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CA 3101662 C 2023/03/07

(11)(21) 3 101 662

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2019/05/24
(87) Date publication PCT/PCT Publication Date: 2019/12/05
(45) Date de délivrance/Issue Date: 2023/03/07
(85) Entrée phase nationale/National Entry: 2020/11/26
(86) N° demande PCT/PCT Application No.: CN 2019/088333
(87) N° publication PCT/PCT Publication No.: 2019/228260
(30) Priorité/Priority: 2018/05/28 (CN201810521893.2)

(51) Cl.Int./Int.Cl. *C07H 17/08* (2006.01)

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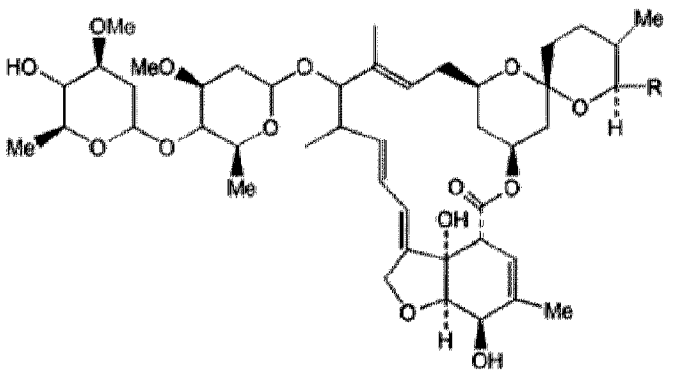
(54) Titre : FORME CRISTALLINE DE LA TENVERMECTINE B, SON PROCEDE DE PREPARATION ET SON UTILISATION

(54) Title: CRYSTAL FORM OF TENVERMECTIN B, PREPARATION METHOD THEREFOR, AND USE THEREOF



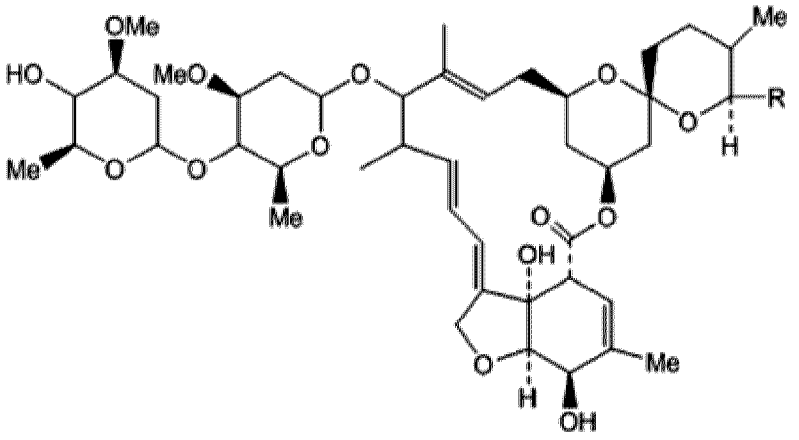
(57) Abrégé/Abstract:
The present invention relates to a crystal form I of tenvermectin B, which can be characterized by X-ray powder diffraction (XRPD) pattern, Infrared (IR) absorption spectrum, Differential scanning calorimetry (DSC) thermogram and the like. Meanwhile, the present invention also relates to a method for preparing the crystal form I of tenvermectin B and a use thereof. The crystal form I of tenvermectin B or the composition comprising the crystal form I of tenvermectin B can be used for preparing agents for controlling parasites and harmful insects. Tenvermectin B has the following structure:

(see above formula)

wherein R is C_2H_5.

ABSTRACT

The present invention relates to a crystal form I of tenvermectin B, which can be characterized by X-ray powder diffraction (XRPD) pattern, Infrared (IR) absorption spectrum, Differential scanning calorimetry (DSC) thermogram and the like. Meanwhile, the present invention also relates to a method for preparing the crystal form I of tenvermectin B and a use thereof. The crystal form I of tenvermectin B or the composition comprising the crystal form I of tenvermectin B can be used for preparing agents for controlling parasites and harmful insects. Tenvermectin B has the following structure:



wherein R is C_2H_5.

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CRYSTAL FORM OF TENVERMECTIN B, PREPARATION METHOD THEREFOR, AND USE THEREOF

TECHNICAL FIELD

The present invention relates to the field of pharmaceuticals. More specifically, the present invention relates to a crystal form of tenvermectin B and a preparation method therefor and use thereof.

BACKGROUND

Sixteen-membered macrolides produced by streptomyces have high activity and broad spectrum activity. In addition, this kind of compounds is closely combined with soil in natural environment and is not easy to be washed and infiltrate. The compounds rapidly degrade into inactive compounds under light conditions or actions of soil microbes, and their molecular fragments are finally decomposed and utilized by plants and microbes as carbon sources without any residual toxicity. This kind of compounds has become a high-efficient biological pesticides used in agriculture and animals.

Due to remarkable properties of this kind of compounds, the homologues of the compounds have been extensively studied over the world. On one hand, molecular structure modification has been carried out by synthesis, and on the other hand, strains produced thereby are mutated by genetic improvement methods, in order to find new compounds with higher activity. A team of Zhejiang Hisun Pharmaceutical Co., Ltd. integrated Milbemycins PKS gene into the initial module of avermectins-producing strains Streptomyces avermitilis by a technology for seamless splicing large fragments of DNA, and obtained gene engineering strains MA220 for producing tenvermectins (see WO2015135242). Tenvermectin have main components: tenvermectin A and tenvermectin B (their structures are shown below). CN201410208660.9 and WO2015135467 disclose that tenvermectin A and tenvermectin B can control pests and mites of agricultural and forestry crops, such as tetranychus cinnabarinus, tetranychus urticae, plutella xylostella, spodoptera exigua, spodoptera litura, cotton

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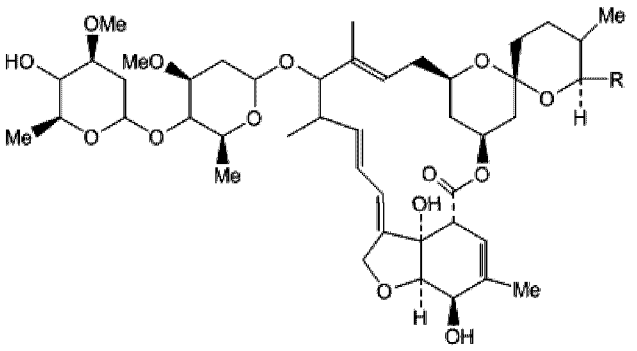
bollworm, agrotis ypsilon, wireworm, armyworm, pine caterpillar, pine wood nematode and rice borer.



Tenvermectin A: R=CH_3; Tenvermectin B: R=C_2H_5

In addition, CN201410208660.9 and WO2015135467 also disclose a method for preparing tenvermectin A and B, wherein fermentation liquor of gene engineering strain MA220 is filtered with filter cloth to obtain a filter cake which is extracted with ethanol twice to obtain an ethanol extract; thus obtained ethanol extract is concentrated to dryness under vacuum, then separated by silica gel column chromatography, and then separated and purified by semi-preparative high-pressure liquid phase (eluent: methanol/acetonitrile/water =46/46/8), and thus obtained fractions are directly concentrated to dryness to obtain tenvermectin A and tenvermectin B. DE4031039 discloses a preparation method of tenvermectin B. According to the method, a crude product of tenvermectin B is synthesized, and then purified by silica gel (ethyl acetate/hexene=2: 1) to obtain tenvermectin B. However, neither of the two preparation methods has studied the crystal form of the obtained tenvermectin B.

After a large number of experiments, firstly, the inventors of the present invention found that the product obtained according to the above two preparation methods is amorphous powder of tenvermectin B; secondly, during their study, the inventors found that the amorphous powder is very unstable, which leads to an increased cost for subsequent development of product. Finally, the inventors discovered a crystal form of tenvermectin B which is named as crystal form I. It is more stable and not easy to degrade, thus laying a solid foundation for the subsequent

development of tenvermectin B.

SUMMARY

One object of the present invention is to provide a crystal form of tenvermectin B with good chemical and physical stability, which is named as crystal form I. The crystal form is more stable and not easy to degrade, and is more advantageous for the subsequent development of products.

The crystal form I of tenvermectin B according to the present invention exhibits characteristic peaks at 2θ degree of 9.62±0.20°, 11.33±0.20°, 11.79±0.20°, 12.48±0.20°, 13.48±0.20°, 21.12±0.20° and 23.70±0.20° in X-ray powder diffraction pattern using Cu-Kα radiation.

Preferably, the crystal form I of tenvermectin B according to the present invention further exhibits characteristic peaks at 2θ degree of 6.71±0.20°, 9.22±0.20°, 12.02±0.20°, 14.95±0.20°, 17.39±0.20°, 18.33±0.20°, 22.97±0.20°, 26.53±0.20° and 27.16±0.20° in X-ray powder diffraction pattern using Cu-Kα radiation.

More preferably, the crystal form I of tenvermectin B according to the present invention further exhibits characteristic peaks at 2θ degree of 4.63±0.20°, 15.45±0.20°, 15.80±0.20°, 16.64±0.20°, 17.74±0.20°, 19.20±0.20°, 19.75±0.20°, 22.14±0.20°, 22.52±0.20°, 25.01±0.20°, 25.54±0.20° and 29.60±0.20° in X-ray powder diffraction pattern using Cu-Kα radiation.

Furthermore, the 2θ degree at which the characteristic peaks are exhibited, d value and relative intensity data of the characteristic peaks shown in the X-ray powder diffraction pattern of the crystal form I of tenvermectin B according to the present invention are shown in table 1 below.

Table 1 2θ degree, d value and relative intensity of the crystal form I of tenvermectin B

Peak No.	2θ(°)	d (Interplanar spacing)	Relative intensity (%)
1	4.63	19.0699	5.9
2	6.71	13.1625	29.7
3	9.22	9.5837	28.4
4	9.62	9.1862	100.0
5	11.33	7.8034	42.3
6	11.79	7.4998	43.8
7	12.02	7.3569	31.4
8	12.48	7.0867	49.7
9	13.48	6.5631	90.6

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10	14.95	5.9209	34.0
11	15.45	5.7304	10.9
12	15.80	5.6043	14.0
13	16.64	5.3232	6.1
14	17.39	5.0953	26.6
15	17.74	4.9956	15.6
16	18.33	4.8360	29.4
17	19.20	4.6188	16.8
18	19.75	4.9515	12.5
19	21.12	4.2031	42.4
20	22.14	4.0117	18.5
21	22.52	3.9449	16.9
22	22.97	3.8686	20.4
23	23.70	3.7511	42.2
24	25.01	3.5575	11.5
25	25.54	3.4848	4.7
26	26.53	3.3570	22.4
27	27.16	3.2805	21.7
28	29.60	3.0154	10.5

The XRPD pattern of the crystal form I of tenvermectin B according to the present invention is shown in Figure 1.

In addition, the crystal form I of tenvermectin B according to the present invention can be characterized by the infrared absorption spectrum measured by KBr pellet, and according to the spectrum, there are characteristic peaks at 3481 cm^{-1}, 2930 cm^{-1}, 1732 cm^{-1}, 1678 cm^{-1}, 1371 cm^{-1}, 1183cm^{-1}, 1124 cm^{-1}, 984 cm^{-1}, 877 cm^{-1} and 761 cm^{-1}.

The infrared spectrum of the crystal form I of tenvermectin B according to the present invention is shown in Figure 2.

There is an exothermic peak at 160.8±2°C in differential scanning calorimetry (DSC) thermogram of the crystal form I of tenvermectin B according to the present invention.

The DSC thermogram of the crystal form I of tenvermectin B according to the present invention is shown in Figure 3.

The thermogravimetric analysis (TGA) thermogram of the crystal form I of tenvermectin B according to the present invention is shown in Figure 4.

Another object of the present invention is to provide a method for preparing the crystal form I of tenvermectin B, which comprises a step of precipitating the crystal form I of tenvermectin B from a solvent system containing formamide.

Preferably, the solvent system containing formamide is a combination of lower alcohol, formamide and water or a combination of lower ketone, formamide and water, wherein the lower alcohol is preferably selected from a group consisting of methanol, ethanol or isopropanol; and the lower ketone is preferably acetone. More preferably, the solvent system containing formamide is preferably a combination of ethanol, formamide and water.

Preferably, the ratio of the mass of the tenvermectin B to the volume of the lower alcohol to the volume of formamide and to the volume of water is 1g : 2ml: 4-5ml : 2ml, and the ratio of the mass of the tenvermectin B to the volume of the lower ketone to the volume of formamide and to the volume of water is 1g : 2ml: 4-5ml : 2ml.

Preferably, the method for preparing the crystal form I of tenvermectin B according to the present invention comprises steps of: dissolving tenvermectin B with ethanol, then sequentially adding formamide and water thereto, stirring and crystallizing the thus obtained mixture to obtain the crystal form I of tenvermectin B, wherein, more preferably, the ratio of the mass of the tenvermectin B to the volume of ethanol to the volume of formamide to the volume of water is 1 g : 2 ml : 4-5 ml : 2 ml.

Yet another object of the present invention is to provide a composition containing the crystal form I of tenvermectin B, which further comprises a pharmaceutically acceptable carrier, excipient or the combination thereof.

Yet another object of the present invention is to provide use of the crystal form I of tenvermectin B or the composition containing the crystal form I of tenvermectin B in preparing agents for controlling parasites and harmful insects. Objects to be controlled involve parasites and harmful insects in crops, humans, animals, aquatic products and the like. Reference can be made to CN201610213645.2 (use of tenvermectin for controlling parasites in human or animal) and CN201610211064.5 (use of tenvermectin for controlling harmful insects in agricultural and forestry crops).

The present invention also relates to use of raw materials containing the crystal form I of tenvermectin B in preparing preparation of anti-parasitic and preparation for controlling harmful insect. The preparation includes pour-on solution, tablet, injection and dry suspension.

In one embodiment, the tenvermectin B is dissolved with ethanol, then water is added thereto, and the amorphous powder of tenvermectin B is obtained by stirring and crystallizing. In another embodiment, the tenvermectin B is dissolved with

ethanol, then formamide and water are added thereto in sequence, and the crystal form I of tenvermectin B is obtained by stirring and crystallizing. In yet another embodiment, the amorphous tenvermectin B is compared with the crystal form I of tenvermectin B regarding stability and it is found that the stability of the crystal form I of tenvermectin B is much better than that of amorphous tenvermectin B.

The crystal form I of tenvermectin B provided by the present invention is the only crystal form of tenvermectin B that is known now, and it is a significant breakthrough over prior art. Product of this crystal form has higher purity, and has more uniform crystal, subsequent drying process of the product is more controllable. In addition, the crystal transformation phenomenon does not occur and no degradation is found in the stability experiment of the product of this crystal form.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an X-ray powder diffraction pattern of the crystal form I of tenvermectin B obtained in Example 1.

Figure 2 is an infrared absorption spectrum of the crystal form I of tenvermectin B obtained in Example 1.

Figure 3 is a DSC thermogram of the crystal form I of tenvermectin B obtained in Example 1.

Figure 4 is a TGA thermogram of the crystal form I of tenvermectin B obtained in Example 1.

Figure 5 is an X-ray powder diffraction pattern of amorphous powder of tenvermectin B obtained in Comparative Example 1.

EMBODIMENTS

The following examples further illustrate the present invention, but do not limit the present invention.

Tenvermectin B raw material used in the present invention is prepared according to Example 1 in CN201410208660.9, and it is confirmed to be amorphous tenvermectin B by X-ray powder diffraction test.

The X-ray powder diffractometer and test conditions in the present invention are as follows. X-ray powder diffractometer model: Rigaku D/max-2200 Cu target; operation condition: scanning speed, 4°/min, scanning step width, 0.01°.

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The infrared spectrophotometer and test conditions in the present invention are as follows. Infrared spectrophotometer model: BRWKER VECTOR 22; operation method: KBr pellet method, scanning range 400-4000 cm^{-1}.

The differential scanning calorimeter and test conditions are as follows. differential scanning calorimeter model: PERKIN ELMER DSC8000; operation condition: heating rate, 10°C/min, temperature range, 20°C-280°C.

Thermal gravimetric analyzer and test conditions are as follows. thermal gravimetric analyzer model: PerkinElmer TGA400; operation condition: heating rate, 10°C/min, temperature range, 30°C-300°C.

High performance liquid chromatograph (HPLC) and test conditions in the present invention are as follows. High performance liquid chromatograph, Agilent1100; Chromatographic column: C18, 4.6mm × 250mm; mobile phase, acetonitrile : 0.1% phosphoric acid aqueous solution = 65 : 35 (v : v); Detection wavelength: 240 nm; flow rate, 1.0ml/min; Column temperature: 25°C.

Unless otherwise specified, the dissolution and crystallization steps involved in the present invention generally require stirring, which can be carried out in known ways, such as magnetic stirring, mechanical stirring, etc.

EXAMPLE 1: Preparation of crystal form I of tenvermectin B

5.0 g of tenvermectin B raw material (purity 98.2% by HPLC) was dissolved in 10 ml of ethanol, and then filtered to obtain transparent filtrate. 25 ml of formamide and 10 ml of water were added thereto, thus obtained mixture was placed on a magnetic stirrer to stir at 200 r/min. After stirring at 25°C for 10 h, the mixture was filtered to obtain a solid which was dried at 50°C under vacuum for 48 h to obtain crystal form I of tenvermectin B (purity 99.1% by HPLC). The obtained product was sampled for testing. X-ray powder diffraction pattern was shown in Figure 1, infrared absorption spectrum of the product was shown in Figure 2, DSC thermogram of the product was shown in Figure 3 and TGA thermogram of the product was shown in Figure 4.

EXAMPLE 2: Preparation of crystal form I of tenvermectin B

5.0 g of tenvermectin B raw material (purity 98.2% by HPLC) was dissolved in 10 ml of acetone, and then filtered to obtain transparent filtrate. 20 ml of formamide

and 10 ml of water were added thereto, thus obtained mixture was placed on a magnetic stirrer to stir at 200 r/min. After stirring at 25°C for 10 h, the mixture was filtered to obtain a solid which was dried at 50°C under vacuum for 48 h to obtain crystal form I of tenvermectin B (purity 98.8% by HPLC). The obtained product was sampled for testing. X-ray powder diffraction pattern confirmed that the product is crystal form I.

Comparative Example 1: preparation of amorphous powder of tenvermectin B

5.0 g of tenvermectin B raw material (purity 98.2% by HPLC) was dissolved in 200 ml of ethanol solvent, and then filtered to obtain transparent filtrate. 800 ml of water were dropwisely added thereto in 20 minutes, thus obtained mixture was placed on a magnetic stirrer to stir at 200 r/min. After stirring at 25°C for 10 h, the mixture was filtered to obtain a solid which was dried at 50°C under vacuum for 48 h. The product was sampled for testing. X-ray powder diffraction pattern of the product was shown in Figure 5 and showed that the sample was amorphous powder of tenvermectin B (purity 98.3% by HPLC).

Comparative Example 2: preparation of amorphous tenvermectin B

1.0 g of tenvermectin B raw material (purity 98.2% by HPLC) was dissolved in 200 ml of ethyl acetate, and then 100 ml of hexene was added thereto. After being mixed evenly, thus obtained mixture was filtered to obtain transparent filtrate which was concentrated under vacuum at 50°C to dryness. Thus obtained solid was dried under vacuum at 50°C for 48h. The obtained product was sampled for testing. Test result of the X-ray powder diffraction pattern of the product showed that the product was amorphous powder of tenvermectin B (purity 97.6% by HPLC). The X-ray powder diffraction pattern of the product was consistent with that of the amorphous tenvermectin B obtained in Comparative Example 1.

Stability test

10 gram of the crystal form I of tenvermectin B obtained in Example 1 and 10 gram of the amorphous tenvermectin B obtained in Comparative Example 1 were taken and placed into double-layer PE bags by 1 g per bag. The bags were

electrothermally sealed, and then placed into aluminum foil bags which were also electrothermally sealed. The sealed foil bags were placed at conditions of a temperature of 40±2°C and a humidity of 75±5% for accelerated experiment, wherein, the amorphous tenvermectin B sample was degraded by 15% in the first month, while the crystal form I of tenvermectin B was basically stable after a storage of 6 months, and the data were shown in Table 2 below.

Table 2, Stability data of crystal form I of tenvermectin B

Item	Initial data	First month	Second month	Third month	Sixth month
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
XRPD	crystal form I	crystal form I	crystal form I	crystal form I	crystal form I
Purity by HPLC	99.1	99.0	99.1	98.9	98.9

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Claims

1. A crystal form I of tenvermectin B, wherein the crystal form I of tenvermectin B exhibits characteristic peaks at 2θ degree of 9.62±0.20°, 11.33±0.20°, 11.79±0.20°, 12.48±0.20°, 13.48±0.20°, 21.12±0.20° and 23.70±0.20° in X-ray powder diffraction pattern using Cu-Kα radiation.

2. The crystal form I of tenvermectin B according to claim 1, wherein the crystal form I of tenvermectin B further exhibits characteristic peaks at 2θ degree of 6.71±0.20°, 9.22±0.20°, 12.02±0.20°, 14.95±0.20°, 17.39±0.20°, 18.33±0.20°, 22.97±0.20°, 26.53±0.20° and 27.16±0.20° in X-ray powder diffraction pattern using Cu-Kα radiation.

3. The crystal form I of tenvermectin B according to claim 2, wherein the crystal form I of tenvermectin B further exhibits characteristic peaks at 2θ degree of 4.63±0.20°, 15.45±0.20°, 15.80±0.20°, 16.64±0.20°, 17.74±0.20°, 19.20±0.20°, 19.75±0.20°, 22.14±0.20°, 22.52±0.20°, 25.01±0.20°, 25.54±0.20° and 29.60±0.20° in X-ray powder diffraction pattern using Cu-Kα radiation.

4. A method for preparing the crystal form I of tenvermectin B as defined in any one of claims 1 to 3, wherein the method comprises a step of precipitating the crystal form I of tenvermectin B from a solvent system containing formamide.

5. The method according to claim 4, wherein the solvent system containing formamide is a combination of lower alcohol, formamide and water or a combination of lower ketone, formamide and water.

6. The method according to claim 5, wherein the lower alcohol is selected from a group consisting of methanol, ethanol and isopropanol; and the lower ketone is acetone.

7. The method according to claim 4 or 5, wherein the solvent system containing formamide is a combination of ethanol, formamide and water.

8. The method according to any one of claims 4 to 7, wherein the method comprises steps of: dissolving tenvermectin B with ethanol, then adding formamide and water in sequence, stirring and crystallizing the thus obtained mixture to obtain the crystal form I of tenvermectin B.

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9. The method according to claim 8, wherein a ratio of the mass of the tenvermectin B to the volume of ethanol to the volume of formamide to the volume of water is 1 g : 2 ml : 4-5 ml : 2 ml.

10. A composition comprising the crystal form I of tenvermectin B as defined in any one of claims 1 to 3 and a pharmaceutically acceptable carrier, excipient or the combination thereof.

11. Use of the crystal form I of tenvermectin B as defined in any one of claims 1 to 3 or the composition containing the crystal form I of tenvermectin B as defined in claim 10 in preparing agents for controlling parasites and harmful insects.

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Fig. 1

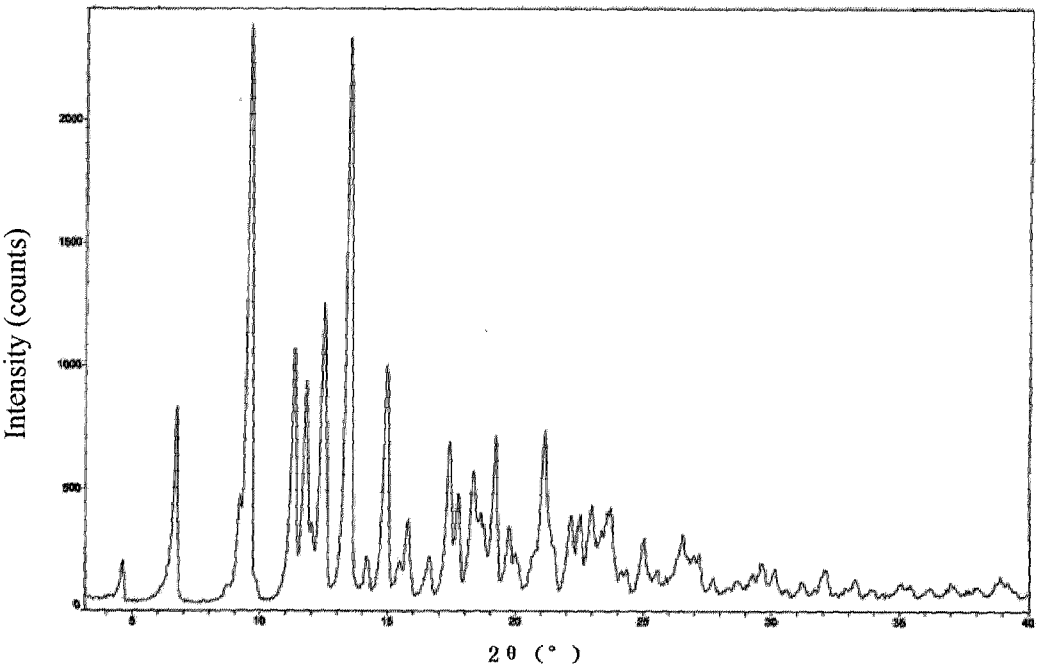
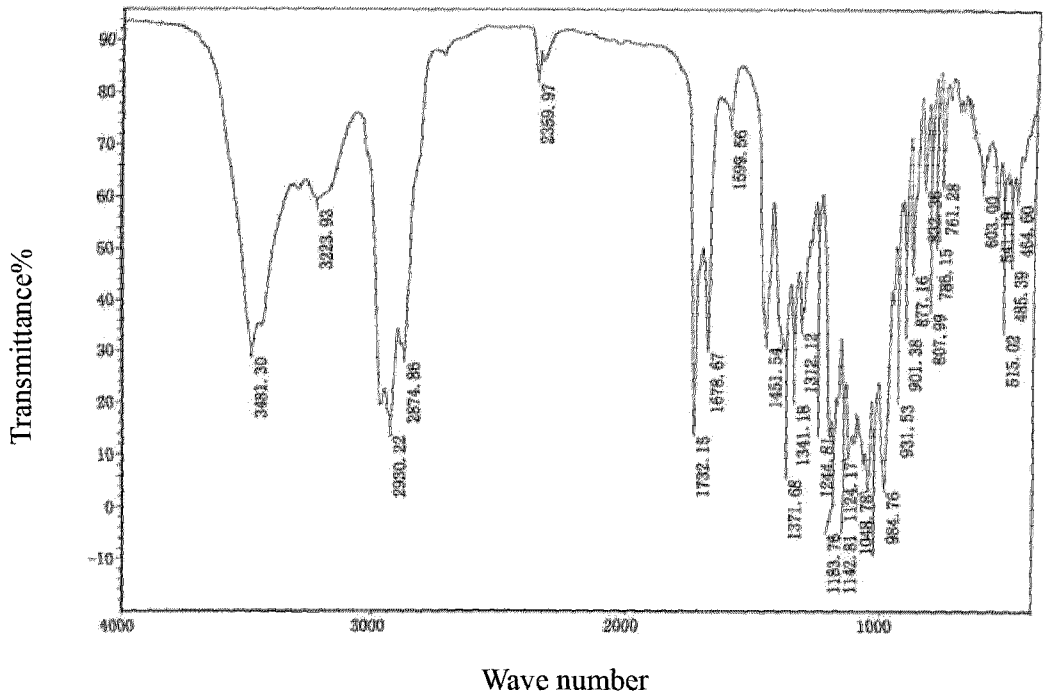

Intensity (counts)
2θ (°)

Fig. 2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2930.22
2874.86
2359.97
1732.15
1678.67
1596.56
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1371.68
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1312.12
1266.81
1183.76
1142.81
1124.17
1048.78
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Fig. 3

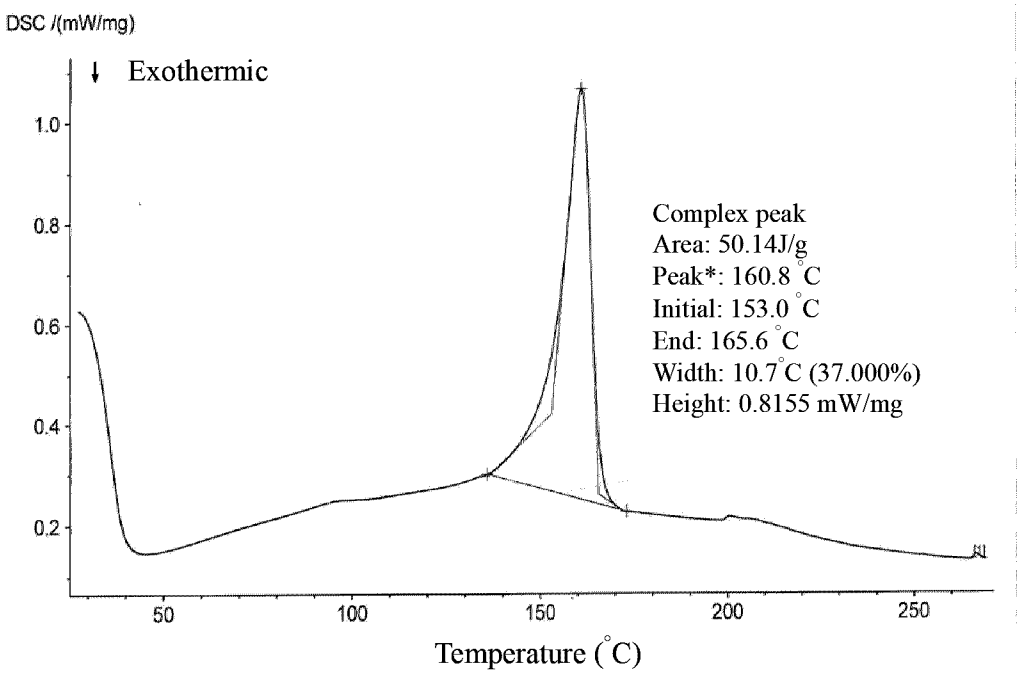

Fig. 4

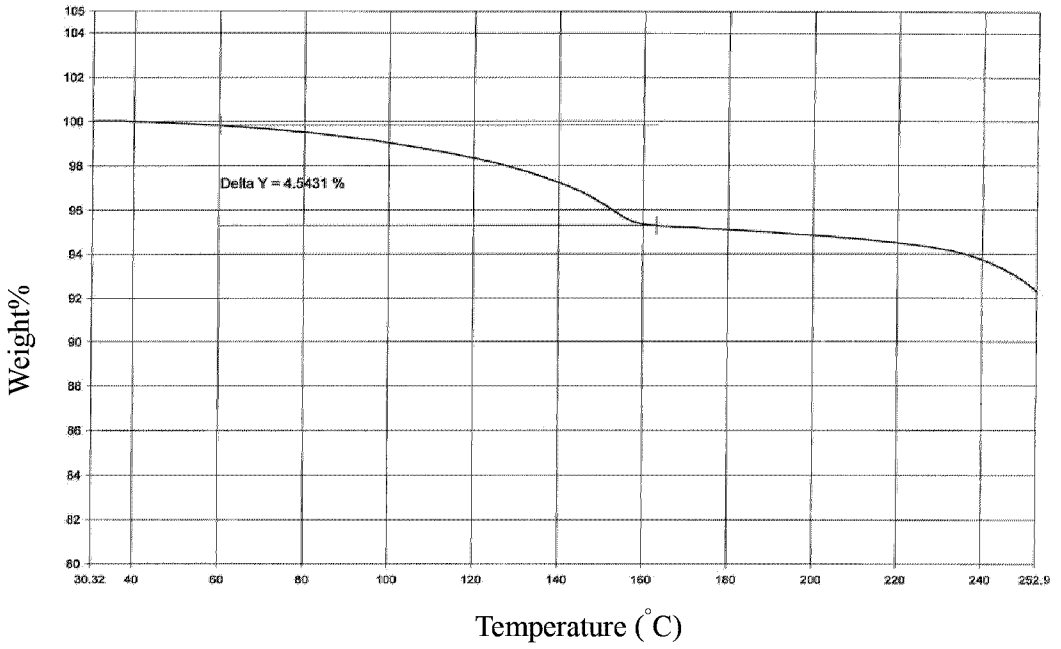

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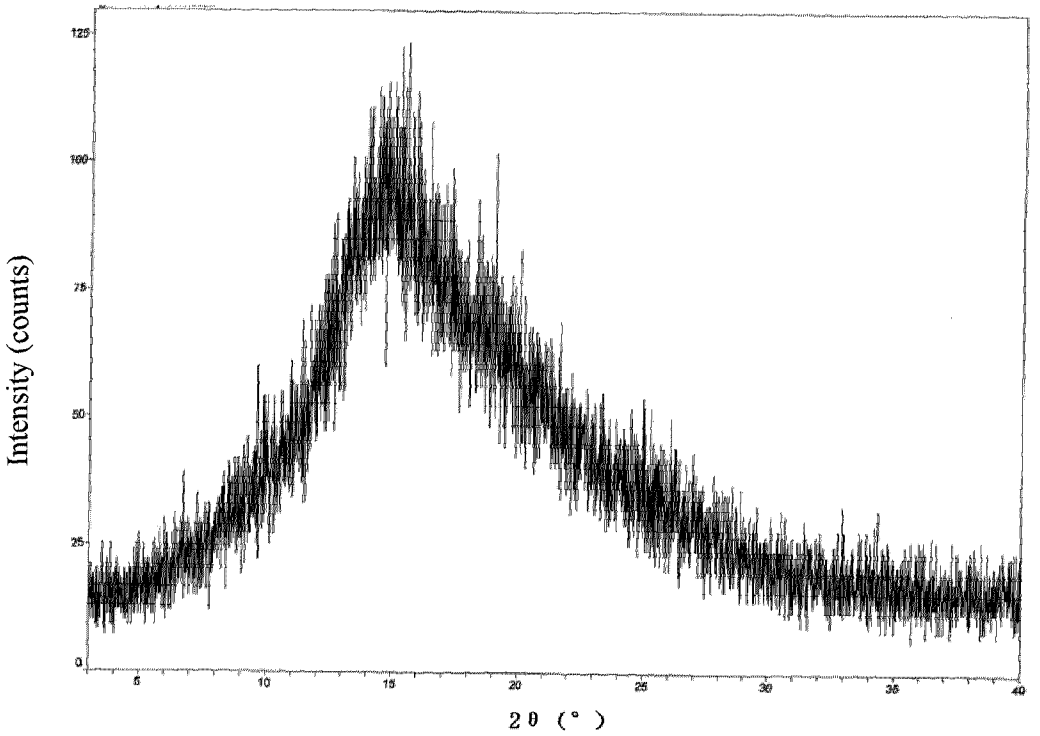
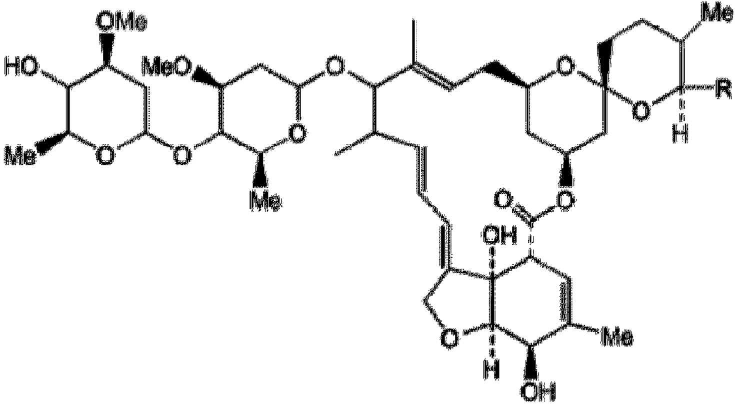
Fig. 5





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