4,10β-Diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate and its process of preparation

The present invention relates to 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate and to its preparation.

4,10β-Diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate exhibits notable anticancer and antileukaemic properties.

4,10β-Diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate is prepared according to the process which is described more particularly in International Application PCT WO 94/13654.

It has been found that 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate exhibits a stability which is markedly superior to that of the anhydrous product.

According to the invention, 4,10β-diacetoxy-

2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate can be obtained by crystallization of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate or by recrystallization of the dihydrate from a mixture of water and an aliphatic alcohol containing 1 to 3 carbon atoms or from a mixture of water and a ketone containing 3 to 4 carbon atoms, followed by drying the isolated product under reduced pressure and by optionally maintaining in a relative humidity greater than or equal to 40 % or by direct drying in an atmosphere with a relative humidity greater than or equal to 40 %.

In order to carry out the process according to the invention, it may be particularly advantageous

- to dissolve 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate or its hydrate in an aliphatic alcohol containing 1 to 3 carbon atoms or in a ketone containing 3 to 4 carbon atoms,
- to treat the solution with water,
- to seed the solution with the dihydrate and then to treat again with water,
- to separate the crystals obtained, then

- to dry them under reduced pressure and then optionally to maintain them in an atmosphere with a relative humidity greater than or equal to 40 %, that is to say, by way of example for a relative humidity of 40 %, drying under a residual pressure of approximately 1.33 kPa for a temperature of 25°C and under a residual pressure of approximately 3.86 kPa for a temperature of 45°C,

- or to dry them directly in an atmosphere with a relative humidity greater than or equal to 40 %, that is to say, by way of example for a relative humidity of 40 %, drying under a residual pressure of approximately 1.33 kPa for a temperature of 25°C and under a residual pressure of approximately 3.86 kPa for a temperature of 45°C.

Generally, 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate or its hydrate is dissolved in an excess of aliphatic alcohol, preferably ethanol, or in an excess of a ketone, preferably acetone. Preferably, the amount of alcohol or ketone is between 4 and 16 parts by volume with respect to the weight of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate used.

Generally, the water is added such that the water/alcohol or water/ketone final volume ratio is

between 1/3 and 3/1.

The 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate which crystallizes is separated, preferably by filtration or centrifuging. The drying or the optional maintaining in an atmosphere with a relative humidity greater than or equal to 40 % is carried out under reduced pressure, generally of between 0.5 and 30 kPa, preferably in the region of 5 kPa, at a temperature of between 10 and 70°C, preferably in the region of 40°C.

The water activity isotherm of the product was studied. Thus, 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate samples, intentionally dehydrated by forced drying, were maintained in atmospheres of controlled relative humidity (HR) at 25°C. The water contents, determined by TGA, show that the products stabilize at contents in the region of 4 % (between 3 and 5 %) when the relative humidity is greater than or equal to 40 % (theoretical water content of 4.15 % for a dihydrate).

Water activity isotherm at 25°C

Initial drying conditions	Initial form by XD	Initial TGA	RH %	Residence time	Loss by TGA	Form by XD
20°C, 48 hours 1.3 kPa	Dihydrate	1.6 % / 32 to 71°C Stability by weight from 71 to 200°C	0	18 days 31 days	0.8 % / 34 to 170°C 0.99 % / 33 to 55°C	Dihydrate Dihydrate
			5	55 days	1.69 % / 32 to 63°C	Dihydrate
			11	55 days	1.17 % / 33 to 65°C	Dihydrate
			20	19 days 31 days	1.96 % / 33 to 68°C 2.06 % / 34 to 61°C	Dihydrate Dihydrate
			42	19 days 31 days	3.77 % / 33 to 65°C 4.07 % / 34 to 60°C	Dihydrate Dihydrate
			58	19 days 31 days	3.30 % / 34 to 63°C 3.59 % / 34 to 65°C	Dihydrate Dihydrate
			80	19 days 31 days	3.98% / 34 to 63°C 4.22 % / 34 to 65°C	Dihydrate Dihydrate
			98	19 days 31 days	4.61 % / 34 to 68°C 4.63 % / 34 to 65°C	Dihydrate Dihydrate
60°C, 60 hours 4.6 kPa	Dihydrate	0.76 % / 32 to 170°C	0	35 days	0.85% / 31 to 60°C	Dihydrate
			11	37 days	0.90 % / 31 to 60°C	Dihydrate
			20	35 days	0.95 % / 32 to 54°C	Dihydrate
			42	35 days	3.51 % / 32 to 60°C	Dihydrate
			58	35 days	2.35 % / 32 to 60°C	Dihydrate
			80	35 days	3.75 % / 32 to 70°C	Dihydrate
			98	37 days	3.97 % / 32 to 60°C	Dihydrate

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In order to use the process according to the invention, it is possible to work directly on the ethanolic solution of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate obtained after deprotection in acid medium of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,4S,5R)-3-tert-butoxycarbonyl-2-(4-methoxyphenyl)-4-phenyloxazolidine-5-carboxylate.

The following examples illustrate the present invention.

EXAMPLE 1

2 g of crude 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate and 20 cm^3 of absolute ethanol are introduced into a three-necked flask. 20 cm^3 of water are added over approximately 45 minutes to the stirred solution at 50°C and the suspension obtained is then cooled to a temperature in the region of 20°C. After filtration, the product is washed on the filter with 20 cm^3 of an absolute ethanol/water (1/1 by volume) mixture and the product is then dried at 40°C under reduced pressure (5.3 kPa). 1.64 g of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-

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butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate, containing 3.8 % by TGA (Figure 5) of water (theoretical value of the water content of the dihydrate of 4.15 %), are obtained. The yield obtained is approximately 80 %. The XRPD (X-ray powder diagram) diagram represented by Figure 1 shows that the product thus obtained exhibits the characteristics of the dihydrate.

The X-ray powder diagram is obtained using a Philips PW 1700® device with a cobalt anti-cathode tube (λ $K_{\alpha 1}$ = 1.7889 Å), the sweeping being performed at an initial sweep angle of 5° 2-Θ, final sweep angle of 40° 2-Θ, with an increment of 0.02° 2-Θ, at a rate of 1 second per increment and using a silicon pastille.

Thermogravimetric analysis (TGA) is carried out using a Perkin-Elmer TGA 7® thermobalance at the initial temperature of 25°C and at the final temperature of 300°C with a temperature gradient of 10°C per minute.

EXAMPLE 2

515 g of crude 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate and 4 litres of absolute ethanol are introduced into a reactor purged with nitrogen. The suspension is heated at 35-40°C until dissolution is complete and the reactor is placed under

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a reduced pressure of 8 kPa. Approximately 8 litres of solvent are distilled off at constant volume, the reactor being continuously supplied with ethanol (8 litres). After returning the reactor to atmospheric pressure, the solution is clarified by passing through a filter equipped with a 0.45 µm filter cloth. After rinsing the filter with 1 litre of absolute ethanol, approximately 1.8 litres of water are added at 40°C over 1 hour to the whole of the solution. After initiating with 8 g of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate, the mixture is cooled at a temperature in the region of 20°C for 15 hours. The suspension is then heated to 40°C and then 1.66 litres of water are added over 4 hours. The mixture is cooled to a temperature in the region of 20°C and left stirring for 17 hours. The suspension is filtered on sintered glass and the filtered product is washed with 1.25 litres of an alcohol/water (50/50 by volume) mixture. The product is dried in an oven at 35°C at 5.3 kPa for 72 hours in the presence of a stock of water and then at 35°C at 2.7 kPa for 8 hours without a stock of water and again at 35°C at 5.3 kPa for 16 hours in the presence of a stock of water. 491 g of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate

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dihydrate containing 4.0 % of water (Karl Fischer) are thus obtained. The XRPD (X-ray powder diagram) diagram represented by Figure 2 shows that the product thus obtained exists in the form of a dihydrate (theoretical value of the water content of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate of 4.15 %).

The X-ray powder diagram is obtained under the conditions described in Example 1.

EXAMPLE 3

2 g of crude 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate and 20 cm^3 of acetone are introduced into a three-necked round-bottomed flask. The solution obtained, which is stirred at a temperature in the region of 20°C, is treated with 20 cm^3 of water over approximately 35 minutes. The suspension obtained is stirred for approximately 15 minutes. After filtration, the product is washed twice on the filter with 20 cm^3 of an acetone/water (1/1 by volume) mixture and the product is then dried at 40°C under reduced pressure (5.3 kPa). 1.28 g of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-

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3-phenylpropionate dihydrate are thus obtained. The yield obtained is approximately 75 %. The XRPD (X-ray powder diagram) diagram represented by Figure 3 shows that the product thus obtained exhibits the characteristics of the dihydrate.

The X-ray powder diagram is obtained under the conditions described in Example 1.

EXAMPLE 4

120 g of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,4S,5R)-3-tert-butoxycarbonyl-2-(4-methoxyphenyl)-4-phenyloxazolidine-5-carboxylate and 1.08 litres of ethyl acetate are introduced into a reactor purged with nitrogen. The suspension is stirred at a temperature in the region of 20°C and then a solution of 3.25 cm^3 of 36 % hydrochloric acid in 17.6 cm^3 of water is introduced. After maintaining for 3 hours 30 minutes, a solution of 3.5 g of sodium hydrogencarbonate in 350 cm^3 of water is charged to the reactor and stirring is carried out for 15 minutes. After separation by settling and drawing off the aqueous phase, washing is carried out twice in succession with 350 cm^3 of water. The organic phase is concentrated under reduced pressure at approximately 25°C until a residual volume of 350 cm^3 is reached and then 350 cm^3 of absolute ethanol are introduced. Distillation under reduced pressure is resumed at

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approximately 30°C until the ethyl acetate has been exhaustively removed, while supplying with 1.5 litres of absolute ethanol. The solution is heated to 40°C and then 470 cm^3 of water are added over 15 minutes. The solution is seeded with a suspension of 1 g of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate in a mixture of 40 cm^3 of water and 40 cm^3 of absolute ethanol. After maintaining for 15 hours at approximately 40°C, 410 cm^3 of water are added over 4-5 hours and the suspension is then cooled to 20°C. After filtration and washing the product on the filter, the product is dried for 15 hours under reduced pressure (2.7 kPa) at 25°C and then for 24 hours at 35°C. 102 g of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate, containing 4.3 % of water (Karl Fischer), are thus obtained with a yield of approximately 93 %. The XRPD (X-ray powder diagram) diagram represented by Figure 4 shows that the product thus obtained exhibits the characteristics of the dihydrate.

The X-ray powder diagram is obtained under the conditions described in Example 1.

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CLAIMS

1. 4,10β-Diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate.

2. Process for the preparation of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate dihydrate, characterized in that 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate or its hydrate is crystallized from a mixture of water and an aliphatic alcohol containing 1 to 3 carbon atoms or from a mixture of water and a ketone containing 3 to 4 carbon atoms, in that the product obtained is dried under reduced pressure and in that it is optionally maintained under relative humidity conditions greater than or equal to 40 %, or in that the product is dried directly under relative humidity conditions greater than or equal to 40 %.

3. Process according to claim 2, characterized in that the water/alcohol and water ketone final ratio by volume is between 3/1 to 1/3.

4. Process according to either of claims 2 and 3, characterized in that the alcohol is ethanol and

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the ketone is acetone.

5. Process according to claim 2, characterized in that the drying or the optional maintaining under a relative humidity greater than or equal to 40 % is carried out at a temperature in the region of 40°C under a pressure in the region of 5 kPa and that the product stabilizes under these conditions between 3 and 5 % of water.

6. Process according to claim 2, characterized in that the preparation is carried out directly from the ethanolic solution of 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate obtained by deprotection in acid medium of the ester 4,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1-hydroxy-9-oxo-19-norcyclopropa[g]tax-11-en-13α-yl (2R,4S,5R)-3-tert-butoxycarbonyl-2-(4-methoxyphenyl)-4-phenyloxazolidine-5-carboxylate.

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