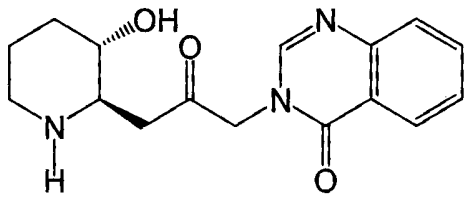
Sample	P. falciparum EC_{50}	FM3A EC_{50}	Ratio of Selective Toxicity for Malarial Protozoan <chemotherapeutic coefficient>
A	3.0×10^{-10}	8.0×10^{-7}	2667
A_0	1.9×10^{-7}	2.0×10^{-5}	105
B	7.6×10^{-11}	2.2×10^{-7}	2895
B_0	2.0×10^{-7}	2.2×10^{-5}	110

As can be seen from Table 1, febrifugine (A) and isofebrifugine (B) of the present invention showed selective malarial protozoan growth inhibiting activity, while formerly known substances only showed low activity.

As described in detail above, the present invention provides a febrifugine and an isofebrifugine as novel compounds having extremely strong activities against tropical malarial protozoan. The present invention also provides a novel production method which enables efficient large scale synthesis to establish a total synthesis route.

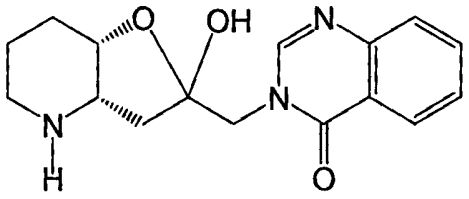
CLAIMS:

1. A febrifugine having formula (A):



(A)

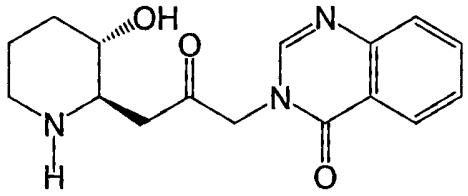
2. An isofebrifugine having formula (B):



(B)

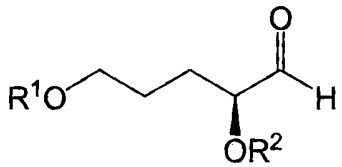
3. A pharmaceutical composition having an anti-malarial activity, which contains as active ingredient a febrifugine according to claim 1, or an isofebrifugine according to claim 2, together with a pharmaceutically acceptable carrier therefor.

4. A method for producing a febrifugine of Formula (A) comprising:



(A)

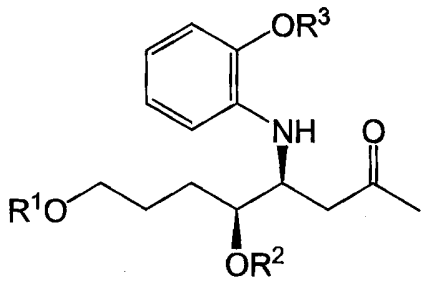
providing an S-aldehyde compound represented by Formula (C):



(C)

wherein R^1 represents a silyl group and R^2 represents a cyclic hydrocarbon group,

subjecting the S-aldehyde compound to a Mannich reaction with a 2-alkoxyaniline compound and a 2-alkoxypropene compound in the presence of a water soluble Lewis acid of a rare earth metal, in an aqueous solvent, to form a diastereomeric mixture of a β-aminoketone compound represented by Formula (D):

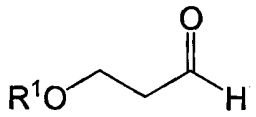


(D)

wherein R^1 and R^2 are as defined above, and R^3 represents an alkyl group which forms an alkoxy group of the 2-alkoxyaniline, the diastereomeric mixture comprising an anti-diastereomer which is isolated and thereafter cyclized to form a piperidine compound, and

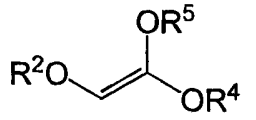
reacting the piperidine compound with a quinazoline compound to obtain the febrifugine of Formula (A).

5. The method according to claim 4, wherein the S-aldehyde compound of formula (C) is obtained by subjecting a silyloxypropanal represented by Formula (E):



(E)

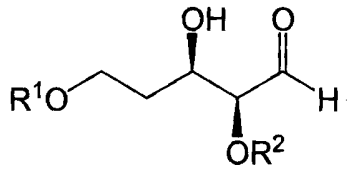
wherein R^1 represents a silyl group, and an ethene compound represented by Formula (F):



(F)

wherein R^4 represents an aromatic hydrocarbon group and R^5 represents a silyl group, and R^2 is as defined in formula (C),

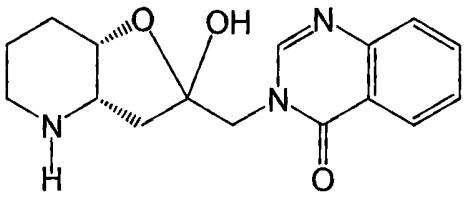
to an asymmetric aldol condensation in the presence of a chiral tin (II) Lewis acid catalyst, to form an addition reaction product represented by Formula (G),



(G)

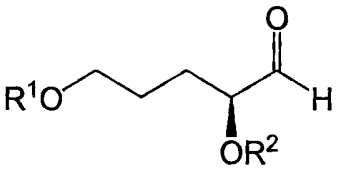
wherein R^1 and R^2 are as defined above, which is thereafter dehydroxylated, and reduced to form the S-aldehyde compound of Formula (C), which is then subjected to the Mannich reaction.

6. A method for producing isofebrifugine of Formula (B):



(B)

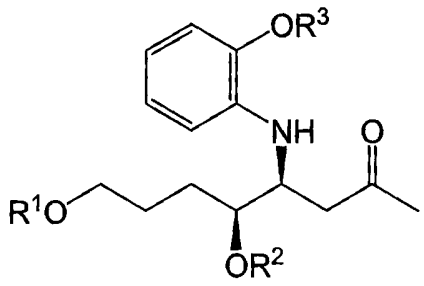
providing an S-aldehyde compound represented by Formula (C):



(C)

wherein R^1 represents a silyl group and R^2 represents a cyclic hydrocarbon group,

subjecting the S-aldehyde to a Mannich reaction with a 2-alkoxyaniline compound and a 2-alkoxypropene compound in the presence of a water soluble Lewis acid of a rare earth metal, in an aqueous solvent, to form a diastereomeric mixture of a β-aminoketone compound represented by Formula (D):

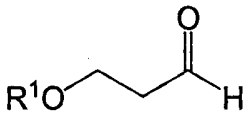


(D)

wherein R^1 and R^2 are as defined above, and R^3 represents an alkyl group which forms an alkoxy group of the 2-alkoxyaniline, the diastereomer mixture comprising a syn-diastereomer which is isolated and thereafter cyclized to form a piperidine compound, and

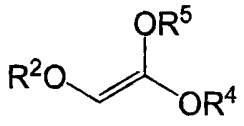
reacting the piperidine compound with a quinazoline compound to obtain a febrifugine represented by Formula (B).

7. A method according to claim 6, wherein the S-aldehyde compound of formula (C) is obtained by subjecting a silyloxypropanal represented by Formula (E):



(E)

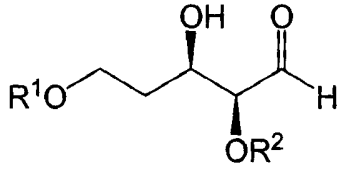
wherein R^1 represents a silyl group, and an ethene compound represented by Formula (F):



(F)

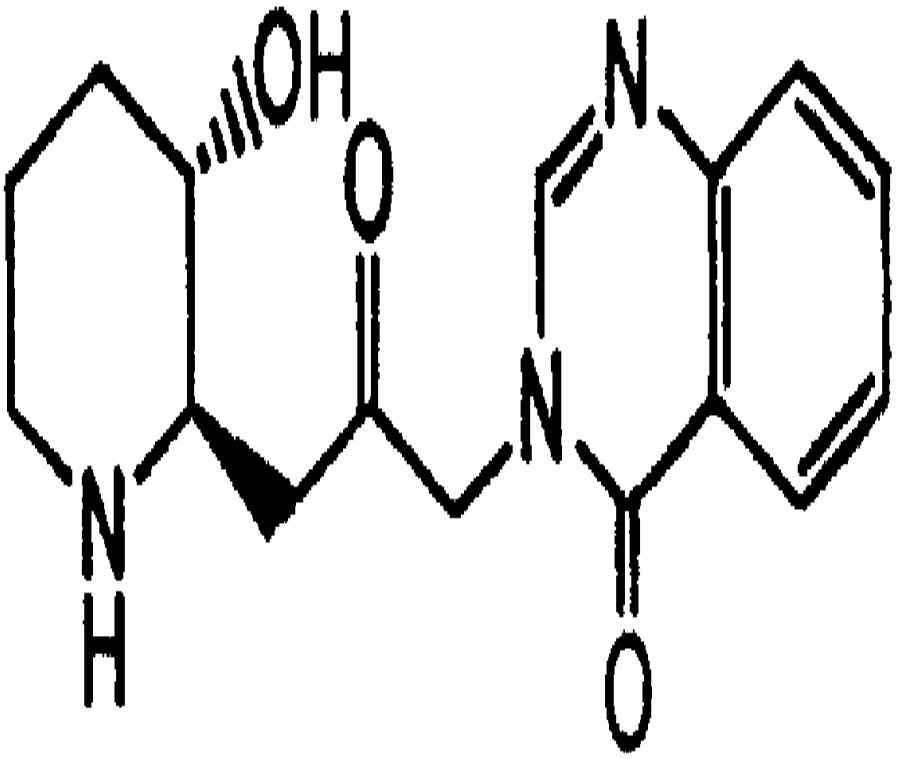
wherein R^2 represents a hydrocarbon group, R^4 represents an aromatic hydrocarbon group and R^5 represents a silyl group,

to an asymmetric aldol condensation in the presence of a chiral tin (II) Lewis acid catalyst, to form an addition reaction product represented by Formula (G),

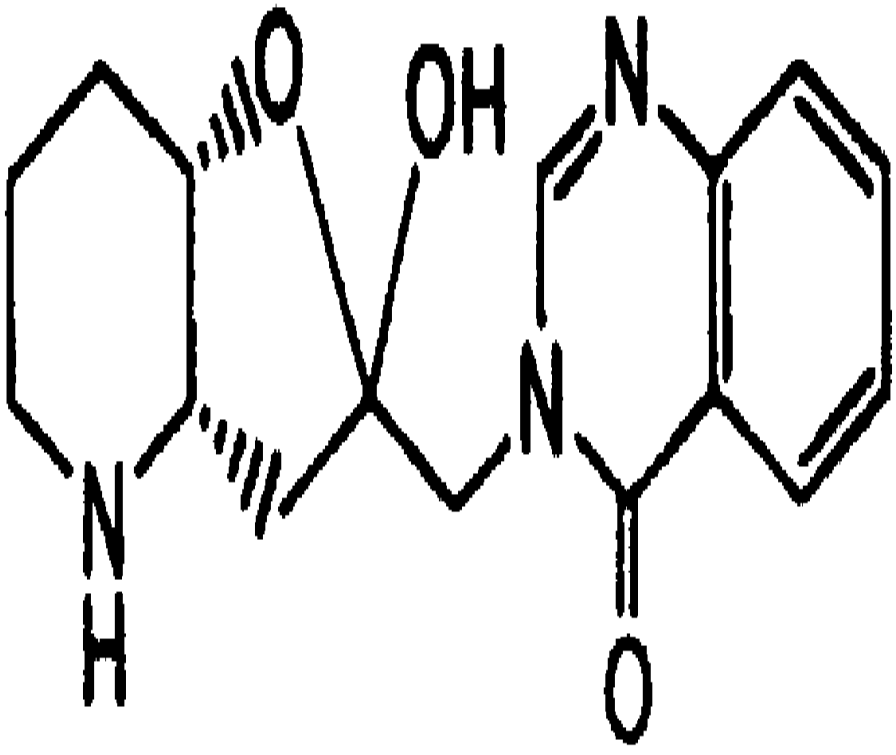


(G)

wherein R^1 and R^2 are as defined above, which is thereafter dehydroxylated, and reduced to form the S-aldehyde compound of Formula (C), which is then subjected to the Mannich reaction.



(A)



(B)