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(54) Titre : CRISTAUX DE SELS STEREOISOMERES NON NATURELS DE MONATINE ET LEUR UTILISATION
(54) Title: CRYSTALS OF NON-NATURAL STEREOISOMER SALTS OF MONATIN AND UTILIZATION THEREOF

(57) **Abrégé/Abstract:**

It is intended to newly provide crystals of non-natural stereoisomer salts of monatin which are usable as sweeteners. Because of being stable and having a high degree of sweetness, these crystals are expected as useful as sweeteners or components thereof as well as agents for imparting sweetness to foods and drinks. It is also possible to provide, for example, sweeteners and sweetened foods and drinks with the use of the above salt crystals as novel sweetening substances. These stereoisomer salts include (2S,4R), (2R,4R) and (2R,4S) isomer salts of monatin. Among all, the (2R,4R) isomer salts of monatin are particularly favorable.



Abstract

Crystals of salt of a stereoisomer which is a non-natural type of monatin which are applicable as a sweet taste substance are newly provided. The crystals are stable, have a high degree of sweet taste (high sweetening potency) and are expected for use as a sweetener or an ingredient thereof and also as a component for giving sweet taste to beverage, food, etc.

A sweetener using the above-mentioned crystals of the salt which is a novel sweet taste substance and a product such as beverage and food to which sweet taste is given are able to be provided.

The stereoisomer salt includes salts of a (2S,4R) substance, a (2R,4R) substance and a (2R,4S) substance of monatin and the salt of a (2R,4R) substance of monatin is particularly preferred.

Description

CRYSTALS OF NON-NATURAL-TYPE

STEREOISOMER SALT OF MONATIN AND USE THEREOF

Technical Field

The present invention relates to crystals of salt of a non-natural-type stereoisomer of monatin and to the use thereof and, more particularly, it relates to crystals of a stereoisomer salt (a non-natural-type stereoisomer salt) of a natural-type monatin (a (2S,4S) substance) being excellent as a sweetener or an effective ingredient (sweetening agent) thereof, to crystals of a mixture of the non-natural-type stereoisomer salts, to crystals of a mixture containing at least one member of such non-natural-type stereoisomer salts and a natural-type monatin (a (2S,4S) substance) salt and to the use of crystals of such salts. The non-natural-type stereoisomer salts as such include salts of various stereoisomers of non-natural type such as a (2S,4R) substance, a (2R,4R) substance and a (2R,4S) substance of monatin and representative examples of such salts are a single substance of such a salt (a salt of any one member of the non-natural-type stereoisomers), a mixture of plural salts and a mixture (composition) containing at least one of these salts.

Background Art

In recent years, as a result of advance of eating habits, obesity by an excessive ingestion of sugars and various diseases accompanied thereby particularly have become a problem and there has been a strong demand for developing a low-calorie sweetener in place of sugar. In addition to the intensity of sweet taste, the sweetener demanded is requested to have many characteristics and essential features such as low calorie, safety, stability to heat and acid, quality of sweet taste, cost, etc.

At present, various kinds of sweeteners are used or proposed. For example, as a sweetener which is practically used having a strong intensity of sweet taste (degree of sweet taste) and being able to be mass-produced in an industrial scale and is widely used, there is aspartame which is excellent in terms of safety and quality of sweet taste. In addition, studies for derivatives of aspartame have been intensively carried out. Besides those, sweet taste substances having various characteristics have been proposed as sweeteners and investigation for practical use has been conducted. Further, thaumatin, glycyrrhizin, stevioside, etc. derived from plants which are present in nature and able to be collected in large quantities are now used as natural sweeteners. Under such circumstances, there has been a demand for developing a sweet taste substance being able to be expected for practical use

as a sweetener and having a strong degree of sweet taste.

Monatin is an amino acid derivative derived from nature being isolated from root skin of *Schlerochiton ilicifolius* which is a plant naturally grown in the area of the northern Transvaal of South Africa and, with regard to its structure, it was reported to be (2S,4S)-2-amino-4-carboxy-4-hydroxy-5-(3-indolyl)-pentanoic acid ((2S,4S)-4-hydroxy-4-(3-indolylmethyl)-glutamic acid; refer to the structural formula (1)) by R. Vleggaar, et al. (cf. R. Vleggaar, et al., J. Chem. Soc. Perkin Trans., 3095-3098 (1992)). According to the above document, etc., intensity of the sweet taste of a (2S,4S) substance (natural-type monatin) derived from the natural plant is reported to be 800- to 1400-fold of sucrose. Although various methods have been reported as a synthetic method for monatin, many of them relate to synthetic methods for a mixture of stereoisomers and there has been nearly no report where each of four stereoisomers having the same chemical structural formulae as the natural-type monatin is synthesized and isolated as a pure substance and properties thereof are investigated in detail. (With regard to examples of synthesis thereof, P. J. van Wyk, et al., ZA 87/4288, ZA 88/4220; Holzapfel, et al., Synthetic Communications, 24(22), 3197-3211 (1994); E. Abushanab, et al., US 5,994,559 (1999); K. Nakamura, et al., Organic Letters, 2, 2967-2970 (2000), etc. may be referred to.)

In accordance with the patent documents by P. J. van Wyk, et al. mentioning monatin for the first time, although the inventors determined by means of an X-ray crystal structure analysis that a stereoisomer being present in nature and having a strong sweet taste is a (2S,4S) substance or a (2R,4R) substance, they reported that, by combining with the result of synthesis of a mixture of a (2S,4S) substance and a (2S,4R) substance from (2S)-aspartic acid, there is a high possibility that a stereoisomer being present in nature and having a strong sweet taste is a (2S,4S) substance. Then, in accordance with the above-mentioned document by R. Vleggaar, et al., there is reported that a stereoisomer (steric structure) of monatin being present in natural plant is a (2S,4S) substance only and its intensity of sweet taste is 800- to 1400-fold of sucrose. On the basis of the information as such, it is proper to conclude that a stereoisomer (steric structure) of monatin which is present in nature and is a substantial body of a strong sweet taste is a (2S,4S) substance.

In the above-mentioned document, K. Nakamura, et al. isolated hydrochlorides of a (2S,4S) substance of monatin and of a (2S,4R) substance of monatin and reported that, with regard to the intensity of sweet taste thereof, they showed a similar sweet taste to a natural specimen of monatin (a (2S,4S) substance) and a slight sweet taste presumably due to monatin (a (2S,4S) substance) which is thought to be present as an

impurity, respectively although the specific intensity of the sweet taste was not reported. Thus, although this document is the first example referring to the intensity of the sweet taste of a non-natural-type stereoisomer (other than a (2S,4S) substance) of monatin, it is reported that hydrochloride of a (2S,4R) substance of monatin has almost no sweet taste.

On the other hand, T. Kitahara, et al. report a selective synthetic method for each stereoisomer of monatin as a sweetener (sweetening agent) but do not report the degree of sweet taste of each stereoisomer (refer to T. Kitahara, et al., Abstracts of Papers Presented at the General Meeting in 2000 of the Agricultural Chemical Society of Japan, 3B128β (page 221)) .

When the information as mentioned above is taken into consideration as a whole, the followings have been noted.

(1) A stereoisomer (steric structure) of monatin being present in nature and having an intensity of sweet taste of as strong as 800- to 1400-fold is a (2S,4S) substance; and

(2) With regard to other non-natural-type stereoisomers of monatin, although there are examples where a part of them is isolated in small quantities, there is no example where the pure substance is isolated and purified and then the effect for sweet taste is confirmed.

Thus, up to now, there have been no clear data for the intensity of sweet taste of each stereoisomer of monatin in

practical concentrations corresponding to 5 to 10% sucrose concentrations except monatin which is derived from nature (a (2S,4S) substance). Accordingly, it is not possible to know from the already-available documents, patent documents, etc. whether non-natural-type stereoisomers of monatin except monatin (a (2S,4S) substance) are able to be used as a sweetener. In other words, reliable information for the degree of sweet taste (intensity of sweet taste) except monatin derived from nature (a (2S,4S) substance) is little and, when the prior art documents information is considered as a whole, the present situation is nothing but to conclude that non-natural-type monatin stereoisomers except monatin (a (2S,4S) substance) have low degree of sweet taste and are unable to be expected for their utility as sweeteners.

One of the causes therefor is that, until now, there has been found no method whereby each of the above-mentioned stereoisomers is synthesized, isolated and purified.

Accordingly, there has been demanded that at least several hundred milligrams or more each of a natural-type monatin and three kinds of non-natural-type stereoisomers thereof are isolated and purified as pure substances, that optical purity and intensity of sweetness of each of them are made clear and that utility of monatin (a (2S,4S) substance) and stereoisomers thereof (i.e., non-natural-type stereoisomers) as sweeteners is made clear whereby a sweetener

containing a component having a high utility is developed.

With regard to a method for the separation of each stereoisomer in a high purity, there will be firstly a crystallizing method. Therefore, some explanations will be made for crystals of monatin (including the forms of free compounds, salts, etc.) as follows.

According to the reports up to now, there is reported in the above-mentioned document by R. Vleggaar, et al. that crystals of a free compound of monatin (a (2S,4S) substance) are prepared from a mixed solvent of water, acetic acid and ethanol (1:1:5) and its melting point is described as 216-220°C. In the patent document by P. J. van Wyk, et al., melting point of a free compound (crystalline solid) of monatin (a (2S,4S) substance) is described as 247-265°C (decomposition) while, with regard to various salts, they are reported to be amorphous solids. In the above-mentioned document by C. W. Holzapfel, et al., crystals of a free compound of a mixture of a (2S,4S) substance and a (2R,4R) substance of synthetic monatin are prepared by crystallization for two times from a mixed solvent of water and acetic acid (10:1) and the melting point is reported to be 212-214°C. Accordingly, with regard to non-natural-type stereoisomers of monatin and a mixture of such a plurality of stereoisomers except the above two examples such as a free compound of a (2S,4S) substance of monatin, not only free compound but also various salts have not been isolated

in a crystalline state whereby their physical property data and other information have not been known at all. Thus, with regard to monatin, there has been no knowledge for a crystallizing method which is the simplest and most effective method as a purifying means as compared with conventional purifying methods such as ion-exchange chromatography and also for crystals prepared thereby except the two cases of a free compound of monatin (a (2S,4S) substance) and a mixture of free compounds of (2S,4S) substance and (2R,4R) substance of monatin. There is no knowledge at all particularly for crystals of salts. Accordingly, in view of a practical application of various stereoisomers of monatin as sweeteners, it has been demanded to clarify various physical properties of those crystals prior to others.

Disclosure of the Invention

(Problems that the Invention is to Solve)

A problem that the present invention is to solve is to provide a sweet taste substance which is able to be expected as a practical application as a sweetener and has a strong intensity of sweet taste. To be more specific, the problem is to separate and purify each of natural-type monatin and three non-natural-type stereoisomers as a pure substance, particularly as crystals, to establish a method for confirming the optical purity thereof and to confirm the intensity of sweet

taste (sweetening potency; degree of sweet taste) of a natural-type monatin (a (2S,4S) substance) which has been said to be 800- to 1400-fold of sucrose as well as to determine the intensity of sweet taste of other three stereoisomers whereby the practical value of those stereoisomers as sweeteners are made clear. Further, it is also an important problem that, for crystals of each of the salts of those stereoisomers, characteristics such as various physical properties are made clear so that practical value from the view of physical properties is made clear.

In the meanwhile, as mentioned already, the natural-type monatin shows a (2S,4S) substance in its steric structure while, in the present invention, all compounds having the same chemical structure formula are called "monatin" and, therefore, the non-natural-type stereoisomers of monatin are called "stereoisomer of the natural-type monatin", "non-natural-type monatin", "(2S,4R) substance of monatin", "(2R,4S) substance of monatin" or "(2R,4R) substance of monatin", etc. Further, monatin (a (2S,4S) substance) is added to such stereoisomers and they are called "four kinds of stereoisomers" and, particularly, the natural-type monatin is called "monatin", "monatin (a (2S,4S) substance)" or "a (2S,4S) substance of monatin", etc.

(Means for Solving the Problems)

In order to solve the above-mentioned problems, the

present inventors have carried out intensive investigations for monatin. Firstly, according to a method by K. Nakamura, et al., stereoisomers (a mixture of isomers) of a (2S,4S) substance of monatin with a (2S,4R) substance of monatin and those of a (2R,4R) substance of monatin with a (2R,4S) substance of monatin were synthesized and, from those stereoisomer mixtures, the aimed stereoisomer was separated and prepared by means of a reversed phase HPLC (high-performance liquid chromatography). A stereoisomer prepared by such a method was also crystallized as a salt with various kinds of bases by the method which will be mentioned later whereby crystals of higher purity were able to be prepared. After that, optical purity of those stereoisomers was confirmed by means of an optically active column HPLC method. Further, intensity of sweet taste of the resulting four kinds of stereoisomers of monatin was measured and determined using sucrose as a standard solution.

As a result, it has been found that the intensity of the sweet taste of the natural-type monatin (a (2S,4S) substance) is far lower than the data which were reported already, that all of other non-natural-type stereoisomers have stronger intensity of sweet taste than monatin (a (2S,4S) substance) and that some of them show far better intensity of sweet taste as compared with the (2S,4S) substance and are excellent as sweeteners.

Thus, it has been newly found that a (2R,4S) substance

of monatin and a (2R,4R) substance of monatin show about 1300-fold and about 2700-fold intensity of sweet taste, respectively, as compared with a 5% aqueous solution of sucrose and that, among the non-natural-type stereoisomers, the (2R) substances are particularly excellent as sweeteners.

Then, a method where a mixture of stereoisomers of monatin prepared by the above synthetic method, etc. is crystallized as salts with various bases, a method where the mixture is crystallized as derivatives, a method where the derivative is separated by means of an optical isomer separation column, etc. were investigated and we have also succeeded in preparing each stereoisomer as an optically pure substance by those methods as well. We have found various crystallizing conditions for each stereoisomer and a mixture thereof prepared as such, succeeded in preparing crystals of various salts for the first time and been able to measure various physical property data such as intensity of sweet taste. It has been found that crystals of those salts with bases which have been firstly prepared at this time, particularly, crystals of non-natural-type stereoisomer salts of monatin have excellent properties in view of easiness in separation/purification, stability in the crystalline state, etc. as compared with conventionally known crystals of free compounds such as a (2S,4S) substance mentioned in the report by R. Vleggaar, et al. and amorphous substances of various salts

which were known only as amorphous solid by the patent document of P. J. van Wyk, et al. Potassium salt of a (2R,4R) substance of monatin having a particularly strong intensity of sweet taste is excellent as the most practical product form in such views that an organic solvent used for crystallization is not contained as an adhered solvent (such as an alcohol of crystallization), that stability upon heating is excellent, that the product is hardly colored as compared with a free compound where crystallization is conducted under an acidic condition, etc.

It has been further found that sweeteners, beverages/foods, etc. can be provided using the crystals of non-natural-type stereoisomer salts of monatin.

The present invention has been achieved on the basis of various findings as mentioned above.

As such, in accordance with the present invention, separation and purification of monatin (a (2S,4S) substance) which is a natural stereoisomer, and separation and purification of four kinds of stereoisomers (optical isomers) having the same chemical structural formula including that and confirmation of their optical purities have been carried out for the first time and, at the same time, more correct sweetness intensity of all of stereoisomers (four kinds) of monatin has been made clear. As a result,, it has been found that novel crystals of the non-natural-type stereoisomer salts of monatin

are excellent as sweet taste substances (sweeteners) and also as a practical product form as sweet taste substances (sweeteners) as compared with the conventionally prepared crystals of free compounds such as a (2S,4S) substance and amorphous solid of salts mentioned in the above patent and accordingly that crystals of non-natural-type stereoisomer salts of monatin are useful as sweeteners. It has been also confirmed that crystals of a salt of a (2R,4R) substance of monatin have sweetness intensity which is the strongest among the four stereoisomers and is unable to be predicted from the conventional findings and that they are most suitable as sweeteners or ingredients therefor which are subjected to a practical application.

Thus, as an embodiment, the present invention relates to crystals of salt of non-natural-type stereoisomer of monatin. The present invention relates to crystals of salt of non-natural-type stereoisomer of monatin which is a sweetener (sweet taste substance) of an amino acid type and the stereoisomer salt includes at least one member of salt of a (2R,4R) substance, salt of a (2R,4S) substance and salt of a (2S,4R) substance of monatin.

In the present invention, a compound which constitutes the crystals of the stereoisomer salt is a salt of the above-mentioned non-natural-type stereoisomer and it may be in a form of hydrate, solvate, etc. thereof. Further, the

compound may be in a form of lactone or lactam of such a monatin salt cyclized in a molecule and/or in a form where at least one of functional groups contained therein is protected.

There is no particular limitation for type, form, etc. of the salt contained in the crystals of the present invention. When they are used for food as the final product, it goes without saying that a salt which is able to be used as food is adopted. Besides the above, salt which is suitable as an intermediate is also useful. Such a thing (the above-mentioned form of the crystals and type, form, etc. of the salt) is not only applied to the case of the form of non-natural-type stereoisomer salt (crystals) of monatin but also similarly applied to that of the form of natural-type stereoisomer salt (crystals) of monatin when used by mixing which will be mentioned later.

The form of such a salt includes, for example, a salt with alkaline metal such as lithium, sodium and potassium, a salt with alkaline earth metal such as calcium and magnesium, an ammonium salt with ammonia, etc., a salt with amino acid such as lysine and arginine, a salt with inorganic acid such as hydrochloric acid and sulfuric acid, a salt with organic acid such as citric acid and acetic acid and a salt with other sweetener or a component thereof such as saccharin, acesulfame, cyclamic acid, glycyrrhizic acid and aspartame and such a thing is also applicable, as mentioned above, to a salt of the stereoisomer used in the present invention and to a salt of

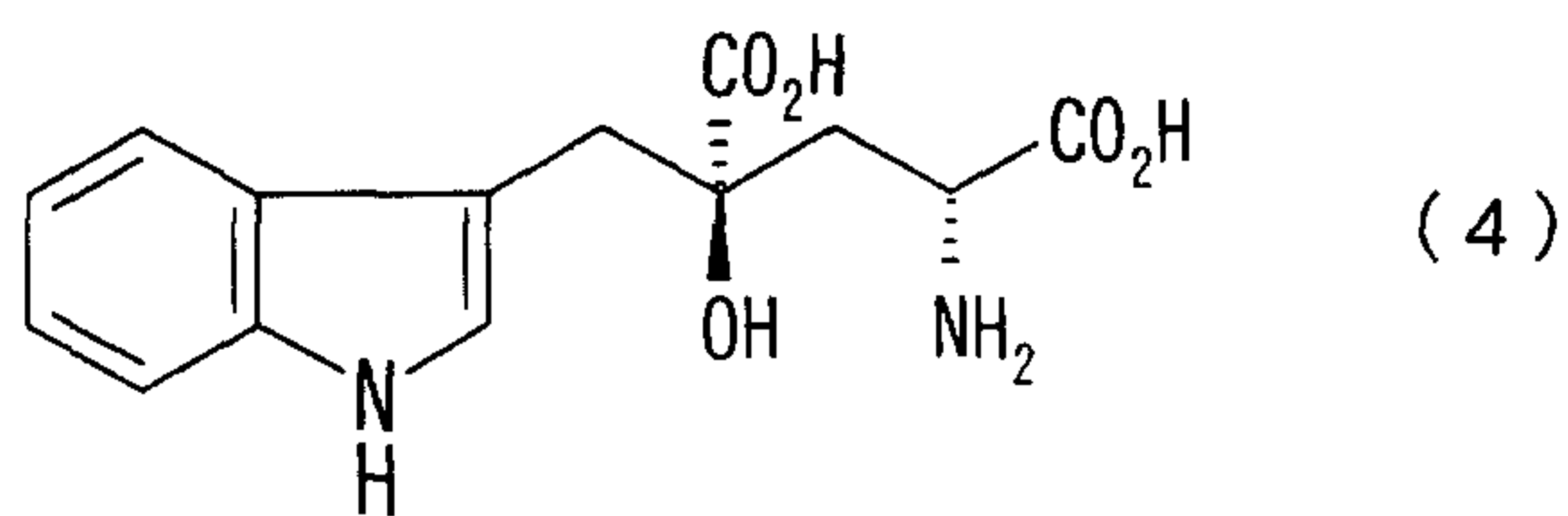
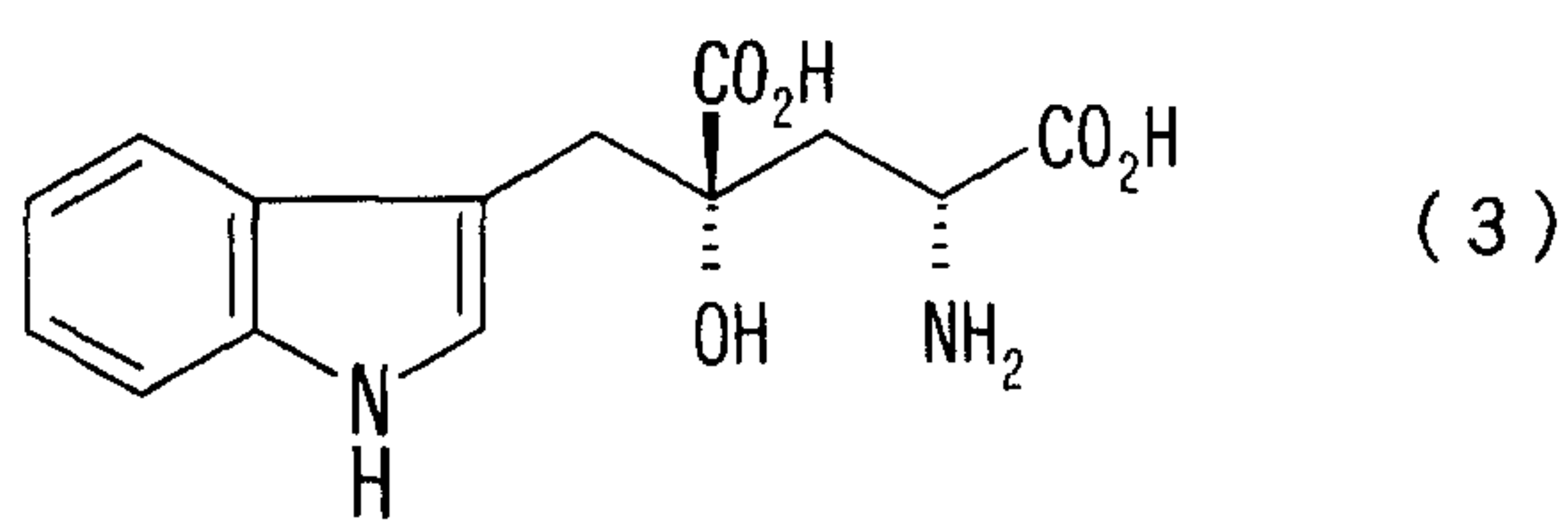
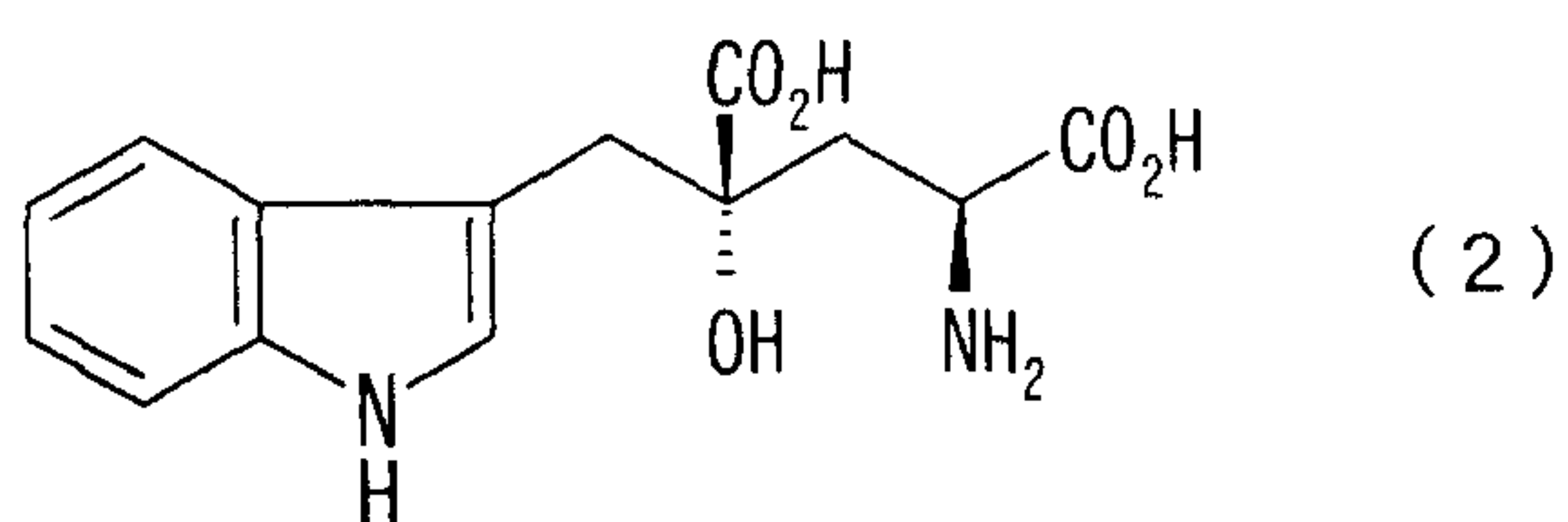
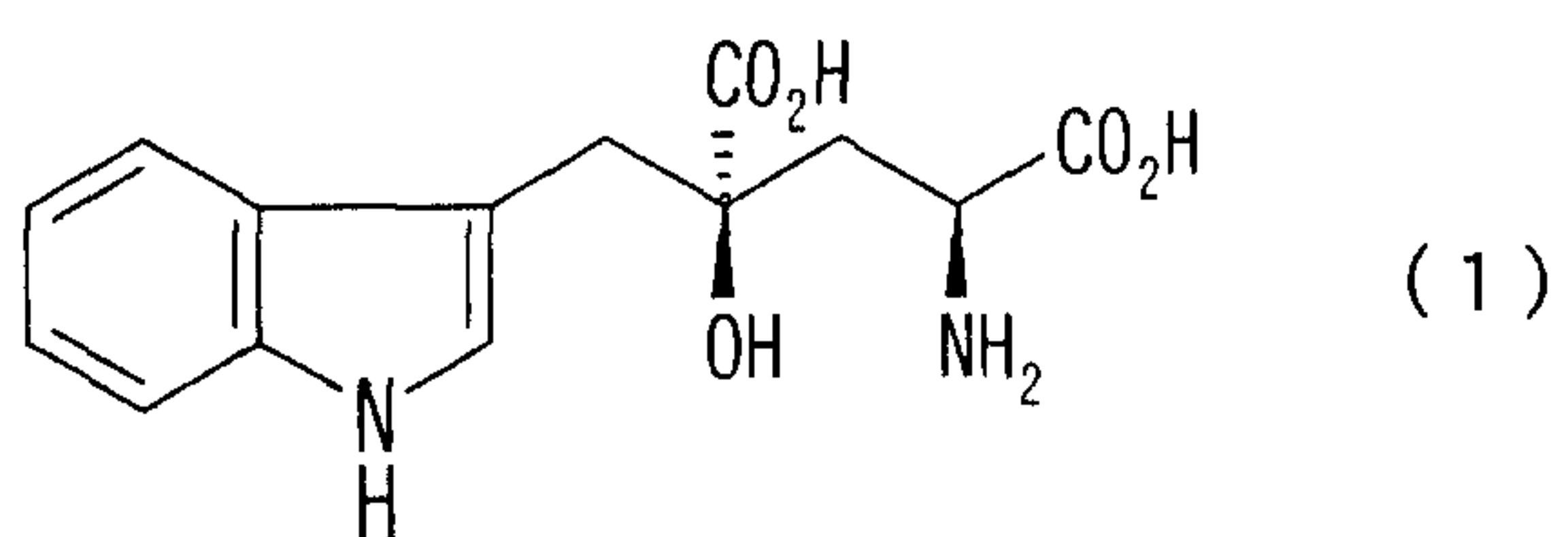
a natural-type monatin when used.

With regard to a method for the production of the above salt, a desired salt may be prepared on the basis of the explanation (such as Examples) for the present invention utilizing, if necessary, commonly-used or publicly-known method for the production of salts.

The above-mentioned stereoisomer including the natural-type monatin when used contains functional groups such as carboxyl group, hydroxyl group, amino group and indolyl (indole) group and such a functional group may be protected. With regard to the protecting group, it is possible to use a protecting group which is commonly used in organic chemistry, particularly in amino acid and peptide chemistry as a protecting group for each of them.

The crystals of the present invention include the following contents [1] to [6].

Incidentally, a (2S,4S) substance which is a natural-type monatin and a (2S,4R) substance, a (2R,4R) substance and a (2R,4S) substance which are non-natural-type monatins are represented by the following structural formulae (1), (2), (3) and (4).



[1]

With regard to the non-natural-type stereoisomer salt, salt of a (2S,4R) substance, salt of a (2R,4R) substance and salt of a (2R,4S) substance of monatin are listed. Crystals of the salt may be in a form of a hydrate or a solvate or the like.

Among those stereoisomer salts, a (2R,4S) substance and a (2R,4R) substance of monatin are more preferred and a (2R,4R) substance of monatin is most preferred. Crystals of the salt as such covered by the present invention are preferred because of their easiness in isolation and purification and excellence in stability upon preservation.

[2]

The above-mentioned crystals of the non-natural-type stereoisomer salt of the present invention may have the salt of the stereoisomer (including the form of hydrate, solvate, etc.) preferably at least about 95% or, more preferably, at least about 97% of chemical purity.

[3]

The above-mentioned crystals of the non-natural-type stereoisomer salt of the present invention may have preferably at least about 90%, more preferably at least about 94% and, still more preferably at least about 98% of optical purity. For example, a highly optically pure product of salt of a (2R,4R) substance of monatin (including the form of hydrate, solvate and a mixture of the salts) may be listed.

[4]

With regard to the above-mentioned crystals of stereoisomer salt of the present invention, crystals of a mixture of at least two members selected from stereoisomer salts of a (2S,4R) substance, a (2R,4R) substance and a (2R,4S)

substance of monatin may be adopted.

[5]

With regard to the above-mentioned crystals of non-natural-type stereoisomer salt of the present invention, those having sweetness intensity of preferably at least about 200-fold or, more preferably, at least about 1000-fold of that of sucrose in a practical concentration of 5-10% may be adopted.

[6]

The above-mentioned crystals of the stereoisomer salt of the present invention may be used in a mixed form with crystals of salt of a natural-type monatin (a (2S,4S) substance). In that case, a (2S,4S) substance of the monatin may be contained preferably in not more than about 70% or, more preferably, in not more than about 50% in the total monatin.

A compound which constitutes the crystals of the natural-type monatin salt is a salt of a natural-type monatin and it is also possible to use in a form of hydrate, solvate, etc. thereof. It is still also possible that the compound is used as a salt of monatin as such in a form of lactone or lactam where cyclization takes place in a molecule and/or in a form where at least one of the functional groups contained therein is protected.

Even in that case, it is possible to prepare the aimed mixture crystals in preferably at least about 200-fold or, more preferably, at least about 1000-fold of sucrose of the

above-mentioned practical concentration in terms of sweetness intensity of the mixture crystals in a state of crystals of salt including the natural-type stereoisomer.

With regard to the preferred examples of the above-mentioned ones, crystals of a mixture of salts of a (2S,4S) substance of monatin with a (2R,4R) substance of monatin or, particularly, crystals of a mixture of a (2S,4S) substance salt of monatin with a (2R,4R) substance salt of monatin in a ratio (by weight) of about 1:0.5-2 or, particularly, preferably about 1:1 may be exemplified.

As another embodiment, the present invention also relates to a sweetener which is characterized in containing the above-mentioned crystals of the non-natural-type stereoisomer salts of the present invention (including any of the above [1] to [6]).

The sweetener may contain a carrier and/or a filler (bulking agent), etc. for sweeteners.

There may be contained a carrier or a filler which has been known to be used for sweeteners or will be developed for such a purpose. It is also and of course possible to contain an additive which is able to be used for sweeteners. The sweetener is used for animals such as mammals, particularly for humans.

As still another embodiment, the present invention also relates to a product such as beverage and food to which sweet

taste is given which is characterized in containing the above-mentioned crystals of salt of non-natural-type stereoisomer of the present invention (including any of the above-mentioned [1] to [6]).

The crystals may be used at least as a part of a sweetener for products for animals, particularly for beverages and foods for humans to which sweet taste is requested. Besides the above, the crystals may be also used for the purpose of oral hygiene such as dentifrice and drugs or for orally-used products with the purpose of giving sweet taste.

The above-mentioned products such as sweetener and beverage/food according to the present invention may also contain at least one member (one compound) selected from other sweetener components (sweetening agents), particularly saccharides and other artificial and natural sweeteners. For example, sucrose, aspartame, acesulfame, sucralose, saccharin, stevioside, xylose, trehalose, sorbitol, maltitol and the like may be used together.

Brief Description of the Drawings

[Fig. 1]

Fig. 1 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized solution of potassium salt crystals of a (2R,4R) substance of monatin in Example 13.

[Fig. 2]

Fig. 2 shows an optical microphotograph (magnification: 200-power) after drying of potassium salt crystals of a (2R,4R) substance of monatin in Example 13.

[Fig. 3]

Fig. 3 is a powder X-ray diffraction chart after drying of potassium salt crystals of a (2R,4R) substance of monatin in Example 13.

Ordinate and abscissa show diffraction intensity and diffraction angle 2θ [deg], respectively (they are the same in the following powder X-ray diffraction charts as well).

[Fig. 4]

Fig. 4 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized solution of sodium salt crystals of a (2R,4R) substance of monatin in Example 14.

[Fig. 5]

Fig. 5 shows an optical microphotograph (magnification: 200-power) after drying of sodium salt crystals of a (2R,4R) substance of monatin in Example 14.

[Fig. 6]

Fig. 6 is a powder X-ray diffraction chart after drying of sodium salt crystals of a (2R,4R) substance of monatin in Example 14.

[Fig. 7]

Fig. 7 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized solution of ammonium salt crystals of a (2R,4R) substance of monatin in Example 15.

[Fig. 8]

Fig. 8 shows an optical microphotograph (magnification: 200-power) after drying of ammonium salt crystals of a (2R,4R) substance of monatin in Example 15.

[Fig. 9]

Fig. 9 is a powder X-ray diffraction chart after drying of ammonium salt crystals of a (2R,4R) substance of monatin in Example 15.

[Fig. 10]

Fig. 10 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized solution of crystals of a free (2R,4R) substance of monatin in Comparative Example 1.

[Fig. 11]

Fig. 11 shows an optical microphotograph (magnification: 200-power) after drying of crystals of a free (2R,4R) substance of monatin in Comparative Example 1.

[Fig. 12]

Fig. 12 is a powder X-ray diffraction chart after drying of crystals of a free (2R,4R) substance of monatin in Comparative Example 1.

[Fig. 13]

Fig. 13 shows an optical microphotograph (magnification: 200-power) after drying of amorphous solid of potassium salt of a (2R,4R) substance of monatin in Comparative Example 2.

[Fig. 14]

Fig. 14 is a powder X-ray diffraction chart after drying of amorphous solid of potassium salt of a (2R,4R) substance of monatin in Comparative Example 2.

[Fig. 15]

Fig. 15 is a powder X-ray diffraction chart after drying of crystals of potassium salt of a (2R,4R) substance of monatin in Example 17.

[Fig. 16]

Fig. 16 is a powder X-ray diffraction chart after drying of crystals of potassium salt of a (2R,4R) substance of monatin in Example 18.

[Fig. 17]

Fig. 17 shows an optical microphotograph (magnification: 200-power) after drying of ammonium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin in Example 2.

[Fig. 18]

Fig. 18 is a powder X-ray diffraction chart after drying of ammonium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin in Example 2.

[Fig. 19]

Fig. 19 shows an optical microphotograph (magnification: 200-power) after drying of ammonium salt crystals of [(2S,4R) + (2R,4S)] substances of monatin in Example 2.

[Fig. 20]

Fig. 20 is a powder X-ray diffraction chart after drying of ammonium salt crystals of [(2S,4R) + (2R,4S)] substances of monatin in Example 2.

[Fig. 21]

Fig. 21 shows an optical microphotograph (magnification: 200-power) after drying of sodium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin in Example 3.

[Fig. 22]

Fig. 22 is a powder X-ray diffraction chart after drying of sodium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin in Example 3.

[Fig. 23]

Fig. 23 shows an optical microphotograph (magnification: 200-power) after drying of potassium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin in Example 4.

[Fig. 24]

Fig. 24 is a powder X-ray diffraction chart after drying of potassium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin in Example 4.

[Fig. 25]

Fig. 25 shows an optical microphotograph (magnification:

200-power) after drying of sodium salt crystals of a (2R,4S) substance of monatin in Example 11.

[Fig. 26]

Fig. 26 is a powder X-ray diffraction chart after drying of sodium salt crystals of a (2R,4S) substance of monatin in Example 11.

[Fig. 27]

Fig. 27 shows an optical microphotograph (magnification: 200-power) after drying of sodium salt crystals of a (2S,4R) substance of monatin in Example 12.

[Fig. 28]

Fig. 28 is a powder X-ray diffraction chart after drying of sodium salt crystals of a (2S,4R) substance of monatin in Example 12.

[Fig. 29]

Fig. 29 is a drawing which shows steam adsorption and desorption (adsorption/desorption) curves of potassium salt (crystallizing temperature: 10°C, 35°C, 60°C) of a (2R,4R) substance of monatin in Example 19.

Ordinate: Moisture (% by weight); abscissa: relative humidity (%)

Δ: adsorbed at 10°C; ▲: desorbed at 10°C; ◇: adsorbed at 35°C; ◆: desorbed at 35°C; ○: adsorbed at 60°C; ●: desorbed at 60°C.

[Fig. 30]

Fig. 30 is a powder X-ray diffraction chart after drying of sodium salt crystals of a (2R,4R) substance of monatin in Example 20.

[Fig. 31]

Fig. 31 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example A.

[Fig. 32]

Fig. 32 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example B.

[Fig. 33]

Fig. 33 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example C.

[Fig. 34]

Fig. 34 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example D.

[Fig. 35]

Fig. 35 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example E.

[Fig. 36]

Fig. 36 is a powder X-ray diffraction chart of wet

potassium salt crystals of a (2R,4R) substance of monatin in Example F.

[Fig. 37]

Fig. 37 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example G.

[Fig. 38-1]

Fig. 38-1 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example H.

[Fig. 38-2]

Fig. 38-2 is a powder X-ray diffraction chart after drying of potassium salt crystals of a (2R,4R) substance of monatin in Example H.

[Fig. 39-1]

Fig. 39-1 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example I.

[Fig. 39-2]

Fig. 39-2 is a powder X-ray diffraction chart after drying of potassium salt crystals of a (2R,4R) substance of monatin in Example I.

[Fig. 40-1]

Fig. 40-1 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in

Example J.

[Fig. 40-2]

Fig. 40-2 is a powder X-ray diffraction chart after drying of potassium salt crystals of a (2R,4R) substance of monatin in Example J.

[Fig. 41-1]

Fig. 41-1 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example K.

[Fig. 41-2]

Fig. 41-2 is a powder X-ray diffraction chart after drying of potassium salt crystals of a (2R,4R) substance of monatin in Example K.

[Fig. 42]

Fig. 42 is a powder X-ray diffraction chart of wet potassium salt crystals of a (2R,4R) substance of monatin in Example L.

[Fig. 43]

Fig. 43 is a powder X-ray diffraction chart of potassium salt solid of a (2R,4R) substance of monatin in Example M.

[Fig. 44]

Fig. 44 is a powder X-ray diffraction chart of potassium salt crystals of a (2R,4R) substance of monatin in Example N.

[Fig. 45]

Fig. 45 is a powder X-ray diffraction chart of potassium

salt crystals of a (2R,4R) substance of monatin in Example O.

[Fig. 46]

Fig. 46 is a powder X-ray diffraction chart of potassium salt crystals of a (2R,4R) substance of monatin in Example P.

[Fig. 47]

Fig. 47 is a powder X-ray diffraction chart of potassium salt crystals of a (2R,4R) substance of monatin in Example Q.

Mode for Carrying Out the Invention

As hereunder, mode for carrying out the present invention will be illustrated.

Monatin of (2S) substances (a mixture of a (2S,4S) substance and a (2S,4R) substance of monatin), monatin of (2R) substances (a mixture of a (2R,4R) substance and a (2R,4S) substance of monatin) and a mixture of the four stereoisomers can be synthesized according to the above-mentioned method by K. Nakamura, et al. and each stereoisomer is able to be separated therefrom and, with regard to a synthetic method for monatin containing various stereoisomers, other methods may be adopted and it is not limited to the above-mentioned method by K. Nakamura, et al.

Hereinafter, examples for the case where each stereoisomer is separated and purified from monatin containing various stereoisomers will be briefly illustrated. They are illustrated as examples and the present invention is not

limited to those examples.

With regard to stereoisomers of monatin of (2S) substances and monatin of (2R) substances synthesized according to a method by K. Nakamura, et al., they are able to be separated and prepared by means of a reversed phase HPLC but a method for resolving the stereoisomers of monatin is not limited thereto.

It is also possible that the four stereoisomers synthesized by the already-reported method, etc. are separated to give each stereoisomer. For example, it is possible to use a method where the four stereoisomers of monatin are crystallized as a salt or a derivative and a method where the derivatives are separated by a column of separation of optical isomers and the resulting isomer is returned to monatin again although the resolving method for the stereoisomers is not limited thereto.

The component which constitutes the crystals of the present invention is the above-mentioned monatin salt and, as mentioned above, it is also possible to use in other forms such as a lactone derivative or a lactam derivative thereof. For example, in forming a lactone or lactam derivative where cyclization takes place in a molecule in the monatin salt, that can be carried out by utilizing a method which has been known as a method for forming lactone or lactam being cyclized in a molecule. Further, in the case of formation of a derivative

where at least one of functional groups is protected, it is also possible to do that by utilizing a method which has been known as a method for protection of functional group.

Optical purity of the crystals of each stereoisomer obtained is able to be determined by means of an optical activity column HPLC method but a method for determination of optical purity is not limited thereto.

Intensity of sweet taste (sweetening potency; degree of sweet taste) of the crystals of each stereoisomer obtained is able to be determined by comparing with a 5% aqueous solution of sucrose but a method for the determination of intensity of sweet taste is not limited thereto.

Now the relation between the structure of each stereoisomer and the intensity of sweet taste or the optical purity is shown in Tables 1 and 2. Tables 1 and 2 show the evaluated results for the samples which are prepared by means of an HPLC, purified by ion-exchange resin and freeze-dried as an ammonium salt and for those which are crystallized and purified as a sodium salt, respectively.

[Table 1]

Stereoisomers	Sweetness Intensity ¹⁾	Optical Purity ²⁾
(2S,4S)	ca. 300	94
(2S,4R)	ca. 300	94
(2R,4R)	ca. 2000	94
(2R,4S)	ca. 800	96

1) The data as compared with a 5% aqueous solution of sucrose.

2) Most of other stereoisomers contained were enantiomers.

[Table 2]

Stereoisomers	Sweetness Intensity ¹⁾	Optical Purity
(2S,4S)	ca. 50	99.8
(2S,4R)	ca. 300	99.4
(2R,4R)	ca. 2700	99.3
(2R,4S)	ca. 1300	99.2

1) The data as compared with a 5% aqueous solution of sucrose.

As shown in Table 2, it is noted that the sweetness intensity of monatin being present in nature (a natural-type one) (a (2S,4S) substance) is about 50-fold and is lower than the already-reported data. Interestingly, all of other three stereoisomers of monatin show higher sweetness intensity than monatin (a (2S,4S) substance) and, particularly, a (2R,4R) substance of monatin shows the strongest sweetness intensity of as high as about 2700-fold.

From various stereoisomers which are separated by chromatography, etc. or prepared by a synthetic method, etc., it is possible to separate a salt of each stereoisomer as crystals of a high purity by means of further crystallization.

In the comparison of the results of evaluation as shown in the above Tables 1 and 2, purity including optical purity is improved by crystallization for the (2R) substances whereby the utility by making into salt crystals is shown.

Incidentally, when the crystals of the non-natural-type

stereoisomer salt of the present invention (including hydrate, solvate, etc.) (the stereoisomer includes a form of lactone or lactam where cyclization takes place in a molecule, a form where at least one of the contained functional groups is protected, etc.) are used as a sweetener, it goes without saying that they may be used together with other sweetener(s) unless there is any particular inconvenience.

When crystals of the stereoisomer salt of the present invention (including hydrate, solvate, etc.) are used as a sweetener, it is possible to use a carrier and/or a filler (bulking agent) if necessary and, for example, it is possible to use a carrier, a filler, etc. which have been known or used for sweeteners already.

Although crystals of stereoisomer salt of the present invention (including hydrate, solvate, etc.) may be used as a sweetener or a component for a sweetener, it is also possible that the crystals may be used as a sweetener or a sweet taste ingredient by compounding with the products such as beverage and food which are needed to be given with a sweet taste such as confectionery, chewing gum, sanitary product, cosmetics, drugs and also with products for animals except humans. It is further possible that crystals of the non-natural-type stereoisomer salt are used as a form of a product to which sweet taste is given by containing or using the non-natural-type stereoisomer salt crystals of the present invention or as a

sweetness-giving component in a method for giving sweet taste to the product which is needed to be given with a sweet taste. With regard to a method for using them, etc., that may be conducted by a conventional method which has been commonly used as a method for using or producing a sweetener or by other known method.

Preferred Embodiments of the Invention

The present invention will now be illustrated in detail by way of the following Examples and Comparative Examples although the present invention is not limited to those Examples.

¹H-NMR spectrum was measured by a Bruker Avance 400 (400 MHz) while MS spectrum was measured by a Thermo Quest TSQ 700. With regard to cation-exchange resin, Amberlite IR120B H AG and Diaion PK 228 were used. With regard to powder X-ray diffraction, measurement was carried out by PW 3050 manufactured by Phillips. With regard to melting point, measurement was carried out by Micro Melting Point Apparatus of Yanaco. With regard to optical rotary power, measurement was carried out by DIP-370 Digital Polarimeter manufactured by Nippon Bunko (Jasco Engineering).

(Example 1)

Monatin was synthesized according to a method by K. Nakamura, et al. (cf. K. Nakamura, et al., Organic Letters,

2, 2967-2970 (2000)), finally adsorbed with a cation-exchange resin (H^+ type) and subjected to purification by means of elution with a 3% aqueous ammonia and freeze-drying to give 2.92 g of ammonium salts of monatin of (2S) substances (a mixture of a (2S,4S) substance and a (2S,4R) substance of monatin) and 711 mg of ammonium salts of monatin of (2R) substances (a mixture of a (2R,4R) substance and a (2R,4S) substance of monatin).

Resolution of 660 mg of monatin of (2S) substances and 711 mg of monatin of (2R) substances respectively was carried out according to the following preparative condition to give 207 mg of a (2S,4S) substance of monatin, 233 mg of a (2S,4R) substance of monatin, 261 mg of a (2R,4R) substance of monatin and 254 mg of a (2R,4S) substance of monatin as amorphous solid in a form of ammonium salts.

(Preparative Conditions)

Guard column: Inertsil ODS-3 30 × 50 mm;

Column: Inertsil ODS-3 30 × 250 mm;

Detection: UV 210 nm;

Eluent: <A> acetonitrile 0.05% TFA, H_2O 0.05% TFA;

Flow rate: 28 ml/min;

Gradient: 12% to 18% of <A> within 25 minutes;

Loaded amount: 10-13 mg; and

Temperature upon working: 25°C.

(Treatment after the Preparation)

The preparation fraction was neutralized with aqueous ammonia and concentrated. The preparation fractions were combined, concentrated and adsorbed with a cation-exchange resin (Amberlite IR 120B H AG; H⁺ type). This was eluted with a 5% aqueous ammonia and the eluted fraction was freeze-dried.

Optical purity (enantiomer purity) of each stereoisomer was tested by the following analytical conditions.

(Analytical Conditions)

Column: Crownpak CR(+) 4 × 150 mm;

Detection: UV 210 nm;

Eluent: Aqueous perchloric acid (pH 2.0)/methanol = 90/10;

Flow rate: 1.2 ml/min; and

Temperature for analysis: 25°C.

Optical purity of each stereoisomer was as follows. Data in () show the eluting time for each peak.

(2S,4S) substance of monatin: 94% (45.0 min);

(2S,4R) substance of monatin: 94% (26.1 min);

(2R,4R) substance of monatin: 94% (20.9 min); and

(2R,4S) substance of monatin: 96% (16.1 min).

The main impurity contained in each stereoisomer was an enantiomer thereof. For example, a (2S,4S) substance of monatin contained a few amount of a (2R,4R) substance of monatin.

Intensity of sweet taste for each stereoisomer obtained

was measured by the following method.

A 0.05% aqueous solution of each stereoisomer was prepared and diluted to an appropriate extent to prepare an aqueous solution of a sample of a predetermined concentration. Separately, a 5% aqueous solution of sucrose was prepared and used as a standard solution. The diluted aqueous solution of the sample and the standard aqueous sucrose solution were tasted one after another and the sweetness rate was determined from the sample concentration where the intensities of sweetness were noted to be same. The evaluation was carried out by seven panelists.

Intensity of the sweet taste for each stereoisomer was decided to be as follows.

(2S,4S) substance of monatin: about 300-fold;
(2S,4R) substance of monatin: about 300-fold;
(2R,4R) substance of monatin: about 2000-fold; and
(2R,4S) substance of monatin: about 800-fold.

Result of measurement of optical rotary power ($[\alpha]_D^{25}$ (c = 0.5)) for each stereoisomer was as follows.

(2S,4S) substance of monatin: -44.1 (H₂O), -7.5 (3% aqueous ammonia);

(2S,4R) substance of monatin: -7.8 (H₂O);

(2R,4R) substance of monatin: +3.7 (H₂O), +8.7 (3% aqueous ammonia); and

(2R,4S) substance of monatin: +11.1 (H₂O).

^1H -NMR of each stereoisomer was as follows (400 MHz, D_2O).

Ammonium salts of (2S,4S) substance and (2R,4R) substance of monatin:

1.96 (1H, dd, $J = 11.8 \text{ Hz}$), $J = 15.2 \text{ Hz}$), 2.57 (1H, dd, $J = 1.9 \text{ Hz}$, $J = 15.2 \text{ Hz}$), 3.00 (1H, d, $J = 14.6 \text{ Hz}$), 3.20 (1H, d, $J = 14.6 \text{ Hz}$), 3.54 (1H, d, $J = 10.2 \text{ Hz}$), 7.04 (1H, t, $J = 7.2 \text{ Hz}$), 7.10 (1H, t, $J = 7.2 \text{ Hz}$), 7.10 (1H, s), 7.38 (1H, d, $J = 8.0 \text{ Hz}$), 7.62 (1H, d, $J = 8.0 \text{ Hz}$).

Ammonium salts of (2S,4R) substance and (2R,4S) substance of monatin:

2.11 (1H, dd, $J = 10.4 \text{ Hz}$, $J = 15.0 \text{ Hz}$), 2.37 (1H, d, $J = 15.4 \text{ Hz}$), 3.13 (2H, s), 3.88 (1H, d, $J = 9.8 \text{ Hz}$), 7.05 (1H, d, $J = 7.6 \text{ Hz}$), 7.14 (2H, s), 7.38 (1H, d, $J = 7.9 \text{ Hz}$), 7.63 (1H, d, $J = 7.9 \text{ Hz}$).

MS spectrum of each stereoisomer was as follows.

ESI-MS: 291 (M-H^-).

(Example 2)

[Resolution of a mixture of ammonium salts of four stereoisomers of monatin into racemic crystals of a (2S,4S) substance and a (2R,4R) substance and racemic crystals of a (2S,4R) substance and a (2R,4S) substance]

Ammonium salts (10.00 g; 32.33 mmol) of a mixture of steric (optical) isomers of monatin (monatin [(2S,4S) + (2R,4R) substances] : monatin [(2S,4R) + (2R,4S) substances] = 6:4) were dissolved in 100 ml of a 2.5% aqueous ammonia and the

resulting solution was concentrated to an extent of 20 ml. Newly, 3 ml of a 5% aqueous ammonia were added thereto and the mixture was homogenized and allowed to stand at room temperature for 30 minutes. After the crystals were separated out (produced), the mixture was made into a slurry by addition of 80 ml of an aqueous solution comprising a 5% aqueous ammonia and ethanol (25/75) and crystals of ammonium salts of (2S,4S) and (2R,4R) substances of monatin were filtered. The resulting crystals were dissolved in 30 ml of a 2.5% aqueous ammonia once again, concentrated and recrystallized from 0.5 ml of a 5% aqueous ammonia and 30 ml of ethanol to give 4.80 g (15.52 mmol; reversed phase HPLC purity (hereinafter, referred to as "HPLC purity"): 98.0%) of crystals of ammonium salts of (2S,4S) and (2R,4R) substances of monatin.

$^1\text{H-NMR}$ (D_2O) δ : 1.95-2.02 (m, 1H), 2.58-2.62 (m, 1H), 3.01-3.05 (m, 1H), 3.21-3.24 (m, 1H), 3.55-3.58 (m, 1H), 7.07-7.11 (m, 1H), 7.14-7.18 (m, 2H), 7.42-7.44 (d, 1H), 7.66-7.68 (d, 1H).

ESI-MS: 291.39 (M-H) $^-$.

Melting point: 182-186°C.

Degree of sweet taste: about 1300-fold (as compared with a 5% aqueous solution of sucrose).

Fig. 17 shows an optical microphotograph (magnification: 200-power) after drying of ammonium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin.

Fig. 18 shows a powder X-ray diffraction chart after drying of ammonium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin. Characteristic diffraction X-ray peaks were noted at the angles of diffraction (2θ , CuK α line) of 6.0°, 12.1°, 15.2°, 18.6°, 21.3°, 23.2° and 25.0°.

The filtrate ([(2S,4S) + (2R,4R) substances of monatin] : [(2S,4R) + (2R,4S) substances of monatin] = 3:10) obtained in the above operation was concentrated to an extent of 5 ml. Newly, 3 ml of a 5% aqueous ammonia were added thereto and the mixture was homogenized and allowed to stand at room temperature for 10 minutes. After the crystals were separated out, the mixture was made into a slurry by addition of 80 ml of ethanol and crystals of ammonium salts of [(2S,4R) + (2R,4S) substances of monatin] were filtered. The resulting crystals were dissolved in 30 ml of a 2.5% aqueous ammonia once again, concentrated and recrystallized for three times from 0.5 ml of a 5% aqueous ammonia and 30 ml of ethanol to give 3.10 g (10.02 mmol; HPLC purity: 98.2%) of crystals of ammonium salts of [(2S,4R) + (2R,4S) substances of monatin]. The overall recovery rate was 79.0%.

$^1\text{H-NMR}$ (D_2O) δ : 2.11-2.17 (m, 1H), 2.38-2.43 (m, 1H), 3.16 (s, 2H), 3.90-3.93 (m, 1H), 7.06-7.10 (m, 1H), 7.13-7.17 (m, 2H), 7.41-7.43 (d, 1H), 7.66-7.68 (d, 1H).

ESI-MS: 291.19 (M-H) $^-$.

Melting point: 167.2-168.4°C.

Degree of sweet taste: about 800-fold (as compared with a 5% aqueous solution of sucrose).

Fig. 19 shows an optical microphotograph (magnification: 200-power) after drying of ammonium salt crystals of [(2S,4R) + (2R,4S)] substances of monatin.

Fig. 20 shows a powder X-ray diffraction chart after drying of ammonium salt crystals of [(2S,4R) + (2R,4S)] substances of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.4°, 10.2°, 19.7°, 21.0° and 21.8°.

(Example 3)

[Preparation of crystals of sodium salts of racemic compounds (or a racemic mixture) comprising (2S,4S) and (2R,4R) substances of monatin]

Ammonium salts (1.00 g; 3.23 mmol; HPLC purity: 100%) of [(2S,4S) + (2R,4R)] substances of monatin were dissolved in 10 ml of water, an aqueous solution of sodium hydroxide (20 equivalents) was added thereto and the mixture was concentrated. An operation of addition of 20 ml of water followed by concentrating was repeated for three times, then the concentrate was dissolved in 20 ml of water again and ion-exchange resin (Amberlite IR 120B H AG (H⁺)) was added to make the solution weakly alkaline followed by stirring to remove an excessive sodium. After the resin was filtered off, the filtrate was concentrated in vacuo. The concentrate was

crystallized using a 95% aqueous solution of ethanol at room temperature to give 0.680 g (2.14 mmol) of crystals of a solvate of sodium salt of [(2S,4S) + (2R,4R)] substances of monatin with 0.05 molar equivalent of ethanol in a yield of 66.3%.

$^1\text{H-NMR}$ (D_2O) δ : the same as those in Example 2.

ESI-MS: 291.19 (M-H) $^-$.

Melting point: 201.7-203.2°C.

Fig. 21 shows an optical microphotograph (magnification: 200-power) after drying of sodium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin.

Fig. 22 shows a powder X-ray diffraction chart after drying of sodium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin. Characteristic diffraction X-ray peaks were noted at the angles of diffraction (2θ , $\text{CuK}\alpha$ line) of 4.4°, 13.6°, 15.2°, 16.7°, 22.2° and 24.4°.

(Example 4)

[Preparation of crystals of potassium salts of racemic compounds (or a racemic mixture) comprising (2S,4S) and (2R,4R) substances of monatin]

The same operation as in Example 3 was carried out except that an aqueous solution of potassium hydroxide was used in place of an aqueous solution of sodium hydroxide to give 0.71 g (2.13 mmol) of crystals of a solvate of potassium salt of [(2S,4S) + (2R,4R)] substances of monatin with 0.05 molar equivalent of ethanol in a yield of 65.9%.

^1H -NMR (D_2O) δ : the same as those in Example 2.

ESI-MS: 291.49 (M-H) $^-$.

Melting point: 223.8-224.7°C.

Fig. 23 shows an optical microphotograph (magnification: 200-power) after drying of potassium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin.

Fig. 24 shows a powder X-ray diffraction chart after drying of potassium salt crystals of [(2S,4S) + (2R,4R)] substances of monatin. Characteristic diffraction X-ray peaks were noted at the angles of diffraction (2θ , $\text{CuK}\alpha$ line) of 5.9°, 18.7°, 20.1° and 23.8°.

(Example 5)

[Preparation of Z-lactone (2-benzyloxycarbonylamino-4-(3-indolylmethyl)-4-carboxy- γ -butyrolactone) of a racemic compound (or a racemic mixture) of (2S,4S) substance with (2R,4R) substance of monatin]

An ammonium salt (19.51 g; 63.07 mmol; HPLC purity: 99.2%) of [(2S,4S) + (2R,4R) substances] of monatin was dissolved in 94.6 ml of a 2N aqueous solution of sodium hydroxide (189.2 mmol) and 90 ml of water. Benzyloxycarbonyl chloride (12.61 ml; 88.30 mmol) was added followed by stirring for 2 hours at room temperature and then 15.8 ml (31.54 mmol) of a 2N aqueous solution of sodium hydroxide and 4.50 ml (31.54 mmol) of benzyloxycarbonyl chloride were added followed by stirring for one night at room temperature. The resulting

aqueous reaction solution was subjected to an extracting operation with 50 ml of ether for three times to remove an excessive benzyloxycarbonyl chloride. The reaction solution was adjusted to pH 3 by hydrochloric acid and subjected to an extracting operation with 100 ml of ethyl acetate for three times and the organic layer was dried over anhydrous magnesium sulfate. Magnesium sulfate was removed by filtration and the filtrate was concentrated in vacuo to give 27.93 g (65.50 mmol) of Z-monatin (2-benzyloxycarbonylamino-4-hydroxy-4-carboxy-5-(3-indolyl)pentanoic acid), [(2S,4S) + (2R,4R)] substances. The Z-monatin [(2S,4S) + (2R,4R)] substances (27.93 g; 65.50 mmol) was dissolved in 400 ml of ethyl acetate and heated at 75°C for 3 hours after addition of 1.25 g (6.55 mmol) of p-toluenesulfonic acid thereto. The resulting reaction solution was washed with water and a saturated saline solution and dried over anhydrous magnesium sulfate. The magnesium sulfate was removed by filtration and the filtrate was concentrated in vacuo. To the residue were added 100 ml of chloroform and the crystals separated out therefrom were collected by filtration to give 17.64 g (43.19 mmol; HPLC purity: 99.6%) of Z-lactone [(2S,4S) + (2R,4R)] substances in an overall yield of 68.5%.

¹H-NMR (DMSO-d₆) δ: 2.36-2.42 (m, 1H), 2.64-2.70 (m, 1H), 3.24-3.41 (m, 2H), 3.71-3.81 (m, 1H), 4.98 (s, 2H), 6.97-7.00 (m, 1H), 7.04-7.09 (m, 1H), 7.21 (s, 1H), 7.30-7.33 (m, 5H),

7.54-7.56 (d, 1H), 7.66-7.69 (d, 1H), 11.03 (s, 1H).

ESI-MS: 409.68 (M + H)⁺.

Melting point: 195.5-196.9°C.

(Example 6)

[Preparation of Z-lactone (2-benzyloxycarbonylamino-4-(3-indolylmethyl)-4-carboxy-γ-butyrolactone) of a racemic compound (or a racemic mixture) of a (2S,4R) substance with a (2R,4S) substance of monatin]

The same operation as in Example 5 was carried out except that 15.00 g (48.49 mmol; HPLC purity: 99.5%) of ammonium salt of [(2S,4R) + (2R,4S)] substances of monatin was used in place of ammonium salt of [(2S,4S) + (2R,4R)] substances of monatin and that heating was carried out at 75°C for 2 hours after addition of p-toluenesulfonic acid to give 12.10 g (29.64 mmol; HPLC purity: 100%) of the Z-lactone [(2S,4R) + (2R,4S)] substances in an overall yield of 61.1%.

¹H-NMR (DMSO-d₆) δ: 2.31-2.37 (m, 1H), 2.71-2.76 (m, 1H), 3.19-3.23 (m, 1H), 3.43-3.47 (m, 1H), 4.34-4.41 (m, 1H), 5.05 (s, 1H), 6.96-7.00 (m, 1H), 7.04-7.08 (m, 1H), 7.14 (s, 1H), 7.32-7.37 (m, 5H), 7.53-7.55 (d, 1H), 7.85-7.87 (d, 1H), 10.95 (s, 1H).

ESI-MS: 409.58 (M + H)⁺.

Melting point: 156.7-159.1°C.

(Example 7)

[Resolution into Z-lactone (2S,4S) substance and Z-

lactone (2R,4R) substance]

Resolution by an optical isomer resolution column was carried out from 1.17 g (2.86 mmol; HPLC purity: 99.7%) Z-lactone [(2S,4S) + (2R,4R)] substances. At that time, CHIRALPAK AS (20 × 50 mm) and CHIRALPAK AS (20 × 250 mm) were used as a guard column and a preparative column, respectively. The preparation was carried out where eluent was hexane/ethanol/acetic acid (40/60/0.5), flow rate was 10 ml/minute, detection was by UV 210 nm, temperature was 40°C and loaded amount was 25 mg. Time for the elution was 13 minutes for Z-lactone (2S,4S) substances and 23 minutes for Z-lactone (2R,4R) substances, respectively. Each preparative fraction was concentrated, dissolved in 50 ml of ethyl acetate and concentrated again. The residue was crystallized from 30 ml of chloroform to give 428 mg (1.05 mmol) of Z-lactone (2S,4S) substance and 399 mg (0.977 mmol) of Z-lactone (2R,4R) substance (an overall recovery yield: 70.7%).

(Z-lactone (2S,4S) substance)

¹H-NMR (DMSO-d₆) δ: the same as those in Example 5.

ESI-MS: 409.68 (M + H)⁺.

Melting point: 179.8-182.0°C.

(Z-lactone (2R,4R) substance)

¹H-NMR (DMSO-d₆) δ: the same as those in Example 5.

ESI-MS: 409.88 (M + H)⁺.

Melting point: 179.2-182.8°C.

(Example 8)

[Resolution into Z-lactone (2S,4R) substance and Z-lactone (2R,4S) substance]

Resolution by an optical isomer resolution column was carried out from 9.89 g (24.22 mmol; HPLC purity: 100%) Z-lactone [(2S,4R) + (2R,4S)] substances. At that time, CHIRALCEL OJ (20 × 50 mm) and CHIRALCEL OJ (20 × 250 mm) were used as a guard column and a preparative column, respectively. The preparation was carried out where eluent was hexane/ethanol/trifluoroacetic acid (40/60/0.1), flow rate was 8 ml/minute, detection was by UV 210 nm, temperature was 40°C and loaded amount was 50 mg. Time for the elution was 16 minutes for Z-lactone (2R,4S) substances and 21 minutes for Z-lactone (2S,4R) substances, respectively. Each preparative fraction was concentrated after being neutralized with an aqueous ammonia. The concentrate was dissolved in 150 ml of ethyl acetate, washed with an aqueous solution of hydrochloric acid adjusted to pH 3, then washed with a saturated saline solution and dried over anhydrous magnesium sulfate. The magnesium sulfate was removed by filtration and the filtrate was concentrated in vacuo. The residue was crystallized with 100 ml of hexane to give 4.88 g (11.45 mmol; HPLC purity: 97.3%) of a solvate of Z-lactone (2R,4S) substance with 0.2 molar equivalent of ethyl acetate and 5.41 g (12.70 mmol; HPLC purity: 96.9%) of a solvate of Z-lactone (2S,4R) substance with 0.2

molar equivalent of ethyl acetate (an overall recovery yield: 99.7%).

(Solvate of Z-lactone (2R,4S) substance with 0.2 molar equivalent of ethyl acetate)

$^1\text{H-NMR}$ (DMSO-d_6) δ : 2.21-2.28 (m, 1H), 2.64-2.70 (m, 1H), 3.18-3.22 (m, 1H), 3.40-3.44 (m, 1H), 4.42-4.45 (m, 1H), 5.04 (s, 2H), 6.95-7.00 (m, 1H), 7.03-7.07 (m, 1H), 7.15 (s, 1H), 7.32-7.35 (m, 5H), 7.52-7.55 (d, 1H), 7.80-7.82 (d, 1H), 10.92 (s, 1H).

ESI-MS: 409.58 ($\text{M} + \text{H}$) $^+$.

Melting point: 109.1-110.8°C.

(Solvate of Z-lactone (2S,4R) substance with 0.2 molar equivalent of ethyl acetate)

$^1\text{H-NMR}$ (DMSO-d_6) δ : the same as those in above-mentioned (2R,4S) substance.

ESI-MS: 409.58 ($\text{M} + \text{H}$) $^+$.

Melting point: 116.1-116.8°C.

(Example 9)

[Conversion of Z-lactone (2R,4R) substance into sodium salt crystals of monatin (2R,4R) substance (Preparation of crystals of sodium salt. No. 1)]

A Z-lactone (2R,4R) substance (14.24 g; 34.85 mmol; HPLC purity: 99.5%) was dissolved in 400 ml of methanol and 40 ml of water, 3 g of 10% palladium-carbon were added thereto and reduction was carried out in a hydrogen atmosphere at room

temperature for 2 hours. After the reduction, 100 ml of water and 19.2 ml of a 4N aqueous solution of sodium hydroxide (76.67 mmol) were added thereto followed by stirring for a while, the catalyst was removed by filtration and the filtrate was concentrated. The residue was dissolved in 160 ml of water and an ion-exchange resin (Amberlite IR 120 B H AG (H⁺)) was added thereto little by little until the solution became weakly acidic whereupon an excessive sodium was removed. To this were added 34.8 ml of a 28% aqueous ammonia and the ion-exchange resin was removed by filtration. The ion-exchange resin was washed with an 5% aqueous ammonia and the resulting aqueous solution after washing was combined with the filtrate followed by concentrating. The residue after concentration was dissolved in 100 ml of water and 1 g of active carbon was added to the solution followed by stirring for 10 minutes. The active carbon was removed by filtration, the filtrate was concentrated and the concentrate was crystallized by adding a 90% aqueous solution of ethanol thereto at room temperature to give 6.55 g (20.19 mmol; optical activity column HPLC purity: 99.3%) of a solvate of sodium salt crystals of monatin (2R,4R) substance with 0.2 molar equivalent of ethanol in an overall yield of 57.9%.

¹H-NMR (D₂O) δ: 1.95-2.02 (m, 1H), 2.58-2.62 (m, 1H), 3.01-3.05 (m, 1H), 3.21-3.24 (m, 1H), 3.55-3.58 (m, 1H), 7.07-7.11 (m, 1H), 7.14-7.18 (m, 2H), 7.42-7.44 (d, 1H),

7.66-7.68 (d, 1H).

ESI-MS: 291.49 (M - H)⁻.

Melting point: 197.1-198.3°C.

Specific rotation (5% NH₃ water, c = 0.5): $[\alpha]_D^{25} = +0.64$.

Degree of sweet taste: about 2700-fold (as compared with a 5% aqueous solution of sucrose).

(Example 10)

[Conversion of Z-lactone (2S,4S) substance into sodium salt crystals of monatin (2S,4S) substance]

The same operation as in Example 9 was carried out except that 5.00 g (12.25 mmol; HPLC purity: 99.8%) of Z-lactone (2S,4S) substance were used in place of Z-lactone (2R,4R) substance and that reduction was carried out in a hydrogen atmosphere at room temperature for 1 hour to give 3.15 g (9.71 mmol; optical activity column HPLC purity: 99.8%) of a solvate of sodium salt crystals of monatin (2S,4S) substance with 0.2 molar equivalent of ethanol in an overall yield of 79.3%.

¹H-NMR (D₂O) δ: the same as those in Example 9.

ESI-MS: 291.59 (M - H)⁻.

Melting point: 196.1-197.9°C.

Specific rotation (5% NH₃ water, c = 0.5): $[\alpha]_D^{25} = -1.67$.

Degree of sweetness: about 50-fold (as compared with a 5% aqueous solution of sucrose).

(Example 11)

[Conversion of Z-lactone (2R,4S) substance into sodium

salt crystals of monatin (2R,4S) substance]

The same operation as in Example 9 was carried out except that 3.66 g (8.59 mmol; HPLC purity: 97.3%) of a solvate of Z-lactone (2R,4S) substance with 0.2 molar equivalent of ethyl acetate were used in place of Z-lactone (2R,4R) substance and that reduction was carried out in a hydrogen atmosphere at room temperature for 1 hour to give 2.23 g (7.07 mmol; optical activity column HPLC purity: 99.2%) of sodium salt crystals of monatin (2R,4S) substance in an overall yield of 82.3%.

$^1\text{H-NMR}$ (D_2O) δ : 2.11-2.17 (m, 1H), 2.38-2.43 (m, 1H), 3.16 (s, 2H), 3.90-3.93 (m, 1H), 7.06-7.10 (m, 1H), 7.13-7.17 (m, 2H), 7.41-7.43 (d, 1H), 7.66-7.68 (d, 1H).

ESI-MS: 291.19 ($\text{M} - \text{H}$) $^-$.

Melting point: 227.5-229.2°C.

Specific rotation (5% NH_3 water, $c = 0.5$): $[\alpha]_{\text{D}}^{25} = +11.08$.

Degree of sweetness: about 1300-fold (as compared with a 5% aqueous solution of sucrose).

Fig. 25 shows an optical microphotograph (magnification: 200-power) after drying of sodium salt crystals of a (2R,4S) substance of monatin.

Fig. 26 shows a powder X-ray diffraction chart after drying of sodium salt crystals of a (2R,4S) substance of monatin. Characteristic diffraction X-ray peaks were noted at the angles of diffraction (2θ , $\text{CuK}\alpha$ line) of 4.4°, 13.7°, 16.6°, 17.9°, 18.6°, 20.2° and 22.6°.

(Example 12)

[Conversion of Z-lactone (2S,4R) substance into sodium salt crystals of monatin (2S,4R) substance]

The same operation as in Example 9 was carried out except that 5.23 g (12.28 mmol; HPLC purity: 96.9%) of a solvate of Z-lactone (2S,4R) substance with 0.2 molar equivalent of ethyl acetate were used in place of Z-lactone (2R,4R) substance and that reduction was carried out in a hydrogen atmosphere at room temperature for 1 hour followed by recrystallizing from a 90% aqueous solution of ethanol twice to give 2.57 g (8.14 mmol; optical activity column HPLC purity: 99.4%) of sodium salt crystals of monatin (2S,4R) substance in an overall yield of 66.3%.

$^1\text{H-NMR}$ (D_2O) δ : same as those in Example 11.

ESI-MS: 291.49 ($\text{M} - \text{H}$) $^-$.

Melting point: 227.1-229.4°C.

Specific rotation (5% NH_3 water, $c = 0.5$): $[\alpha]_{\text{D}}^{25} = -9.57$.

Degree of sweetness: about 300-fold (as compared with a 5% aqueous solution of sucrose).

Fig. 27 shows an optical microphotograph (magnification: 200-power) after drying of sodium salt crystals of a (2S,4R) substance of monatin.

Fig. 28 shows a powder X-ray diffraction chart after drying of sodium salt crystals of a (2S,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at the angles

of diffraction (2θ , CuK α line) of 4.4° , 13.7° , 16.6° , 17.9° , 18.6° , 20.2° and 22.6° .

(Example 13)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin (No. 1)]

An ammonium salt (1.5 g) of (2R,4R) substance of monatin was dissolved in 10 ml of water, passed through a column filled with 25 ml of a cation-exchange resin (Diaion PK 228 (potassium type; manufactured by Mitsubishi Chemical)) to exchange with a desired ion and the eluate was concentrated to an extent of 11.5 g. The resulting concentrate was heated up to 60°C and 60 ml of ethanol were added thereto. The resulting aqueous ethanolic solution was cooled down to 10°C at the rate of $5^\circ\text{C}/\text{hour}$ and stirred at 10°C for one night. Crystals were separated from the resulting crystallized solution and the wet crystals were dried in a vacuum drier to give 1.1 g of potassium salt crystals of (2R,4R) substance of monatin.

Melting point: $213.3\text{-}214.7^\circ\text{C}$.

Fig. 1 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized solution of potassium salt crystals of a (2R,4R) substance of monatin.

Fig. 2 shows an optical microphotograph (magnification: 200-power) after drying of potassium salt crystals of a (2R,4R) substance of monatin.

Fig. 3 shows a powder X-ray diffraction chart after drying of potassium salt crystals of a (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at the angles of diffraction (2θ , CuK α line) of 5.7°, 6.1°, 6.6°, 11.5°, 11.8°, 12.2°, 13.9°, 17.2°, 18.3°, 20.6°, 22.9°, 24.5° and 26.3°.

(Example 14)

[Preparation of sodium salt crystals of a (2R,4R) substance of monatin (No. 2)]

An ammonium salt (1.5 g) of (2R,4R) substance of monatin was dissolved in 10 ml of water, passed through a column filled with 25 ml of a cation-exchange resin (Diaion PK 228 (Na-type; manufactured by Mitsubishi Chemical)) to exchange to a desired ion and the eluate was concentrated to an extent of 11.5 g. The resulting concentrate was heated up to 60°C and 60 ml of ethanol was added thereto. The aqueous ethanolic solution was cooled down to 10°C at the rate of 5°C/hour and then stirred at 10°C for one night.

Crystals were separated from the resulting crystallized solution and, then the wet crystals were dried in a vacuum drier to give 1.2 g of crystals of a solvate of sodium salt of a (2R,4R) substance of monatin with 0.2 molar equivalent of ethanol.

Melting point: 193.5-195.1°C.

Fig. 4 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized

solution of sodium salt crystals of a (2R,4R) substance of monatin.

Fig. 5 shows an optical microphotograph (magnification: 200-power) after drying of sodium salt crystals of a (2R,4R) substance of monatin.

Fig. 6 shows a powder X-ray diffraction chart after drying of sodium salt crystals of a (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 4.4°, 15.3°, 17.5°, 19.1° and 24.6°.

(Example 15)

[Preparation of ammonium salt crystals of (2R,4R) substance of monatin]

An ammonium salt (1.5 g) of (2R,4R) substance of monatin was dissolved in 10 ml of water. The solution was heated up to 60°C and 60 ml of ethanol were added thereto. The aqueous ethanolic solution was cooled down to 10°C at the rate of 5°C/hour and stirred at 10°C for one night. Crystals were separated from the resulting crystallized solution and the wet crystals were dried in a vacuum drier to give 0.77 g of ammonium salt crystals of (2R,4R) substance of monatin.

Melting point: 172.1-172.8°C.

Fig. 7 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized solution of ammonium salt crystals of a (2R,4R) substance of

monatin.

Fig. 8 shows an optical microphotograph (magnification: 200-power) after drying of ammonium salt crystals of a (2R,4R) substance of monatin.

Fig. 9 shows a powder X-ray diffraction chart after drying of ammonium salt crystals of a (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 6.1° , 11.6° , 18.1° , 19.6° and 25.0° .

(Comparative Example 1)

[Preparation of crystals of a free (2R,4R) substance of monatin]

An ammonium salt (0.5 g) of (2R,4R) substance of monatin was dissolved in 10 ml of a 50% aqueous solution of acetic acid and 25 ml of ethanol were added thereto at 25°C during one hour. Stirring was further carried out for 4.5 hours at 25°C . Crystals were separated from the resulting crystallized solution and the wet crystals were dried in a vacuum drier to give 0.38 g of crystals of a free (2R,4R) substance of monatin.

Melting point: $175.2\text{--}176.1^\circ\text{C}$.

Fig. 10 shows an optical microphotograph (magnification: 200-power) immediately before separation of a crystallized solution of crystals of a free (2R,4R) substance of monatin.

Fig. 11 shows an optical microphotograph (magnification: 200-power) after drying of crystals of a free (2R,4R) substance

of monatin.

Fig. 12 shows a powder X-ray diffraction chart after drying of crystals of a free (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.9°, 17.9°, 19.2°, 23.9° and 27.8°.

(Comparative Example 2)

[Preparation of amorphous solid of potassium salt of (2R,4R) substance of monatin]

A potassium salt (1.0 g) of a (2R,4R) substance of monatin manufactured according to Example 13 was dissolved in 10 ml of water and freeze-dried to give 0.97 g of amorphous solid of potassium salt of a (2R,4R) substance.

Melting point: 183.2-184.8°C.

Fig. 13 shows an optical microphotograph (magnification: 200-power) after drying of amorphous solid of potassium salt of a (2R,4R) substance of monatin.

Fig. 14 shows a powder X-ray diffraction chart after drying of amorphous solid of potassium salt of a (2R,4R) substance of monatin. As will be apparent from this chart, the solid was amorphous.

(Example 16)

[Comparison of thermostability of the crystals]

Each 50 mg of the crystals and the amorphous solid prepared by the above-mentioned methods were placed in a 4-ml

vial and preserved (kept) at the temperature of 120°C. At that time, the vial was made in an open state. Each 2 mg of the sample were taken out after retention time of 3 hours, 7 hours and 24 hours and the rate (ratio) of decomposed products was determined by means of HPLC (high-performance liquid chromatography). Relation between the retention time and the rate of the decomposed products is shown in the following Table 3 and Table 4.

Table 3. Rate of Decomposed Product A

(Area % to residual monatin)

	Retention Time (hours)		
	3	7	24
Samples	Rate of Decomposed Product A (%)		
Potassium salt crystals	0.9%	0.8%	0.6%
Sodium salt crystals	3.4%	4.6%	6.7%
Ammonium salt crystals	1.9%	3.8%	11.8%
Free substance crystals	2.0%	3.8%	5.3%
Amorphous solid	3.4%	3.9%	8.9%

Table 4. Rate of Decomposed Product B

(Area % to residual monatin)

	Retention Time (hours)		
	3	7	24
Samples	Rate of Decomposed Product B (%)		
Potassium salt crystals	0.1%	0.2%	0.3%
Sodium salt crystals	2.1%	3.6%	7.5%
Ammonium salt crystals	1.2%	2.3%	7.6%
Free substance crystals	2.1%	4.3%	11.7%
Amorphous solid	3.4%	4.3%	11.1%

From the result of the above tables, it was noted that

potassium salt shows minimum production of the decomposed products A and B and was particularly superior to other crystals in terms of stability. With regard to the decomposed product B, its production is less in crystals of various salts than free substance crystals and amorphous solid and, therefore, it is noted that crystals of such salts are superior in terms of stability.

(Example 17)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin (No. 2)]

An ammonium salt (10 g) of a (2R,4R) substance of monatin was dissolved in 20 ml of water and passed through a column filled with 50 ml of a cation-exchange resin (Diaion PK 228; potassium-type, manufactured by Mitsubishi Chemical) to exchange with a desired ion and an eluate was concentrated to an extent of 23.48 g. The resulting concentrate was heated up to 35°C and 84 ml of ethanol were dropped thereinto during about 3 hours. The aqueous ethanolic solution was cooled down to 10°C at the rate of 5°C/hour and stirred at 10°C for one night. Crystals were separated from the resulting crystallized solution and the wet crystals were dried in a vacuum drier to give 9.3 g of potassium salt crystals of a (2R,4R) substance of monatin.

Melting point: 220.0-222.3°C.

Fig. 15 shows a powder X-ray diffraction chart of the

potassium salt crystals of a (2R,4R) substance of monatin after dried. Characteristic diffraction X-ray peaks were noted at the angles of diffraction (2θ , CuK α line) of 5.7°, 6.1°, 11.5°, 12.2°, 18.3°, 20.6° and 24.5°.

(Example 18)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin (No. 3)]

Potassium salt crystals (1.5 g) of a (2R,4R) substance of monatin prepared according to Example 17 were dissolved in 10 ml of water, the solution was cooled down to 10°C and 60 ml of ethanol were dropped thereinto during about 2.5 hours. The aqueous ethanolic solution was stirred at 10°C for one night. Crystals were separated from the resulting crystallized solution and the wet crystals were dried in a vacuum drier to give 1.2 g of potassium salt crystals of a (2R,4R) substance.

Melting point: 213.2-215.6°C.

Fig. 16 shows a powder X-ray diffraction chart of the potassium salt crystals of a (2R,4R) substance of monatin after dried. Characteristic diffraction X-ray peaks were noted at the angles of diffraction (2θ , CuK α line) of 5.7°, 6.6°, 11.5°, 11.8°, 17.2°, 22.9° and 23.1°.

(Example 19)

[Steam adsorption/desorption curve of the crystals]

Steam adsorption/desorption curve for each of the potassium salt crystals (crystallized at 10°C, 35°C and 60°C)

of a (2R,4R) substance of monatin prepared by the above-mentioned methods was determined. The measurement data are shown in Fig. 29. Conditions for the measurement are as follows.

Apparatus: Automatic measuring apparatus for amounts of adsorption of steam, Belsorp 18, manufactured by Nippon Bell K. K.

Measuring method: Constant-volume gas adsorption method

Measuring conditions:

Adsorption gas: H_2O

Air thermostat temperature: 323K

Adsorbing temperature: 298K

Initial introducing pressure: 1.0 torr

Introducing pressure difference: 0 torr

Saturated steam pressure: 23.76 torr

Adsorption cross section: 0.125 nm^2

Maximum adsorbing pressure: 0.90

Minimum adsorbing pressure: 0.10

Equilibrium time: 300 sec

(Example 20)

The sodium salt of (2R,4R) substance of monatin prepared in Example 14 was stored for two days in a constant-moisture and constant-temperature device of 40°C temperature and 75% humidity to give crystals in which ethanol in the crystals disappeared.

Fig. 30 shows a powder X-ray diffraction chart of the resulting crystals. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 4.4° , 15.2° , 17.8° , 20.6° and 24.1° .

(Example A)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (400 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 2.5 ml of water, the solution was heated up to 40°C and 15 ml of methanol were slowly dropped thereinto with stirring. The aqueous methanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 1.03 g of wet crystals of potassium salt of a (2R,4R) substance of monatin.

Fig. 31 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7° , 11.5° , 11.8° , 17.2° and 23.1° .

(Example B)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The same operation as in Example A was carried out except that ethanol was used in place of methanol to give 0.38 g of

wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 5.97%).

Fig. 32 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 6.1°, 11.5°, 11.8°, 12.2°, 17.2°, 18.3°, 20.6°, 23.1° and 24.5°.

(Example C)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The same operation as in Example A was carried out except that isopropyl alcohol was used in place of methanol to give 0.38 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 9.85%).

Fig. 33 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 11.5°, 11.8°, 17.2° and 23.1°.

(Example D)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The same operation as in Example A was carried out except that acetone was used in place of methanol to give 0.30 g of wet crystals of potassium salt of a (2R,4R) substance of monatin

(water content: 10.64%).

Fig. 34 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 11.5°, 11.8°, 17.2° and 23.1°.

(Example E)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (400 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 5 ml of water, the solution was heated up to 35°C and 30 ml of ethanol were slowly dropped thereinto with stirring. The aqueous ethanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.28 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 9.75%).

Fig. 35 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 11.5°, 11.8°, 17.2° and 23.1°.

(Example F)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (400 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 1.7 ml of water, the solution was heated up to 35°C and 10 ml of ethanol were slowly dropped thereinto with stirring. The aqueous ethanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.38 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 9.45%).

Fig. 36 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 11.5°, 11.8°, 17.2° and 23.1°.

(Example G)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (400 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 1.25 ml of water, the solution was heated up to 35°C and 7.5 ml of ethanol were slowly dropped thereinto with stirring. The aqueous ethanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.42 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 9.67%).

Fig. 37 shows a powder X-ray diffraction chart of the

wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 11.5°, 11.8°, 17.2° and 23.1°.

(Example H)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (475 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 5 ml of water, the solution was cooled down to 10°C and 30 ml of ethanol were slowly dropped thereinto with stirring. The aqueous ethanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.36 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 8.26%).

Fig. 38-1 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 6.1°, 11.5°, 11.8°, 12.2°, 17.2°, 18.3°, 20.6°, 23.1° and 24.5°.

The crystals were dried at 60°C in vacuo to give crystals (water content: 2.11%) of potassium salt of a (2R,4R) substance of monatin.

Fig. 38-2 shows a powder X-ray diffraction chart of the crystals of the potassium salt of the (2R,4R) substance of

monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 6.1° , 6.6° , 12.2° , 13.9° , 18.3° , 20.6° , 22.9° , 24.5° and 26.3° .

(Example I)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (400 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 2.5 ml of water, the solution was heated up to 25°C and 15 ml of ethanol were slowly dropped thereinto with stirring. The aqueous ethanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.40 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 10.39%).

Fig. 39-1 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7° , 6.1° , 11.5° , 11.8° , 12.2° , 17.2° , 18.3° , 20.6° , 23.1° and 24.5° .

The crystals were dried at 60°C in vacuo to give crystals (water content: 1.69%) of potassium salt of a (2R,4R) substance of monatin.

Fig. 39-2 shows a powder X-ray diffraction chart of the crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted

at angles of diffraction (2θ , CuK α line) of 5.7° , 6.1° , 6.6° , 11.5° , 11.8° , 12.2° , 13.9° , 18.3° , 20.6° , 22.9° , 24.5° and 26.3° .

(Example J)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (213 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 1.25 ml of water, the solution was heated up to 40°C and 7.5 ml of ethanol were slowly dropped thereinto with stirring. The aqueous ethanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.19 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 6.43%).

Fig. 40-1 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7° , 6.1° , 11.5° , 11.8° , 12.2° , 17.2° , 18.3° , 20.6° , 23.1° and 24.5° .

The crystals were dried at 60°C in vacuo to give crystals (water content: 5.43%) of potassium salt of a (2R,4R) substance of monatin.

Fig. 40-2 shows a powder X-ray diffraction chart of the crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 6.1° , 6.6° , 11.5° ,

12.2°, 13.9°, 18.3°, 20.6°, 22.9°, 24.5° and 26.3°.

(Example K)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

The potassium salt crystals (217 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were dissolved in 1.25 ml of water, the solution was heated up to 60°C and 7.5 ml of ethanol were slowly dropped thereinto with stirring. The aqueous ethanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.20 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 7.25%).

Fig. 41-1 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 6.1°, 11.5°, 11.8°, 12.2°, 17.2°, 18.3°, 20.6°, 23.1° and 24.5°.

The crystals were dried at 60°C in vacuo to give crystals (water content: 4.79%) of potassium salt of a (2R,4R) substance of monatin.

Fig. 41-2 shows a powder X-ray diffraction chart of the crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 6.1°, 6.6°, 11.5°, 12.2°, 13.9°, 18.3°, 20.6°, 22.9°, 24.5° and 26.3°.

(Example L)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

An ammonium salt (420 mg) of a (2R,4R) substance of monatin was dissolved in 25 ml of methanol at 45°C and 0.3 ml of a 20 % by weight methanolic solution of potassium hydroxide was slowly dropped thereinto with stirring. The methanolic solution was allowed to stand in a refrigerator of 10°C for one night. The resulting crystals were separated to give 0.22 g of wet crystals of potassium salt of a (2R,4R) substance of monatin (water content: 5.64%).

Fig. 42 shows a powder X-ray diffraction chart of the wet crystals of the potassium salt of the (2R,4R) substance of monatin. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 6.1°, 6.6°, 11.5°, 11.8°, 12.2°, 13.9°, 17.2°, 18.3°, 22.9°, 23.1° and 26.3°.

(Example M)

[Preparation of potassium salt crystals of (2R,4R) substance of monatin]

Methanol (25 ml) and 0.38 ml of a 20% by weight solution of potassium hydroxide in methanol were mixed and 400 mg of an ammonium salt of a (2R,4R) substance of monatin were added thereto. The methanolic solution was stirred for one night at room temperature. The resulting slurry was separated to give 0.25 g of solid of potassium salt of a (2R,4R) substance

of monatin (water content: 5.42%).

Fig. 43 shows a powder X-ray diffraction chart of the solid of the potassium salt of the (2R,4R) substance of monatin. As will be apparent from this chart, the solid was amorphous.

(Example N)

Potassium salt crystals (150 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were allowed to stand in a constant-temperature vessel of 60°C for one night.

Fig. 44 shows a powder X-ray diffraction chart of potassium salt crystals (water content: 7.04%) of a (2R,4R) substance of monatin stored at 60°C. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 6.1°, 12.2°, 18.3°, 20.6° and 24.5°.

(Comparative Example N)

The same operation as in Example N was carried out except that a refrigerator of 10°C was used in place of the constant-temperature vessel of 60°C and the crystals were allowed to stand for one night. A powder X-ray diffraction chart of the crystals (water content: 6.96%) was the same as that of Fig. 15.

(Example O)

The same operation as in Example N was carried out except that a constant-temperature vessel of 120°C was used in place of the constant-temperature vessel of 60°C and the crystals were allowed to stand for one night.

Fig. 45 shows a powder X-ray diffraction chart of potassium salt crystals (water content: 4.73%) of a (2R,4R) substance of monatin stored at 120°C. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 6.1°, 12.2°, 18.3°, 20.6° and 24.5°.

(Example P)

Potassium salt crystals (150 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were allowed to stand in a desiccator kept at relative humidity of 97% for one night.

Fig. 46 shows a powder X-ray diffraction chart of potassium salt crystals (water content: 8.61%) of a (2R,4R) substance of monatin stored at relative humidity of 97%. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , CuK α line) of 5.7°, 11.5°, 11.8°, 17.2° and 23.1°.

(Comparative Example P1)

Potassium salt crystals (150 mg) of a (2R,4R) substance of monatin manufactured according to Example 17 were allowed to stand for one night in a constant-temperature and constant-humidity device of relative humidity of 75%. A powder X-ray diffraction chart of the crystals (water content: 5.43%) was the same as that of Fig. 15.

(Comparative Example P2)

Potassium salt crystals (200 mg) of a (2R,4R) substance

of monatin manufactured according to Example G were allowed to stand for one night in a desiccator kept at relative humidity of 10%. A powder X-ray diffraction chart of the crystals was the same as that of Fig. 37.

(Example Q)

Potassium salt crystals (240 mg) of a (2R,4R) substance of monatin manufactured according to Example F were dried in vacuo at 60°C.

Fig. 47 shows a powder X-ray diffraction chart of potassium salt crystals (water content: 1.04%) of a (2R,4R) substance of monatin which were dried in vacuo at 60°C. Characteristic diffraction X-ray peaks were noted at angles of diffraction (2θ , $\text{CuK}\alpha$ line) of 6.6°, 13.9°, 22.9° and 26.3°.

(Comparative Example Q)

Potassium salt crystals (150 mg) of a (2R,4R) substance of monatin manufactured according to Example F were dried in vacuo at 40°C. A powder X-ray diffraction chart of the crystals was the same as that of Fig. 36.

Advantage of the Invention

In accordance with the present invention, a novel sweet taste substance in the crystals of a non-natural-type stereoisomer salt of monatin is used as an effective ingredient whereupon it is possible to provide a novel sweetener or a product such as beverage and food to which sweet taste is given.

Crystals of the stereoisomer salt are excellent particularly in stability upon preservation, show a strong intensity of sweet taste and express an excellent taste as a sweetener. Among them, crystals of salts (such as potassium salt) of a (2R,4R) substance of monatin are preferred as crystals of the non-natural-type stereoisomer salts and they have particularly excellent taste and stability upon preservation.

It is further possible in accordance with the present invention to provide a novel sweet taste substance (the above-mentioned crystals of the non-natural-type stereoisomer salt) having an excellent property as a sweetener or an ingredient thereof and as a component for giving sweet taste to beverage, food, etc.

Consequently, the present invention is quite useful in industry, particularly in a field of foods.

Claims

1. Crystals of salt of a non-natural-type stereoisomer of monatin.

2. The crystals according to claim 1, wherein the crystals are crystals of any of salts of a (2S,4R) substance, a (2R,4R) substance and a (2R,4S) substance of monatin.

3. The crystals according to claim 2, wherein the crystals are crystals of a salt of a (2R,4R) substance of monatin.

4. The crystals according to claim 1, wherein the salt is selected from ammonium salt, sodium salt, potassium salt, lithium salt, calcium salt and magnesium salt.

5. The crystals according to claim 1 or 3, wherein the crystals contain the stereoisomer salt in a chemical purity of at least 95%.

6. The crystals according to claim 1, 3 or 5, wherein the crystals have an optical purity of at least 94%.

7. The crystals according to claim 1, wherein the crystals are those of salts of a mixture of at least two isomers selected from stereoisomers of a (2S,4R) substance, a (2R,4R) substance and a (2R,4S) substance of monatin.

8. The crystals according to claim 1, wherein the crystals have intensity of sweet taste (sweetening potency) of at least 1000-fold of sucrose.

9. The crystals according to claim 1 or 3, wherein the crystals are in a state of being mixed with a salt of a (2S,4S) substance of monatin.

10. The crystals according to claim 9, wherein the crystals contain not more than 70% of the salt of the (2S,4S) substance of monatin to the total monatin salts.

11. The crystals according to claim 9, wherein the crystals are in a state of mixed crystals of the salt of the (2S,4S) substance of monatin and the salt of the (2R,4R) substance of monatin.

12. The crystals according to claim 11, wherein the ratio of the salt of the (2S,4S) substance of monatin to the salt of the (2R,4R) substance of monatin is 1:1.

13. A sweetener which is characterized in containing crystals mentioned in any of claims 1 to 12 where the sweetener may optionally contain a carrier and/or a filler (a bulking agent) for sweeteners.

14. A product such as beverage or food to which sweet taste is given which is characterized in containing the crystals mentioned in any of claims 1 to 12 or in being prepared by using the same.

15. The sweetener or the product such as beverage or food according to claim 13 or 14, wherein at least one member (compound) selected from saccharides, and other artificial sweeteners and natural sweeteners is contained.

Application number numéro de demande: JP 02/12472

Figures: 1, 2, 4, 5, 7, 8, 10, 11, 13, 17
~~19, 21, 23, 25, 27~~

Unscannable items
received with this application.

(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au
10^{ème} étage)

Fig. 3

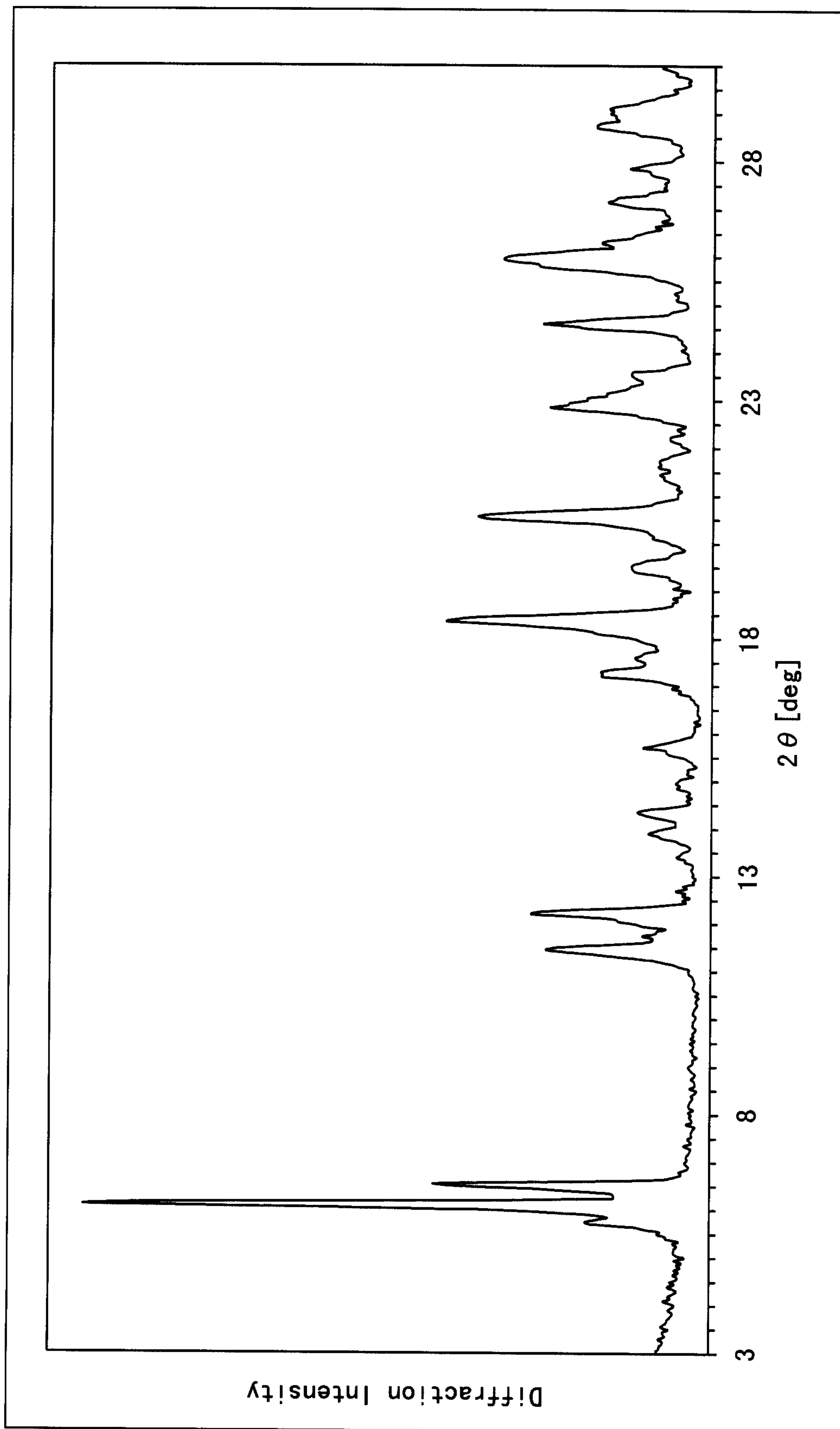


Fig. 6

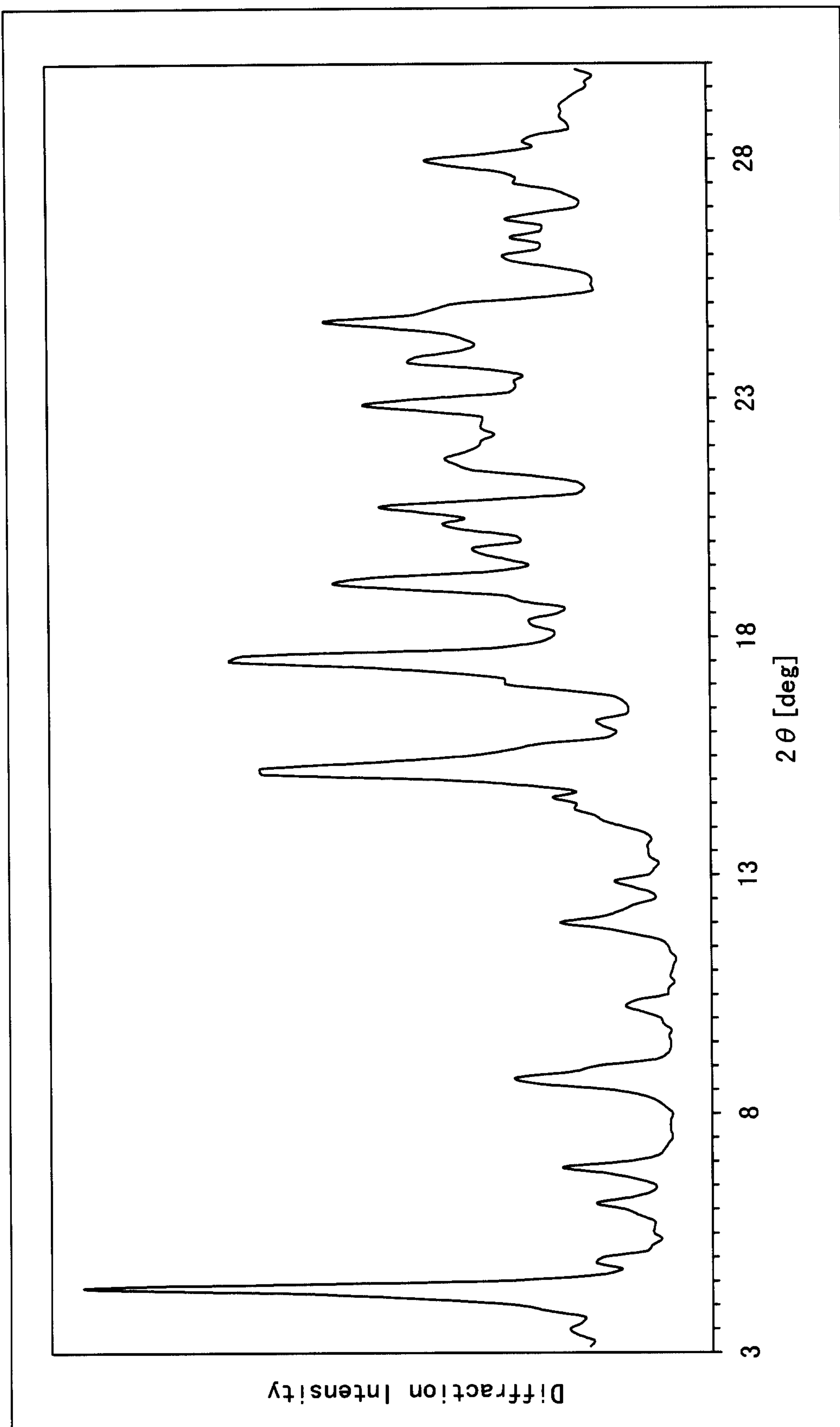


Fig. 9

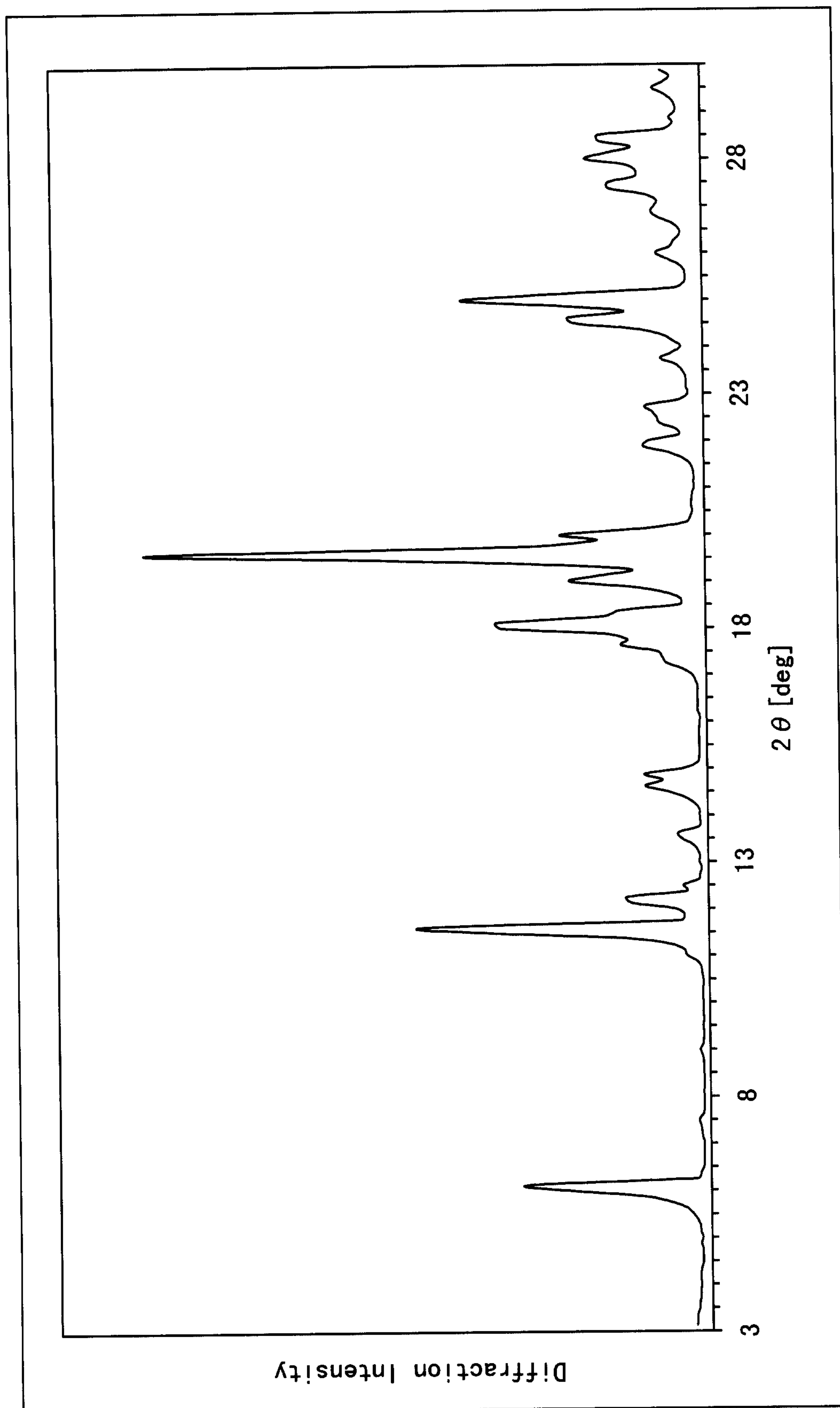


Fig. 12

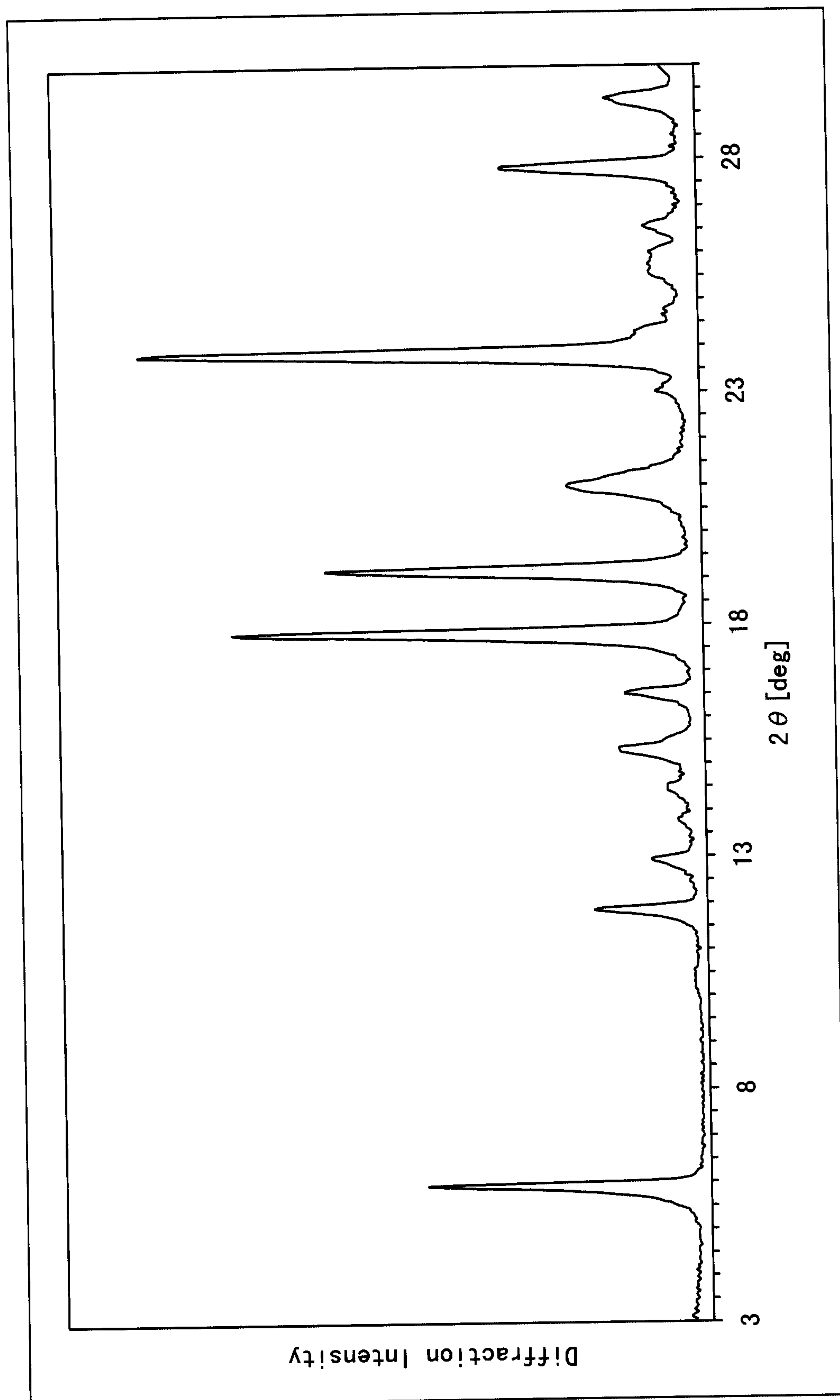


Fig. 14

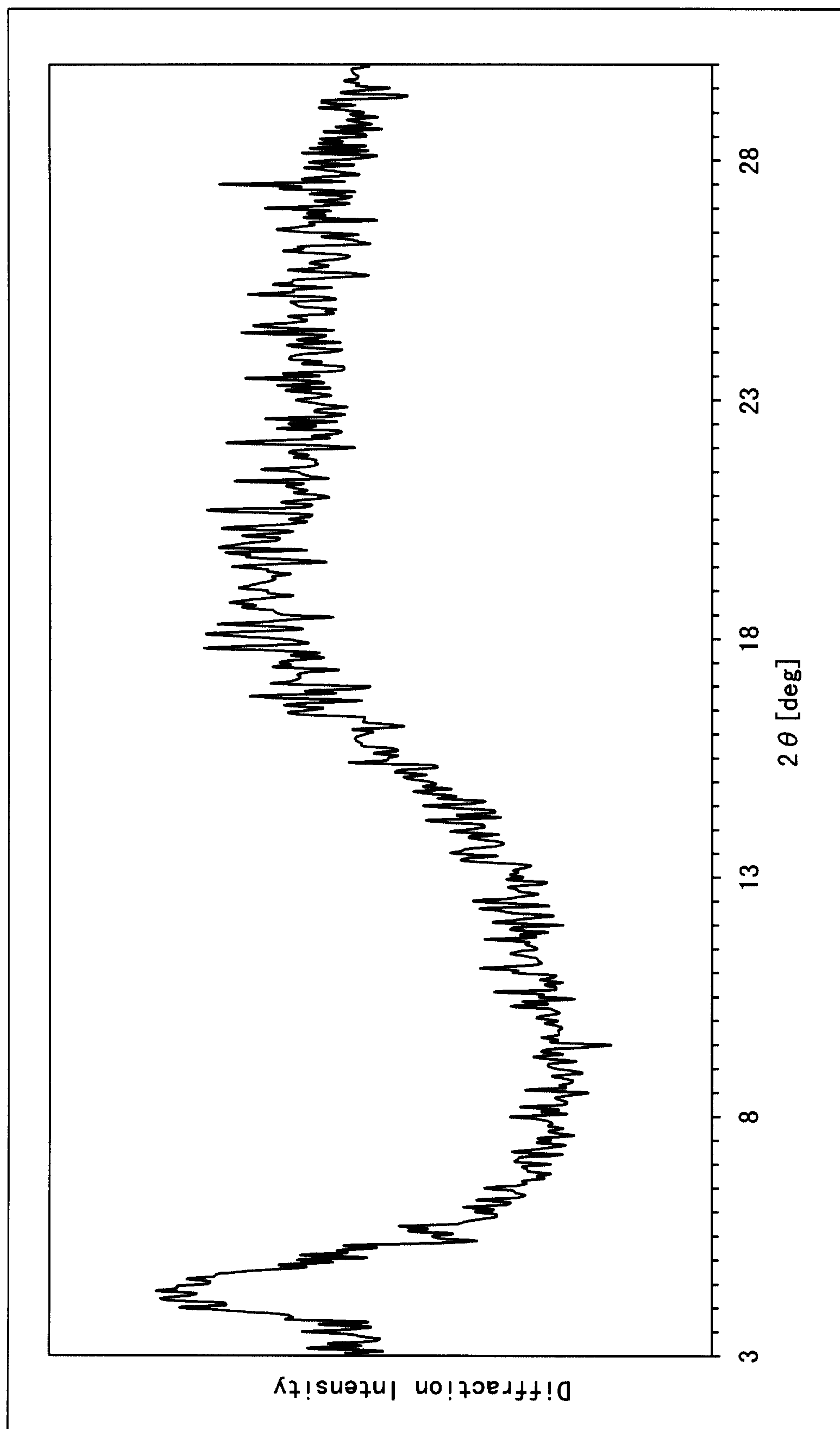


Fig. 15

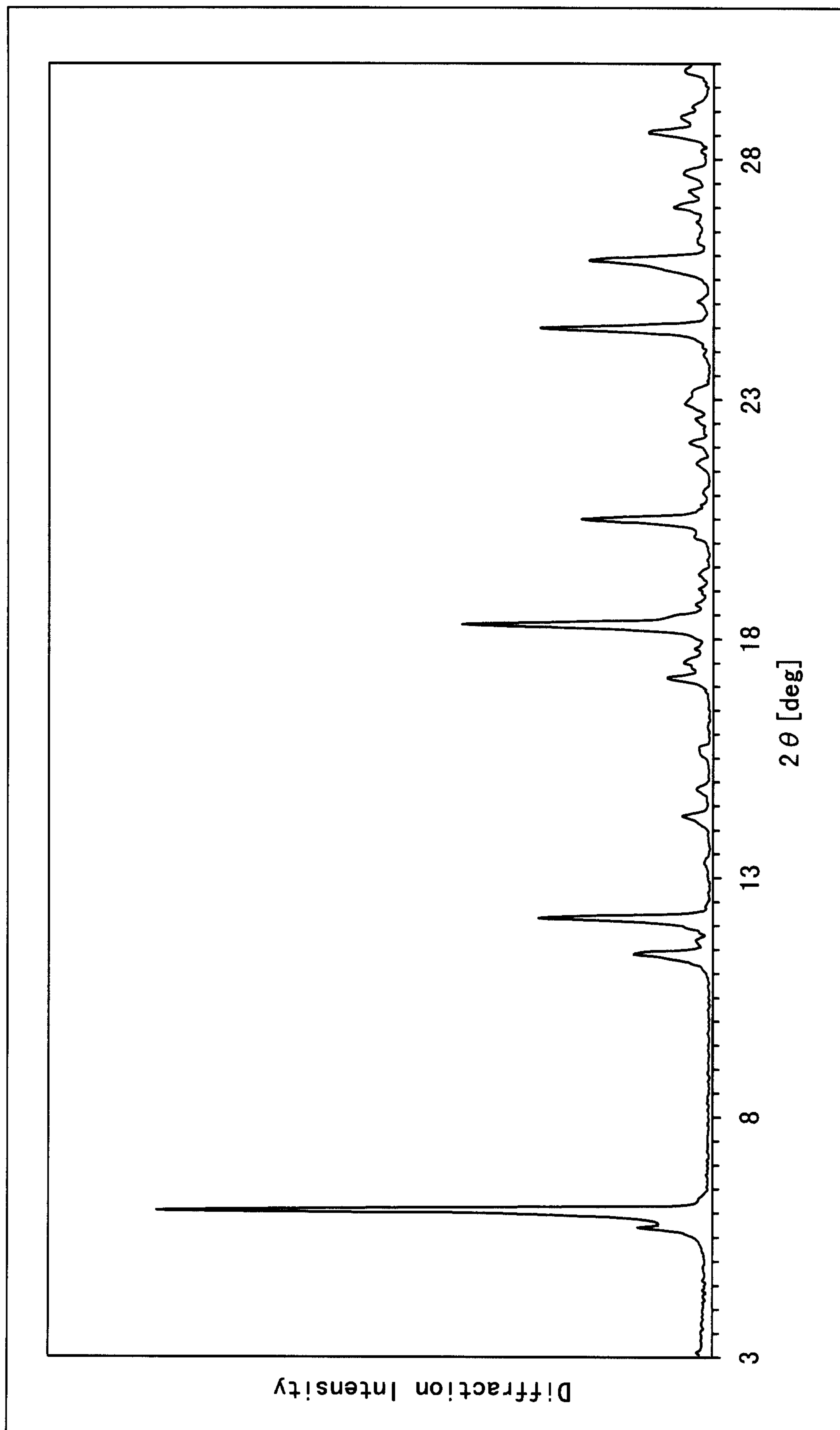


Fig. 16

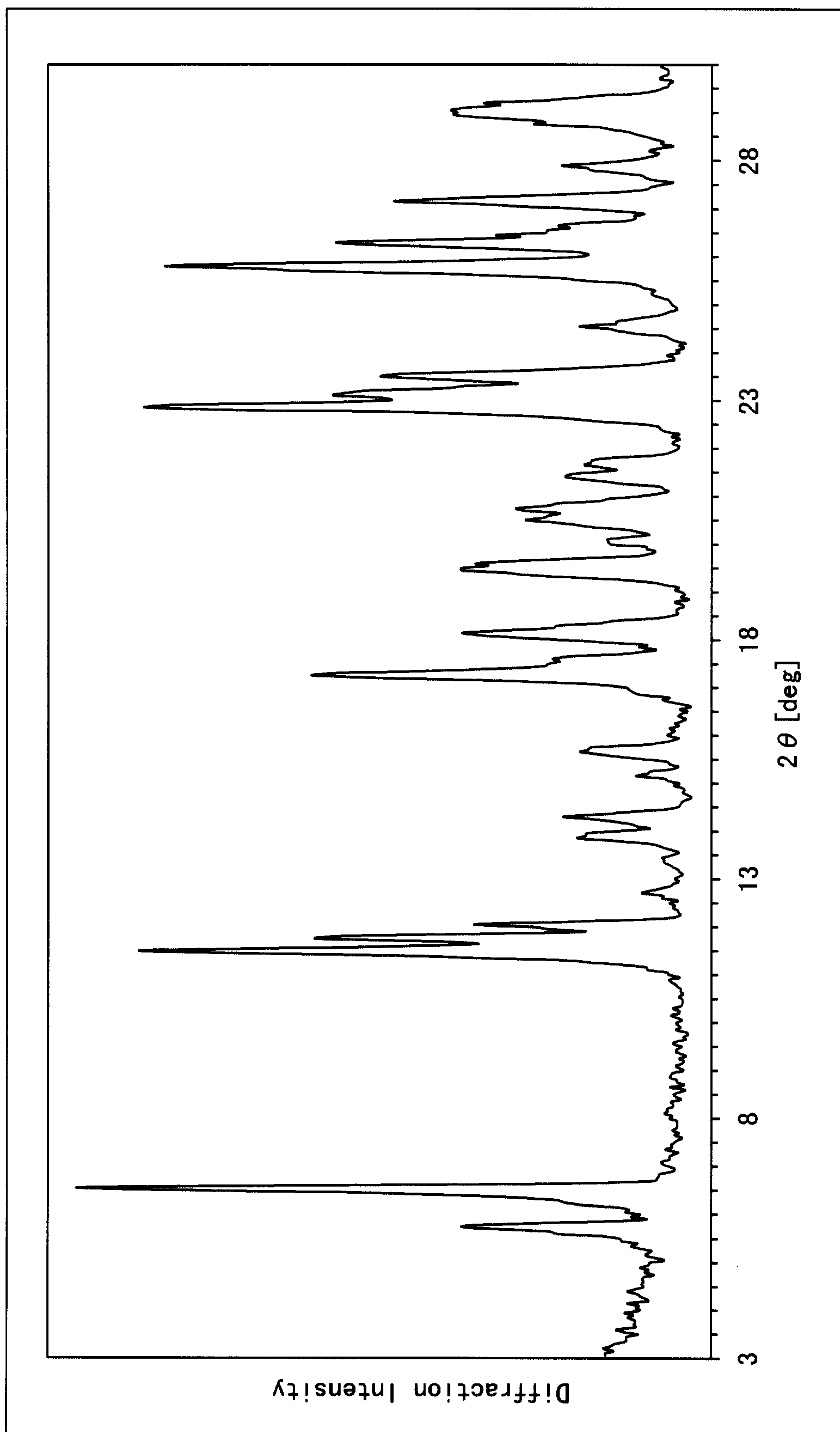


Fig. 18

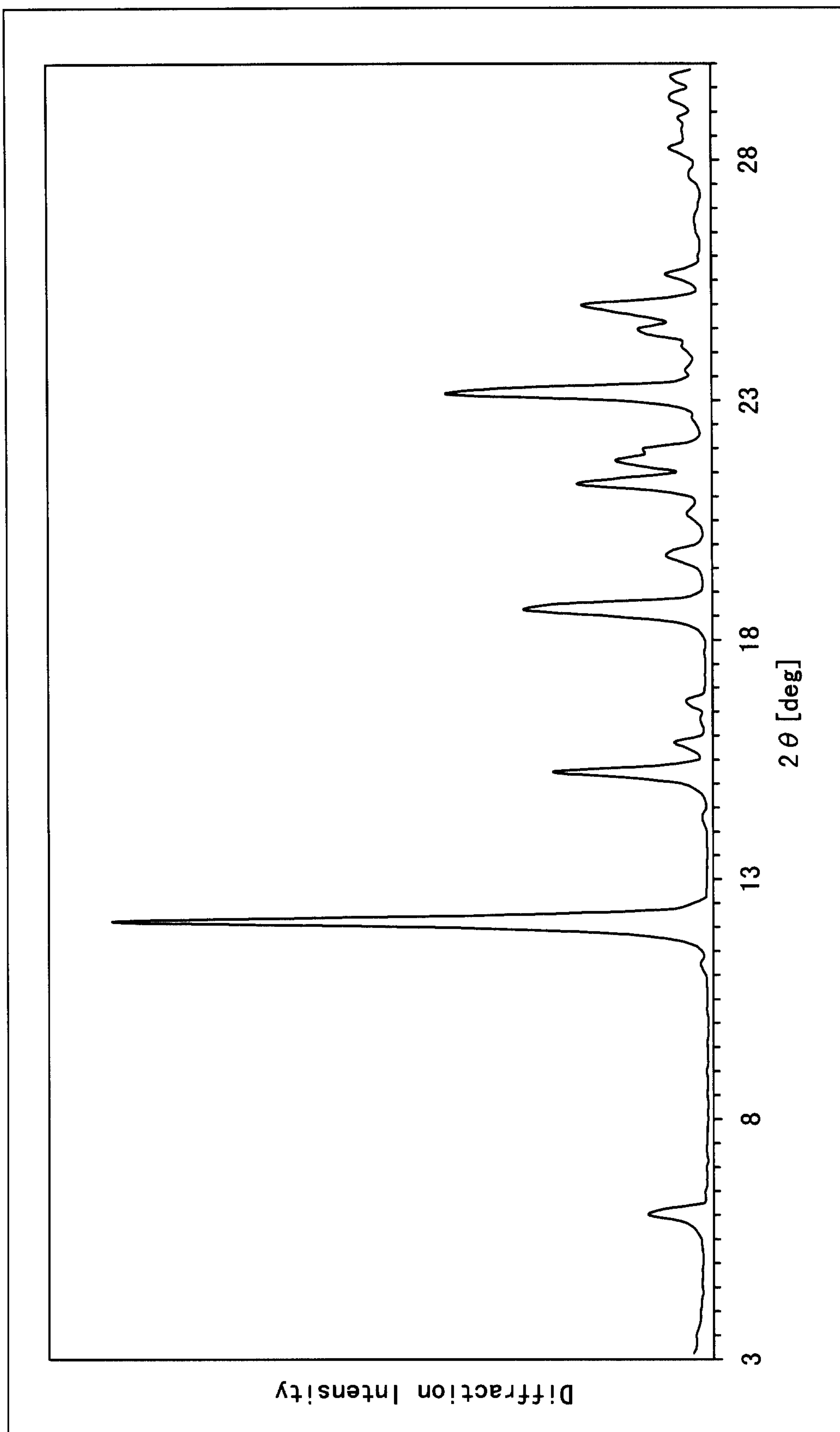


Fig. 20

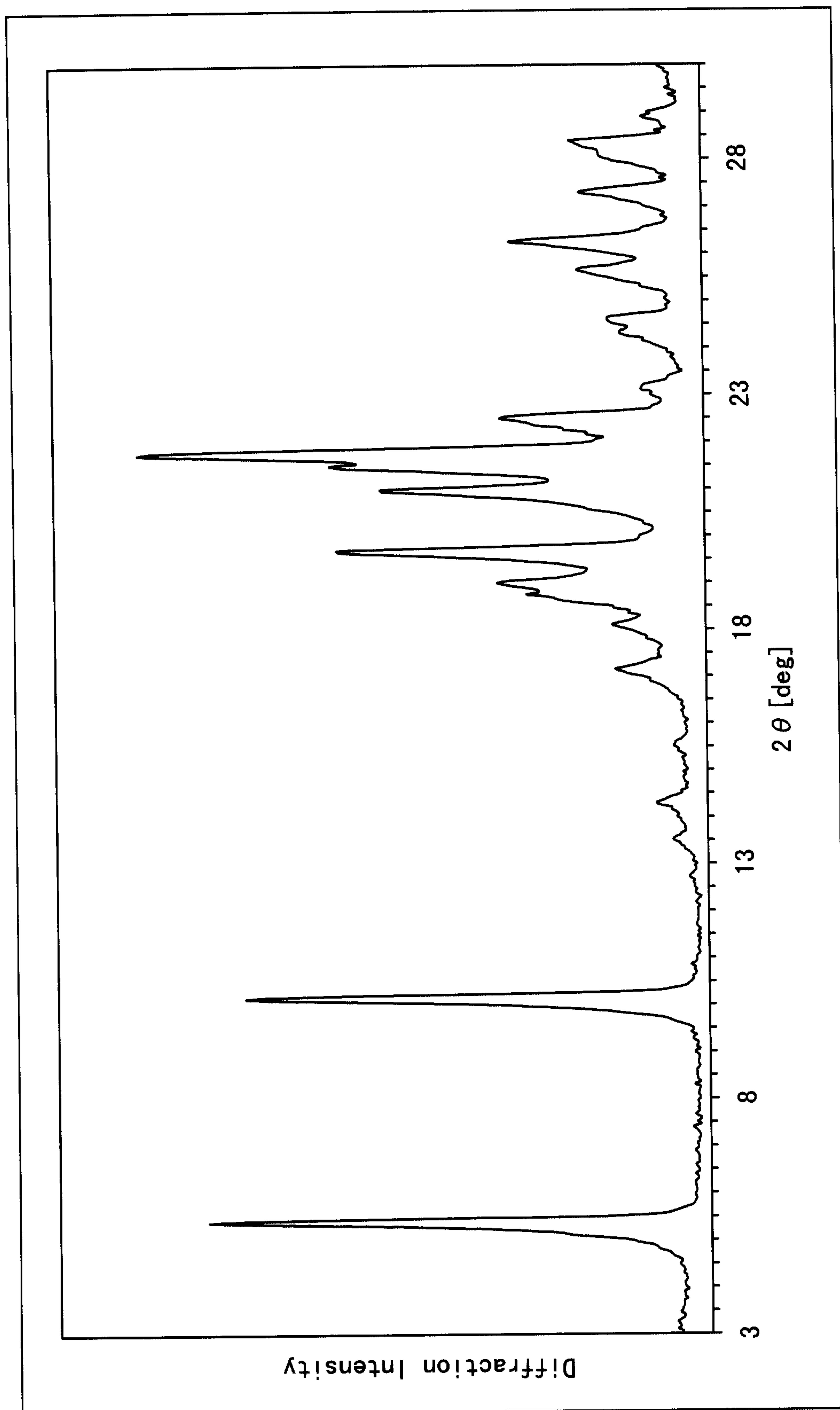


Fig. 22

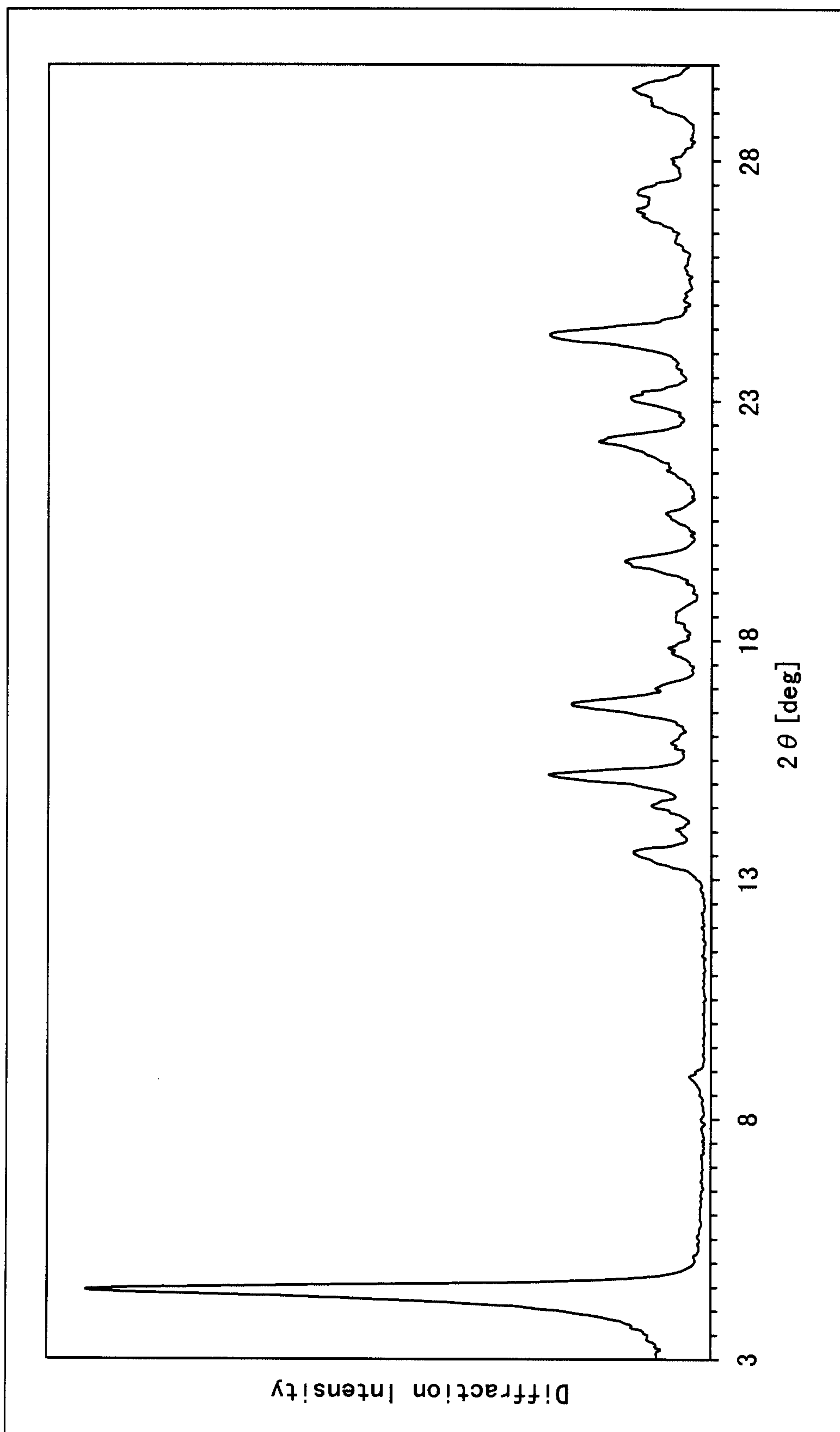


Fig. 24

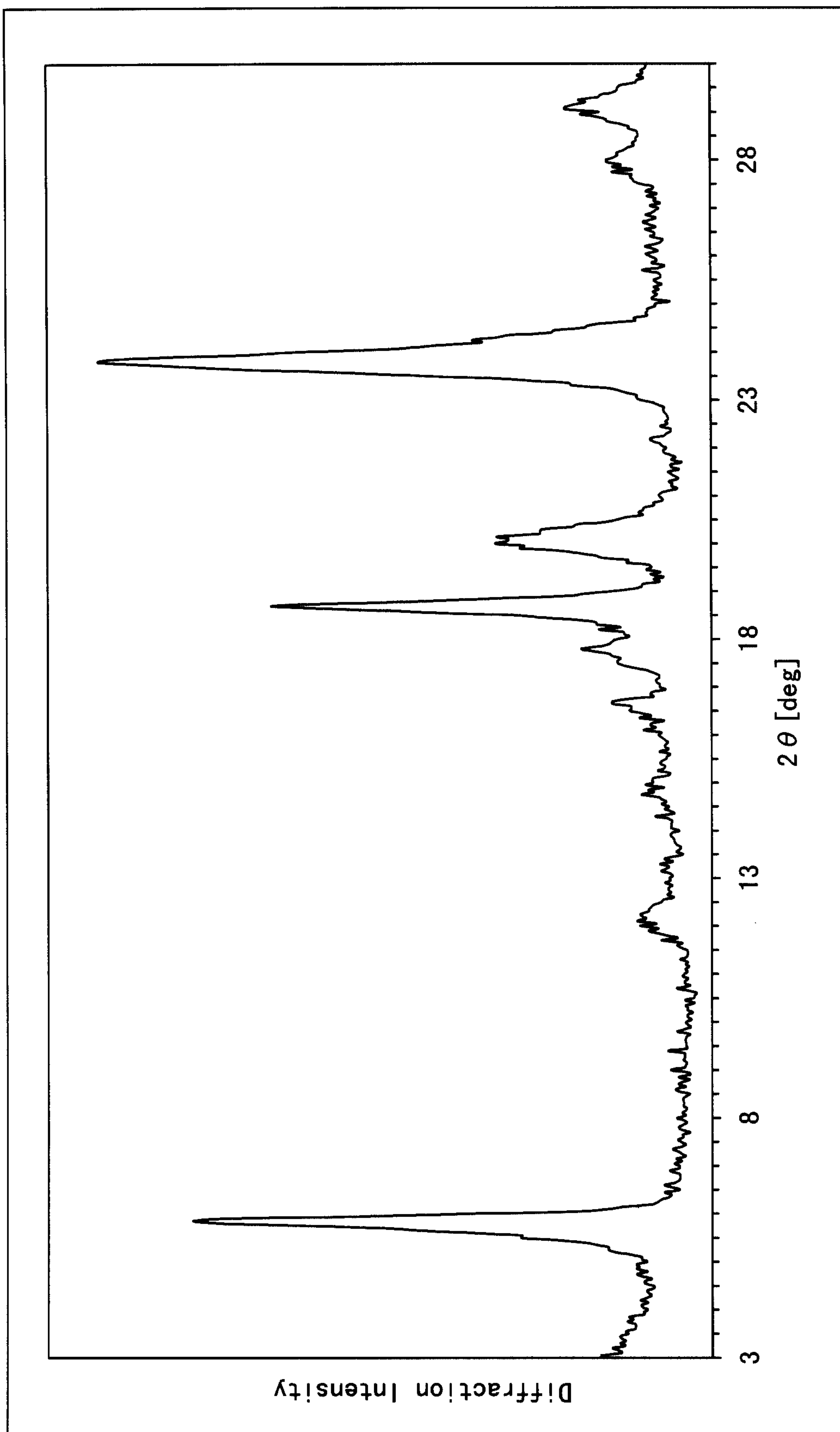


Fig. 26

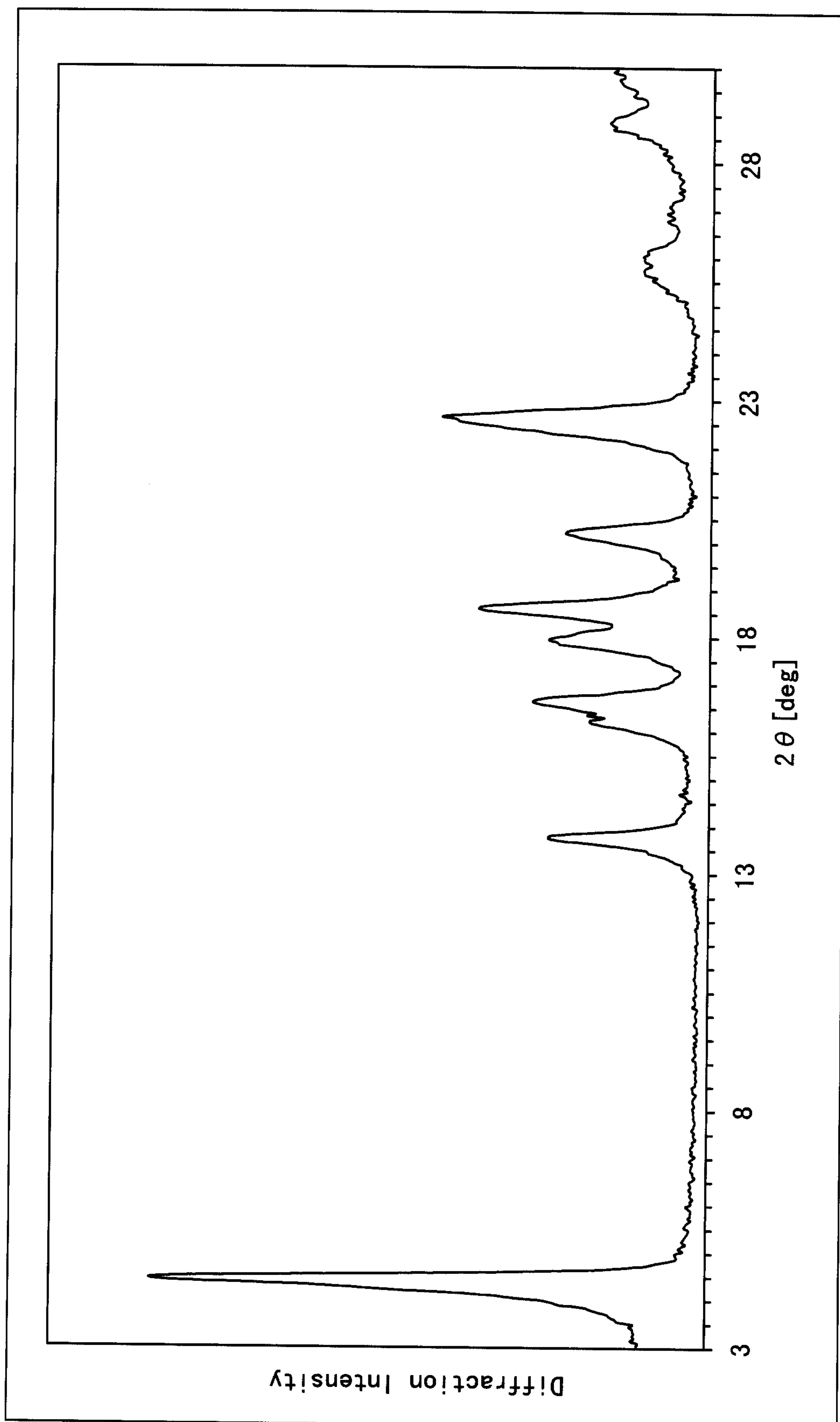


Fig. 28

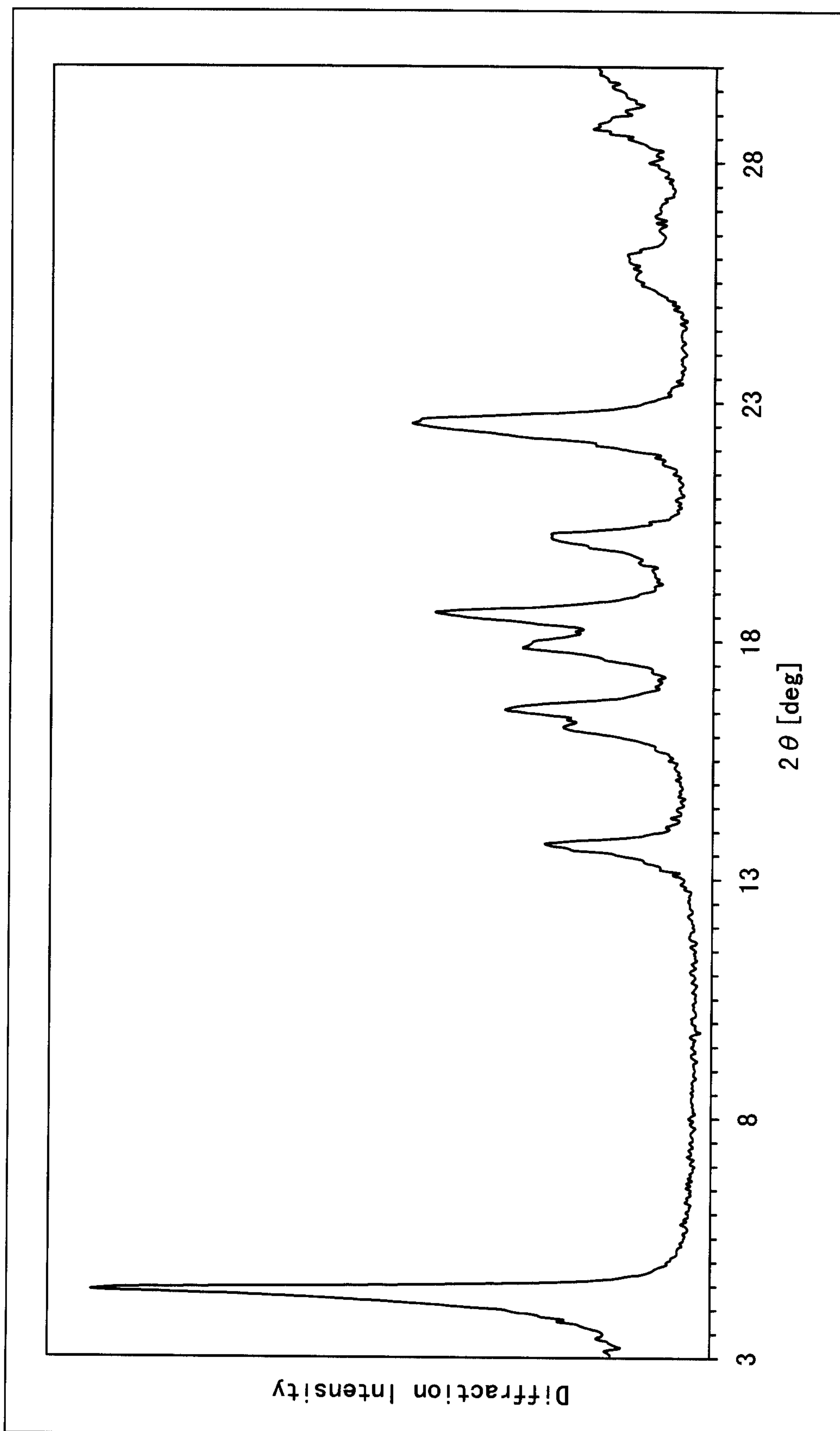


Fig. 29

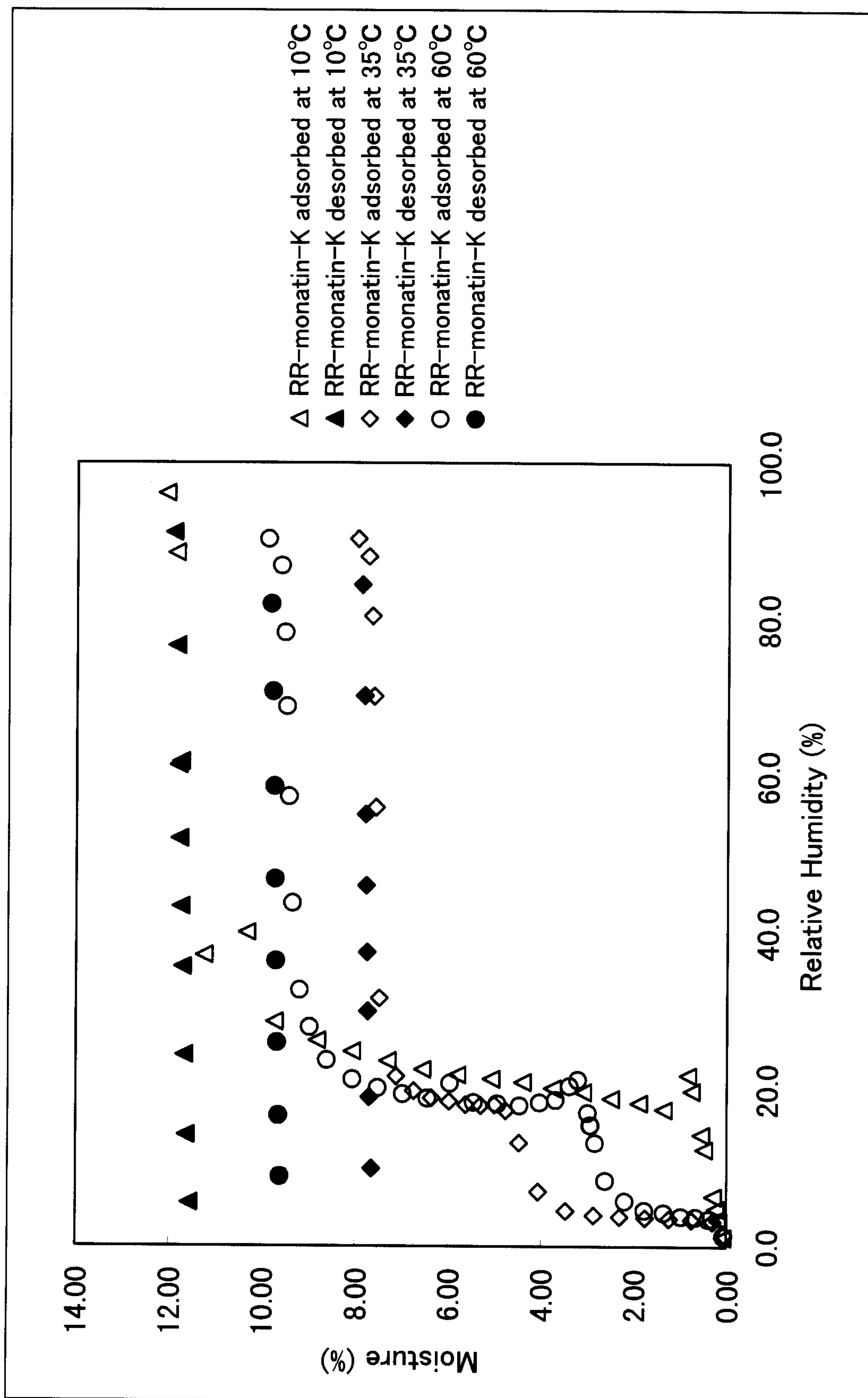


Fig. 30

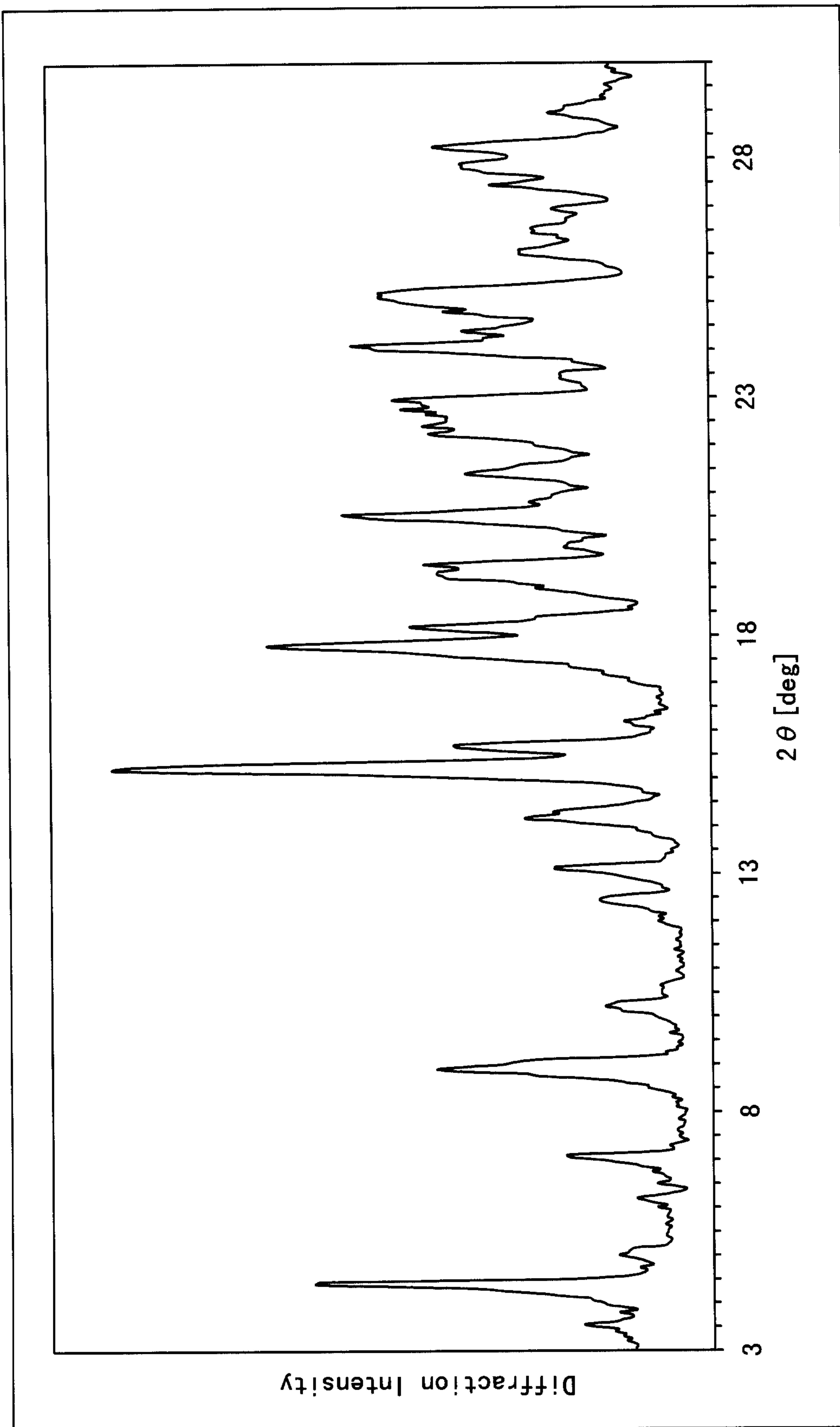


Fig. 31

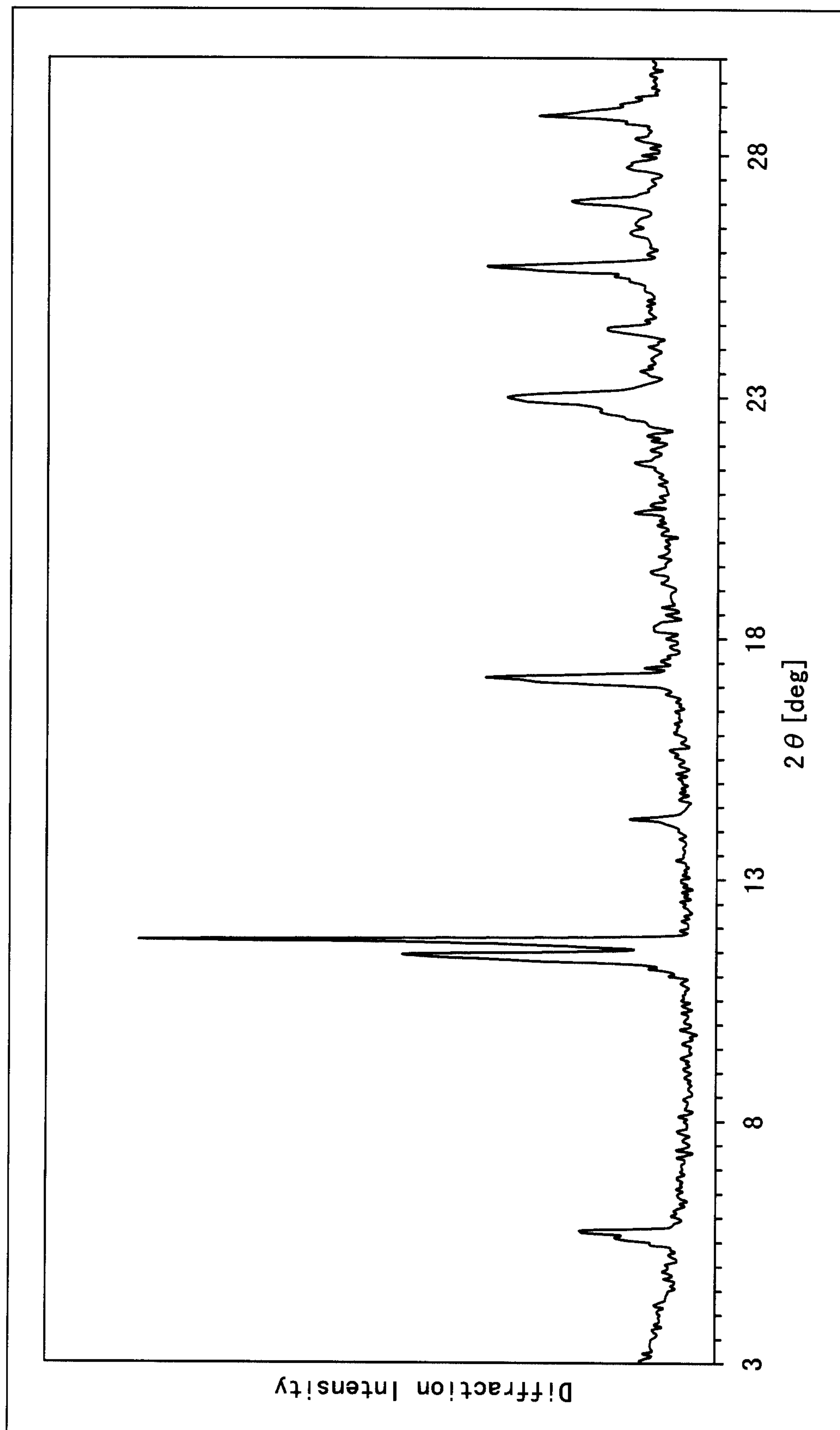


Fig. 32

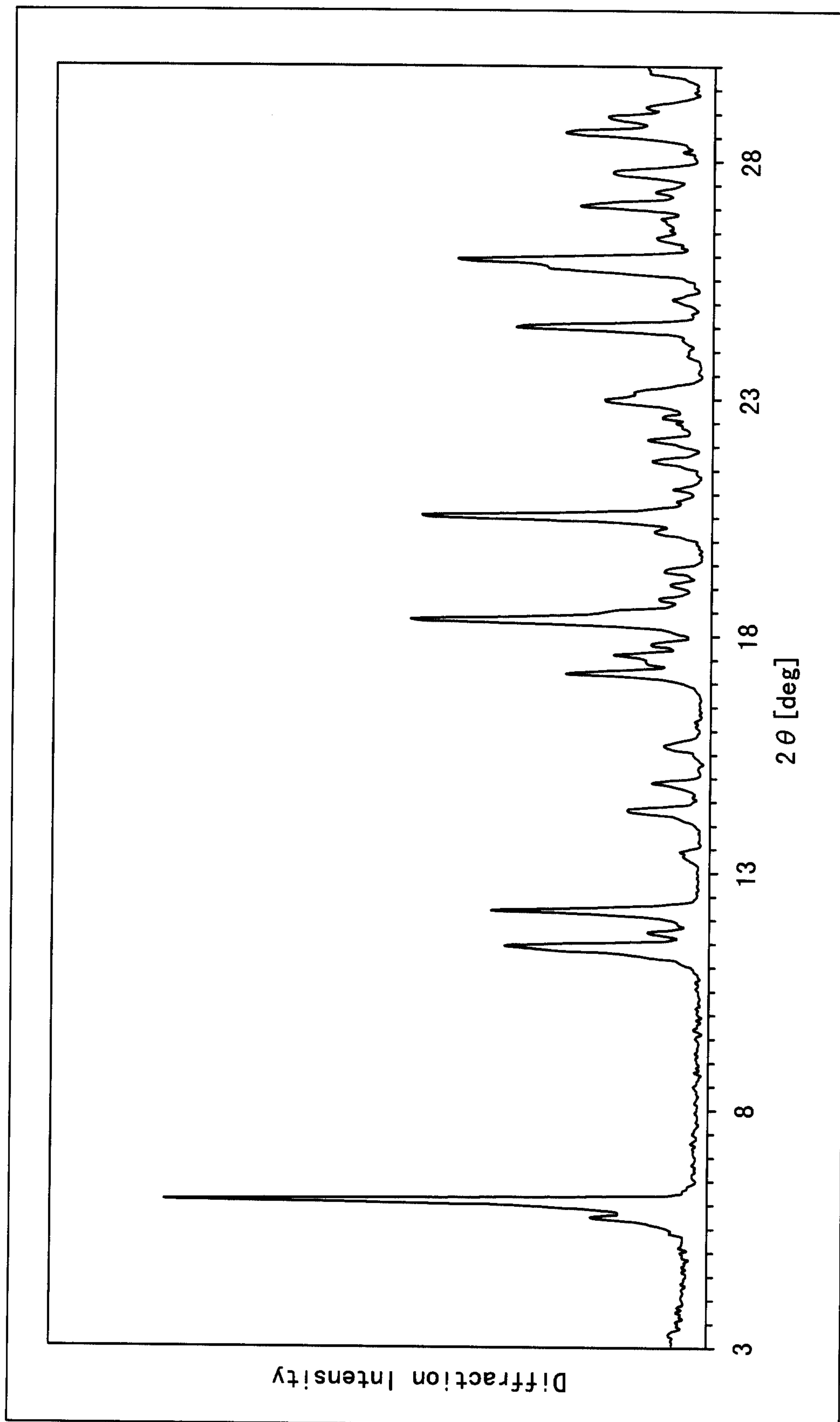


Fig. 33

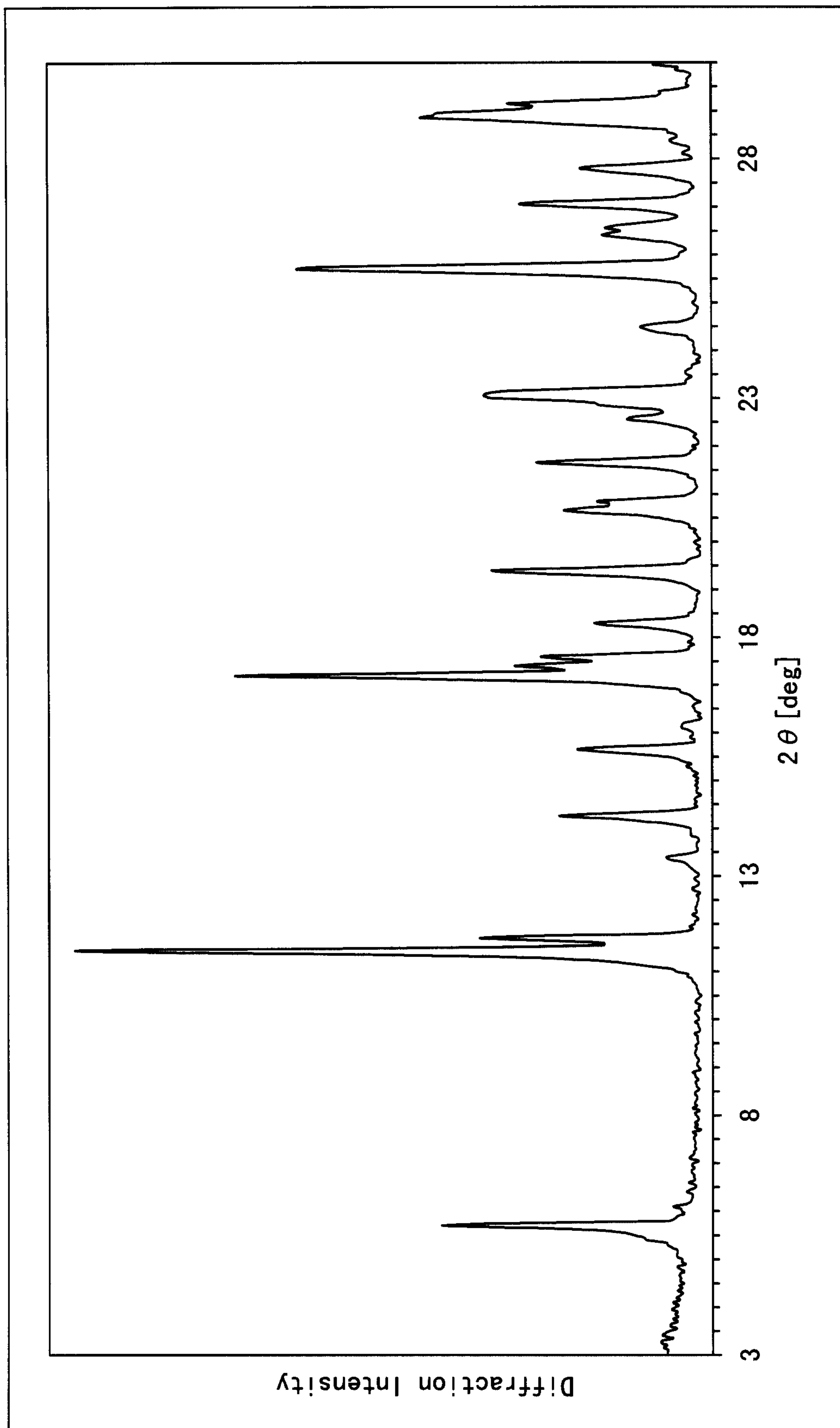


Fig. 34

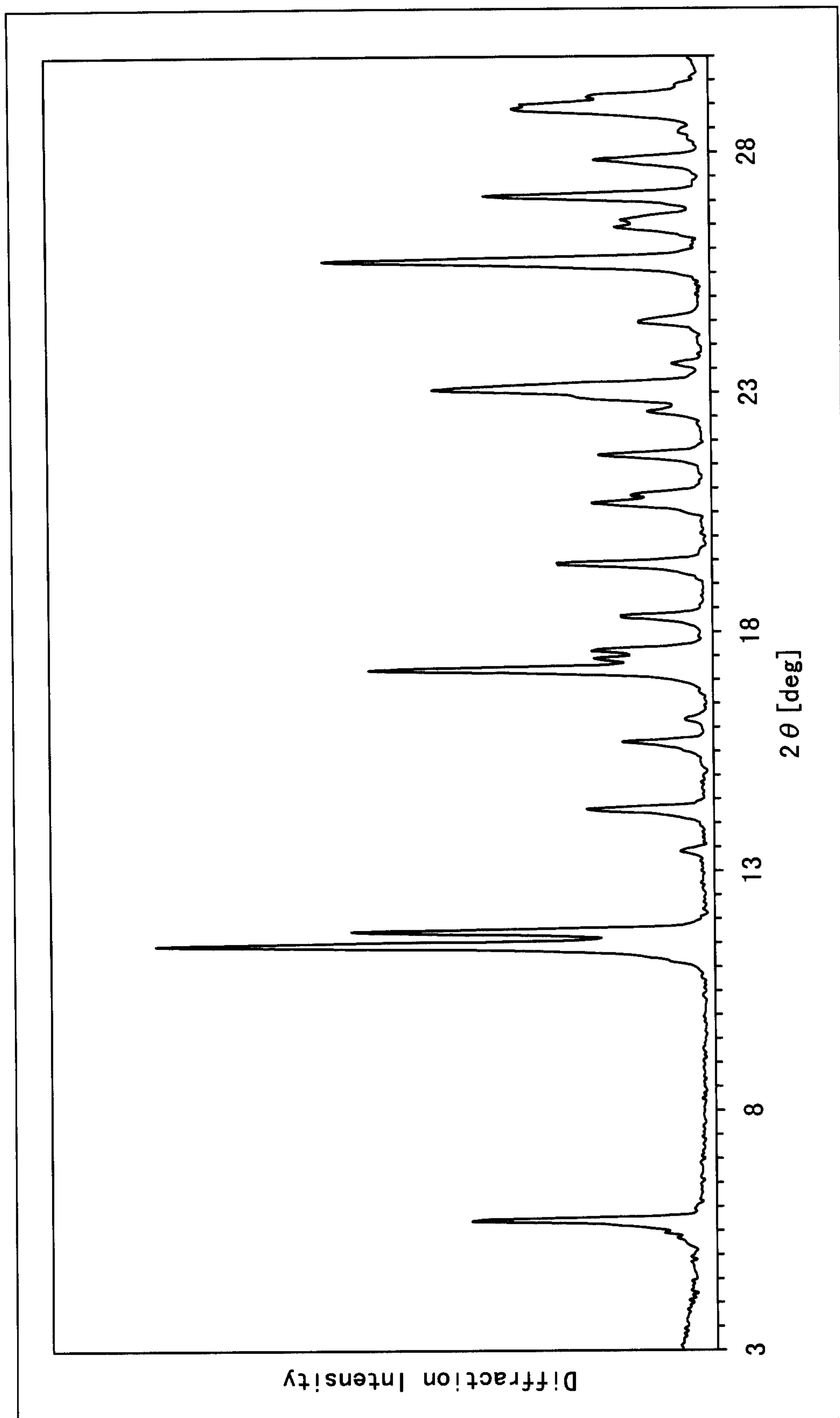


Fig. 35

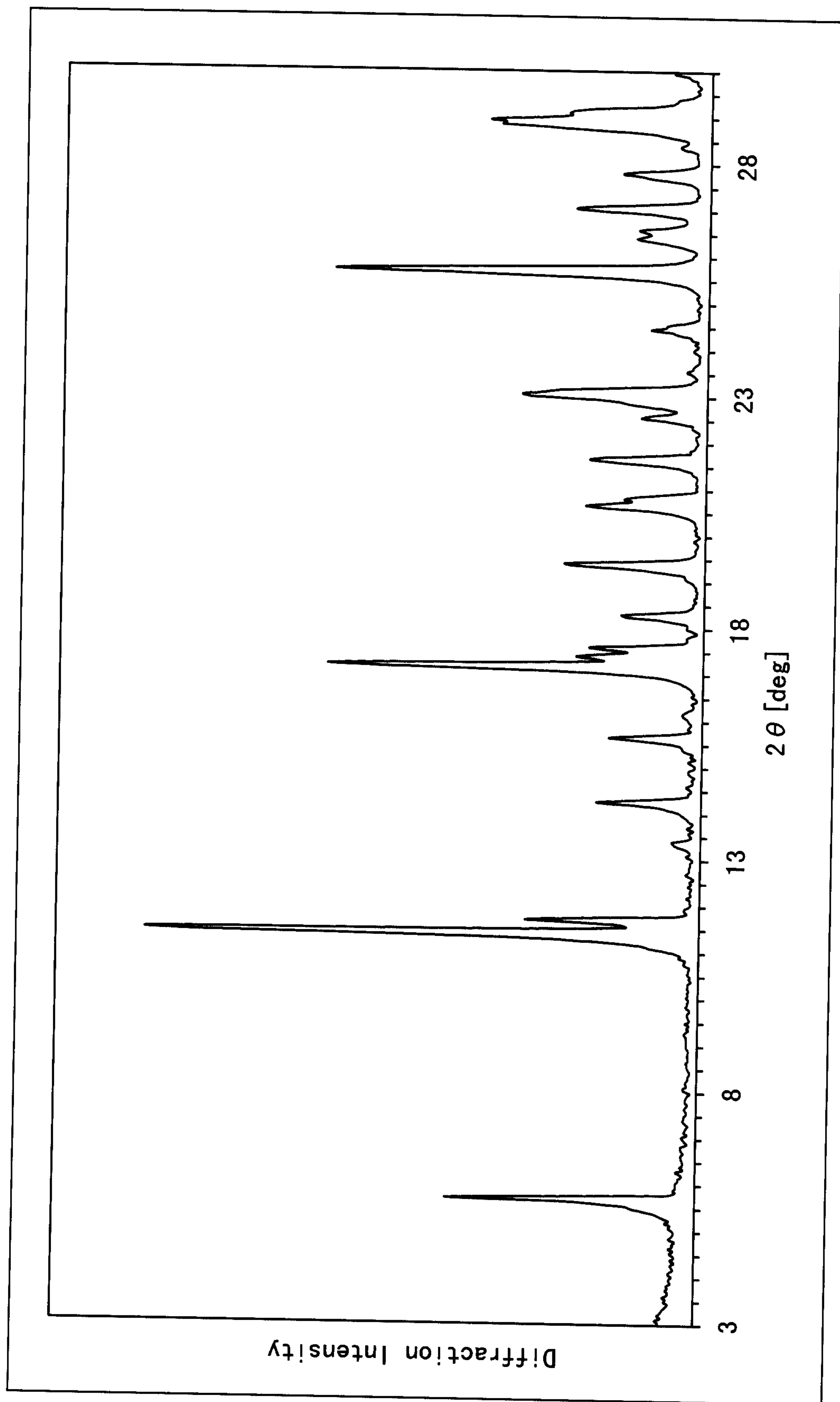


Fig. 36

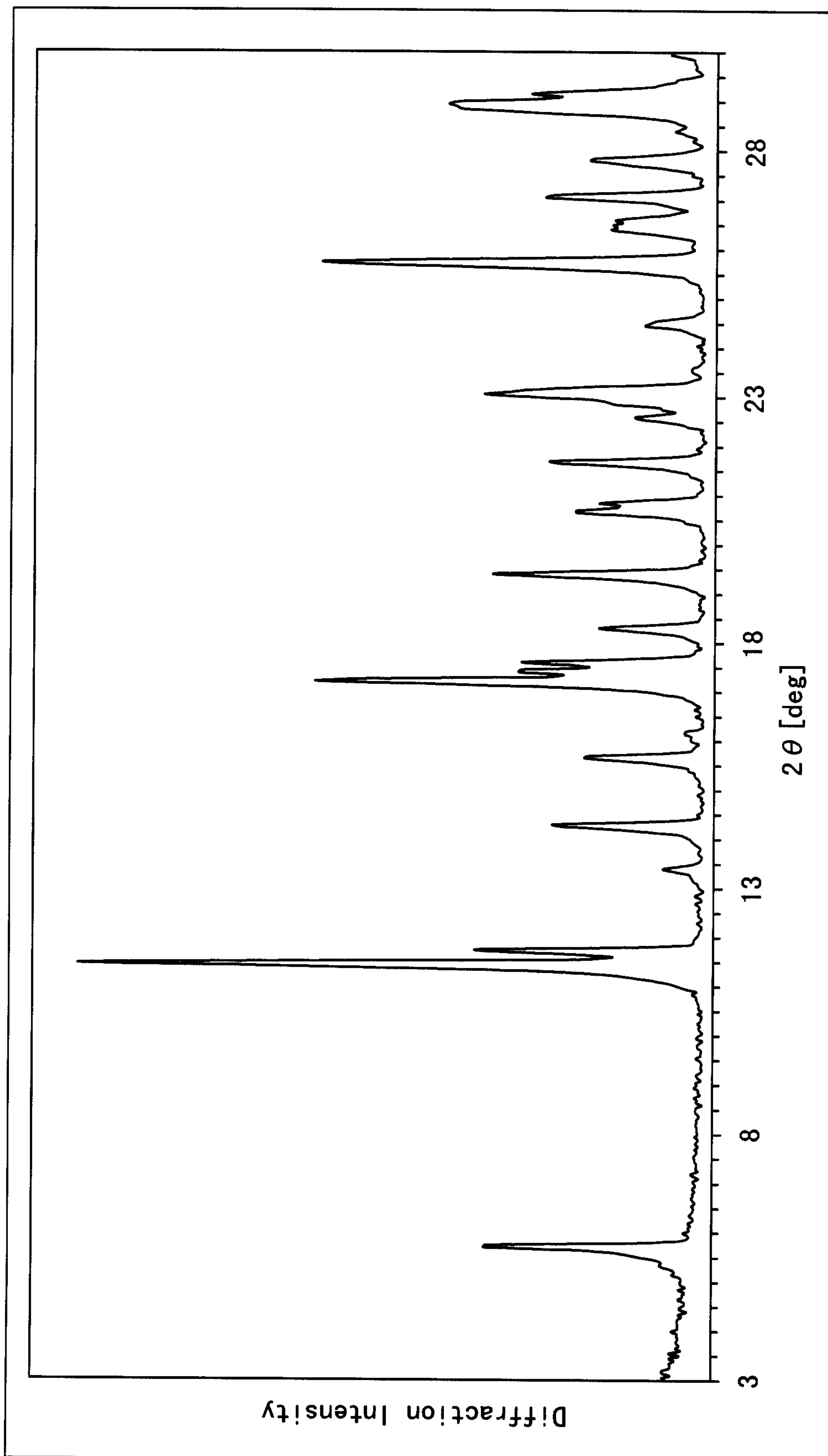


Fig. 37

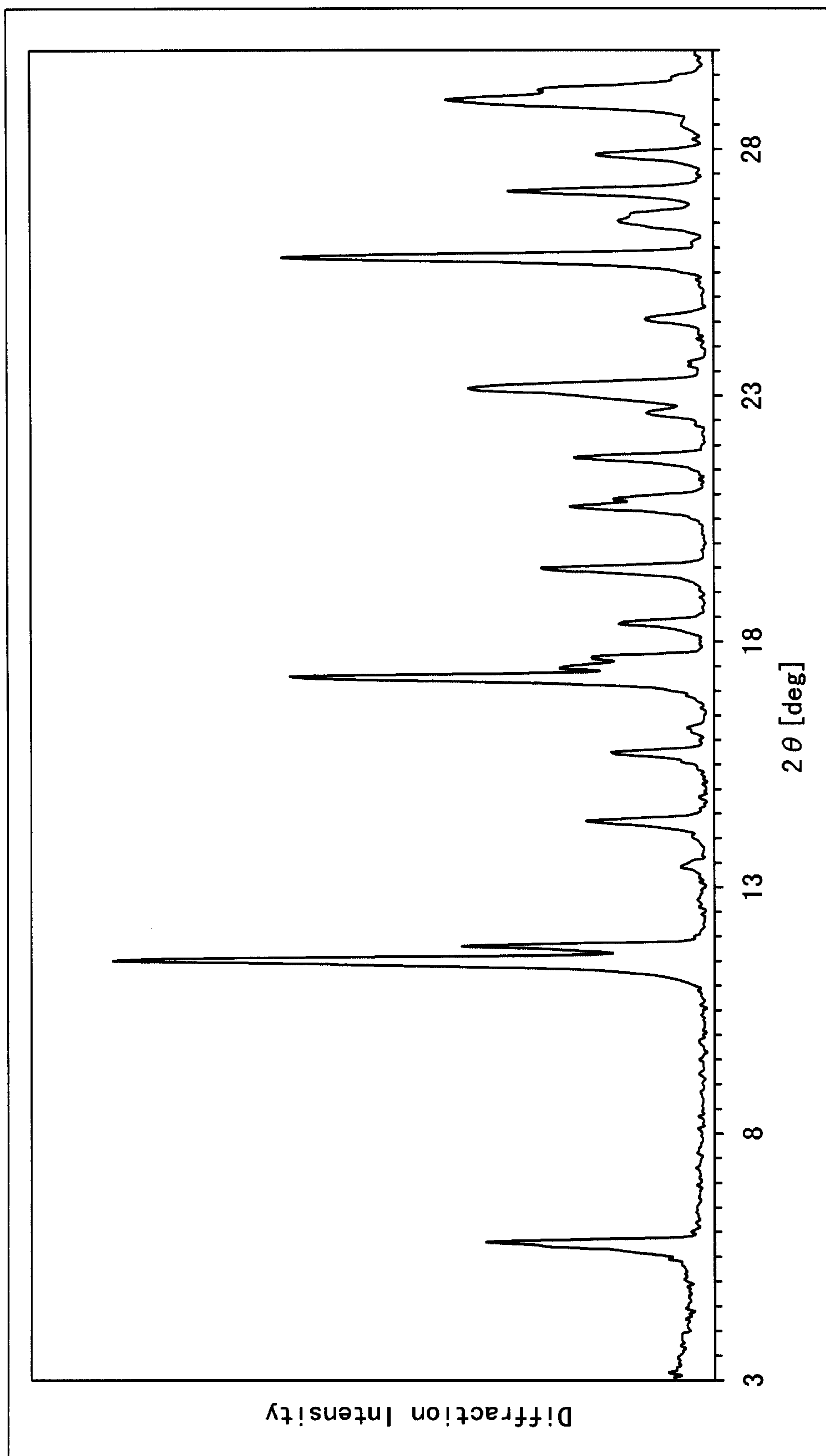


Fig. 38-1

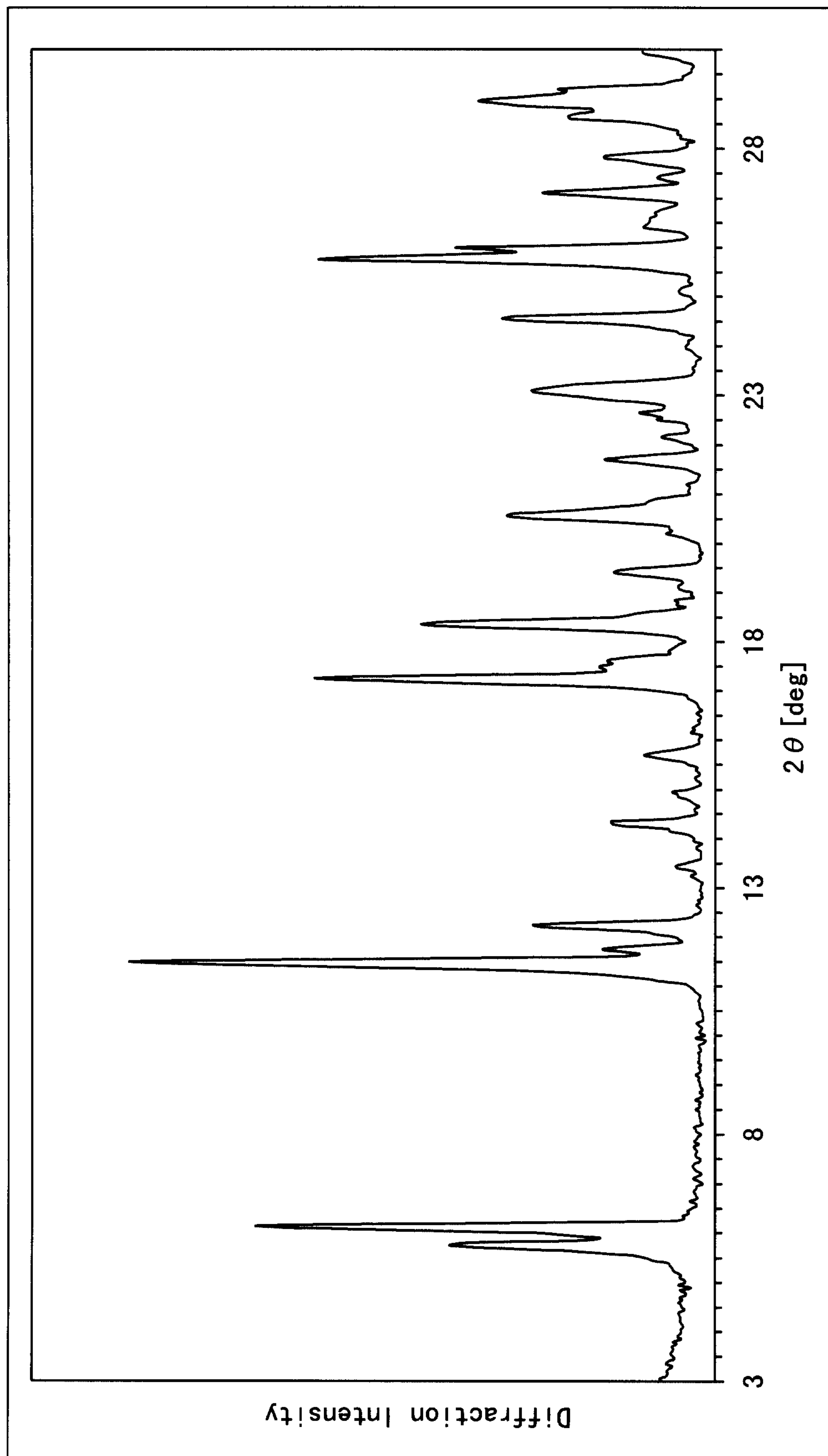


Fig. 38-2

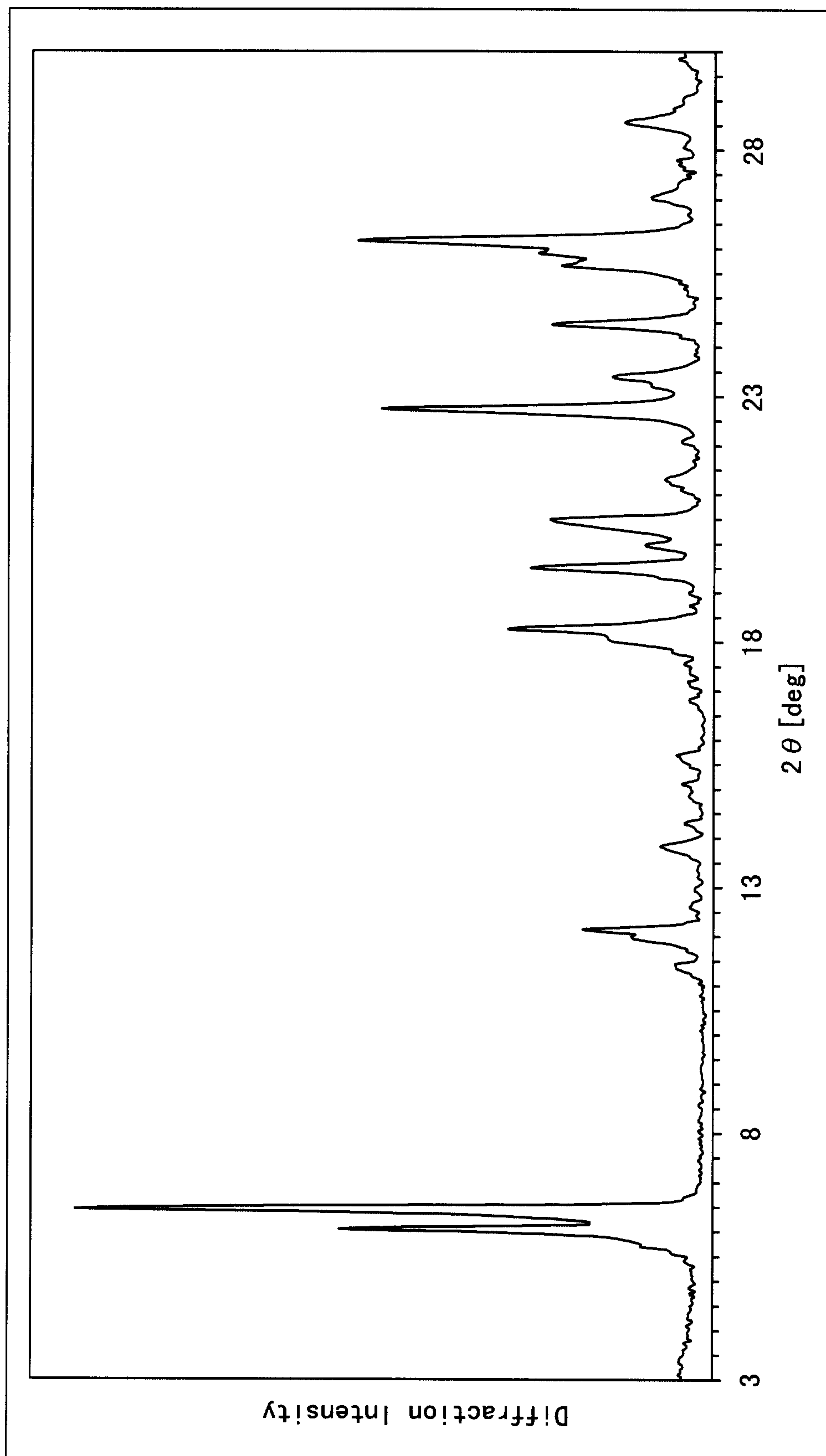


Fig. 39-1

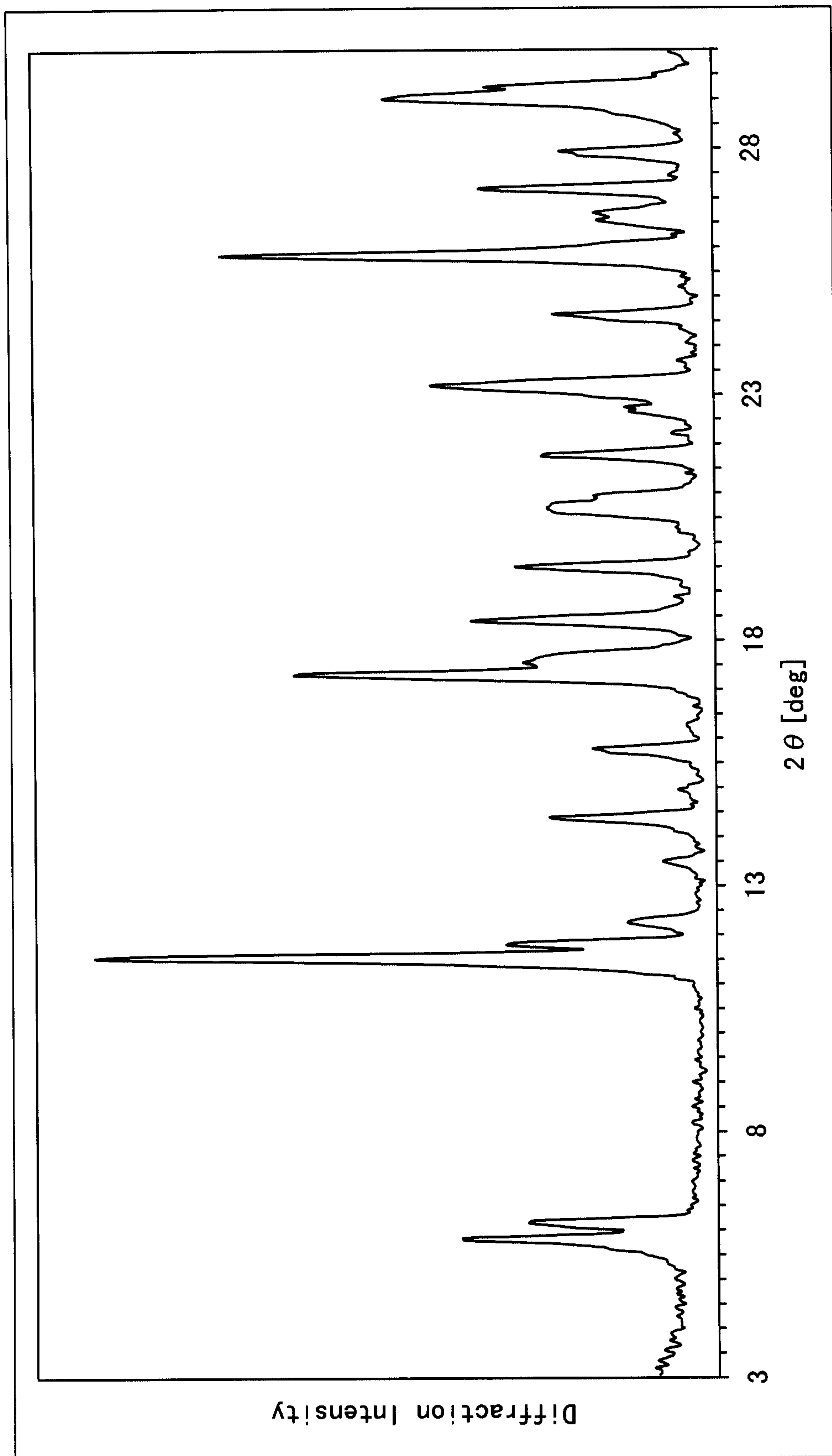


Fig. 39-2

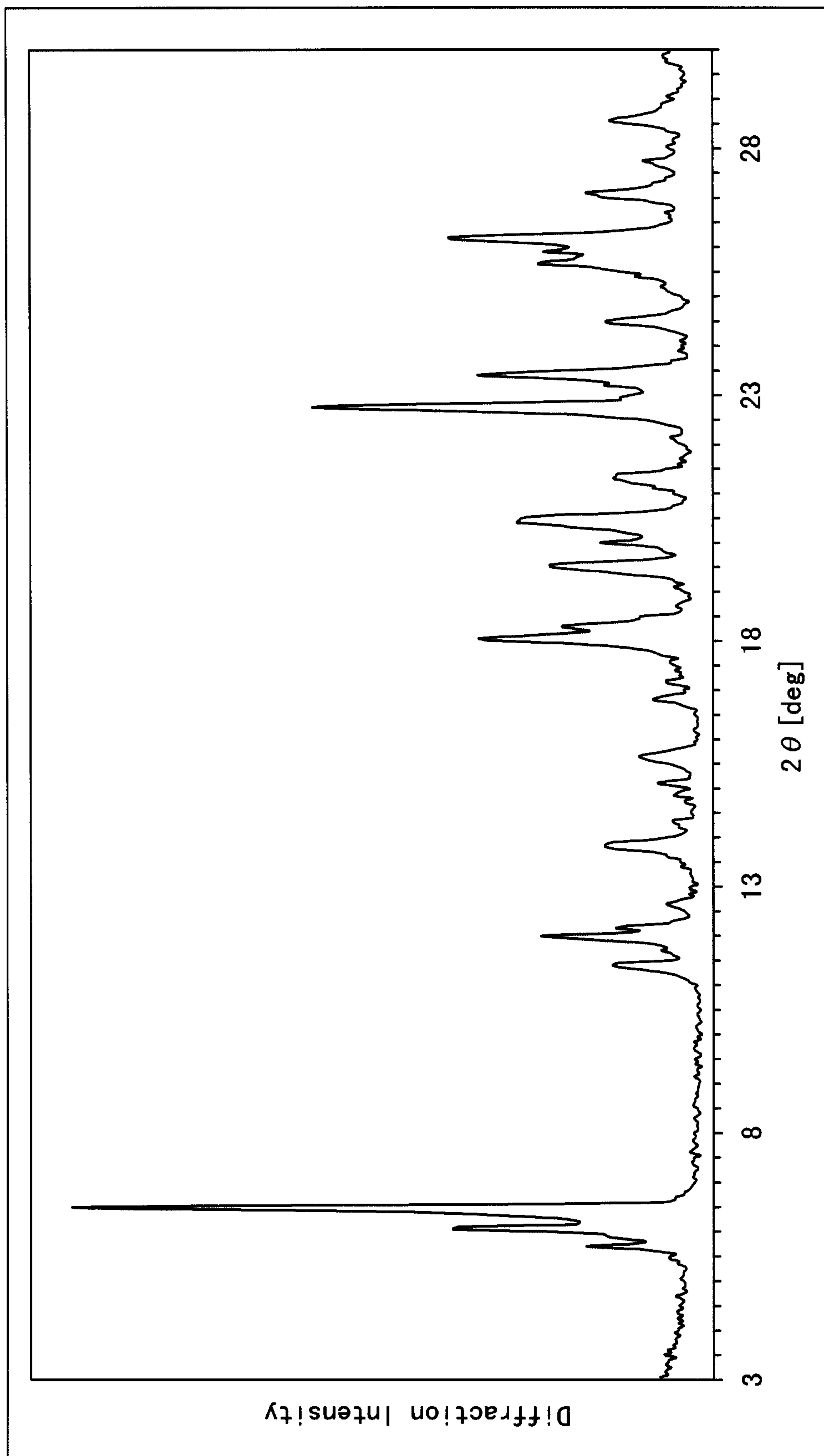


Fig. 40-1

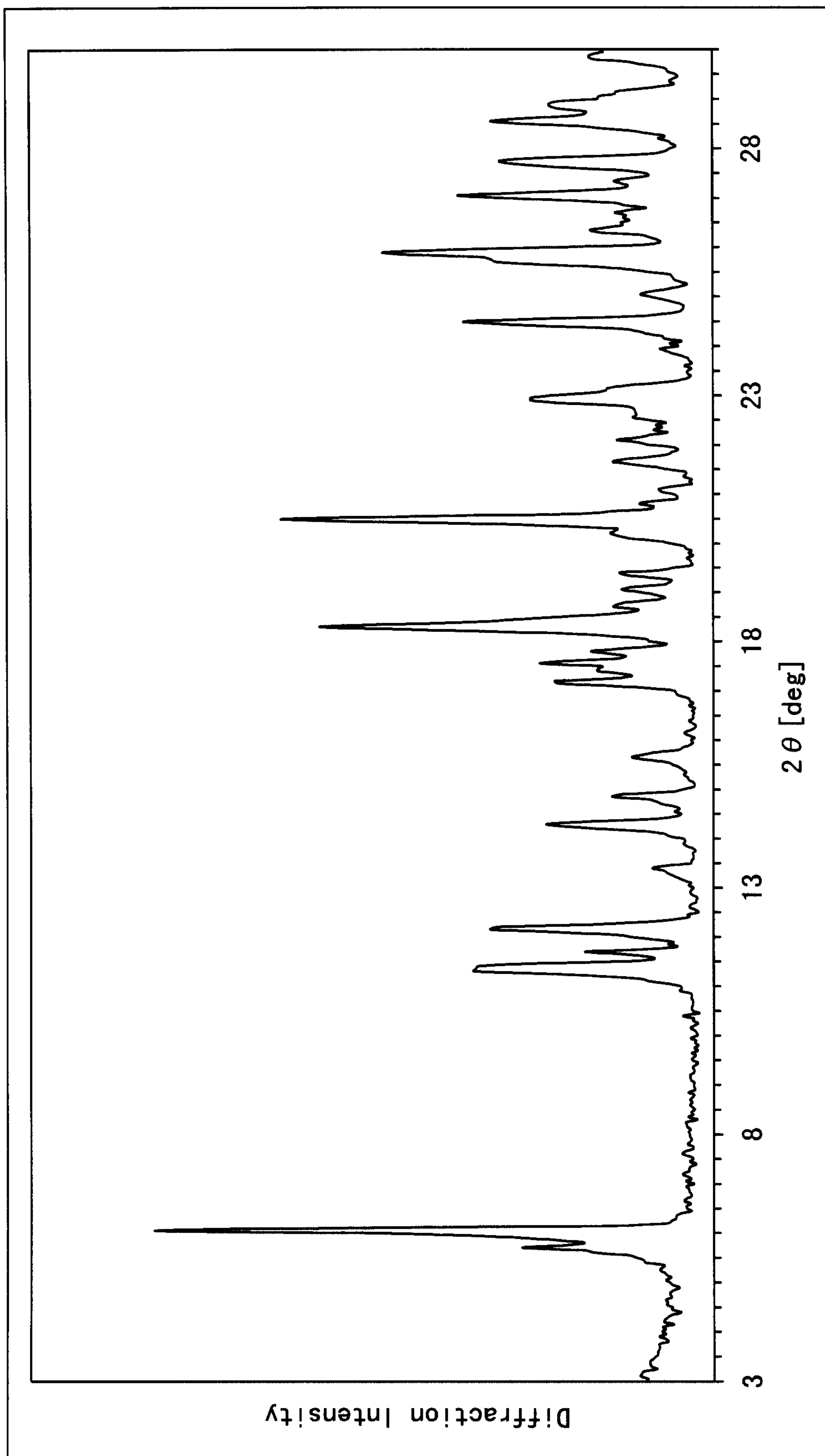


Fig. 40-2

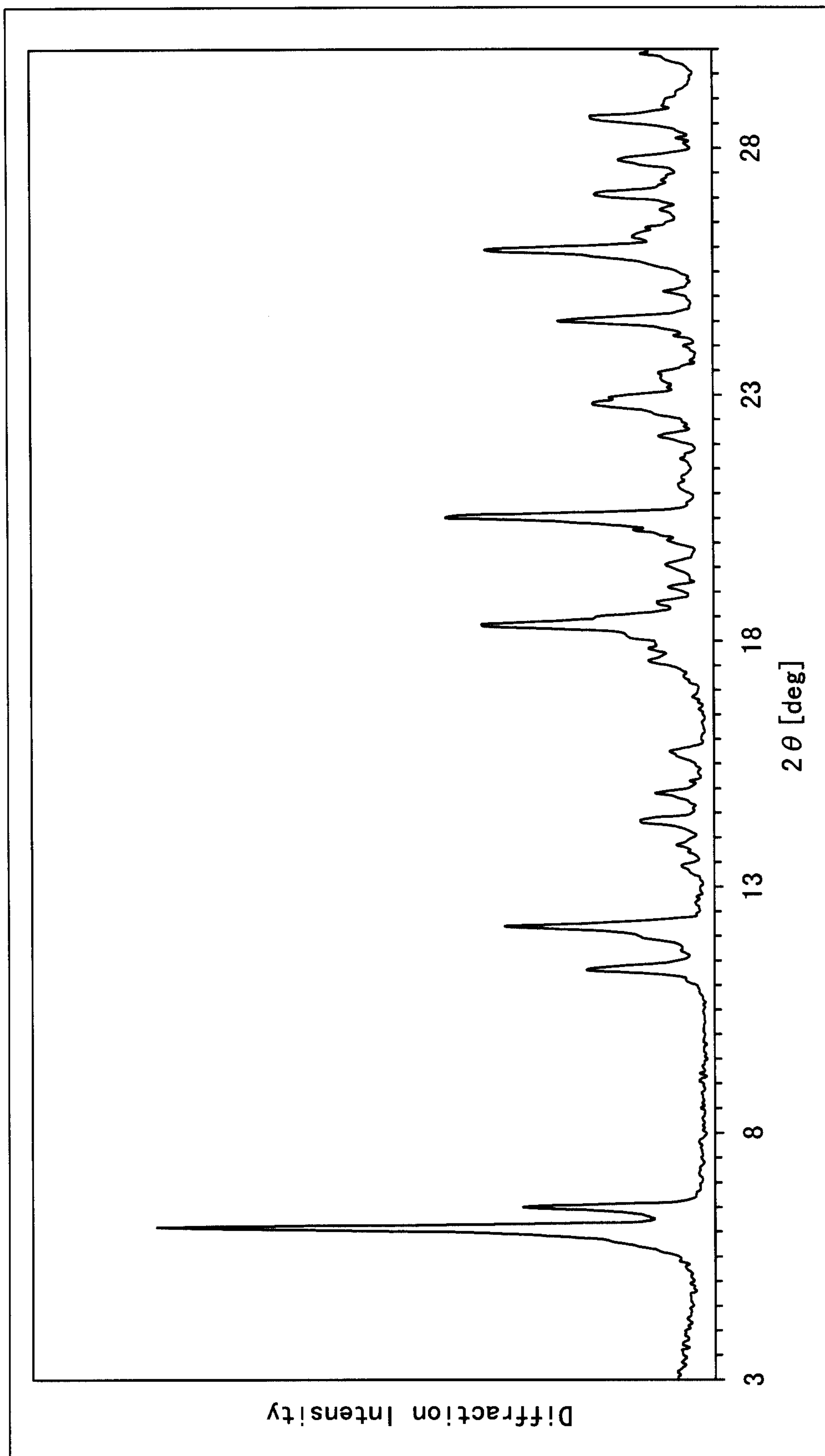


Fig. 41-1

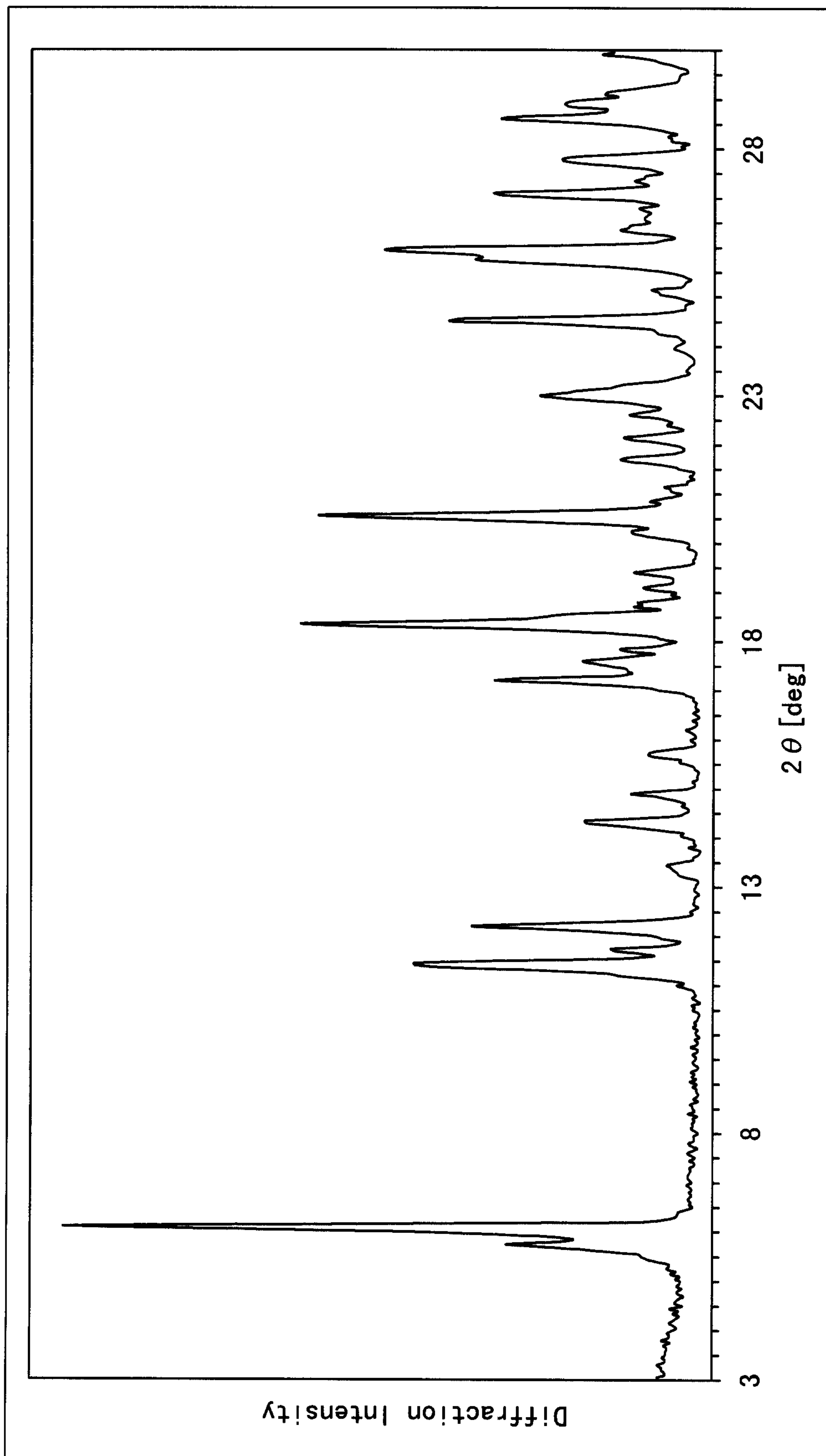


Fig. 41-2

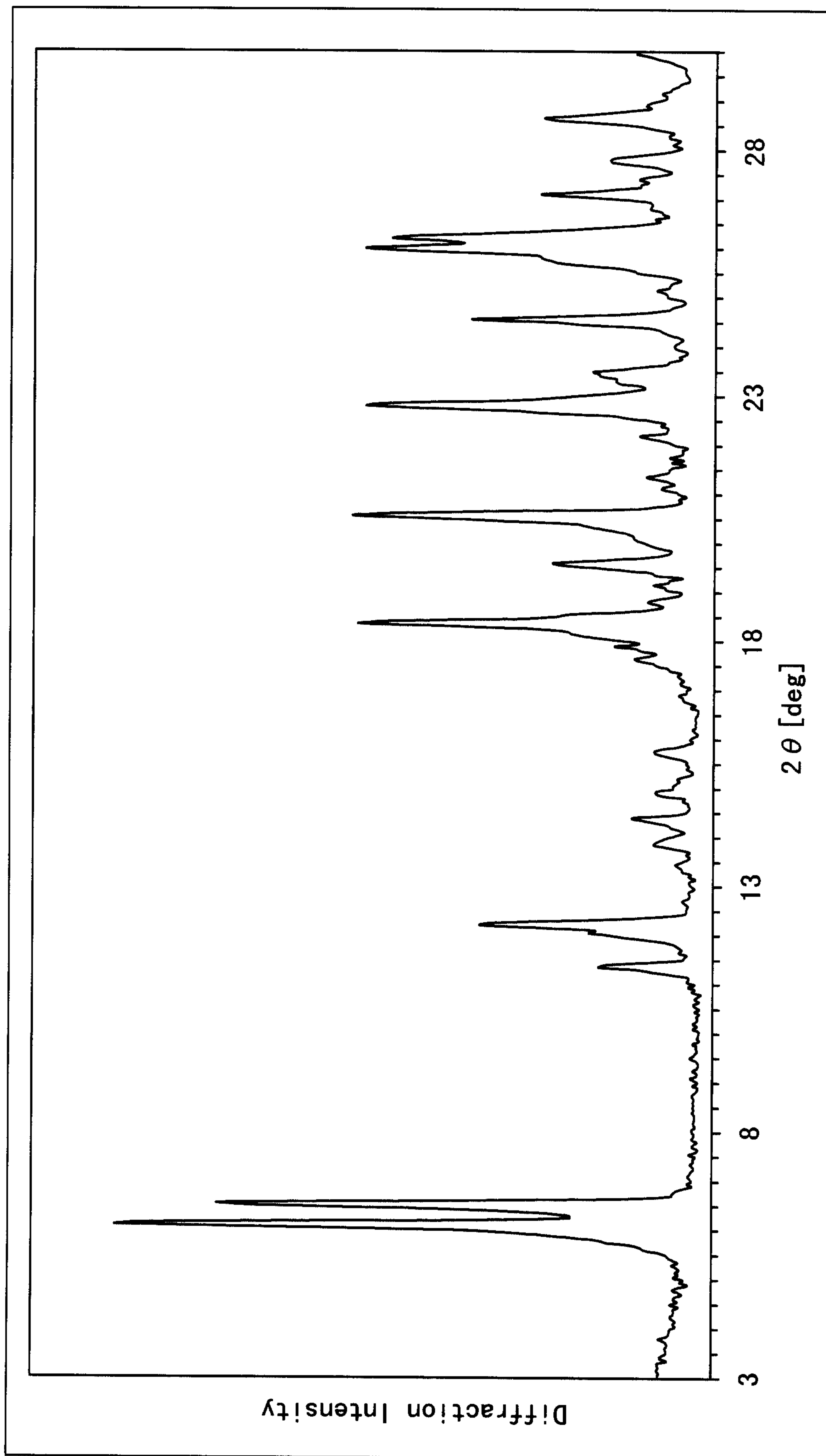


Fig. 42

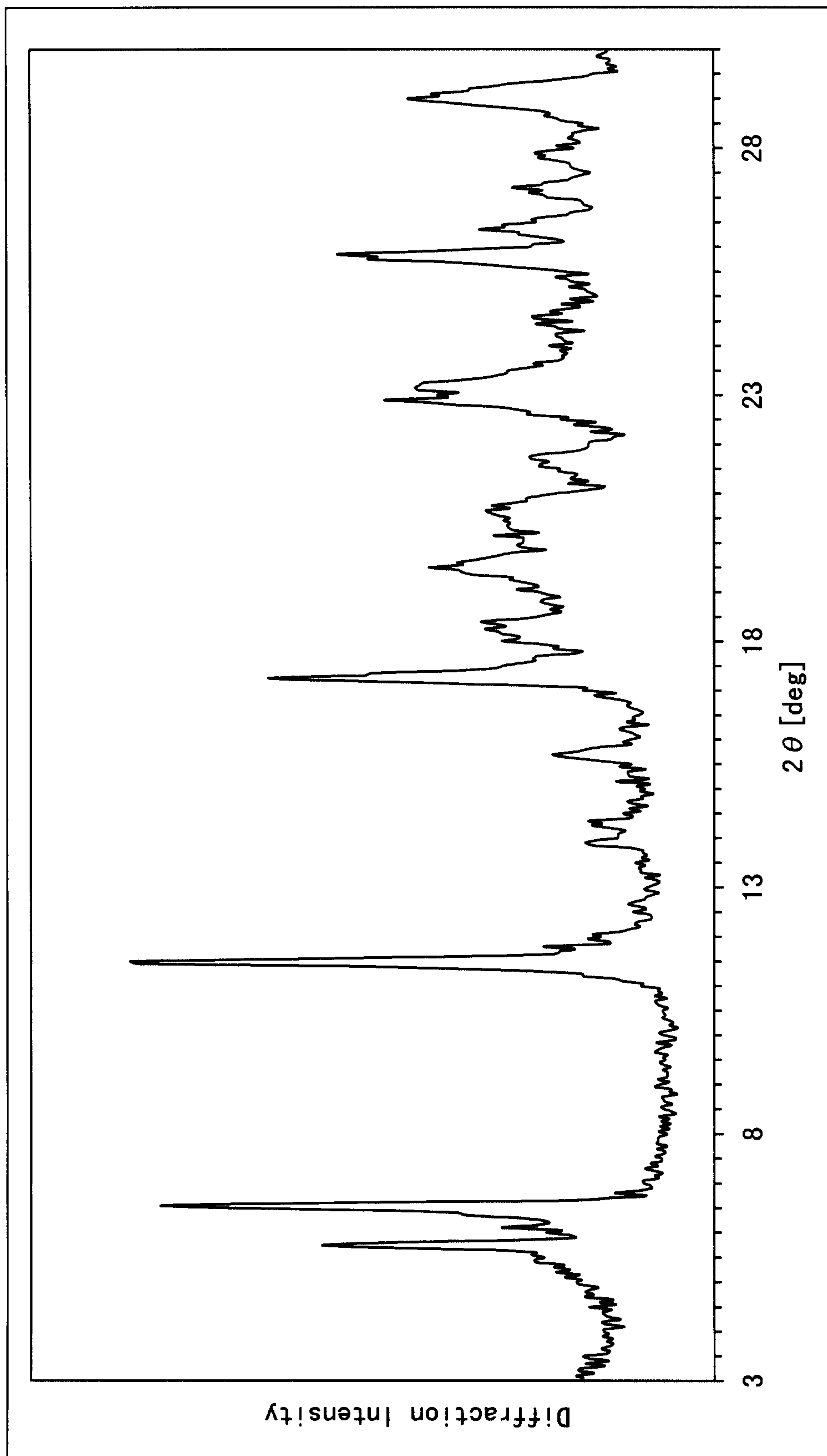


Fig. 43

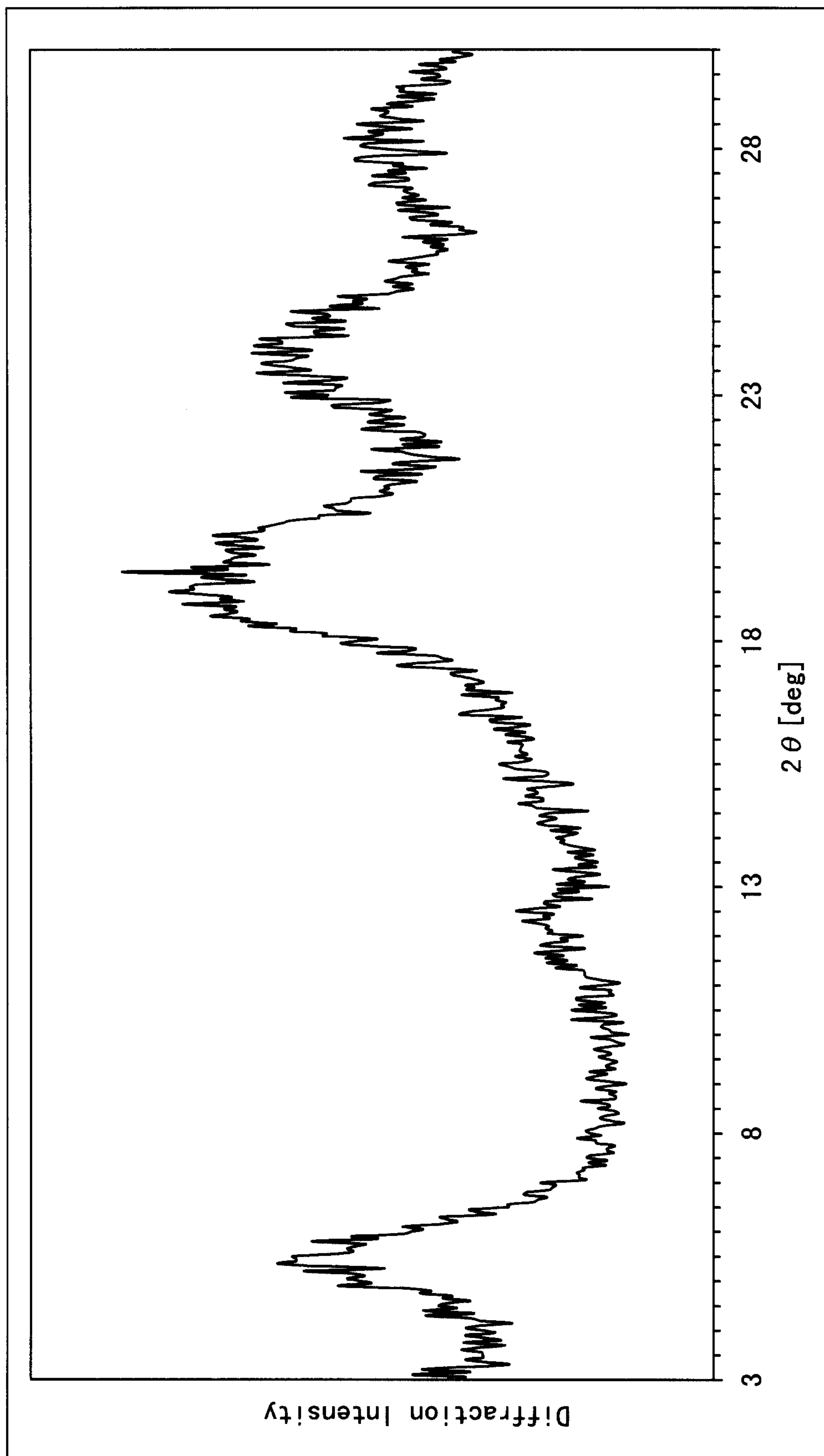


Fig. 44

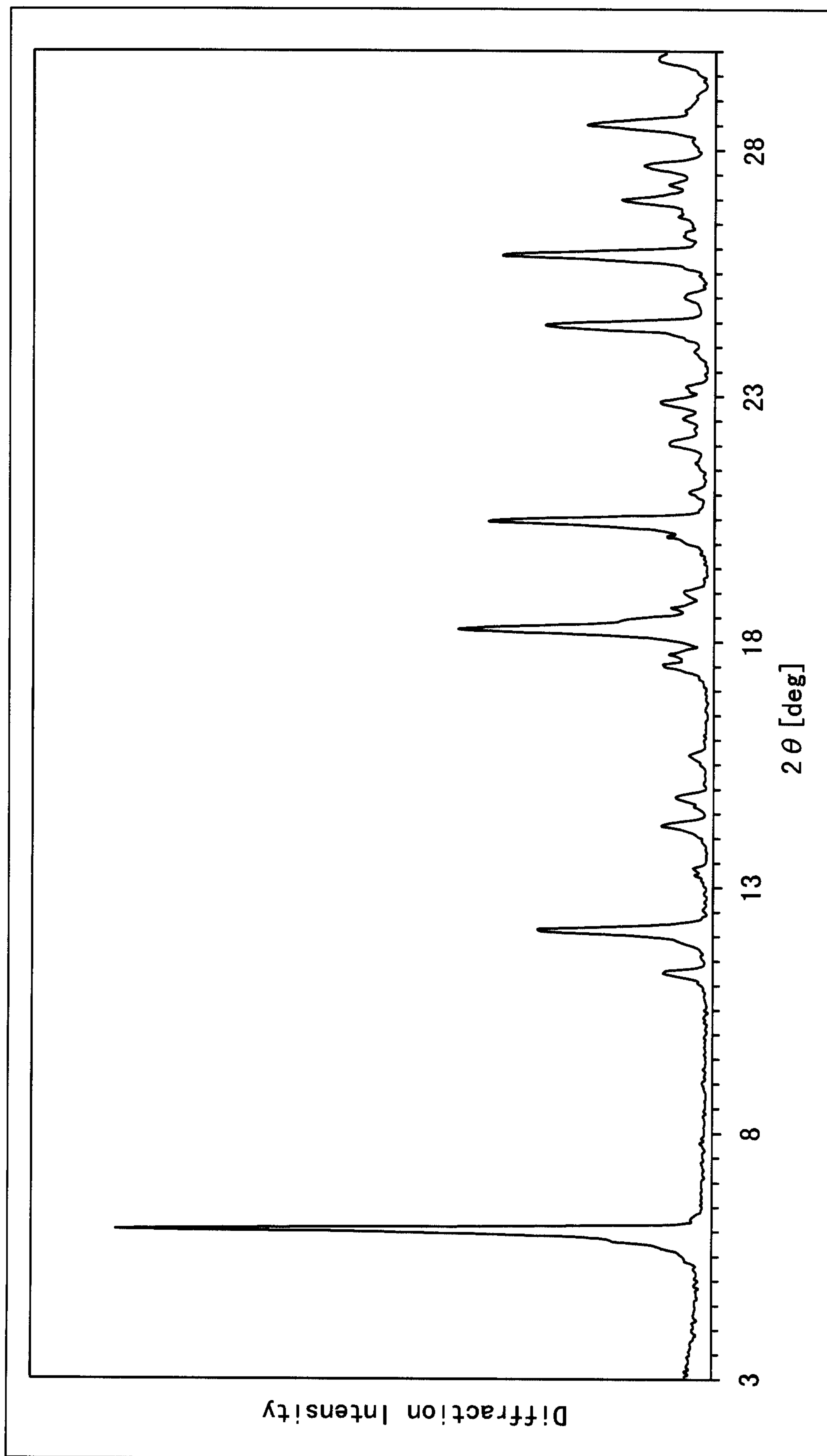


Fig. 45

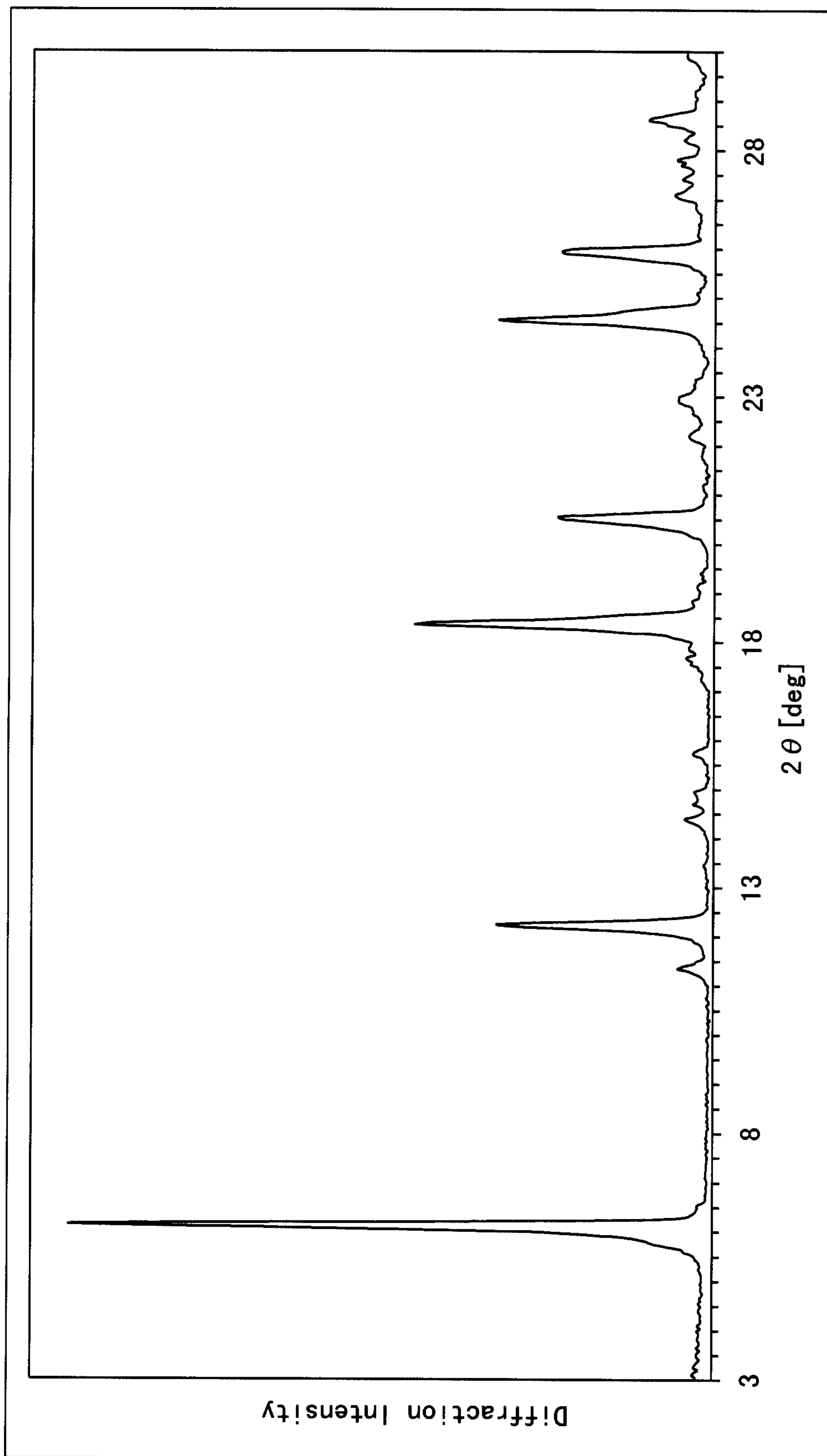


Fig. 46

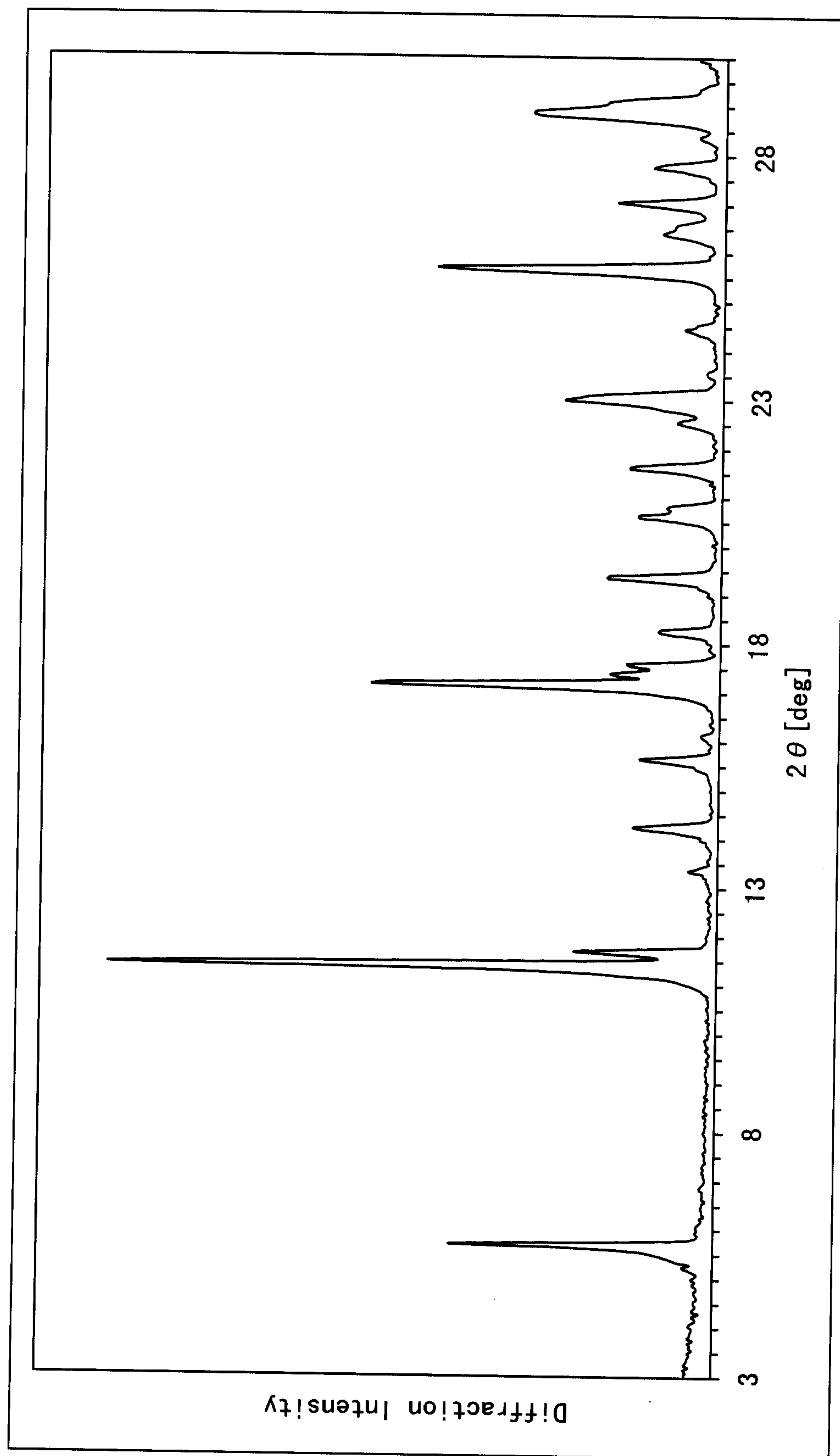


Fig. 47

