

# United States Patent [19]

## Sumikawa et al.

## [54] CRYSTALS OF N-(TRANS-4-ISOPROPYLCYCLOHEXYCARBONYL)-D-PHENYLALANINE AND METHODS FOR PREPARING THEM

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- [21] Appl. No.: 166,144
- [22] Filed: Dec. 14, 1993

#### **Related U.S. Application Data**

[63] Continuation of Ser. No. 921,224, Jul. 29, 1992, abandoned.

#### [30] Foreign Application Priority Data

Jul. 30, 1991	[JP]	Japan	
Aug. 8, 1991	[JP]	Japan	

- [51] Int. Cl.<sup>6</sup> ...... C07C 239/00
- [58] Field of Search ...... 562/450; 514/563

## [56] **References Cited**

#### U.S. PATENT DOCUMENTS

196,222	1/1886	Toyoshima et al.	 562/450
4,816,484	3/1989	Toyoshima et al.	 514/563

## [11] **Patent Number:** 5,488,150

## [45] **Date of Patent:** Jan. 30, 1996

#### OTHER PUBLICATIONS

Diabetes Research and Clinical Practice, 12 (1991) 53-60 "Possibility of ideal blood glucose control by a new oral hypoglycemic agent,

N–[Trans–4–isopropylcyclohexyl)–carbonyl] –D–phenylalanine (A–4166), and its stimulatory effect on insulin secretion in animals" Yusuke Sato, Masahiko Nishikawa, Hisashi Shinkai and Eiji Sukegawa, Central Research Laboratories, Ajinomoto Co. Inc., Life Sciences Laboratories, Yokohama, Japan.

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#### [57] ABSTRACT

Stable crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine may be produced by treating this compound with a solvent at a temperature of at least 10° C. and forming crystals in the solvent at a temperature of at least 10° C. For example, crystals may be formed by crystallization out of solution, or may be formed from solid particles of the compound suspended in a solvent. Crystals formed in this way have different melting point, infra red spectrum and X-ray diffraction patterns from previously known forms of the compound and have enhanced processability, eg. stability to grinding.

### 13 Claims, 5 Drawing Sheets













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## CRYSTALS OF N-(TRANS-4-ISOPROPYLCYCLOHEXYCARBONYL)-D-PHENYLALANINE AND METHODS FOR PREPARING THEM

This application is a Continuation of application Ser. No. 07/921,224, filed on Jul. 29, 1992, now abandoned.

#### BACKGROUND OF THE INVENTION

The present invention relates to a crystalline form of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine and to methods for the production of that crystalline form.

N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine of formula (1) is a known substance having therapeutic 15 utility in depressing blood glucose levels.



N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine is disclosed in Japanese Patent Application Laid Open No. 63-54321 (equivalent to EP-A-196222 and U.S. Pat. No. 4,816,484) and in J. Med Chem 32, 1436. The Japanese application describes how the compound may be crystallised from aqueous methanol to yield crystals having a melting point of 129° to 130° C. These crystals are in a crystalline form referred to herein as "B-type". The X-ray powder diffraction pattern and infra-red spectrum of B type crystals are shown in FIGS. 1 and 2 respectively.

The known B-type crystals suffer from problems of 40 instability, especially when subjected to mechanical grinding. The instability results, for example, in conversion of the B-type crystals into other forms. The instability of the B-type crystals means that they are not ideal for use in medicine. It is in general desirable that a medicinal product 45 containing a crystalline active ingredient have a composition which is well defined and stable in terms of the crystalline form of the active ingredient. Conversion of one crystalline form into unknown amounts of different, or amorphous, forms during processing or storage is undesirable and in 50 many cases would be regarded as analogous to the appearance of unquantified amounts of impurities in the product.

The present inventors have discovered a method for producing a crystalline form of N-(trans-4-isopropylcyclo-hexylcarbonyl)-D-phenylalanine having improved stability <sup>55</sup> over the known B-type. For instance, the crystals according to one aspect of the present invention have enhanced stability to grinding. Such crystals are therefore more suitable for use in medicines than those of the B-type. The crystals having enhanced stability have been designated "H-type" by <sup>60</sup> the inventors.

## SUMMARY OF THE INVENTION

According to a first aspect of the invention there is 65 provided a method for the production of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine comprising treat-

ing N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine with a solvent at a temperature of at least  $10^{\circ}$  C. and forming said crystals in said solvent at a temperature of at least  $10^{\circ}$  C.

In one embodiment of this method N-(trans-4-isopropy-lcyclohexylcarbonyl)-D-phenylalanine is dissolved in the solvent at a temperature of at least  $10^{\circ}$  C. to form a solution and crystals are then crystallised from the solution at a temperature of at least  $10^{\circ}$  C.

Alternatively, N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine is combined at a temperature of at least 10° C. with a solvent in which it is incompletely soluble at that temperature, to form a suspension of solid N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine, and said suspension is maintained at a temperature of at least 10° C.

According to a second aspect of the invention there are provided crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine as obtainable by the method of the first aspect.

According to a still further aspect crystals of N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine are provided which have at least one, and preferably all, of the following properties:

- (a) a melting point in the range of 136° to 142° C.;
  - (b) an X-ray diffraction pattern having maxima at approximately  $2\theta$ =8.1°, 13.1°, 19.6° and 19.9°; and
  - (c) an infra red spectrum having absorptions at about 1714, 1649, 1542 and 1214 cm<sup>-1</sup>. Such crystals are designated "H-type" herein.

Crystals of the second aspect of the invention desirably comprise enhanced amounts of H-type crystals relative to the starting N-(trans-4-isopropylcyclohexylcarbonyl)-Dphenylalanine.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a powder X-ray diffraction pattern of B-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phe-nylalanine;

FIG. **2** shows an infra red absorption spectrum of B-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phe-nylalanine;

FIG. **3** shows a powder X-ray diffraction pattern of H-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phe-nylalanine;

FIG. 4 shows an infra red absorption spectrum of H-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phe-nylalanine.

FIGS. 5A-C shows differential scanning calorimeter (DSC) traces of: B-type crystals before grinding (FIG. 5a); H-type crystals before grinding (FIG. 5b); B-type crystals after grinding (FIG. 5c).

# DETAILED DESCRIPTION OF THE INVENTION

As was indicated above one aspect of the present invention provides N-(trans-4-isopropylcyclohexylcarbonyl)-Dphenylalanine in H-type crystalline form. Examples of the physical properties of the H-type crystals are as follows.

The inventors have measured the melting point of H-type crystals and found it to be in the range of  $136^{\circ}$  to  $142^{\circ}$ . By contrast, when the melting point of B-type crystals was measured by the same technique a melting point of  $128^{\circ}$  to  $131^{\circ}$  C. was found.

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Examples of powder X-ray diffraction patterns of H-type and B-type crystals may be found at FIGS. 3 and 1 respectively. The diffraction pattern of the H-type cystals shows maxima at 20 values of 8.1°, 13.1°, 19.6° and 19.9° where  $2\theta$  is the angle between the primary beam projection and the diffracted beam. There are no reflections at these 20 values in the diffraction pattern of the B-type crystals. The diffraction pattern of H-type crystals also displays strong reflections at 20 values between about  $15^{\circ}$  and  $17^{\circ}$  while the B-type crystals give only weak reflections in this range of 10 20. H-type crystals of the present invention preferably display a powder X-ray diffraction pattern substantially the same as that shown in FIG. 3.

Table 1 below sets out the principal reflections in the powder pattern of H-type crystals in terms of 20 values and intensity. The data were obtained using a Philips PW1700<sup>15</sup> powder diffractometer and a scan speed of 0.05°/sec.

TA	BL	Æ	1
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20	intensity	degree	intensity	degree	intensity	degree
	s	8.1	М	5.7	S	5.5
	w	10.4	W	9.0	w	8.5
	w	12.0	S	11.5	М	11.1
	S	15.2	W	14.3	S	13.1
•	S	16.2	S	15.9	М	15.4
25	w	18.2	W	17.3	w	17.0
	S	19.6	w	18.9	w	18.6
	w	21.5	w	21.1	S	19.9
	w	23.7	W	23.1	w	22.1
			W	29.9	w	24.5

S; strong, M; medium, W; weak

An example of an infra red adsorption spectrum of H-type crystals, obtained by the KBr method is shown at FIG. 4, and that of B-type crystals as obtained by the same method is shown at FIG. 2. The infra red spectrum of the H-type 35crystals is characterised by absorptions at around 714 cm<sup>-</sup> 1649 cm<sup>-1</sup>, 1542 cm<sup>-1</sup> and 1214 cm<sup>-1</sup>, which absorptions are not present in the spectrum of the B-type crystals. H-type crystals of the present invention preferably display an infra red spectrum substantially the same as that shown in FIG. 4.

40 The inventors carried out elementary analysis of both H-type and B type crystals and the results are shown in Table 2. These confirm that the two crystal types have the same chemical composition  $(C_{19}H_{27}NO_3)$ 

TABLE 2

	С	Н	Ν
Calculated data (H- or B-type)(%)	71.89	8.57	4.41
Measured data (H-type)(%)	71.98	8.69	4.33
Measured data (B-type)(%)	71.82	8.66	4.27

H-type crystals are preferably substantially stable to grinding. Stability to grinding may be assessed by measurement of an appropriate physical property before and after grinding. Where the physical property remains substantially 55 unchanged substantial stability to grinding is indicated. Suitable physical properties for measurement include melting point, differential scanning calorimeter trace X-ray diffraction pattern and infra red absorption spectrum, particularly the X-ray diffraction pattern.

As mentioned above the first aspect of the invention provides a method for the production of crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine method comprising treating N-trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine with a solvent at a temperature 65 of at least 10° C. and forming crystals in the solvent at a temperature of at least 10° C.

In one embodiment -4-isopropylcyclohexylcarbonyl)-Dphenylalanine is dissolved in the solvent at a temperature of at least 10° C. The solution may be produced by dissolving in a solvent any one or more of amorphous N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine, B-type crystals of the compound, and solvates of the compound such as hydrates, methanolates, ethanolates, isopropanolates and acetonitrilates.

Crystals may then be formed by crystallisation from solution, the crystallisation from solution taking place at a temperature between 10° C. and the boiling point of the solvent. The crystals thus formed generally comprise enhanced amounts of H-type crystals relative to the starting N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine. Preferably, the product is predominantly H-type crystals.

The dissolution and crystallisation at a temperature of at least 10° C. may be carried out in several ways as will be apparent to those of skill in the art. For instance, N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine may be dissolved in a solvent, or mixture of solvents in which it is readily soluble at elevated temperatures but in which it is only sparingly soluble at lower temperatures (which are still at least 10° C.). Dissolution at elevated temperature is, in this case, followed by cooling during which the desired H-type crystals crystallise out of solution. Solvents which are suitable for use in this way include esters, such as methyl acetate and ethyl acetate, toluene and acetonitrile. Mixed solvents comprising a good solvent in which N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine is readily soluble, preferably, in amounts of at least 1 weight % at 30° C., and a poor solvent in which it is more sparingly soluble, preferably, in amounts of not more than 0.01% at 30° C., may also be employed provided that crystallisation from the mixture at a temperature of at least 10° C. is possible using the selected solvent mixture.

An alternative way of achieving crystallisation from solution at a temperature of 10° C. is to utilise the difference in solubility of the crystals in different solvents. For example, N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine may be dissolved in a good solvent in which it is highly soluble such as one in which it is soluble in amounts of at least 1 weight % at 30° C. and the solution subsequently mixed with a poor solvent in which it is more sparingly soluble, such as one in which it is soluble in amounts of not more than 0.01% at 30° C. Thus, the solution of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine in the good solvent might be added to the poor solvent, while maintaining a temperature in excess of 10° C., or the poor solvent might be added to the solution of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine in the good solvent, again while maintaining a temperature in excess of 10° C. Examples of good solvents include lower alcohols, such as methanol, ethanol and isopropanol, as well as acetone, tetrahydrofuran and dioxane. Examples of poor solvents are water, hexane and diethyl ether.

Whichever of the two alternative crystallisation methods is employed it is important that the crystallisation temperature be at least 10°  $\bar{C}.$  up to the boiling point of the solvent. If the temperature employed is lower than 10° C. it is not possible to obtain good yields of H-type crystals. Preferably, crystallisation is effected at a temperature in the range of 10° to 60° C., especially preferably from 20° to 60° C.

Crystals which have come out of solution are preferably separated from the solvent e.g. by filtration or centrifuging and are desirably then dried for example at a temperature in the range of from 20° C. to 100° C.

In an alternative embodiment of the first aspect of the invention solid N-(trans-4-isopropylcyclohexylcarbonyl)-D-

phenylalanine is suspended at a temperature of at least 10° C. in a solvent in which it is incompletely soluble, preferably only sparingly soluble, at that temperature. A suspension results in which particles of solid are dispersed, and remain incompletely dissolved in the solvent. Preferably the solids 5 are maintained in a state of suspension by agitation e.g. by shaking or stirring. The suspension is kept at a temperature of 10° C. or higher thereby to effect a transformation of the starting solids into product crystals.

The solid N-(trans-4-isopropylcyclohexylcarbonyl)-D- 10 phenylalanine suspended in suitable solvent may be of any type, such as amorphous, or in the form of B-type crystals and may be a solvate, e.g. hydrate, methanolate, ethanolate, isopropanolate or acetonitrilate. The amorphous powder may be derived by drying a solvate. Preferably, the suspen-15 sion is maintained at a temperature of at least 10° C. for sufficiently long that the product crystals contain enhanced amounts of H-type crystals relative to the starting N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine.

Solvents suitable for use in this embodiment of the 20 invention include water, esters such as methyl acetate and ethyl acetate, as well as toluene. Good solvents in which N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine is more readily soluble for example in amounts of at least 1% by weight at 30° C., such as lower alcohols e.g. 25 methanol, ethanol and isopropanol, as well as acetone, acetonitrile, tetrahydrofuran and dioxane may also be used provided they are used in combination with a solvent in which N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine is only poorly soluble for example in amounts of 30 0.01 weight % or less, e.g. water, hexane or diethyl ether. Where a mixed solvent is employed the concentration of good solvent is generally 70% by volume or less. Where it exceeds 70% by volume the solubility of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine in the mixed sol- 35 vent would be so high that the yield of desired H-type crystals would be disadvantageously low. Generally, the use of a mixed solvent gives rise to a favourable result. Preferably, the amount of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine dispersed in the solvent is from 0.5% 40 to 30% by weight of the resulting suspension. If it is more than 30% by weight then the slurry properties of the suspension are poor and it will be difficult to agitate. On the other hand, it is not efficient in terms of the volume of solvent required to use less than 0.5% by weight. Preferably 45 the suspension includes from 1% to 15% by weight.

The suspension is maintained at a temperature from 10° C. to the boiling point of the solvent, in general from 20° C. to 70° C. Temperatures below 10° C. do not facilitate transformation of the solids to H-type crystals. The time for 50 which the suspension is left before H-type crystals may be collected from it varies depending on the nature of the solvent(s) used, the temperature and other factors, such as the quantity of solids in suspension and the size of the solid particles.

Generally, however, it may be in the range of from 10 minutes to 48 hours. By adding H-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine to the dispersion as seed crystals, the time required to form H-type crystals may be shortened. The end point in formation of 60 H-type crystals can be determined by sampling crystals from the suspension, for example by filtration during the course of the conversion followed by measuring the powder X-ray diffraction pattern or infra red absorption spectrum of the crystals. 65

The H-type crystals as obtained in the manner mentioned above can be separated from suspension by filtration or

centrifugation. In isolating them, cooling may be effected, if desired. In that case, the cooling temperature is preferably no lower than 10° C. The isolated crystals are dried, for example at a temperature in the range of from 20° to 120° C.

According to another aspect of the invention there is provided a pharmaceutical composition comprising crystals as obtainable by the method of the first aspect, in particular H-type crystals, and a pharmaceutically acceptable excipient, diluent or carrier.

According to a still further aspect of the invention there is provided a method of manufacture of a pharmaceutical composition comprising mixing an effective amount of crystals as obtainable by the method of the first aspect of the invention, in particular H-type crystals and a pharmaceutically acceptable excipient diluent or carrier.

According to a still further aspect of the invention there is provided a method for treatment of a human or other mammal to depress its blood glucose level comprising administering an effective amount of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine crystals as obtainable by the methods of the present invention, in particular B-type crystals.

#### **EXAMPLES**

Embodiments of the invention are illustrated below by way of example only.

N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine for use in the following examples was obtained by the method described in Example 3 of Japanese patent application laid open no. 63-54321. The product contained crystals of B-type.

#### (A) H-Type Crystals by Crystallisation from Solution

#### Example A1

20 ml of an acetone solution of 5 g of N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine were added dropwise to a stirred mixture of acetone (40 ml) and water (60 ml) at 25° C. After cooling to 10° C., the precipitated crystals were filtered and dried at 90° C. at reduced pressure overnight. 4.5 g of dry crystals were obtained. The crystals had a melting point of 138° to 141° C. The powder X-ray diffraction pattern and the infra-red absorption spectrum were measured and the crystals were thus identified as H-type.

#### Example A2

N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (4 g) was dissolved in a mixture of ethanol (50 ml) and water (50 ml) at 45° C. The solution was cooled with stirring. H-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (0.1 g) prepared in Example A1 were added at a temperature of 37° C. and the solution was cooled further to 25° C. The crystals were filtered and dried at 60° C. overnight and at reduced pressure. 2.5 g of dry crystals were obtained. The crystals had a melting point of 138° to 141° C. The powder X-ray diffraction pattern and the infra-red absorption spectrum enabled the crystals to be identified as H-type.

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### Example A3

N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (0.5 g) was dissolved in acetonitrile at 45° C. and the solution was cooled to 25° C. The precipitated crystals were filtered and dried at 90° C. under reduced pressure, 0.48 g of dry crystals were obtained. The crystals had a melting point of 138° to 141° C. Their powder X-ray diffraction pattern and infra red absorption spectrum were consistent with their being H-type crystals.

#### Comparative Example A1

The procedure of Example A1 was followed but cooling to 5° C. was employed, 4.6 g of dry crystals were obtained. <sup>15</sup> The crystals had a melting point of 128° to 131° C. The powder X-ray diffraction pattern and the infra-red absorption spectrum of the crystals were measured and the crystals were identified as B-type.

#### Comparative Example A2

N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (5 g) was dissolved in a mixture of ethanol (60 ml) and water (40 ml) at 30 ° C. The solution was cooled to 5° C. 25 with stirring. The precipitated crystals were filtered and dried at 90° C. under reduced pressure, and overnight. 3.3 g of dried crystals were obtained. The crystals had a melting point of 128° to 131° C. and their powder X-ray diffraction pattern and infra-red absorption spectrum indicated that they 30 were of the B-type.

#### Comparative Example A3

N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (5 g) was dissolved in a mixture of methanol (70 ml) and water (30 ml) at 40° C. The solution was cooled to 5° C. with stirring. The precipitated crystals were filtered and dried at 90° C. under reduced pressure overnight. 3.5 g of dry crystals were obtained. Once again, the crystals had a 40 melting point of 128° to 131° C. The powder X-ray diffraction pattern and the infra-red absorption spectrum were consistent with the crystals being B-type.

#### (B) H-Type Crystals from Suspension

#### Example B1

B-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (3 g) were dispersed in 300 ml of water and stirred at 30 ° C. for 1 day. The crystals were filtered and dried at 90° C. under reduced pressure overnight. 2.9 g of dry crystals were obtained. The powder X-ray diffraction pattern and the infra-red absorption spectrum 55 were recorded and indicated that the crystals were of H-type.

#### Example B2

B-type crystals of N-(trans-4-isopropylcyclohexylcarbo- 60 nyl)-D-phenylalanine (4 g) were dispersed in a mixture of acetone (40 ml) and water (60 ml) and stirred at 20° C. overnight. The crystals were subsequently filtered off and dried at 90° C. under reduced pressure overnight. 3.6 g of dry crystals were obtained. Powder X-ray diffraction and 65 infra red absorption spectroscopy indicated that the product crystals were of H-type.

## Example B3

Hydrate of N-(trans-4-isopropylcyclohexylcarbonyl)-Dphenylalanine for use in this example was prepared as follows. 20 g of B-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine were dissolved in a mixture of ethanol (300 ml) and water (200 ml) at 30° C. The solution was cooled to 5° C. with stirring. The precipitated crystals were filtered off and dried at 40° C. under reduced pressure for 2 hours. 13.9 g of dried crystals resulted.

4.2 g of the hydrate was dispersed in a mixture of ethanol (30 ml) and water (70 ml) and stirred at  $45^{\circ}$  C. overnight. The crystals were filtered off and dried at  $90^{\circ}$  C. under reduced pressure overnight. 3.8 g of dried crystals were obtained. These were found to be of the H-type by powder X-ray diffraction and infra red spectroscopy.

#### Example B4

4.2 g of the hydrate of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine prepared as in example B3 was dispersed in a mixture of ethanol (30 ml) and water (70 ml). 40 mg of H-type crystals were added and the dispersion was stirred at 45° C. for 1 hour. The crystals were filtered off and dried at 90° C. under reduced pressure overnight. 3.9 g of dry crystals were obtained. These were found to be of the H-type by X-ray diffraction and infra red absorption spectroscopy.

#### Example B5

B-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (5 g) were dispersed in a mixture of isopropanol (25 ml) and water (75 ml) and stirred at 50° C. for 10 hours. The resulting crystals were filtered off and dried at 90 ° C. under reduced pressure overnight. 4.4 g of dry crystals were obtained. These were found to be of the H-type by X-ray diffraction and infra red spectroscopy.

#### Comparative Example B1

4 g of B-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine were dispersed in a mixture of acetone (40 ml) and water (60 ml) and stirred at 5° C. overnight. The crystals were filtered off and dried at 90° C. under reduced pressure overnight. 3.6 g of dry crystals were obtained. The powder X-ray diffraction pattern and the infra red absorption spectrum were measured and the crystals were found to be of B-type.

## (C) Stability to Grinding

In order to demonstrate the stability of H-type crystals to grinding the following experiment was carried out.

H-type crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine and the previously known B-type crystals were each mechanically ground in a grinder, and the X-ray diffraction pattern of each powder was measured and compared with the spectrum before grinding. No change was observed in the H-type crystals before and after grinding but changes were observed in the diffraction pattern of the B-type crystals.

Similarly, the differential scanning calorimeter (DSC) trace for each of the H-type, and B-type crystals was measured before and after grinding. FIG. 5a shows the DSC trace of B-type crystals before grinding. The crystals show a sharp melting point at around 130° C. The DSC trace of H-type crystals before grinding is shown in FIG. 5b. These

crystals also demonstrated a sharp melting point at around  $140^{\circ}$  C. The DSC trace of the H-type crystals was unchanged by grinding. By contrast, the trace shown in FIG. **5***c* for B-type crystals after grinding differs from the trace obtained before grinding and new troughs are visible in the 5 trace.

We claim:

**1**. A method for the production of crystals of N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine having at least one of the following properties:

(a) a melting point in the range of 136°-142° C.;

(b) a powder X-ray diffraction pattern with reflection maxima at 20 of about 8.1°, 13.1°, 19.6° and 19.9°; comprising dissolving or suspending N-(trans-4-iso-propylcyclohexylcarbonyl)-D-phenylalanine in a sol-15 vent in which it is completely or incompletely soluble at a temperature of at least 10° C. and up to the boiling point of the solvent and permitting said crystals to crystallize from or form in said solvent at a temperature of 10° C. or in excess thereof.

2. A method according to claim 1 wherein N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine is dissolved in a solvent in which it is soluble at a temperature of at least  $10^{\circ}$  C. to form a solution and crystals are then crystallised from the solution at a temperature of  $10^{\circ}$  C. or in excess<sup>25</sup> thereof by addition of a poorer solvent or by lowering the temperature of the solution.<sup>25</sup>

3. A method according to claim 2 wherein crystallisation from solution is effected by reducing the temperature of the solution to a temperature of  $10^{\circ}$  C. or in excess thereof. <sup>30</sup>

4. A method according to claim 2 wherein the crystallisation from solution is effected at a temperature of 10° C. or in excess thereof by adding to the solution a further solvent selected such that the solubility of said N-(trans-4-isopropylcyclohexylcarbonyl)-C-phenylalanine in the mixture of the solvent and further solvent in less than in the solvent, the solubility being reduced to an extent such that crystalls crystallize from the solvent mixture at a temperature in excess of 10° C.

**5.** A method according to claim **1** wherein N-(trans-4isopropylcyclohexylcarbonyl)-D-phenylalanine is combined at a temperature of at least 10° C. with a solvent in which it is incompletely soluble at that temperature to form a suspension of solid N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine, and said suspension is maintained at a temperature of at least 10° C., thereby to form said crystals from said solids.

6. A method according to claim 1 further comprising separating said crystals from the solvent at a temperature in excess of  $10^{\circ}$  C.

7. Crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-Phenylalanine obtained by the method of claim **1**.

8. Crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine having at least one of the following properties:

(a) a melting point in the range of 136°-142° C.; and

(b) a powder X-ray diffraction pattern with reflection maxima at 2θ of about 8.1°, 13.1°, 19.6° and 19.9°.

**9**. Crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine according to claim **8** having both of the properties (a) and (b).

**10.** Crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine having an X-ray powder diffraction pattern as shown in FIG. **3**.

11. A method according to claim 1 wherein the crystals formed contain a greater percentage content of crystals having at least one of the specified properties relative to the starting N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine.

12. A pharmaceutical composition comprising an effective amount of crystals according to claim 8 and a pharmaceutically acceptable excipient, diluent or carrier.

13. A pharmaceutical composition comprising an effective amount of crystals according to claim 10 and a pharmaceutically acceptable excipient, diluent or carrier.

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## UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO. : 5,488,150 DATED : January 30, 1996 INVENTOR(S) : Michito Sumikawa, et. al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Title page, item [54] and col. 1, line 1, the title should read--

CRYSTALS OF N-(TRANS-4-ISOPROPYLCYCLOHEXYLCARBONYL)-D-PHENYLALANINE AND METHODS FOR PREPARING THEM--

> Signed and Sealed this Ninth Day of April, 1996

Since Tehman

BRUCE LEHMAN Commissioner of Patents and Trademarks

Attest:

Attesting Officer