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[54] **METHOD OF PRODUCTION OF ESSENTIALLY PURE MELATONIN AND THE METHOD OF SOLUBILIZING MELATONIN IN WATER**

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**Related U.S. Patent Documents**

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Sep. 8, 1988 [IT] Italy ..... 21872 A/88

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[52] **U.S. Cl.** ..... **514/415**; 514/46; 536/27.6; 548/504; 548/507

[58] **Field of Search** ..... 544/276; 514/46, 514/415; 548/504, 508, 507; 536/27.3

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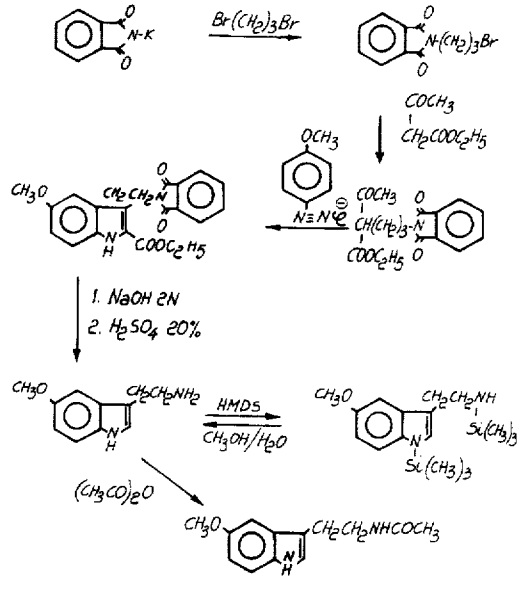
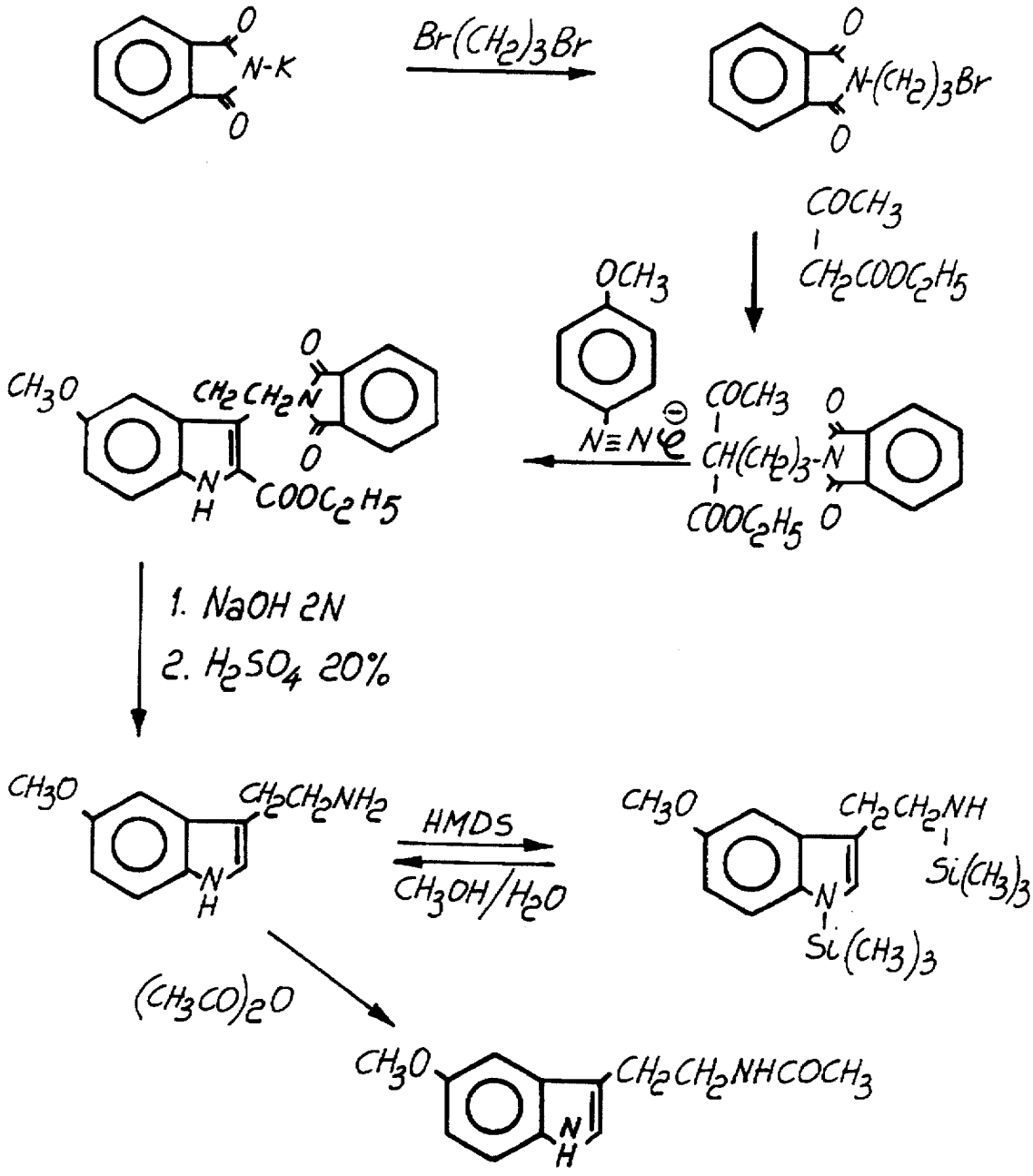
[57] **ABSTRACT**

A method of synthesizing an indole derivative of the tryptamine type particularly [melatonine,] *melatonin* comprising the steps of 1) reacting potassium phthalimide and 1,3-di-bromopropane to obtain 3-bromopropylphthalimide; 2) reacting 3-bromopropylphthalimide with sodium acetoacetic ester in ethanol to obtain ethyl-2-acetyl-5-phthalimidopentanoate; 3) reacting the product from step 2) with diazo-p-anisidine to obtain 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole; 4) reacting the 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole with 2N/NaOH and then 20% $H_2SO_4$ to obtain impure 5-methoxytriptamine, which is purified by means of hexamethyldisilazane. The mono and disubstituted derivatives are obtained and the monosubstituted derivative is hydrolyzed with aqueous methanol and then recrystallized from ethanol. The N-acetyl derivative is prepared by reaction with acetic anhydride. [Melatonine] *Melatonin* of high purity is obtained for prophylaxy and also against AIDS (Acquired Immuno Deficiency Syndrome).

**6 Claims, 1 Drawing Sheet**




N-K
Br(CH2)3Br
N-(CH2)3Br
COCH3
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OCH3
N≡N⊖
CH(CH2)3-N
COOC2H5
CH3O
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COOC2H5
1. NaOH 2N
2. H2SO4 20%
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CH3O
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CH2CH2NHCOCH3

# METHOD OF PRODUCTION OF ESSENTIALLY PURE MELATONIN AND THE METHOD OF SOLUBILIZING MELATONIN IN WATER

**Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.**

## BACKGROUND OF THE INVENTION

The present invention relates to a total synthesis method for preparing an indole structure derivative product class, of the tryptamine type, in particular of [melatonine] *melatonin* or N-acetyl-5-methoxytriptamine type, having a high purity degree and easily soluble, for therapeutic use against acquired immuno-deficiency syndromes or so-called AIDS.

As is known, it has been found that [melatonine] *melatonin* (MTL), administered with suitable doses and at given times, is able of reducing proteic synthesis of hypothalamus and hypophisis and that it, moreover, may inhibit the synthesis of gonadostymulines.

Such an action is probably exerted by means of a modulation of genic transcription and repression, as well as on the incretion of the two GH and PRL growth factors, under particular conditions.

The above mentioned overall effects, which are associated with other particular actions, as disclosed in a more detailed way hereinafter, justify as useful, even if not indispensable, the use of [melatonine] *melatonin* against tumours.

In fact one may reasonably think that [melatonine] *melatonin* pertains to that class of drugs which interfere with the growth of neoplastic cells and reduce the life time thereof.

On the other hand, also known is the fact that presently available methods for making the tryptamine structure having the hydrogen atom at the 5-position replaced by the group $OCH_3$, are based on a series of chemical reactions providing 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole, therefrom there is obtained 5-methoxytryptamine by means of a plurality of comparatively complex and low yield processing steps.

More specifically, known prior art methods comprise an alkaline saponifying step providing 2-carboxy-3-(2-O-carboxybenzamidoethyl)-5-methoxy-indole acid which is then dry decarboxylated at 250° C. in order to form phthalimidoethyl-5-methoxy-indole, which is then water hydrazinolized to provide 5-methoxytryptamine.

In order to obtain pure N-acetyl-5-methoxytryptamine or [melatonine] *melatonin* with a high yield, it is necessary to have a high purity starting product, that is 5-methoxytryptamine.

Known conventional purifying methods, based on the use of solvents or mixtures thereof, on the other hand, have not provided a sufficiently high purity degree with a contemporaneous high production yield.

## SUMMARY OF THE INVENTION

Accordingly, the main object of the present invention is to overcome the above mentioned drawback by providing a method for making, with high production yields, very pure 5-methoxytryptamine, which method essentially comprises a synthesis known per se with respect to the reagents, but carried out by new techniques starting from 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole.

Another object of the invention is to provide such a method which, in addition to simplifying the processing steps, can provide [melatonine] *melatonin* starting both from 5-methoxytryptamine, in raw form, and from pure 5-methoxytryptamine.



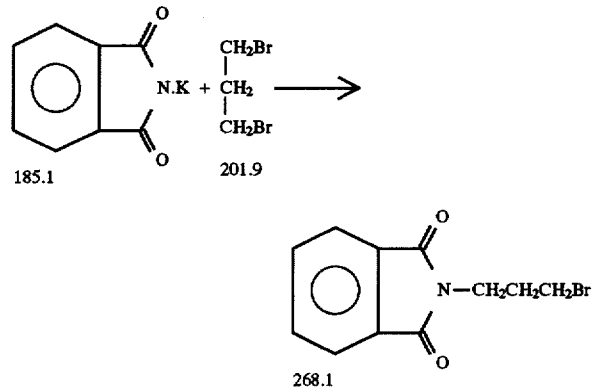
Yet another object of the present invention is to provide a total synthesis method which provides a very pure and reliable product, with consistent curative properties.

According to one aspect of the present invention, the above mentioned objects, as well as yet other objects, which will become more apparent hereinafter, are achieved by a total synthesis method for making an indole structure derivative product class, of the tryptamine type, in particular of [melatonine] *melatonin* or N-acetyl-5-methoxytryptamine type, having a high purity degree and easily water soluble for therapeutic use against acquired immuno-deficiency syndromes comprising the steps of combining potassium phthalimide and dibromopropane, to form 3-bromopropylphthalimide, adding [acetacetic] *acetoacetic* ester in the presence of anhydrous ethanol dissolved sodium to form ethyl-2-acetyl-phthalimidopentanoate, adding diazo-p-anisidine to form 2-carboxyethyl-[3-(2-phtalimidoethyl)-]*3-2-phthalimidoethyl*-5-methoxy-indole, which is processed, in a first step, by NaOH 2N up to a complete solution and, then, by $H_2SO_4$ (at 20%) to form raw 5-methoxytryptamine, which is purified by means of hexamethyldisilazane, so as to form the related mono- and bi-derivative therefrom, by means of an aqueous methanol hydrolysis, there is obtained [the starting compound] *5-methoxytryptamine.*

## BRIEF DESCRIPTION OF THE DRAWING

Further characteristics and advantages of the present invention will become more apparent from the following detailed description of the total synthesis method according to the invention, with reference to the chemical diagram shown in the accompanying drawing table.

## DESCRIPTION OF THE PREFERRED EMBODIMENT




The method according to the invention comprises the step of preparing 3-bromopropylphthalimide as follows: into a 3-neck 1-liter flask, provided with stirrer and cooling medium, there are introduced 101 g (0.5 moles) of 1.3-dibromo-propane, 250 ml acetone and 15 g K-phthalimide, by reflux processing the mixture under stirring. At 1 hour time intervals there are added further (15+10+6.3) g K-phthalimide (46.3 g corresponding to 0.25 moles), by holding under reflux conditions for a total period of 24 hours.

At the end of this period the precipitated KBr is filtered and acetone is evaporated in a rotary evaporating device: the

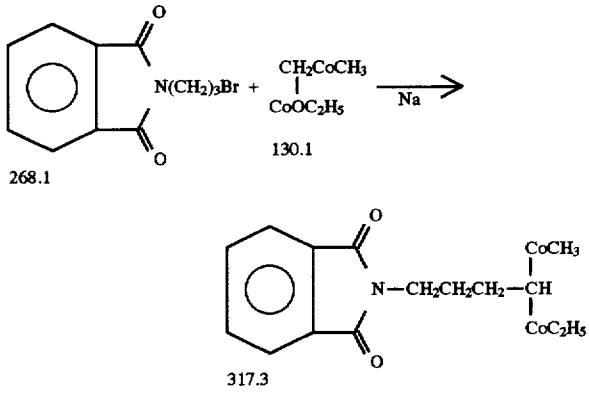
obtained oil is distilled under vacuum (as provided by a water pump) and there are recovered 48.5 g (0.25 moles) of 1,3-dibromopropane, which is distilled at 69°–70° C. The residue (dissolved before solidification in the distillation flask) is [crystalized] *crystallized* twice from ethanol, so as to remove the small amount of formed diphthalimidopropane.

There are thus obtained 48.2 g of a white [crystaline] *crystalline* solid, melting point 72° C., with a yield of 72%.

Purity is controlled for TLC on silica gel, using as eluent benzene-acetone (45:5), freshly prepared, Rf of about 0.95 (diphthalimidopropane having a lower Rf).

By analogous, reactions, in which K-phthalimide is added once, there is obtained a product which contains greater amounts of diphthalimidopropane, thereby it is necessary to purify by distillation (e.g. 150° C./0.25 mm) by using a Vigreux device without cooling, since the distillate tends toward solidification. The yield is substantially equal to the above disclosed yield.

[Preparing] *Preparation* of ethyl-2-acetyl-5-phthalimidopentanoate




In a three-neck flask having a capacity of 500 ml, provided with $CaCl_2$ cooling there are dissolved 4.60 g (0.2 g/A) Na in 100 ml anhydrous ethanol. To the solution, at room temperature, there are added 27.32 (0.21 moles) of [acetacetic esther] *acetoacetic ester* and then, after ten minutes, 40 g of 3-bromopropylphthalimide and, after one hour, further 12.5 g (in total 52.5 g corresponding to 0.196 moles), by holding the reflux processing and continuing for further three hours.

At the end of this period, sodium bromide is filtered, the solution is neutralized by 2N HCl and ethanol is evaporated under reduced pressure. The residue is recovered by ether, washed by $H_2O$×2, dried on anhydrous $Na_2SO_4$ and the solvent is evaporated, thereby providing a light yellow oil which is [crystalized] *crystallized* by dissolving it in a minimum ethanol amount by adding a small amount of ether and upon [ageing] *aging* for a night.

There are thus obtained 45 g (yield 72%) of a white crystalline solid, with m.p. 60° C. Upon [recrystalisation] *recrystallization* there is obtained a m.p. of 63° C. Product also [crystalizes] *crystallizes* from benzene-petroleum ether.

TLC on silica gel, benzene-acetone (45:5), Rf about 0.70.

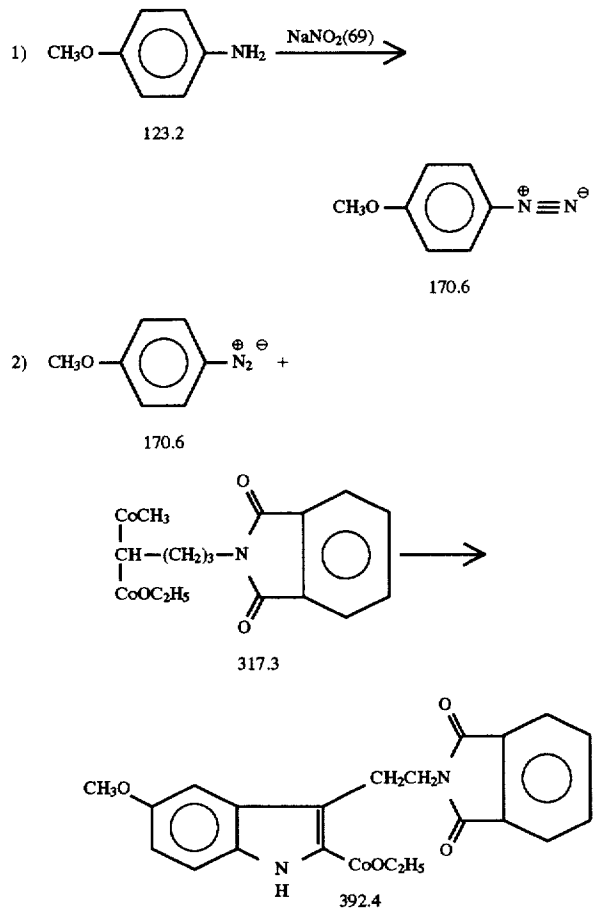
Purification of 4-anisidine

A sample of 4-anisidine, of a very dark colour, is dissolved in an excess of 2N HCl and the solution is repeatedly extracted by chloroform as far as the colour is no longer extracted.



The acid solution is boiled [by] *with* decolorizing charcoal and hot filtered. The strongly cooled filtrate is processed by concentrated NaOH and extracted by chloroform. The chloroform solution is dried on anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue is [crystalized] *crystallized* from benzene thereby providing a white lamellae product with a melting point of 57° C.

[Preparing] *Preparation* of 2-carboxyethyl-5-(2-phthalimidoethyl)-5-methoxy-indole




24.64 g (0.2 moles) p-anisidine in 80 ml ethanol 120 ml water and 80 ml (0.96 moles) 37% HCl are diazotized at 0°–5° C. by 14.5 g (0.21 moles) $NaNO_2$ in 40 ml water; at the end the reaction is continued for other 30 minutes at the same temperature.

The thus obtained diazonium salt solution is added to a solution (stirred and held at 0° C.) of 63.46 g (0.2 moles) of ethyl-2-acetyl-5-phthalimidopentanoate and of 130.64 g (0.96 moles) of sodium acetate trihydrate in 700 ml ethanol. The reaction is continued for 1 hour (the end pH must be included in the 5–6 range); then the solution is brought to room temperature under stirring for other three hours.

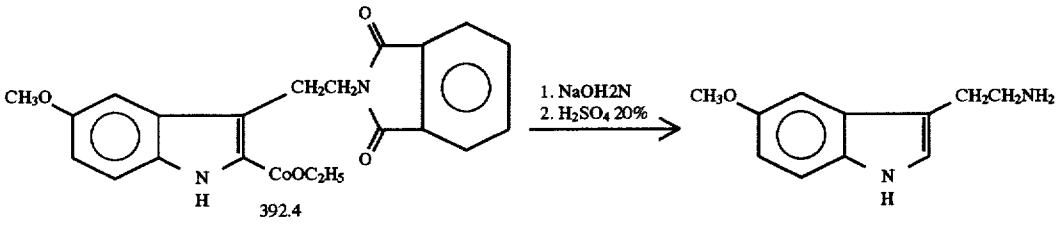
At the end of this period, the mixture is diluted with 2 l water and extracted by $CH_2Cl_2$ three times; the organic phase, after washing by water and drying on anhydrous $Na_2SO_4$, is evaporated, thereby providing 89.2 g of a dark red oil which is dissolved in a minimum amount of ethanol and introduced into a 3-neck 1 liter capacity flask, provided with stirrer, cooler and loading funnel. By stirring and heating there are added in 20 minutes 480 ml of a 10% solution of gaseous HCl in ethanol, by refluxing for 2 hours.

At the end of this period, the mixture is cooled down (for a night in a refrigerator or for 3 hours in an ice bath) and filtered by fully washing with methanol, water and methanol again. The dry solid material has a weight of 57.3 g (yield 73%), with a m.p. of 234°–7° C.

By [recrystalisation] *recrystallization* from glacial acetic acid there are obtained 54.9 g (yield 70%) with m.p. 239°–40° C.

TLC on silica gel, concentrated benzene-methanol-ammonia (50:10:1), Rf about 0.80.

[Preparing] *Preparation* of 5-methoxytryptamine



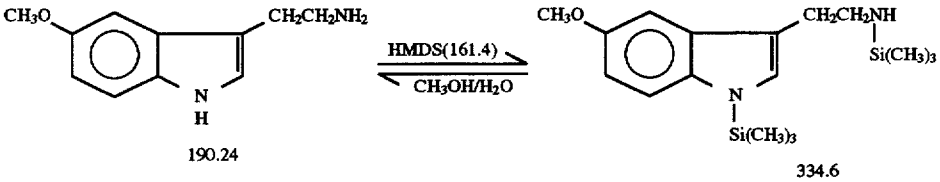

Into a 3-neck 3 liter flask, provided with stirrer, cooler and loading funnel, there are introduced 58.86 g (0.15 moles) of 2-carboxyethyl-3-2-phthalimidoethyl-5-methoxy-indole and 187.5 ml (15 g; 0.375 moles) of 2N NaOH and the mixture is refluxed at 135° C. for 2.5 hours, thereby providing a complete solution.

By holding stirring and temperature, there are added, in 30 minutes, 750 ml of $H_2SO_4$ (at 20%) (v/v), by further reflux processing for 4 hours.

At the end, the solution is cooled (for a night in a refrigerator or for 3 hours in an ice bath), by removing by filtration the precipitated [phtalic] *phthalic* acid. The solution is made alkaline by cooling with 30% NaOH and extracted by $CH_2Cl_2$×3; the collected extracted materials are washed with water, dried on anhydrous $Na_2SO_4$ and evaporated, thereby providing 20.25 g (yield 71%) of crude 5-methoxytryptamine.

TLC on silica gel, sat. $CHCl_3$, $NH_4OH$-methanol (50:2), Rf about 0.65.

[Purifying] *Purification* of 5-methoxytryptamine




To purify 5-methoxytriptamine, 19 g (0.1 moles) of 5-methoxytriptamine (in a raw condition) and 76 ml (58.86–0.36 moles) of hexamethyldisilazane are refluxed for a night in a flask with sodium hydroxide protected cooling.

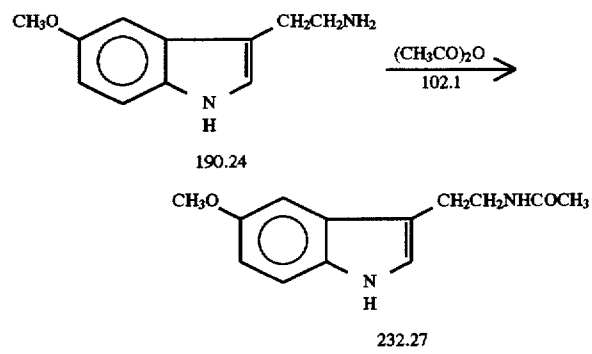
The solution is firstly distilled under normal pressure for recovering excess HMDS (43.6 g; 0.27 moles; m.p. 124°–5° C.) and then under a reduced pressure, thereby providing a mixture of biderivative (20.26 g; m.p 135°–40° C. at 0.1 Torr) and monoderivative material (5.25 g; m.p. 165° C. at 0.1 Torr).



The silyl derivative is [hydrolized] *hydrolyzed* by aqueous methanol, thereby providing 15.36 (0.08 moles) with a yield of 80%. The mixture is [crystalized] *crystallized* from ethanol, so as to provide a white product having a m.p of 120°–1° C.

[Preparing] *Preparation* of N-acetyl-5-methoxytryptamine

Method A




To a suspension, cooled in ice, of 20 g (0.105 moles) of pure 5-methoxytryptamine in 400 ml methylene chloride there is slowly added, under stirring, a cold solution of 20 ml (0.21 moles) of acetic anhydride in 200 ml methylene chloride. Stirring and cooling are continued for 1 hour (the reaction progression can be controlled by TCL) so as to obtain a [full] *complete* solution; then the solution is washed with $Na_2CO_3$×2, under long stirring, and then with water. The organic phase, dried on $Na_2SO_4$ and evaporated, provides 24 g (yield 98%) of lightly colored [melatonine] *melatonin*.

In order to obtain a white product it is sufficient to process, if necessary, by charcoal in acetone and then [crystalize] *crystallize* from acetone-water. There are obtained 20 g (yield 83%) with m.p 116°–7° C. (Tottoli).

TLC on silica gel, chloroform-ethanol (9.1). Rf of about 0.60.

Yield 46.5%, calculated on 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole.

Method B

To a suspension, cooled in ice, of 5 g (26.3 moles) of 5-methoxytryptamine (in a raw condition) in 100 ml methylene chloride there is slowly added, under stirring, a cold solution of 5 ml (52.6 moles) of acetic anhydride in 50 ml of methylene chloride. Stirring and cooling are continued for 1 hour (the reaction progression can be controlled by TLC), so as to obtain a [full] *complete* solution; then the solution is washed by $Na_2CO_3$ 2N×2, under strong stirring, and then by water. The organic phase, dried on anhydrous $Na_2SO_4$ and evaporated, provides 6 g of [raw melatonine,] *crude melatonin* which is purified by chromatography on column (4 cm; diameter/length ratio 1:5) of Merck silica gel (70–230 mesh) (120 g); the solution is eluted by methylene chloride in order to remove the scarcely polar products thereby providing pure [melatonine] *melatonin* by eluting with methylene chloride-acetone (8.2). There are obtained 4 g of product (purifying yield 65%) which are [crystalized] *crystallized* from acetone-water. Melting point 116°–7° C.

TLC on silica gel, chloroform-ethanol (9:1), Rf of about 0.60.

Yield of 46% calculated on 2-carboxyethyl-3-(2-phtalimidoethyl)-5-methoxy-indole.

In order to better disclose the total [synthesis] *synthetic* method according to the present invention, reference is now made to the accompanying drawing, in which there is shown the diagram of the several steps of this method.

The thus obtained [melatonine] *melatonin* has such a purity that it can be used, in suitable packages, both in the tumoural prophylaxis and in the tumoural therapy, as well as against AIDS.

In fact it has been found that this product, administered in suitable doses and with suitable procedures, provides, in addition to the above mentioned effects, also specific effects, such as:

a calming and slightly hypnotic action (which is useful for improving the antipain effect) and an antispasm effect (which is indispensable in the therapy of primitive tumours and brain metastasis tumours);

a mielotropic action, thereby it is possible to use comparatively high doses of radiation and chemical therapeutical substances;

an antimitotic action, perhaps of the same type of those found on the microtubule arrangement and eyelash regeneration;

a modulating action on the NK cell activity.

In this connection it should be pointed out that, in order to obtain the above mentioned effects, there are sufficient very small doses by os or by i.m injection or endovenous injection: from 2 mg/day to 20 mg/day; higher doses should be avoided in order to prevent the antiaggregating action of [melatonine] *melatonin* on the circulating platelets.

In addition there has been recently found a possible relationship between opium peptides and the action mode of [melatonine] *melatonin*.

This fact is very important, since antagonists are able of slowing neoplastic growth, whereas opium agonists seem to have an antimithotic action, both in vivo and in vitro.

In particular, [melatonine] *melatonin* has been found to be of essential importance in the following cases:

neuroblastoms, glioblastoms and astrocytoms;

leio and rabdomions;

condro-osteo-mixo-liposarcomas;

melanomas;

tumours of the respiratory paths and lungs:

tumours of the digestive apparatus;

tumours of the man and woman genital apparatus, loins, and prostate;

spino and basocellular epitheliomas:

malignant lymphomas and, with a less efficacy, in the Hodgkin lymphoma;

plasmocytoms;

thyroid tumours;

[mamma] *mammary* tumours;

linphoblastic leukaemia and cronic limphoides;

mieloblastic leukaemia and cronic mieloid.

Thus, we can reasonably think that the efficacy of [melatonine] *melatonin* in the above very different nature tumours is such as to advise its use [it] because of its general indirect and not specific action, which, on the other hand, is very essential.

In this connection it should be moreover pointed out that in the last ten years, experimental reports have stressed the fact that the [neuroendocrin] *neuroendocrinologic* system and immunity seem to be mutually related and that some diseases, characterized by immunitary disorders, may be due to alterations of this interrelationship.

Among the several modulating [neuroendocrin] *neuroendocrinologic* factors affecting the immunitary system pineal secretions and endogen opium peptides seem to have a very important function.

In fact there has been demonstrated that both pineal gland and opium system are involved in the control of cellular growth and tumoural growth.

At the immunitary system level, the endogen opium peptides seem to provide a stimulating-action; in particular endorphine may, under given conditions, stimulate the NK activity and the interleukine production.

On the other hand, basic data seem to suggest that [melatonine] *melatonin*, i.e. N-acetyl-5-methoxytryptamine has a very important function in maintaining an efficient [immunologic] *immunological* response in rats, under induced immunitary experimental stimulation.

The effect provided by [melatonine] *melatonin*, under the disclosed experimental conditions, is hindered by the simultaneous administration of naltrexone; this suggests that the immunomodulating action of [melatonine] *melatonin* can be controlled by opium mechanisms.

Under basal condition and in the absence of the immunity activity, in rats, the [melatonine] *melatonin* administration has no efficacy.

There has been moreover demonstrated that repeated administrations of pineal extracts induce lymphocytopoiesis and timic hyperplasia, whereas pinealhectomy causes timic atrophy.

In this connection it should moreover be stressed that pineal [endocrin] *endocrinologic* function itself seems to be modulated by opioid tone and that, vice versa, some typical actions of opium substances, such as analgesic action, are

controlled by the activity of pineal gland and follow a [circadic rythm] *circadian rhythm.*

Thus, one may reasonably think that the pineal gland, through its main [melatonine] *melatonin* hormone, as a structure involved into the modulation of the [neuroendocrin] *neuroendocrine* activities, is able of controlling the effects exerted by phychoemotional effects on the immunitary system.

In fact, documented [circadic] *circadian* variations of the NK activity could be related to the [circadic rythm] *circadian rhythm* of [melatonine] *melatonin*, as demonstrated by some recent results.

From a lot of experimental tests, it has been found that surprising results has been obtained in the treatment of patients affected by AIDS.

These patients have been treated [by melatonine] *with melatonin* with doses of 20 mg per day and, after a long therapy, it has been demonstrated that they had a less amount of infections, with a significative increase of the "null cells", as determined by an examination of peripheral blood.

[Melatonine] *Melatonin*, or N-acetyl-5-methoxytryptamine, which has a formulation which constitutes the subject matter of the Italian Patent Application No. 23.323 A/79 in the name of the same [applicant] *applicants*, and which [his] *is* herein included by reference, has been found to provide significative improvements in the treatment of patients affected by AIDS.

The effect of [melatonine] *melatonin* is further increased as [melatonine] *melatonin* is used together with azidotimidine.

In particular, patients affected by AIDS, who were treated by azidotimidine with a dose of 3 mg/kg each four hours, and who required weekly blood transfusions because of the alteration of the coagulation processes, and subjected to a simultaneous treatment by [melatonine] *melatonin*, with a dose of 20 mg per day, provided greatly improved collateral effects affording the possibility of performing blood transfusions at 8 week intervals.

These patients have been treated [by melatonine] *with melatonin*, with the mentioned doses of 20 mg per day, and after a long therapy it has been demonstrated that they had a less amount of infections, with a significative increase of the null cells, as determined by an examination of peripheral blood.

Thus it has been found that [melatonine] *melatonin* can efficiently treat patients affected by AIDS, mainly in combination with other known treatment methods.

In this connection, it should be apparent that all of the administering details and the used doses can be suitably changed depending on each patient.

In particular, for a better use of [melatonine,] *melatonin* the present invention suggests to solubilize it [with] *in* water in order to facilitate the therapeutical applications, by using a particular method.

In fact, as is known [melatonine] *melatonin* is a substance scarcely soluble in water, and satisfactorily soluble at 40°–45° C.

After long experimentation applicant has found that adenosine is adapted to easily dissolve [melatonine] *melatonin* in water.

In particular an optimal ratio has been found i.e.:

for a mole of [melatonine] *melatonin* (252.27 g) must be used four moles of adenosine (267.26 g).

From the above disclosure it should be apparent that the invention fully achieves the intended objects.

While the invention has been disclosed and illustrated with reference to some embodiments thereof, it should be apparent that the disclosed embodiments are susceptible to several modifications and variations all of which will come within the spirit and scope of the invention, as defined in the accompanying claims.

We claim:

1. The method of solubilizing [melatonine] *melatonin* in water which consists of mixing [melatonine] *melatonin* with adenosine in a ratio of one mole of [melatonine] *melatonin* to four moles of adenosine whereby a water soluble product is obtained.

*2. A method of preparation of melatonin having a high degree of purity which consists of the steps of:*

*a) reacting potassium phthalimide with dibromopropane, whereby 3-bromopropylphthalimide is obtained;*

*b) reacting 3-bromopropylphthalimide from step a) with acetoacetic ester in the presence of sodium ethoxide whereby ethyl 2-acetyl phthalimido-pentanoate is obtained;*

*c) reacting said ethyl 2-acetyl phthalimido pentanoate from step b) with the diazonium salt of p-anisidine whereby 2-carboxyethyl 3-(2-phthalimidoethyl) 5-methoxy indole is obtained;*

*d) reacting said 2-carboxyethyl 3-(2-phthalimidoethyl) 5-methoxy-indole from step c) first with sodium hydroxide and then with sulfuric acid whereby crude 5-methoxy-tryptamine is obtained;*

*e) reacting said crude 5-methoxy-tryptamine from step d) with hexamethyl disilazane to obtain a mixture of mono- and disubstitution products and hydrolyzing said mixture with aqueous methanol to obtain essentially pure 5-methoxy-tryptamine;*

*f) reacting said essentially pure 5-methoxy-tryptamine from step c) with acetic anhydride to obtain crude melatonin and purifying said crude melatonin by chromatography on silica gel and first eluting with methylene chloride followed by eluting with methylene chloride and acetone to obtain a solution, concentrating said methylene chloride and acetone solution to obtain a solid and recrystallizing said solid whereby purified melatonin is obtained.*

*3. The method according to claim 2 wherein said step d) is carried out by refluxing at 135° C. for 2½ hours until complete solution is obtained, then adding a 20% (v/v) $H_2SO_4$ solution and further refluxing for four hours.*

*4. The method according to claim 3 wherein after refluxing with said 20% sulfuric acid, the solution is cooled to let phthalic acid precipitate and filtering off said phthalic acid.*

*5. The method according to claim 4 wherein after said phthalic acid is filtered off, sodium hydroxide is added and crude 5-methoxytryptamine is extracted with methylene dichloride.*

*6. The method according to claim 2 wherein said step f) is carried out by refluxing for 12–14 hours said crude 5-methoxytryptamine with hexamethyl-disilazane, to obtain the mono and di-silyl substitution products, then distilling the solution under normal pressure so as to recover excess hexamethyl disilazane and hydrolyzing the silyl substitution products with aqueous methanol whereby essentially pure 5-methoxytryptamine is provided.*

* * * * *

US00RE35631E

# United States Patent [19]

Fraschini et al.

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[45] Reissued Date of Patent: Oct. 14, 1997

[54] **METHOD OF PRODUCTION OF ESSENTIALLY PURE MELATONIN AND THE METHOD OF SOLUBILIZING MELATONIN IN WATER**

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[73] Assignee: **IFLO S.A.S.**, Milan, Italy

[21] Appl. No.: **252,905**

[22] Filed: **Jun. 2, 1994**

**Related U.S. Patent Documents**

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Filed: **Feb. 17, 1989**

[30] **Foreign Application Priority Data**

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Sep. 8, 1988 [IT] Italy ..... 21872 A/88

[51] **Int. Cl.**[6] ..... **C07D 209/16**; C07D 473/34; A61K 31/40; A61K 31/52

[52] **U.S. Cl.** ..... **514/415**; 514/46; 536/27.6; 548/504; 548/507

[58] **Field of Search** ..... 544/276; 514/46, 514/415; 548/504, 508, 507; 536/27.3

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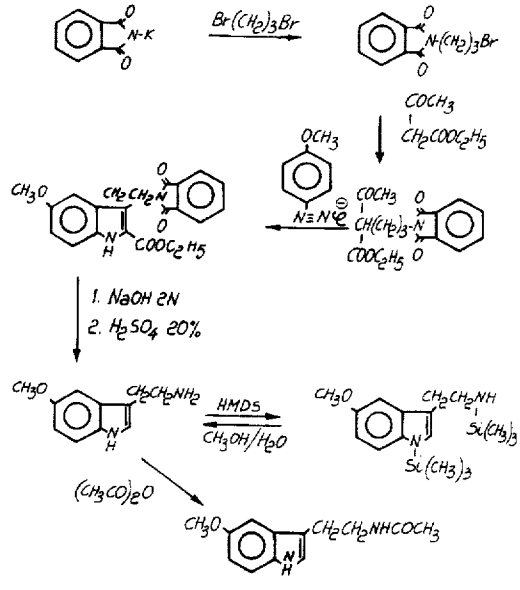
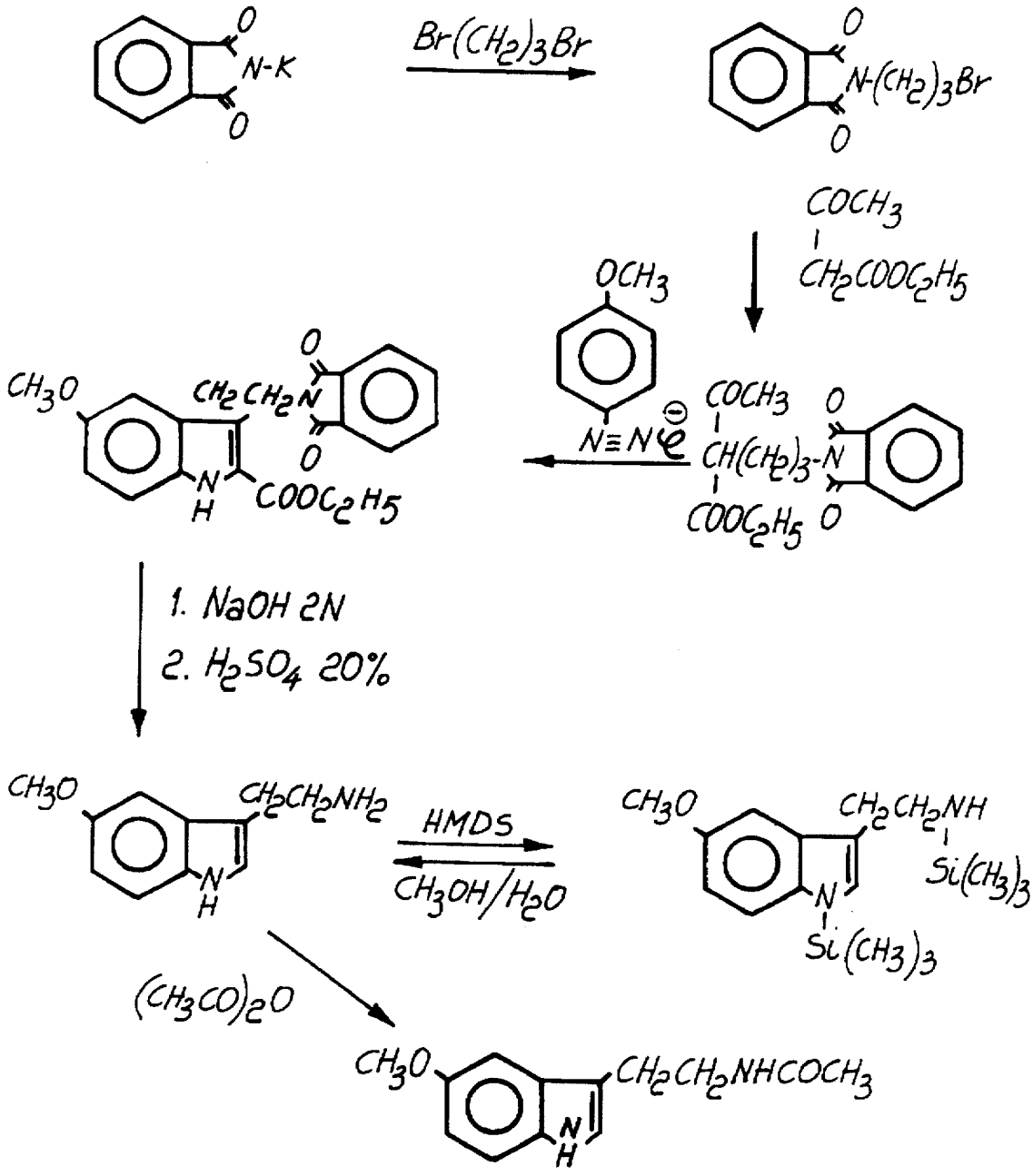
*Attorney, Agent, or Firm*—Bucknam and Archer

[57] **ABSTRACT**

A method of synthesizing an indole derivative of the tryptamine type particularly [melatonine,] *melatonin* comprising the steps of 1) reacting potassium phthalimide and 1,3-di-bromopropane to obtain 3-bromopropylphthalimide; 2) reacting 3-bromopropylphthalimide with sodium acetoacetic ester in ethanol to obtain ethyl-2-acetyl-5-phthalimidopentanoate; 3) reacting the product from step 2) with diazo-p-anisidine to obtain 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole; 4) reacting the 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole with 2N/NaOH and then 20% $H_2SO_4$ to obtain impure 5-methoxytriptamine, which is purified by means of hexamethyldisilazane. The mono and disubstituted derivatives are obtained and the monosubstituted derivative is hydrolyzed with aqueous methanol and then recrystallized from ethanol. The N-acetyl derivative is prepared by reaction with acetic anhydride. [Melatonine] *Melatonin* of high purity is obtained for prophylaxy and also against AIDS (Acquired Immuno Deficiency Syndrome).

**6 Claims, 1 Drawing Sheet**




N-K
Br(CH2)3Br
N-(CH2)3Br
COCH3
CH2COOC2H5
OCH3
N≡N⊖
CH(CH2)3-N
COOC2H5
CH3O
CH2CH2N
COOC2H5
1. NaOH 2N
2. H2SO4 20%
CH3O
CH2CH2NH2
HMDS
CH3OH/H2O
CH3O
CH2CH2NH
Si(CH3)3
Si(CH3)3
(CH3CO)2O
CH3O
CH2CH2NHCOCH3

# METHOD OF PRODUCTION OF ESSENTIALLY PURE MELATONIN AND THE METHOD OF SOLUBILIZING MELATONIN IN WATER

**Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.**

## BACKGROUND OF THE INVENTION

The present invention relates to a total synthesis method for preparing an indole structure derivative product class, of the tryptamine type, in particular of [melatonine] *melatonin* or N-acetyl-5-methoxytriptamine type, having a high purity degree and easily soluble, for therapeutic use against acquired immuno-deficiency syndromes or so-called AIDS.

As is known, it has been found that [melatonine] *melatonin* (MTL), administered with suitable doses and at given times, is able of reducing proteic synthesis of hypothalamus and hypophisis and that it, moreover, may inhibit the synthesis of gonadostymulines.

Such an action is probably exerted by means of a modulation of genic transcription and repression, as well as on the incretion of the two GH and PRL growth factors, under particular conditions.

The above mentioned overall effects, which are associated with other particular actions, as disclosed in a more detailed way hereinafter, justify as useful, even if not indispensable, the use of [melatonine] *melatonin* against tumours.

In fact one may reasonably think that [melatonine] *melatonin* pertains to that class of drugs which interfere with the growth of neoplastic cells and reduce the life time thereof.

On the other hand, also known is the fact that presently available methods for making the tryptamine structure having the hydrogen atom at the 5-position replaced by the group $OCH_3$, are based on a series of chemical reactions providing 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole, therefrom there is obtained 5-methoxytryptamine by means of a plurality of comparatively complex and low yield processing steps.

More specifically, known prior art methods comprise an alkaline saponifying step providing 2-carboxy-3-(2-O-carboxybenzamidoethyl)-5-methoxy-indole acid which is then dry decarboxylated at 250° C. in order to form phthalimidoethyl-5-methoxy-indole, which is then water hydrazinolized to provide 5-methoxytryptamine.

In order to obtain pure N-acetyl-5-methoxytryptamine or [melatonine] *melatonin* with a high yield, it is necessary to have a high purity starting product, that is 5-methoxytryptamine.

Known conventional purifying methods, based on the use of solvents or mixtures thereof, on the other hand, have not provided a sufficiently high purity degree with a contemporaneous high production yield.

## SUMMARY OF THE INVENTION

Accordingly, the main object of the present invention is to overcome the above mentioned drawback by providing a method for making, with high production yields, very pure 5-methoxytryptamine, which method essentially comprises a synthesis known per se with respect to the reagents, but carried out by new techniques starting from 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole.

Another object of the invention is to provide such a method which, in addition to simplifying the processing steps, can provide [melatonine] *melatonin* starting both from 5-methoxytryptamine, in raw form, and from pure 5-methoxytryptamine.



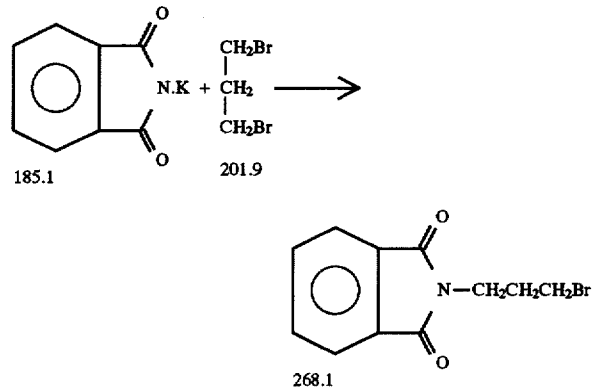
Yet another object of the present invention is to provide a total synthesis method which provides a very pure and reliable product, with consistent curative properties.

According to one aspect of the present invention, the above mentioned objects, as well as yet other objects, which will become more apparent hereinafter, are achieved by a total synthesis method for making an indole structure derivative product class, of the tryptamine type, in particular of [melatonine] *melatonin* or N-acetyl-5-methoxytryptamine type, having a high purity degree and easily water soluble for therapeutic use against acquired immuno-deficiency syndromes comprising the steps of combining potassium phthalimide and dibromopropane, to form 3-bromopropylphthalimide, adding [acetacetic] *acetoacetic* ester in the presence of anhydrous ethanol dissolved sodium to form ethyl-2-acetyl-phthalimidopentanoate, adding diazo-p-anisidine to form 2-carboxyethyl-[3-(2-phtalimidoethyl)-]*3-2-phthalimidoethyl*-5-methoxy-indole, which is processed, in a first step, by NaOH 2N up to a complete solution and, then, by $H_2SO_4$ (at 20%) to form raw 5-methoxytryptamine, which is purified by means of hexamethyldisilazane, so as to form the related mono- and bi-derivative therefrom, by means of an aqueous methanol hydrolysis, there is obtained [the starting compound] *5-methoxytryptamine.*

## BRIEF DESCRIPTION OF THE DRAWING

Further characteristics and advantages of the present invention will become more apparent from the following detailed description of the total synthesis method according to the invention, with reference to the chemical diagram shown in the accompanying drawing table.

## DESCRIPTION OF THE PREFERRED EMBODIMENT




The method according to the invention comprises the step of preparing 3-bromopropylphthalimide as follows: into a 3-neck 1-liter flask, provided with stirrer and cooling medium, there are introduced 101 g (0.5 moles) of 1.3-dibromo-propane, 250 ml acetone and 15 g K-phthalimide, by reflux processing the mixture under stirring. At 1 hour time intervals there are added further (15+10+6.3) g K-phthalimide (46.3 g corresponding to 0.25 moles), by holding under reflux conditions for a total period of 24 hours.

At the end of this period the precipitated KBr is filtered and acetone is evaporated in a rotary evaporating device: the

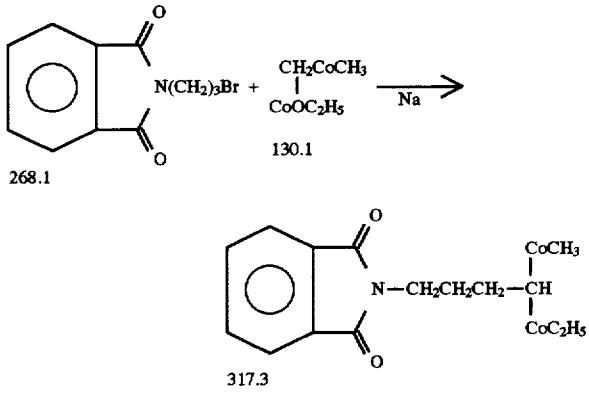
obtained oil is distilled under vacuum (as provided by a water pump) and there are recovered 48.5 g (0.25 moles) of 1,3-dibromopropane, which is distilled at 69°–70° C. The residue (dissolved before solidification in the distillation flask) is [crystalized] *crystallized* twice from ethanol, so as to remove the small amount of formed diphthalimidopropane.

There are thus obtained 48.2 g of a white [crystaline] *crystalline* solid, melting point 72° C., with a yield of 72%.

Purity is controlled for TLC on silica gel, using as eluent benzene-acetone (45:5), freshly prepared, Rf of about 0.95 (diphthalimidopropane having a lower Rf).

By analogous, reactions, in which K-phthalimide is added once, there is obtained a product which contains greater amounts of diphthalimidopropane, thereby it is necessary to purify by distillation (e.g. 150° C./0.25 mm) by using a Vigreux device without cooling, since the distillate tends toward solidification. The yield is substantially equal to the above disclosed yield.

[Preparing] *Preparation* of ethyl-2-acetyl-5-phthalimidopentanoate




In a three-neck flask having a capacity of 500 ml, provided with $CaCl_2$ cooling there are dissolved 4.60 g (0.2 g/A) Na in 100 ml anhydrous ethanol. To the solution, at room temperature, there are added 27.32 (0.21 moles) of [acetacetic esther] *acetoacetic ester* and then, after ten minutes, 40 g of 3-bromopropylphthalimide and, after one hour, further 12.5 g (in total 52.5 g corresponding to 0.196 moles), by holding the reflux processing and continuing for further three hours.

At the end of this period, sodium bromide is filtered, the solution is neutralized by 2N HCl and ethanol is evaporated under reduced pressure. The residue is recovered by ether, washed by $H_2O$×2, dried on anhydrous $Na_2SO_4$ and the solvent is evaporated, thereby providing a light yellow oil which is [crystalized] *crystallized* by dissolving it in a minimum ethanol amount by adding a small amount of ether and upon [ageing] *aging* for a night.

There are thus obtained 45 g (yield 72%) of a white crystalline solid, with m.p. 60° C. Upon [recrystalisation] *recrystallization* there is obtained a m.p. of 63° C. Product also [crystalizes] *crystallizes* from benzene-petroleum ether.

TLC on silica gel, benzene-acetone (45:5), Rf about 0.70.

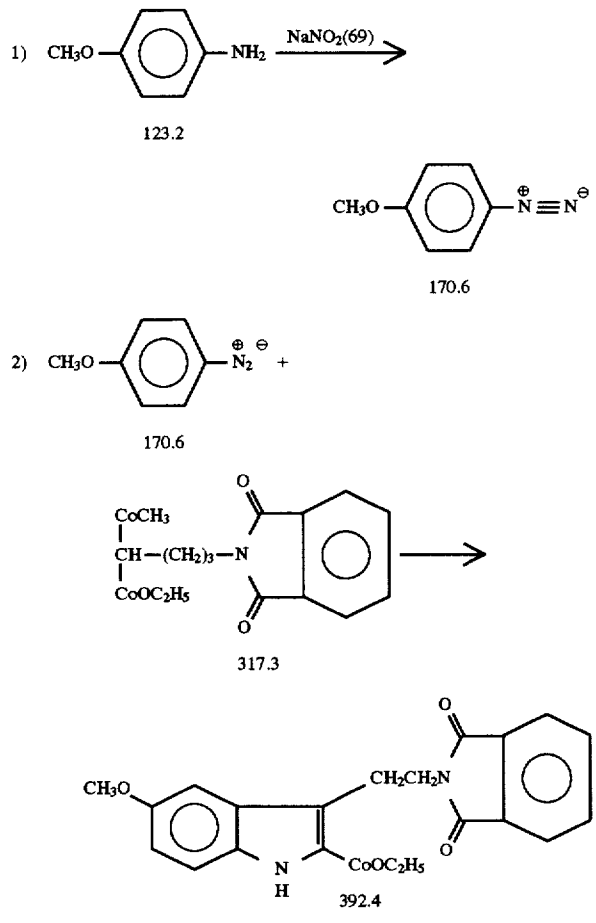
Purification of 4-anisidine

A sample of 4-anisidine, of a very dark colour, is dissolved in an excess of 2N HCl and the solution is repeatedly extracted by chloroform as far as the colour is no longer extracted.



The acid solution is boiled [by] *with* decolorizing charcoal and hot filtered. The strongly cooled filtrate is processed by concentrated NaOH and extracted by chloroform. The chloroform solution is dried on anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue is [crystalized] *crystallized* from benzene thereby providing a white lamellae product with a melting point of 57° C.

[Preparing] *Preparation* of 2-carboxyethyl-5-(2-phthalimidoethyl)-5-methoxy-indole




24.64 g (0.2 moles) p-anisidine in 80 ml ethanol 120 ml water and 80 ml (0.96 moles) 37% HCl are diazotized at 0°–5° C. by 14.5 g (0.21 moles) $NaNO_2$ in 40 ml water; at the end the reaction is continued for other 30 minutes at the same temperature.

The thus obtained diazonium salt solution is added to a solution (stirred and held at 0° C.) of 63.46 g (0.2 moles) of ethyl-2-acetyl-5-phthalimidopentanoate and of 130.64 g (0.96 moles) of sodium acetate trihydrate in 700 ml ethanol. The reaction is continued for 1 hour (the end pH must be included in the 5–6 range); then the solution is brought to room temperature under stirring for other three hours.

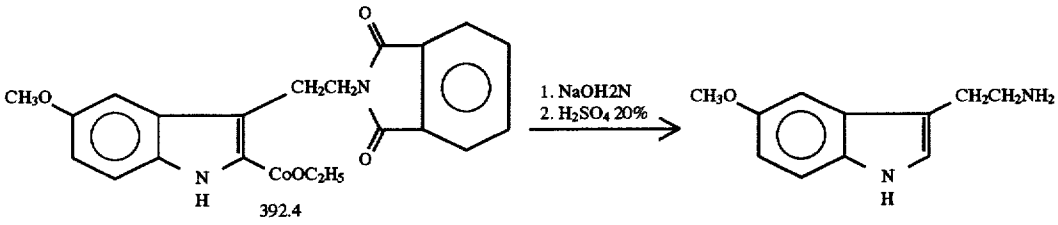
At the end of this period, the mixture is diluted with 2 l water and extracted by $CH_2Cl_2$ three times; the organic phase, after washing by water and drying on anhydrous $Na_2SO_4$, is evaporated, thereby providing 89.2 g of a dark red oil which is dissolved in a minimum amount of ethanol and introduced into a 3-neck 1 liter capacity flask, provided with stirrer, cooler and loading funnel. By stirring and heating there are added in 20 minutes 480 ml of a 10% solution of gaseous HCl in ethanol, by refluxing for 2 hours.

At the end of this period, the mixture is cooled down (for a night in a refrigerator or for 3 hours in an ice bath) and filtered by fully washing with methanol, water and methanol again. The dry solid material has a weight of 57.3 g (yield 73%), with a m.p. of 234°–7° C.

By [recrystalisation] *recrystallization* from glacial acetic acid there are obtained 54.9 g (yield 70%) with m.p. 239°–40° C.

TLC on silica gel, concentrated benzene-methanol-ammonia (50:10:1), Rf about 0.80.

[Preparing] *Preparation* of 5-methoxytryptamine



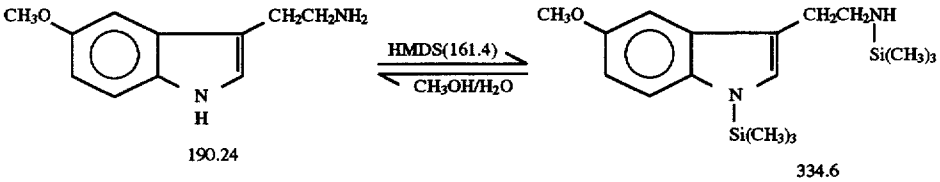

Into a 3-neck 3 liter flask, provided with stirrer, cooler and loading funnel, there are introduced 58.86 g (0.15 moles) of 2-carboxyethyl-3-2-phthalimidoethyl-5-methoxy-indole and 187.5 ml (15 g; 0.375 moles) of 2N NaOH and the mixture is refluxed at 135° C. for 2.5 hours, thereby providing a complete solution.

By holding stirring and temperature, there are added, in 30 minutes, 750 ml of $H_2SO_4$ (at 20%) (v/v), by further reflux processing for 4 hours.

At the end, the solution is cooled (for a night in a refrigerator or for 3 hours in an ice bath), by removing by filtration the precipitated [phtalic] *phthalic* acid. The solution is made alkaline by cooling with 30% NaOH and extracted by $CH_2Cl_2$×3; the collected extracted materials are washed with water, dried on anhydrous $Na_2SO_4$ and evaporated, thereby providing 20.25 g (yield 71%) of crude 5-methoxytryptamine.

TLC on silica gel, sat. $CHCl_3$, $NH_4OH$-methanol (50:2), Rf about 0.65.

[Purifying] *Purification* of 5-methoxytryptamine




To purify 5-methoxytriptamine, 19 g (0.1 moles) of 5-methoxytriptamine (in a raw condition) and 76 ml (58.86–0.36 moles) of hexamethyldisilazane are refluxed for a night in a flask with sodium hydroxide protected cooling.

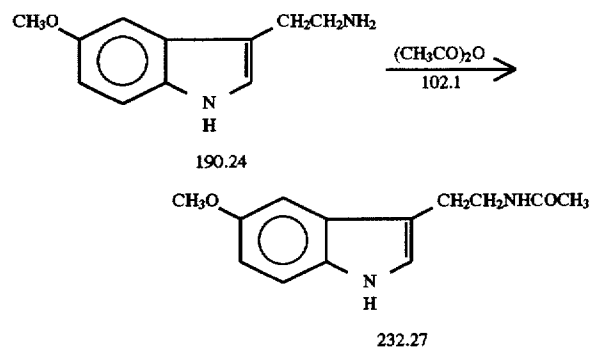
The solution is firstly distilled under normal pressure for recovering excess HMDS (43.6 g; 0.27 moles; m.p. 124°–5° C.) and then under a reduced pressure, thereby providing a mixture of biderivative (20.26 g; m.p 135°–40° C. at 0.1 Torr) and monoderivative material (5.25 g; m.p. 165° C. at 0.1 Torr).



The silyl derivative is [hydrolized] *hydrolyzed* by aqueous methanol, thereby providing 15.36 (0.08 moles) with a yield of 80%. The mixture is [crystalized] *crystallized* from ethanol, so as to provide a white product having a m.p of 120°–1° C.

[Preparing] *Preparation* of N-acetyl-5-methoxytryptamine

Method A




To a suspension, cooled in ice, of 20 g (0.105 moles) of pure 5-methoxytryptamine in 400 ml methylene chloride there is slowly added, under stirring, a cold solution of 20 ml (0.21 moles) of acetic anhydride in 200 ml methylene chloride. Stirring and cooling are continued for 1 hour (the reaction progression can be controlled by TCL) so as to obtain a [full] *complete* solution; then the solution is washed with $Na_2CO_3$×2, under long stirring, and then with water. The organic phase, dried on $Na_2SO_4$ and evaporated, provides 24 g (yield 98%) of lightly colored [melatonine] *melatonin*.

In order to obtain a white product it is sufficient to process, if necessary, by charcoal in acetone and then [crystalize] *crystallize* from acetone-water. There are obtained 20 g (yield 83%) with m.p 116°–7° C. (Tottoli).

TLC on silica gel, chloroform-ethanol (9.1). Rf of about 0.60.

Yield 46.5%, calculated on 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole.

Method B

To a suspension, cooled in ice, of 5 g (26.3 moles) of 5-methoxytryptamine (in a raw condition) in 100 ml methylene chloride there is slowly added, under stirring, a cold solution of 5 ml (52.6 moles) of acetic anhydride in 50 ml of methylene chloride. Stirring and cooling are continued for 1 hour (the reaction progression can be controlled by TLC), so as to obtain a [full] *complete* solution; then the solution is washed by $Na_2CO_3$ 2N×2, under strong stirring, and then by water. The organic phase, dried on anhydrous $Na_2SO_4$ and evaporated, provides 6 g of [raw melatonine,] *crude melatonin* which is purified by chromatography on column (4 cm; diameter/length ratio 1:5) of Merck silica gel (70–230 mesh) (120 g); the solution is eluted by methylene chloride in order to remove the scarcely polar products thereby providing pure [melatonine] *melatonin* by eluting with methylene chloride-acetone (8.2). There are obtained 4 g of product (purifying yield 65%) which are [crystalized] *crystallized* from acetone-water. Melting point 116°–7° C.

TLC on silica gel, chloroform-ethanol (9:1), Rf of about 0.60.

Yield of 46% calculated on 2-carboxyethyl-3-(2-phtalimidoethyl)-5-methoxy-indole.

In order to better disclose the total [synthesis] *synthetic* method according to the present invention, reference is now made to the accompanying drawing, in which there is shown the diagram of the several steps of this method.

The thus obtained [melatonine] *melatonin* has such a purity that it can be used, in suitable packages, both in the tumoural prophylaxis and in the tumoural therapy, as well as against AIDS.

In fact it has been found that this product, administered in suitable doses and with suitable procedures, provides, in addition to the above mentioned effects, also specific effects, such as:

a calming and slightly hypnotic action (which is useful for improving the antipain effect) and an antispasm effect (which is indispensable in the therapy of primitive tumours and brain metastasis tumours);

a mielotropic action, thereby it is possible to use comparatively high doses of radiation and chemical therapeutical substances;

an antimitotic action, perhaps of the same type of those found on the microtubule arrangement and eyelash regeneration;

a modulating action on the NK cell activity.

In this connection it should be pointed out that, in order to obtain the above mentioned effects, there are sufficient very small doses by os or by i.m injection or endovenous injection: from 2 mg/day to 20 mg/day; higher doses should be avoided in order to prevent the antiaggregating action of [melatonine] *melatonin* on the circulating platelets.

In addition there has been recently found a possible relationship between opium peptides and the action mode of [melatonine] *melatonin*.

This fact is very important, since antagonists are able of slowing neoplastic growth, whereas opium agonists seem to have an antimithotic action, both in vivo and in vitro.

In particular, [melatonine] *melatonin* has been found to be of essential importance in the following cases:

neuroblastoms, glioblastoms and astrocytoms;

leio and rabdomions;

condro-osteo-mixo-liposarcomas;

melanomas;

tumours of the respiratory paths and lungs:

tumours of the digestive apparatus;

tumours of the man and woman genital apparatus, loins, and prostate;

spino and basocellular epitheliomas:

malignant lymphomas and, with a less efficacy, in the Hodgkin lymphoma;

plasmocytoms;

thyroid tumours;

[mamma] *mammary* tumours;

linphoblastic leukaemia and cronic limphoides;

mieloblastic leukaemia and cronic mieloid.

Thus, we can reasonably think that the efficacy of [melatonine] *melatonin* in the above very different nature tumours is such as to advise its use [it] because of its general indirect and not specific action, which, on the other hand, is very essential.

In this connection it should be moreover pointed out that in the last ten years, experimental reports have stressed the fact that the [neuroendocrin] *neuroendocrinologic* system and immunity seem to be mutually related and that some diseases, characterized by immunitary disorders, may be due to alterations of this interrelationship.

Among the several modulating [neuroendocrin] *neuroendocrinologic* factors affecting the immunitary system pineal secretions and endogen opium peptides seem to have a very important function.

In fact there has been demonstrated that both pineal gland and opium system are involved in the control of cellular growth and tumoural growth.

At the immunitary system level, the endogen opium peptides seem to provide a stimulating-action; in particular endorphine may, under given conditions, stimulate the NK activity and the interleukine production.

On the other hand, basic data seem to suggest that [melatonine] *melatonin*, i.e. N-acetyl-5-methoxytryptamine has a very important function in maintaining an efficient [immunologic] *immunological* response in rats, under induced immunitary experimental stimulation.

The effect provided by [melatonine] *melatonin*, under the disclosed experimental conditions, is hindered by the simultaneous administration of naltrexone; this suggests that the immunomodulating action of [melatonine] *melatonin* can be controlled by opium mechanisms.

Under basal condition and in the absence of the immunity activity, in rats, the [melatonine] *melatonin* administration has no efficacy.

There has been moreover demonstrated that repeated administrations of pineal extracts induce lymphocytopoiesis and timic hyperplasia, whereas pinealhectomy causes timic atrophy.

In this connection it should moreover be stressed that pineal [endocrin] *endocrinologic* function itself seems to be modulated by opioid tone and that, vice versa, some typical actions of opium substances, such as analgesic action, are

controlled by the activity of pineal gland and follow a [circadic rythm] *circadian rhythm.*

Thus, one may reasonably think that the pineal gland, through its main [melatonine] *melatonin* hormone, as a structure involved into the modulation of the [neuroendocrin] *neuroendocrine* activities, is able of controlling the effects exerted by phychoemotional effects on the immunitary system.

In fact, documented [circadic] *circadian* variations of the NK activity could be related to the [circadic rythm] *circadian rhythm* of [melatonine] *melatonin*, as demonstrated by some recent results.

From a lot of experimental tests, it has been found that surprising results has been obtained in the treatment of patients affected by AIDS.

These patients have been treated [by melatonine] *with melatonin* with doses of 20 mg per day and, after a long therapy, it has been demonstrated that they had a less amount of infections, with a significative increase of the "null cells", as determined by an examination of peripheral blood.

[Melatonine] *Melatonin*, or N-acetyl-5-methoxytryptamine, which has a formulation which constitutes the subject matter of the Italian Patent Application No. 23.323 A/79 in the name of the same [applicant] *applicants*, and which [his] *is* herein included by reference, has been found to provide significative improvements in the treatment of patients affected by AIDS.

The effect of [melatonine] *melatonin* is further increased as [melatonine] *melatonin* is used together with azidotimidine.

In particular, patients affected by AIDS, who were treated by azidotimidine with a dose of 3 mg/kg each four hours, and who required weekly blood transfusions because of the alteration of the coagulation processes, and subjected to a simultaneous treatment by [melatonine] *melatonin*, with a dose of 20 mg per day, provided greatly improved collateral effects affording the possibility of performing blood transfusions at 8 week intervals.

These patients have been treated [by melatonine] *with melatonin*, with the mentioned doses of 20 mg per day, and after a long therapy it has been demonstrated that they had a less amount of infections, with a significative increase of the null cells, as determined by an examination of peripheral blood.

Thus it has been found that [melatonine] *melatonin* can efficiently treat patients affected by AIDS, mainly in combination with other known treatment methods.

In this connection, it should be apparent that all of the administering details and the used doses can be suitably changed depending on each patient.

In particular, for a better use of [melatonine,] *melatonin* the present invention suggests to solubilize it [with] *in* water in order to facilitate the therapeutical applications, by using a particular method.

In fact, as is known [melatonine] *melatonin* is a substance scarcely soluble in water, and satisfactorily soluble at 40°–45° C.

After long experimentation applicant has found that adenosine is adapted to easily dissolve [melatonine] *melatonin* in water.

In particular an optimal ratio has been found i.e.:

for a mole of [melatonine] *melatonin* (252.27 g) must be used four moles of adenosine (267.26 g).

From the above disclosure it should be apparent that the invention fully achieves the intended objects.

While the invention has been disclosed and illustrated with reference to some embodiments thereof, it should be apparent that the disclosed embodiments are susceptible to several modifications and variations all of which will come within the spirit and scope of the invention, as defined in the accompanying claims.

We claim:

1. The method of solubilizing [melatonine] *melatonin* in water which consists of mixing [melatonine] *melatonin* with adenosine in a ratio of one mole of [melatonine] *melatonin* to four moles of adenosine whereby a water soluble product is obtained.

*2. A method of preparation of melatonin having a high degree of purity which consists of the steps of:*

*a) reacting potassium phthalimide with dibromopropane, whereby 3-bromopropylphthalimide is obtained;*

*b) reacting 3-bromopropylphthalimide from step a) with acetoacetic ester in the presence of sodium ethoxide whereby ethyl 2-acetyl phthalimido-pentanoate is obtained;*

*c) reacting said ethyl 2-acetyl phthalimido pentanoate from step b) with the diazonium salt of p-anisidine whereby 2-carboxyethyl 3-(2-phthalimidoethyl) 5-methoxy indole is obtained;*

*d) reacting said 2-carboxyethyl 3-(2-phthalimidoethyl) 5-methoxy-indole from step c) first with sodium hydroxide and then with sulfuric acid whereby crude 5-methoxy-tryptamine is obtained;*

*e) reacting said crude 5-methoxy-tryptamine from step d) with hexamethyl disilazane to obtain a mixture of mono- and disubstitution products and hydrolyzing said mixture with aqueous methanol to obtain essentially pure 5-methoxy-tryptamine;*

*f) reacting said essentially pure 5-methoxy-tryptamine from step c) with acetic anhydride to obtain crude melatonin and purifying said crude melatonin by chromatography on silica gel and first eluting with methylene chloride followed by eluting with methylene chloride and acetone to obtain a solution, concentrating said methylene chloride and acetone solution to obtain a solid and recrystallizing said solid whereby purified melatonin is obtained.*

*3. The method according to claim 2 wherein said step d) is carried out by refluxing at 135° C. for 2½ hours until complete solution is obtained, then adding a 20% (v/v) $H_2SO_4$ solution and further refluxing for four hours.*

*4. The method according to claim 3 wherein after refluxing with said 20% sulfuric acid, the solution is cooled to let phthalic acid precipitate and filtering off said phthalic acid.*

*5. The method according to claim 4 wherein after said phthalic acid is filtered off, sodium hydroxide is added and crude 5-methoxytryptamine is extracted with methylene dichloride.*

*6. The method according to claim 2 wherein said step f) is carried out by refluxing for 12–14 hours said crude 5-methoxytryptamine with hexamethyl-disilazane, to obtain the mono and di-silyl substitution products, then distilling the solution under normal pressure so as to recover excess hexamethyl disilazane and hydrolyzing the silyl substitution products with aqueous methanol whereby essentially pure 5-methoxytryptamine is provided.*

* * * * *

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# METODO DI PRODUZIONE DI MELATONINA SOSTANZA PURA E IL METODO DI SOLUBILIZZARE MELATONINA IN ACQUA.

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## ESTRATTO

Un metodo per sintetizzare un derivato indolico del tipo triptamina, in particolare melatonina, comprendente le fasi 1) reazione del potassio ftalimide e 1,3-di-bromopropano per ottenere 3-bromoprofilftalimide; 2) reazione del 3-bromoprofilftalimide con sodio estere acetoacetico in etanolo per ottenere etil-2-acetil-5-ftalimidopentanoato; 3) reazione del prodotto dalla fase 2 con diazo-p-anisidina per ottenere 2-carbossietil-3-(2-ftalimidoetile)-5-metossi-indolo; 4) reazione del 2-carbossietil-3-(2-ftalimidoetile)-5-metossi-indolo con NaOH 2N e poi con $H_2SO_4$ 20% per ottenere una impura 5-metossitriptamina, che viene purificata mediante ametildisilazano esagonale. Sono ottenuti i derivati mono e disostituito e il derivato monosostituito è idrolisato con metanolo acquoso e quindi ricristallizzato dall'etanolo. Il derivato N-acetil è ottenuto per reazione con anidride acetica. Melatonina di purezza elevata viene usata per profilassi terapeutica e anche contro l'AIDS (Sindrome di Immuno Deficienza Acquisita).

Antefatto dell'invenzione.

La presente invenzione riguarda un metodo di sintesi totale per preparare un prodotto derivato dalla struttura dell'indolo classe tipo triptamina in particolare tipo melatonina o N-acetil-5-metossitriptamina avente un elevato grado di purezza e facilmente solubile per uso terapeutico contro Sindromi da Immuno Deficienza Acquisita o sindromi cosiddetti AIDS.

Come è noto si è trovato che la melatonina (MTL) somministrata in dosi adatte e in tempi giusti è in grado di ridurre la sintesi proteica di ipotalamo e ipofisi e che, inoltre, può inibire la sintesi di gonadostimoline.

Tale azione è probabilmente esercitata mediante una modulazione di trascrizione e repressione genica nonché sulla increzione dei due fattori di crescita GH e PRL in condizioni particolari.

Gli effetti generali sopra citati che sono associati con altre azioni particolari, come descritto in modo più dettagliato in seguito, possono giustificare come utile, anche se non indispensabile, l'uso della melatonina contro i tumori.

In effetti si può ragionevolmente pensare che la melatonina appartenga a quella classe di farmaci che interferiscono con la crescita delle cellule neoplastiche e riducono il tempo di vita dello stesso.

D'altra parte, è anche noto il fatto che i metodi attualmente disponibili per rendere la struttura della triptamina con l'atomo di idrogeno in posizione -5 sostituito dal gruppo $O\text{-}CH_3$, si basano su una serie di reazioni chimiche fornenti 2-carbossietil-3-(2-ftalimidoetile)-5-metossi-indolo, mediante i quali si ottiene 5-metossitriptamina con una pluralità di passaggi relativamente complessi e a basso rendimento di trasformazione.

Più specificamente, metodi di tecnica già noti comprendono un passo di saponificazione alcalina fornendo 2-carbossi-3-(2-O-5-carbossibenzamidoetile)-5-metossi-indolo acido che viene quindi decarbossilato a secco a 250°C, allo scopo di formare ftalimidoetile-5-metossi-indolo, che è poi l'acqua idrazinolizata per ottenere 5-metossitriptamina.

Sommario dell'invenzione.

Pertanto, lo scopo principale della presente invenzione è superare l'inconveniente della purificazione prevedendo la realizzazione, con rese molto elevate di produzione, di 5-

metossitriptamina, il cui metodo comprende essenzialmente una sintesi già nota rispetto ai reagenti, ma effettuata con tecniche nuove a partire da 2-carbossietil-3-(2-ftalimidoetile)-5-metossi-indolo.
Un altro scopo dell'invenzione è quello di realizzare un procedimento che, oltre a semplificare il trattamento, sia in grado di fornire la melatonina a partire dalla 5-metossitriptamina in forma grezza come dalla 5-metossitriptamina in forma pura.
Ancora un altro scopo della presente invenzione è di ottenere un metodo di sintesi totale che fornisca un prodotto molto puro e affidabile, con proprietà curative costanti.

Metodo di solubilizzazione della melatonina in acqua.
1) Per un migliore utilizzo della melatonina, la presente invenzione suggerisce di solubilizzarla in acqua, per facilitare le applicazioni terapeutiche, con un metodo particolare. Infatti come è noto la melatonina è scarsamente solubile in acqua, con solubilità soddisfacente solo a 40-45°C.
Dopo lunga sperimentazione, si è trovato l'adenosina adatta a dissolvere facilmente in acqua la melatonina. In particolare, un rapporto ottimale è stata trovato come segue: per una mole di melatonina (252,27 g) devono essere utilizzate quattro moli di adenosina (267,26 g).

2) Un metodo di preparazione di melatonina avente un elevato grado di purezza consiste nei seguenti passi:
a) reazione della ftalimide di potassio con dibromopropano cui si ottiene 3-bromopropilftalimide;
b) reazione della 3-bromopropilftalimide dal passaggio con uno estere acetoacetico in presenza di etossido di sodio si ottiene etil-2-acetil ftalimido-pentanoato;
c) reazione della etil 2-acetil ftalimido-pentanoato con il sale di diazonio della p-anisidina da cui si ottiene 2-carbossietil-3-(2-ftalimidoetile)-5-metossi-indolo;
d) reazione della 2-carbossietil-3-(2-ftalimidoetile)-5-metossi-indolo con idrossido di sodio e poi con acido solforico da cui si ottiene 5-metossitriptamina grezza;
e) reazione della 5-metossitriptamina grezza con esametil disilazane per ottenere una miscela di prodotti mono e disostituiti e idrolizzando detta miscela con metanolo acquoso per ottenere essenzialmente 5-metossitriptamina pura;
f) reazione della 5-metossitriptamina pura con anidride acetica per ottenere melatonina grezza e purificare detta melatonina grezza mediante cromatografia su gel di silice e una prima separazione con cloruro di metilene seguita da separazione con cloruro di metilene e acetone, ottenendo una soluzione, concentrando detta soluzione di cloruro di metilene e acetone, si ottiene un solido, ricristallizzando detto solido purificato si ottiene melatonina.

3) Il metodo secondo la detta fase d viene eseguita da riflusso a 135°C per 2 ore e mezza fino a soluzione completa, aggiungendo poi un 20% (v/v) di soluzione $H_2SO_4$ ed ulteriore riflusso per quattro ore.

4) Dopo riflusso con detto acido solforico 20%, la soluzione viene raffreddata fino a far precipitare l'acido ftalico e filtrata per l'eliminazione di detto acido ftalico.

5) Dopo filtraggio dell'acido ftalico, è aggiunto idrossido di sodio e la 5-metossitriptamina grezza viene estratta con dicloruro di metilene.

6) Dopo riflusso per 12/14 ore la 5-metossitriptamina grezza è trattata con esametil-disilazano, per ottenere prodotti di sostituzione mono e di silyl, distillare quindi la soluzione sotto pressione normale in modo da recuperare eccesso di esametil-disilazano e idrolizzare i prodotti di sostituzione del silyl con metanolo acquoso allo scopo di ottenere 5-metossitriptamina essenzialmente pura.

*Per Vivere*, **7(2), Settembre 2002, pag.24-26**

# Preparazioni galeniche magistrali e specialità medicinali utilizzate dalla Multiterapia Biologica dei tumori

*Il protocollo terapeutico MDB include preparati farmacologici non reperibili come specialità medicinali e quindi impongono la preparazione di galenici con metodi standard e con necessari controlli chimico-fisici prima della somministrazione.*
*L'importanza di mettere a disposizione del medico gli adeguati preparati per una efficace terapia, ci induce ad esporre di seguito le nozioni fondamentali per preparare in modo adeguato, con cura e diligenza, le principali sostanze da impiegare nella terapia.*

Rif. D.L. 16 giugno 1998 n. 186.

## NORME DI BUONA PREPARAZIONE

L'allestimento della preparazione dovrà essere effettuato dal farmacista su prescrizione del medico e dovrà attenersi, oltre a quanto previsto dal prescrittore nella ricetta, alle più recenti acquisizioni della tecnologia farmaceutica nel rispetto altresì delle precauzioni e norme di conservazione riportate nelle Farmacopee o in testi scientifici autorevoli. Le materie prime devono corrispondere per caratteristiche e purezza a quanto stabilito dalla Farmacopea o da altri testi autorevoli. Tali requisiti debbono essere certificati dal produttore mediante certificato di analisi e scheda tecnica. E' comunque buona norma ricontrollare tali requisiti (in farmacia se attrezzata o presso laboratori idonei), in quanto è responsabilità del farmacista garantire l'identità e la qualità di ogni singola sostanza oltre che del prodotto finito. La legge è uguale per tutti: il prodotto che fa la farmacia deve avere la stessa qualità di quello che fa l'industria.

## MELATONINA CONIUGATA NELLA MULTITERAPIA BIOLOGICA DEL METODO DI BELLA

### Premessa

La melatonina viene assorbita nel nostro organismo legata con l'adenosina; legame che non ha la forza del legame interatomico covalente, ionico metallico, ma è più debole (neben-valenz di Hantzsch & Werner, minor-valenz secondo Huggins). Questo legame d'idrogeno (Hydrogen Bond, Wasserstoffbrűck) rappresenta la maggior forza coesiva tra molecole contenenti gruppi -$NH_2$ e -OH ed altre contenenti gruppi -OH o CO (Pauling); esso è più forte delle cosiddette forze di Van der Walls, ed è relativamente aspecifico, poco energetico (<3Kca/mol), impiega qualche miliardesimo di secondo per disintegrarsi, per cui può intervenire rapidamente nei processi di riconoscimento intermolecolare.

La melatonina con adenosina in rapporto 1 a 4 stabilizzata con circa il 30% di glicocolla viene impiegata nel MDB, nella forma farmaceutica compresse o liofilizzato fiale. Nel preparato magistrale secondo il metodo Di Bella, la melatonina viene coniugata alla adenosina (nucleoside del DNA, ove la adenina funge da base azotata) tramite una miscelazione o liofilizzazione, ciò allo scopo di garantire una migliore biodisponibilità del medicamento.

La melatonina e in grado di formare un complesso, probabilmente di tipo Pi greco per overlap degli orbitali dei sistemi aromatici e dei doppietti elettronici degli atomi di azoto, con l'adenosina. Il complesso e poi stabilizzato dalla glicina, che, dato il pKa piuttosto basso, contribuisce alla

formazione di ponti d'idrogeno. La formazione del complesso comporta una variazione notevole rispetto alle caratteristiche dei singoli componenti: il complesso e infatti completamente idrosolubile, a concentrazioni a cui adenosina e melatonina da sole precipiterebbero o nemmeno si scioglierebbero.

**Ricette**

**Compresse 2 mg**

Melatonina 2 mg
Adenosina 9 mg
Glicina 5 mg
Lattosio 55 mg
Amido di mais 41 mg
Cellulosa micr. 35 mg
Mg stearato 3 mg
TOTALE 150 mg

**Fiale 20 mg**

Melatonina 20 mg
Adenosina 90 mg
Glicina q.b. all'isotonia

**Tecnologia**

Nonostante esso rimanga un preparato galenico, viene procedurato il metodo produttivo per garantire la massima qualità del prodotto. Per le lavorazioni vengono applicate le norme di buona preparazione.
Sono previsti due metodi di allestimento. I1 primo prevede di liofilizzare la soluzione acquosa di melatonina-adenosina-glicina e quindi preparare le compresse con la polvere cosi ottenuta addizionando gli eccipienti. La seconda per compressione diretta, più economica, consiste nel miscelare le polveri della lavorazione della melatonina. A questo proposito e stato fatto presso il laboratorio Provinciale di Trento una prova di idrosolubilità. Sui campioni melatonina—glicina miscelati o granulati e sui campioni di melatonina-adenosina-glicina liofilizzati. E‘ risultato che solo il liofilizzato offre un notevole aumento di idrosolubilità.
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**Percentuali di solubilità**

Campioni: Melatonina/MLT – Adenosina/ADE – Glicina/GLC

A granulare mix: MLT 68,67 - ADE 111,9 - GLC 74,11
B liofilo: MLT 81,91 - ADE 102,07 - GLC 99,12
C granulare: MLT 72,32 - ADE — - GLC —
D liofilo: MLT 79,85 - ADE 98,58 - GLC 96,01
E granulare: MLT 71,1 - ADE 104,18 - GLC 94,62

Il liofilizzato può essere preparato anche in fialette bevibili.

**Lavorazione per miscelazione**

Si pongono nel miscelatore la melatonina e in diluizioni successive 1'adenosina e infine la g1icina. Al termine delle aggiunte si lasciano a miscelare per varie ore. Quindi dopo aver ottimizzato la miscelazione dei principi attivi si aggiungono gli eccipienti e si miscela, per gli ultimi 5 minuti di miscelazione si aggiunge i1 magnesio stearato (lubrificante e glidante in fase di compressione).
I tempi di miscelazione ottimali devono essere standardizzati e determinati con un appropriato studio. Finita la miscelazione si procede alla fase di compressione.
Pesate per lotti standard da 2Kg:
Adenosina base 120 gr
Melatonina 26,7 gr
Glicina 66,7 gr
Lattosio 733,3 gr
Amido di mais 546,7 gr
Magnesio stearato 40 gr

**Lavorazione in liofilizzatrice**

Il procedimento di liofilizzazione permette di rea1izzare su diverse sostanze una efficace disidratazione, tale che i1 prodotto finito mantenga integre le proprie particolari caratteristiche organolettiche e, addizionato anche a distanza di tempo di acqua, ripristini le specifiche proprietà della soluzione primitiva.

**Confezionamento delle compresse**

Il confezionamento delle compresse viene fatto in blister per ripararlo dalla luce e dall'umidità.
Il confezionamento viene effettuato entro un'ora dalla compressione. In ultima fase si procede ad etichettare i blister secondo la legge.

**Lavorazione per fiale orali**

Il liofilizzato ottenuto può essere impiegato anche in fialette orali, in cui 1'acqua viene aggiunta in modo estemporaneo al momento della somministrazione.

**Lavorazione per fiale iniettabili**

La glicina nelle fiale è presente sia come coadiuvante di liofilizzazione che per rendere isotonica la forma farmaceutica stessa. Non essendo veicolata dalla adenosina intestinale, per la melatonina iniettabile è ancora più importante essere legata all'adenosina esogena con legame ad idrogeno.
Le fiale devono rispettare la tecnologia dei preparati STERILI-APIROGENI.
La via parenterale viene utilizzata quando esistono difficoltà di assorbimento della melatonina da parte dell'apparato gastrointestinale, impedimenti all'ingestione della melatonina e sopratutto quando bisogna ricorrere a dosi notevoli di melatonina.
Le fiale iniettabili da 20 mg in MLT liofilizzata, al momento dell'uso vanno sciolte in 10 ml d'acqua per preparazioni iniettabili. Non c'è bisogno di utilizzare la soluzione fisiologica in quanto la presenza di glicina la rende isotonica. Si può eventualmente facilitarne la dissoluzione scaldando leggermente a bagnomaria. La quantità ottenuta si può iniettare tutta in una volta ma molto lentamente, perché l'adenosina è un vaso dilatatore, le arteriole si dilatano e si potrebbe provocare un calo emo-pressorio. Per questo è bene prima saggiare la reattività del paziente, usando da 1 a 5 ml di soluzione. La soluzione può essere iniettata per via intramuscolare o endovenosa.

**Analisi**

Si analizza il prodotto finito eseguendo i seguenti saggi:
1. Uniformità di peso nelle forme farmaceutiche a dose unica: si determina il peso medio di 20 unità di compresse prelevate a caso da uno stesso lotto. Non più di due di tali unità possono presentare uno scarto rispetto al peso medio superiore al 7,5% e nessuna unità uno scarto maggiore del doppio di tale percentuale, considerando il fatto che le pastiglie pesano 150 mg.
2. Determinazione di disaggregazione, nel caso del protocollo Di Bella è richiesto inferiore a 5 minuti.
3. Determinazione quali/quantitativa dei principi attivi.

## SOLUZIONE DI RETINOIDI IN VITAMINA E

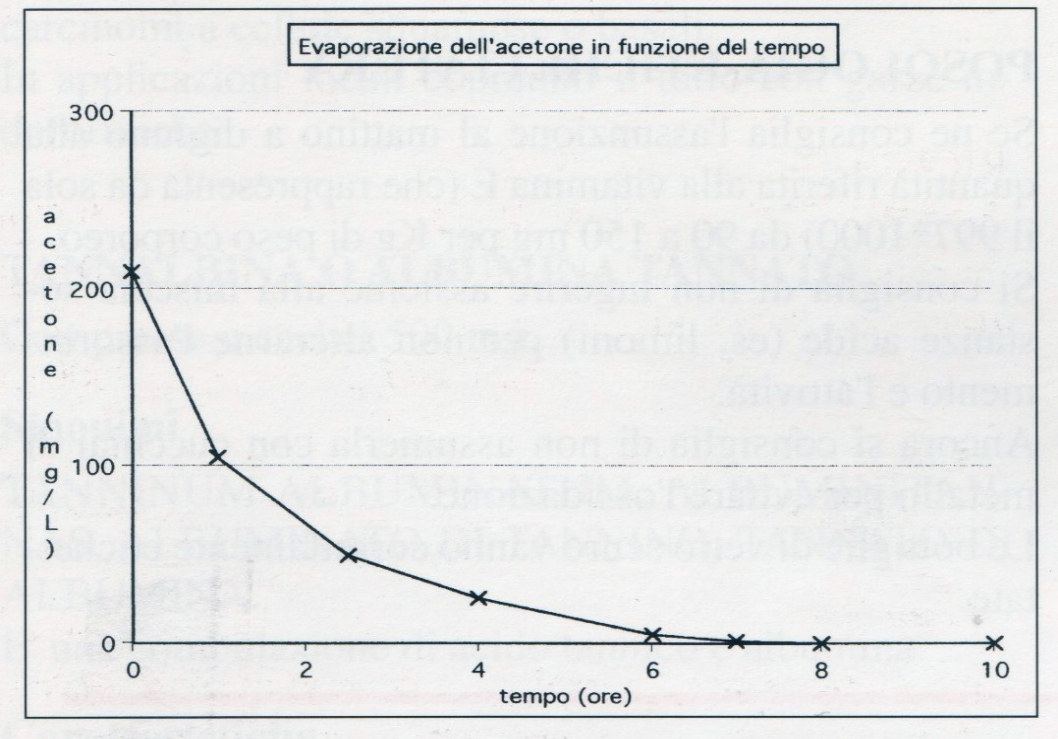
**Premessa**
Nella miscela di vitamine liposolubili MDB la Vitamina A, il Beta-carotene, l'acido Trans-retinoico sono formidabili esoergoni usati nella quantità dell'ordine di millesimi di mg.
Le quantità hanno valore determinante per dare un risultato farmacologico e non creare fenomeni di tossicità.
Agiscono sul processo di crescita nucleare e stabilizzano i potenziali di membrana cellulare.
La Vitamina E esercita, oltre che salvaguardare la membrana cellulare, una azione di difesa antiossidante. La composizione è studiata in modo tale da non arrivare mai ad un accumulo, alle dosi prescritte.
Nel suo insieme la miscela è importante per eliminare i radicali liberi nella microcircolazione, ridurne gli effetti tossici, consistenti nella produzione di stati infiammatori, riperfusione di organi ischemici, aumentata tossicità di alcune sostanze, alterazione del parenchima respiratorio, con produzione a lungo andare di focolai, di enfisema, alterata risposta ai batteri, complessi immunitari, formazione di coaguli intravasali.

**Ricetta**
AXEROFTOLO PALMITATO 0,5 gr
ACIDO RETINOICO 0,5 gr
BETA CAROTENE 2 gr
d,1-alpha-TOCOPHERYL ACETATO 1000 gr

## TECNOLOGIA

**Premessa**
Le materie prime da impiegare devono avere la massima purezza e devono essere conservate alla temperatura richiesta. La loro conservazione dopo la prima apertura del contenitore, comporta l'immissione di azoto come gas inerte e l'osservanza di quanto prescritto dal produttore e dalle farmacopee.
L'acido Trans-retinoico e il Beta-carotene sono allo stato solido mentre la Vitamina A Palmitato e l'alpha-Tocoferile acetato sono liquidi e molto viscosi, a temperatura ambiente. E' possibile mescolarli tra loro per ottenere una dispersione a livello molecolare.
La soluzione può essere allestita mediante agitazione non turbolenta. E' necessario un sistema di agitazione con controller di temperatura efficiente, da scegliere in funzione dell'entità dei lotti di medicamento da preparare. Le metodiche di preparazione differiscono leggermente a seconda delle apparecchiature impiegate, pur rimanendo simili nei principi generali.
Si può usare l'acetone anidro o l'alcool etilico come coadiuvanti della solubilizzazione dell'acido

Trans-retinoico e del Beta-carotene. In tal caso bisogna operare in modo che vengano poi completamente eliminati sotto flusso di azoto.
A questo proposito e stata condotta la seguente prova di evaporazione dell'acetone, presso il laboratorio provinciale di Igiene Pubblica di Trento.
50 gr di soluzione di retinoidi contenente acetone pari a 0,21 gr/l vanno posti in un becker da 100 ml, mantenuto a temperatura costante di 40°C e sotto flusso di azoto di circa 160 ml/minuto.
La determinazione dell'acetone è stata eseguita con tecnica gas-cromatografica con spazio di testa.
Primo risultato: la presenza di acetone non ha nessuna influenza sul titolo dei retinoidi.
Secondo risultato: cui fa riferimento il grafico, l'acetone viene completamente eliminato dopo 6 ore sotto flusso di azoto.




**Azoto**
In tutti i casi è necessario l'azoto.
Le bombole di azoto farmaceutico purissimo possono essere acquistate presso rivenditori di ossigeno terapeutico e/o di altre sostanze gassose. L'azoto contenuto in tali bombole si trova alla pressione di 200 atmosfere; per l'impiego è pertanto necessario collegare alla bombola un riduttore di flusso munito di due manometri, per la misura della pressione nella bombola ed in uscita. Il flusso in uscita viene variato mediante una regolazione fine presente sul riduttore. All'ugello si collega un tubo che porterà in linea un filtro a setto poroso.
Lo scopo del filtro è di trattenere le impurezze metalliche derivanti dalle pareti della bombola, che la corrente di gas potrebbe trascinare; tali impurezze sarebbero un potente catalizzatore di ossidazione, quindi deleterie per la stabilità del prodotto.
Si tenga presente che le sostanze, con esclusione della Vitamina E acetato, sono sensibili:
1) alla luce
2) all'aria, alterazioni ossidative
3) a determinate temperature

**Luce**
Particolare cura deve essere posta nell'evitare l'esposizione alla luce solare diretta attinica, radiazioni nello spettro dell'ultravioletto, delle sostanze. Si operi usando recipienti opachi alla luce e in spazi alla luce rossa.

**Aria**
I componenti sono tutti sensibili all'ossidazione da parte dell'ossigeno presente nell'aria; il componente più sensibile è sicuramente il Beta-carotene, data la presenza di un esteso sistema Pi-greco che conferisce anche il tipico colore rosso mattone. Il Tocoferolo agisce come radical scavenger, in grado di catturare l'ossigeno dell'aria e di formare un legame labile con gli elettroni spaiati della molecola di ossigeno in parte proteggendo dall'ossidazione il Beta-carotene.
E' comunque possibile operare in modo tale da evitare anche questo legame. Allo scopo si sfrutta la maggiore solubilità nei lipidi dell'azoto rispetto all'ossigeno, saturando con azoto il Tocoferolo prima dell'aggiunta del Beta-carotene e degli altri retinoidi.

**Temperatura**
E' da notare che, in assenza di ossidanti, la resistenza alla degradazione termica delle sostanze è relativamente buona ed è comunque in funzione del tempo di lavorazione.
Si consiglia, comunque, di non superare nella lavorazione la temperatura di 40°C.

**Metodo di allestimento D.L. 16-06-98 n.186, g.u. 1706-98 n.139**

Preparazione di 1000 gr di soluzione di retinoidi con solvente organico acetone o alcol etilico 95%.
Il preparatore deve lavorare sotto cappa, a temperatura ambiente, con guanti, mascherina ed occhiali.

**1° fase**
Si pesano 0,5 gr di acido Trans-retinoico, che deve essere ridotto in polvere finissima, e si sciolgono in un mortaio aggiungendo a gocce il solvente organico; sempre in mortaio si sciolgono 2 gr di Beta-carotene in solvente organico, le polveri rimanenti devono essere conservate al freddo e sotto azoto.

**2° fase**

Le polveri disciolte si versano nel miscelatore e si inizia ad inserire, lentamente e sotto agitazione, la Vitamina E fino a 100 cc circa.
Gorgogliare l'azoto a medio flusso fino ad eliminazione del solvente organico nel caso dell'acetone, l'alcol permane in soluzione ma in concentrazione atossica.

**3 ° fase**
Portare gradualmente e lentamente la temperatura fino a 40 ± 2°C, sotto agitazione continua. Lasciare raffreddare per 15-20 minuti e versare lentamente, sempre sotto agitazione, altri 100 cc di Vitamina E; aumentare leggermente la velocità di agitazione per circa 10 minuti.

**4° fase**
Aggiungere 0,5 gr, goccia a goccia, di Axeroftolo palmitato e continuare ad agitate per 10 minuti; versare altri 100 cc di Vitamina E, mantenendo la velocità di agitazione bassissima per almeno 15 minuti; ripetere questa operazione fino a 1000 gr di Vitamina E. Chiudere ermeticamente il miscelatore e lasciare reagire per almeno 6 ore.

**5 ° fase**
Aprire il rubinetto, riempire le bottiglie di vetro scuro e chiuderle immediatamente; conservare al riparo dalle fonti di luce e calore. Se il consumo non è immediato, immettere corrente di azoto prima di chiudere.

**MATERIALI**

Cappa chimica.
Luce rossa.
Miscelatore per liquidi ad alta viscosità con controller di temperatura.
Bombola di azoto da 5 $m^3$, 200 bar, con sistema di flussaggio.
Filtro da gas (Millipore) da 0,22 micron.
Contenitore inox.
Bottiglie di vetro scuro.

**ANALISI**

La soluzione di retinoidi in Tocoferolo deve presentarsi: limpida, di colore rosso mattone scuro, viscosa, inodore, insapore.
Il criterio di giudizio più semplice e diretto è la trasparenza, infatti 1e soluzioni vere sono otticamente vuote, né si ha evidenza di effetto Tyndall come è invece normale nelle forme solubilizzate.
Assenza di acetone.
Verifica della omogeneità di dispersione della Vitamina A, Beta-carotene. Acido trans-retinoico nella Vitamina E.
Titolatura: verifica della eventuale presenza di composti di alterazione formatisi durante l'allestimento.
Stabilità nel tempo.

**CONSERVAZIONE**

La conservazione del prodotto finito può avvenire a temperatura ambiente in flaconi di vetro scuro. Per una migliore conservazione è preferibile confezionare i flaconi insufflandovi azoto.

**POSOLOGIA-ETICHETTATURA**

Se ne consiglia l'assunzione al mattino a digiuno da 90 a 150 mg per Kg di peso corporeo, alla quantità riferita alla Vitamina E che rappresenta da sola il 997 per mille. Si consiglia di non ingerire assieme alla miscela sostanze acide, succo di limone/arancia, per non alterarne l'assorbimento e l'attività.
Ancora si consiglia di non assumerla con cucchiai di metallo per evitarne 1'ossidazione.
Le bottiglie di vetro scuro vanno correttamente etichettate.

Melatonina p. m. 232,27

Triptofano p. m. 267,24

Piridossina p. m. 75,04

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267,24 x 4 = 1068,96

75,04 x 8 = 600,56

232,27 x 1 = 232,27

1901,79

melatonina = 12,203 %